UNIVERSITY OF THESSALY

SCHOOL OF HEALTH SCIENCES

FACULTY OF VETERINARY MEDICINE

CLINIC OF MEDICINE

ADVANCING THE UNDERSTANDING OF ANTIBIOTIC RESISTANCE AND NEONATAL PIGLET DIARRHOEA

A thesis submitted for the degree of Doctor of Philosophy

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Karditsa, 2024

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“Cyclops, if any one of mortal men shall ask thee about the shameful blinding of thine eye, say that Odysseus, the sacker of cities, blinded it, even the son of Laertes, whose home is in Ithaca.”

(Homer, The Odyssey, Book IX, lines 502-505. Translated by A.T. Murray, Harvard University Press, 1919)

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INTRODUCTION

Antibiotic resistance (AMR) is a growing global health crisis that extends beyond human medicine into the realm of veterinary practice and livestock management. Particularly in swine farms, the use of antibiotics has become a double-edged sword, crucial for maintaining animal health and productivity but also a significant driver of AMR. This dissertation explores the prevalence, causes, and implications of antibiotic resistance in livestock, with a focus on swine farms.

Antibiotic resistance is alarmingly prevalent in livestock, especially in swine farms where intensive farming practices necessitate the frequent use of antibiotics. Studies have shown high levels of resistant bacteria in pigs, including strains that produce extended-spectrum beta-lactamases (ESBLs), and enzymes that confer resistance to a broad range of beta-lactam antibiotics, including third-generation cephalosporins. For instance, recent research in the Greek swine industry revealed that 36.5% of isolates were resistant to third-generation cephalosporins, underscoring the extent of the problem.

Several factors contribute to the development of antibiotic resistance in swine farms. One of the primary causes is the overuse and misuse of antibiotics. Antibiotics are often administered not only for therapeutic purposes but also as metaphylactic and growth promoters. This widespread use creates selective pressure that favors the survival and proliferation of resistant bacteria. Furthermore, inadequate dosing and incomplete treatment courses can exacerbate the problem by not fully eradicating pathogens, allowing resistant strains to emerge and multiply.

Another critical factor is horizontal gene transfer among bacteria, where resistance genes are transferred between different bacterial species. This process is facilitated by the close quarters and high-density populations typical of intensive swine farming. Additionally, poor farm management practices, such as insufficient biosecurity measures and hygiene, can increase the spread of resistant bacteria within and between farms.

The implications of antibiotic resistance in swine farms are far-reaching and severe. For the livestock industry, AMR leads to increased morbidity and mortality rates among animals, reducing productivity and causing economic losses. The effectiveness of commonly used antibiotics diminishes, necessitating the use of more potent and often more expensive drugs.

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From a public health perspective, antibiotic resistance in livestock poses a significant threat. Resistant bacteria can be transmitted to humans through direct contact with animals, consumption of contaminated meat, and environmental pathways such as water and soil. This zoonotic transfer can result in infections that are difficult to treat, prolonged illnesses, and increased healthcare costs. Moreover, the presence of multi-drug-resistant bacteria in livestock undermines the effectiveness of critical antibiotics, jeopardizing both human and veterinary medicine.

Addressing antibiotic resistance in swine farms requires a multifaceted approach. Implementing stricter regulations on antibiotic use is essential to ensure that these drugs are used judiciously and only when necessary. Enhanced surveillance systems are needed to monitor antibiotic use and resistance patterns, providing data to inform policy and practice.

Promoting good farm management practices, such as improved hygiene, biosecurity measures, and alternative disease control strategies, can reduce the reliance on antibiotics. The use of probiotics, prebiotics, and other non-antibiotic growth promoters should be encouraged to maintain animal health and productivity without contributing to AMR.

Furthermore, adopting the “One Health” approach, which recognizes the interconnectedness of human, animal, and environmental health, is crucial in tackling antibiotic resistance. This approach advocates for collaboration across disciplines and sectors to develop comprehensive strategies that address the root causes of AMR.

Antibiotic resistance in livestock, particularly in swine farms, is a pressing issue that requires immediate and coordinated action. By understanding the prevalence, causes, and implications of AMR, and by implementing effective mitigation strategies, we can safeguard animal health, protect public health, and ensure the sustainability of the livestock industry. It is imperative to balance the benefits of antibiotic use with the responsibility to preserve their effectiveness for future generations.

On the other hand, neonatal piglet diarrhoea is a significant concern in swine production, posing both economic and animal welfare challenges to veterinarians and farmers. This condition, often caused by infectious agents such as *Escherichia coli* and Clostridia, can lead to high morbidity and mortality rates among newborn piglets. The severe dehydration and nutrient loss associated with diarrhea, can stunt growth and development, leading to reduced weight gain and overall productivity in affected piglets. Furthermore, cases of neonatal piglet diarrhoea require increased veterinary care, medication, and labor, all of which contribute to higher operational costs for swine producers. Effective management strategies, including stringent biosecurity measures,

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optimal nutrition, and preventive measures, are crucial to mitigating the impact of neonatal piglet diarrhea and ensuring the sustainability and profitability of swine production operations.

Our doctoral thesis examines antimicrobial resistance and neonatal piglet diarrhoea in Greek farms. Our investigation produced two publications (Tsekouras et al., 2022; Tsekouras et al., 2023), contributing to the following research questions:

1. What is the prevalence of ESBL-producing *Enterobacteriaceae* in the Greek swine industry, and how do their AMR phenotypes and genotypes vary? (Tsekouras et al., 2022)

2. How do various risk factors, such as past antibiotic use and herd size, contribute to the increased levels of AMR in Greek swine farms? (Tsekouras et al., 2022)

3. What are the specific AMR profiles of ESBL-producing isolates in Greek swine farms, particularly regarding non-β-lactam antibiotics and third-generation cephalosporins? (Tsekouras et al., 2022)

4. How does the implementation of a surveillance system under the One Health approach affect the rational use of antibiotics and the overall public health in relation to the Greek swine industry? (Tsekouras et al., 2022)

5. What is the involvement of Enterotoxigenic (ETEC) *E. coli*, and clostridia in neonatal piglet diarrhoea? (Tsekouras et al., 2023)

6. What is the impact of different intervention strategies, including antibiotics, probiotics, and acidifiers, on the detection of enteric pathogens like *E. coli* and *C. difficile* in neonatal piglets? (Tsekouras et al., 2023)

7. What is the involvement of *C. difficile* in neonatal piglet diarrhoea? (Tsekouras et al., 2023)

These research questions aim to provide a comprehensive understanding of antibiotic resistance and neonatal piglet diarrhoea in the Greek swine industry and inform strategies to mitigate their impact.

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CHAPTER I

Review of literature

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1. Antibiotic use in the swine industry

1.1. History

Since the middle of the 20th century, antimicrobial agents have been widely used in livestock to improve productivity and protect animals' health (Lekagul et al., 2019). The discovery of antimicrobials was essential for veterinary medicine, contributing to the treatment of animal diseases and enhancing animal welfare (Economou and Gousia, 2015). While the initial aim of antimicrobial usage was to treat animals, they were also commonly used as prophylactic agents and growth promoters (Kimera et al., 2020).

The impact of antimicrobials in growth promotion was studied extensively starting in the late 1940s, with Stockstad and Jukes highlighting remarkable results in poultry and pig production after antimicrobial usage as feed additives (Stockstad and Jukes, 1949). From 1949 to the 1960s, around 100,000 studies on the effects of antimicrobials as growth promoters had been published (Thoms, 2012). Alexander Fleming, a few years after the discovery of penicillin, was the first to notice the development of resistance in organisms, leading to the need for prudent use of antimicrobials (Fleming, 1945).

The consequences of excessive antimicrobial usage in animal production were first observed after 1965 (Waluszewski et al., 2018) but were quickly overcome due to increased demand for animal protein and reduced production costs (Kirchhelle, 2018). In the following years, antimicrobials were routinely used in three ways: therapeutically to treat sick animals, metaphylactically to prevent the spread of contagious factors, and prophylactically to prevent diseases in animals of the same age in previous batches on the farm (Callens et al., 2012).

As living standards rose in economically developing nations over the next decades, the consumption of animal protein products increased, leading to intensive livestock production and extensive antimicrobial usage (Van Boeckel et al., 2015; Dall, 2018). Antimicrobials were used not only for treatment and prevention but also became an economic tool for farm management (Begemann et al., 2018). In poultry and swine production, the mass administration of medications via feed or water expedited the treatment and handling of animals, increased animal density on farms, and shortened production cycles (Thoms, 2012; Kirchhelle, 2018).

Around the 1960s, veterinarians in the United Kingdom became concerned about antimicrobial resistance (AMR) and its potential threats to animal and human health. The government established the Swann commission, which concluded in 1969 that

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antimicrobial usage did indeed promote bacterial resistance and could jeopardize animal and human health (Kahn, 2016; Kirchelle, 2018; Begemann et al., 2018). Despite the urgent need for change, the committee acknowledged the economic benefits of antimicrobial usage in animal production (Kahn, 2016).

As the scientific community became aware of the negative implications of antimicrobial usage, particularly regarding bacterial resistance and drug effectiveness, antimicrobials' role in the development of intensive livestock production was brought into question (Kirchhelle, 2018; Wise, 2007; Waluszewski et al., 2018). Since 2006, the European ban on antibiotic growth promoters (AGPs) has been ratified by Regulation (EC) No. 1831/2003 on additives for use in animal nutrition (EC, 2003). Antimicrobials, while instrumental in the evolution of the animal sector, had unintended consequences that required careful consideration (Thoms, 2012).

1.2. Antimicrobial resistance in pigs

The livestock sector is considered to be the primary user of antimicrobials worldwide and is blamed as the main contributor to the development of AMR (DANMAP, 2018). While the principal role of antibiotic use in food animals should be therapeutic, use has also been driven by the objective of improving farm productivity and income in the past where poor management and biosecurity practices were sometimes covered up by high antimicrobial use (Dewulf et al., 2022). In 2013, over 130,000 tons of various antimicrobials were used in animal production, representing more than 60% of the total global antimicrobial usage. Experts predict that antimicrobial usage will exceed 200,000 tons by 2030 (Van Boeckel et al., 2017). For instance, just 42 countries in the world systematically gather data regarding the use of antibiotics in livestock (FAO, 2010). The isolation of antimicrobial-resistant bacteria is now a common occurrence in most livestock animals (Argudin et al., 2017).

Despite religious restrictions worldwide, pork meat accounts for over one-third of human meat consumption (Underwood et al., 2014), and pigs are one of the most numerically and biologically significant farmed animals (Magnusson et al., 2019). Pig production plays a crucial role in the supply chain and has a significant economic impact (Monger et al., 2021). The rapid increase in human population has led to an urgent demand for animal and agricultural production to meet the need for animal protein (Monger et al., 2021).

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The industrialization of pig production has resulted in various changes in pig breeding practices, such as space restrictions, limitations on natural behavior expression, and stressful practices from birth (Schmid et al., 2021; Herskin et al., 2017). This makes pigs more vulnerable to pathogens and predisposed to diseases (Moberg et al., 2000). In Europe, the main antimicrobial classes used in pig production for enteric and respiratory diseases are β-lactams, polymyxins, macrolides, and tetracyclines which are considered highly important for the animal sector (WHO, 2015). Some of these antimicrobials, such as macrolides, certain β-lactams, and polymyxins, are also classified as critically important for humans too (WHO, 2017). Colistin, an essential antimicrobial for human health as a last-resort treatment, is widely used in swine production for treating *Escherichia coli* (*E. coli*) infections (Rhouma et al., 2016).

About 90% of pigs in Europe receive antimicrobial treatment in their lifetime, leading to the transmission of antimicrobial-resistant bacteria between treated and untreated animals (Sarrazin et al., 2019). The discovery of the plasmid-mediated colistin-resistant gene (MCR-1) in isolated *E. coli* strains from pigs raised global concerns (Liu et al., 2016). The isolation of resistant *E. coli* strains has been increasing steadily since 2005 (Chantziaras et al., 2014). Common pathogens in swine clinical cases include *E. coli*, *Actinobacillus pleuropneumoniae* (*A. pleuropneumoniae*), *Streptococcus suis* (*S. suis*), *Staphylococcus hyicus* (*S. hyicus*), and *Bordetella bronchiseptica* (*B. bronchiseptica*), some of which are also found in human diseases (Zimmerman et al., 2012).

ESBL-producing *E. coli* has been characterized as a significant threat to human health globally by the CDC (CDC, 2019). A long-term study in Denmark found that over two-thirds of *E. coli* strains were resistant to tetracycline and streptomycin, with a notable percentage showing resistance to other antimicrobials (Holmer et al., 2019). Similar findings were reported in Quebec, where *E. coli* and *Salmonella* spp. strains showed resistance to three or more antimicrobial classes (MAPAQ, 2020).

In China, a survey found that *E. coli* strains had increased resistance to various antimicrobials, with ESBL-producing strains showing multidrug resistance (Li et al., 2022). Antimicrobial usage is estimated to increase by 11.5% by 2030 (Tiseo et al., 2020). Pig manure, commonly used as fertilizer, has been found to contain antimicrobial residues that contribute to the transfer of resistant bacteria and genes to the environment (Kim et al., 2010).

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Factors such as climate change and decreased biodiversity are believed to promote antimicrobial resistance (Hoberg and Brooks, 2015). Data from developing countries are limited due to a lack of epidemiological research (Van Boeckel et al., 2019).

In conclusion, antimicrobial resistance poses a significant threat to human health, and a better understanding of antimicrobials and their prudent use is needed to minimize their impact on human and animal health (Monger et al., 2021). The current lack of comprehensive knowledge regarding global antibiotic usage underscores the crucial need for an effective strategy that fosters collaboration across various international sectors, including human and veterinary medicine, agriculture, animal production, and consumers (WHO, 2015). Despite regulatory differences among countries, swine production, much like other sectors, employs antibiotics and must strive to balance animal welfare, consumer expectations, and sustainable development. (Monger et al., 2021).

1.3. Public Health issues

Antimicrobial-resistant bacteria from livestock can be transmitted to humans in many ways, including contact (both direct and indirect) among farm workers, farmers, veterinarians, slaughterhouse workers, and animals. Consuming contaminated meat and animal products is the main way these bacteria enter the community (Founou et al., 2016). The excessive and imprudent use of antimicrobials in animals and humans is considered to be the leading cause of AMR worldwide (Goossens et al., 2005). This excessive antimicrobial use not only poses a threat to human health but also to food security (Founou et al., 2021). Currently, there are approximately 700,000 deaths in humans each year due to ineffective antimicrobials resulting from antimicrobial resistance, and the World Health Organization (WHO) predicts that this number could rise to ten million by 2050 (WHO, 2020).

Antimicrobials, which were once considered one of the biggest discoveries of the 20th century, are now ineffective in treating many individual cases due to antimicrobial resistance (Landecker, 2016). Bans on antimicrobial substances, particularly those used in human medicine, began in Europe in the early 1970s (Aarestrup, 2000; Swann, 1969).

Antimicrobial-resistant bacteria transmitted through food can infect humans in various ways, such as in slaughterhouses or during food processing. A recent study from Spain linked one-third of clinical cases of salmonellosis to pig production, noting a high percentage of multidrug-resistant non-Typhoidal *Salmonella* strains in fecal samples and pig carcasses (De Knegt et al., 2015; EFSA, 2019).

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Due to the significant threat of antimicrobial resistance in recent years, veterinary and human medical professionals have chosen to reduce the prescribing and use of antimicrobials in a more rational manner (O'Neill, 2021). In addition to scientific regulations, consumers are demanding a decrease in the use of antimicrobials in pig production, as recent research in Denmark has shown (Denver et al., 2021).

One Health is a comprehensive health framework that advocates for understanding human and animal health as interconnected and mutually reliant, along with their integral connection to the ecosystems they inhabit, all existing in a harmonious relationship (van Bruggen et al., 2019). The One Health approach emphasizes the interconnection of animal, human health, and the environment, highlighting the need for comprehensive efforts against antimicrobial resistance (Unknown author 2016). Various studies have identified different ways in which antimicrobial-resistant bacteria spread in the community, including through the environment, animal products, and pig farm employees. *S. suis*, a potentially zoonotic bacterium with high levels of AMR, can cause severe clinical conditions in humans, such as septicemia, meningitis, and endocarditis, often spread through direct contact between pigs and workers in pig production (Holmer et al., 2019). Furthermore, individuals with chronic illnesses such as asthma or diabetes are also at risk of severe consequences due to AMR (CDC, 2018). Moreover, the CDC (2013) warns that antimicrobial resistance jeopardizes the immune system's ability to combat infectious diseases in vulnerable patients, such as those who are immunocompromised or undergoing surgery.

The excessive and irrational use of antimicrobials in food-producing animals is a key factor in the global increase of AMR (Van Boeckel et al., 2017). To decrease the need for antibiotics globally, new animal health management methods must be introduced in many countries (Davies and Wales, 2019). This may increase production costs, which has been identified as a hindering factor for reducing antibiotic use and for reducing capacity for reinvestment in farm facilities (Hockenhull et al., 2017).

1.4. Economic impact

Antimicrobials were once considered the "wonder drug" of the 20th century, revolutionizing human medicine. However, the success and efficacy of antimicrobials are currently being threatened by the rise of AMR, and new classes of antimicrobials need to be discovered. The impact of AMR on society is not just economic, but also has various social effects (Ahmad and Khan, 2019).

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Shrestha et al. (2018) recently estimated the economic burden of AMR caused by common pathogens such as *Staphylococcus aureus*, *E. coli*, *Klebsiella pneumoniae* (*K. pneumoniae*), and *Pseudomonas aeruginosa* (*P. aeruginosa*) to be approximately $3 billion annually in the USA. AMR is a global phenomenon that affects all people, regardless of social or economic status. It is predicted that increased morbidity and mortality will lead to a loss of human resources in the future (Ahmad and Khan, 2019).

Aside from the human and animal losses related to illness and death caused by AMR, society may be affected by a sense of fear (National Academies of Science, 2018). Economic losses due to AMR can be classified as direct (medical care costs, including treatments and hospitalization) and indirect (societal impacts, such as loss of productivity and human capital) (National Academies of Science, 2018).

The total cost of AMR in the USA is currently estimated to be over $2 billion, with a significant portion of this cost related to medical care and the additional expenses of effective antimicrobials (Innes et al., 2020). By 2050, it is predicted that around 10,000,000 people will die because of AMR, leading to a global economic burden of approximately $100 billion (O'Neill, 2016; O'Neill, 2014).

Furthermore, the inefficacy of current antimicrobials in treating common infections will result in the use of last-line antimicrobials (such as polymyxins or carbapenems) at increased costs and with various side effects. These last-line antimicrobials are not readily available in low-income countries (WHO, 2015). The cost of AMR has recently been estimated to exceed $50 billion in the USA, attributed to losses in productivity and medical care (CDC, 2013; Prestinaci et al., 2015). The changes that AMR also will bring to global trade are expected to be substantial, requiring the establishment of new restrictions (Lekagul et al., 2019).

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2. Neonatal piglet diarrhoea

2.1. Introduction

Neonatal diarrhoea poses a significant challenge in modern swine farming, leading to high mortality rates, decreased growth, and increased treatment expenses (Sjölund et al., 2014). The neonatal period, typically spanning the first few days up to a week after birth, is particularly vulnerable to such outbreaks (Mesonero-Escuredo et al., 2018). In cases of diarrhoea, multiple factors - both infectious and non-infectious - play a role in affecting suckling piglets, with stress, suboptimal management, and nutrition being notable contributors to disease susceptibility (Vidal et al., 2019). Common pathogens implicated in neonatal piglet diarrhoea include *E. coli*, clostridia [*Clostridium perfringens* (*C. perfringens*) type C and type A, *Clostridioides difficile* (*C. difficile*)], *Enterococcus hirae* (*E. hirae*), coronaviruses, rotaviruses and *Cystoisospora suis* (*C. suis*) (Jacobson, 2022). Although these pathogens can individually cause scours in piglets, co-infections are frequently observed (Kongsted et al., 2018). Clinical signs vary depending on the pathogen, infection load, and piglet immunity, making it challenging to attribute specific symptoms to a particular cause (Morin et al., 1983). The diverse agent combinations and disease scenarios detected across swine farms underscore the multifactorial nature of neonatal diarrhoea in pigs, emphasizing the need for tailored diagnosis and management strategies at the farm level. This approach should also account for non-infectious factors that can trigger neonatal diarrhoea (Vidal et al., 2019).

2.2 *Escherichia coli*

The gastrointestinal tract (GIT) of newborn piglets begins as a sterile environment but quickly becomes populated with bacteria, including *E. coli*, through exposure to other piglets, their surroundings, and sow feces (Dubreuil, 2021). Swine colibacillosis, caused by *E. coli* infection, leads to a range of issues such as neonatal diarrhoea, post-weaning diarrhoea (PWD), edema disease (ED), septicemia, polyserositis, coliform mastitis, and urinary tract infections (Fairbrother and Nadeau, 2019). This infection carries substantial financial implications due to decreased body weights, reduced weight gain, and heightened treatment and vaccination expenses (Barros et al., 2023). Various types of *E. coli* pathogens exist, including enteropathogenic (EPEC) *E. coli*, enterohaemorrhagic (EHEC) *E. coli*, enteroaggregative (EAEC) *E. coli*, enteroinvasive (EIEC) *E. coli*, diffusely adherent (DAEC) *E. coli*, and enterotoxigenic (ETEC) *E. coli* (Kaper et al., 2004).

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Of these categories, ETEC is recognized as a primary cause of intestinal issues in neonatal piglets, with F4, F5, F6, and F41 fimbriae specifically associated with diarrhoea (Dubreuil et al., 2016; Luppi et al., 2016). F4 typically colonizes the jejunum and ileum, while F5, F6, and F41 prefer the posterior jejunum and ileum regions (Luppi, 2017). Following attachment to the epithelium, ETEC binds to receptor sites on the epithelial cell surfaces, primarily in the jejunum, triggering enterotoxin Sta production and resulting in excessive fluid and electrolyte secretion in the small intestine. Symptoms of ETEC-induced neonatal diarrhoea include watery to creamy scours, often with a distinct odor and white to yellow color. Post-weaning colibacillosis cases typically present with yellowish, grey, or slightly pink watery diarrhoea persisting for around one week (Luppi, 2017). If the large intestine fails to reabsorb excess fluids, piglets may become dehydrated, leading to potential metabolic acidosis and fatal outcomes (Fairbrother and Nadeau, 2019). Alongside ETEC, EPEC is another major cause of diarrhoea in neonatal piglets (DebRoy and Maddox, 2001), known to colonize the entire intestine (Levine, 1987).

To diagnose neonatal enteric colibacillosis, a combination of diagnostic methods is typically employed, starting with observation of clinical signs and gross lesions, followed by bacteriological investigations and strain typing. Definitive diagnosis necessitates a series of analyses, including quantitative bacteriology, identification of virulence factors using PCR, and histopathology for a comprehensive assessment of observed microscopic lesions alongside the pathogen detected (Luppi, 2017).

Treatment of enteric colibacillosis in swine production involves considerable costs associated with antibiotics such as amoxicillin/clavulanic acid, ceftiofur, sulfonamides, gentamicin, and fluoroquinolones (Luppi, 2017). Typically, antibiotics are reserved for pigs exhibiting clinical signs of colibacillosis; however, in cases of escalating mortality within a production unit, prophylactic treatment may be employed for all animals (García-Meniño et al., 2021). Notably, *E. coli* has been identified as a leading pathogen contributing to antimicrobial resistance-related deaths, underscoring the importance of prudent antimicrobial usage in livestock (Murray et al., 2019). Monitoring antimicrobial resistance in ETEC isolates worldwide highlights the necessity of performing susceptibility testing in farm settings to select appropriate antimicrobial agents for ETEC treatment (Barros et al., 2023).

Despite extensive research into ETEC virulence factors and vaccine development, these bacterial pathogens persist as a significant cause of diarrhoea in pig herds (Wang et al., 2019). Although adhesion-based vaccines offer some protection, a universally effective

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ETEC vaccine against diarrhoea is not yet commercially available (Nadeau et al., 2017). The success of vaccination, particularly in gilts, depends on maternal exposure to ETEC, with piglet susceptibility increasing in the absence of specific antibodies in sow's milk or inadequate milk access (Luppi, 2017).

2.3. Clostridia

Clostridia are a group of gram-positive, spore-forming bacilli that range from strictly anaerobic to partially oxygen-tolerant, and certain species within the *Clostridium* genus are known to cause enteric diseases across various animal species, including humans (Rood, 2016). Among neonatal swine, *Clostridium perfringens* (*C. perfringens*) type C and *Clostridioides difficile* (*C. difficile*; formerly *Clostridium difficile*) are considered the most significant pathogenic clostridia causing enteric issues (Diab et al., 2016). While the involvement of *C. perfringens* type A in piglet diarrhoea has been suggested, definitive evidence is still lacking (Uzal et al., 2016). A recent study by Vidal et al. (2019) demonstrated a significant correlation between clostridia and various pathogens in neonatal piglet diarrhoea, complicating symptoms and presenting challenges for veterinary practitioners and farmers alike (Vidal et al., 2019).

C. perfringens is a critical pathogen affecting both animals and humans, commonly found in soil, decaying plant matter, and the GIT and feces of humans and other animals (McClane et al., 2013). Diseases caused by *C. perfringens* typically manifest at a young age, progress rapidly, exhibit characteristic clinical signs, mortality patterns, and necropsy findings, facilitating diagnosis through bacteriological examination. Nevertheless, in certain scenarios, herd-wide observations may be more informative than individual animal examinations. Effective prevention of clostridial diseases involves vaccinating pregnant sows and reassessing and rectifying environmental conditions and management practices, which significantly influence disease occurrence (Silva et al., 2015).

Despite some researchers considering *C. perfringens* type A as a primary cause of neonatal piglet diarrhoea (Chan et al., 2012), the bacterium's pathogenesis in swine remains poorly understood, posing challenges in diagnosis and prevalence determination (Silva et al., 2015). *C. perfringens* type A infections commonly affect neonates within the first week of life, with sows capable of transmitting the bacterium to piglets. The disease is characterized by non-hemorrhagic mucoid diarrhoea, involving mucosal necrosis and villus atrophy without microorganism invasion (Songer and Uzal, 2005).

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C. perfringens type C strains are distinguished by carrying the toxin genes cpa (encoding α-toxin or CPA) and cpb (encoding β-toxin or CPB) (Rood et al., 2018). Clinical presentations of type C infections can vary from peracute to acute or chronic. Peracute and acute forms primarily affect piglets within the first 3 days of birth, presenting with depression, abdominal pain, and hemorrhagic diarrhoea lasting from a few hours to up to 1-day post-exposure to *C. perfringens* type C (Diab, 2016).

C. difficile is a spore-forming, gram-positive, anaerobic bacillus typically considered harmless in the environment (Brazier and Borriello, 2000). The first report of *C. difficile* isolation from swine dates to 1983 (Jones and Hunter, 1983). In piglets where normal gut flora is absent or disrupted, *C. difficile* can establish, producing toxins in the large intestine and causing pathological changes, typically affecting piglets aged one to seven days, often linked to poor hygiene, stress, or dietary changes (Bilkei et al., 1995).

In certain scenarios, *C. difficile* is suggested as a significant uncontrolled cause of neonatal piglet diarrhoea, with a notably higher prevalence in diarrheic piglets (Kim et al., 2018). Clinical signs include diarrhoea, respiratory distress, and high morbidity and mortality rates. Pathological findings commonly reveal inflammatory changes in the intestine, mesocolonic edema, hydrothorax, ascites, suppuration foci, neutrophil accumulation, and fibrin in the colonic lamina propria (Songer et al., 2000). Improved hygiene and management practices, such as stress reduction and hygiene enhancements, have proven effective in preventing neonatal piglet losses associated with *C. difficile* (Nagy and Bilkei, 2003).

Given that *C. perfringens* types C and A, as well as *C. difficile*, can be present in varying prevalences in the intestines of healthy pigs, diagnosis should not rely solely on clostridia isolation. Several other factors such as farm management practices, intensity of farming, sow/piglet housing, hygiene protocols, birth control, genetics, sow immune status, stress factors, antibiotic use, vaccination programs, clinical signs, lesions, and exposure to other intestinal pathogens must also be considered during the diagnostic process (Uzal et al., 2023).

Effective prevention of clostridial diseases in pigs involves vaccinating sows during the dry period to provide passive protection through colostrum antibodies for piglets. Vaccines against *C. perfringens* typically utilize inactivated culture supernatants as immunogens, containing a mix of antigens, including toxins produced by the vaccine strain. Administering vaccinations following recommended schedules can reduce mortality in sow litters by ensuring sufficient colostrum antibody levels. This regimen typically involves two

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immunizations with an interval of several weeks before farrowing, as per manufacturers' instructions (Posthaus et al., 2020).

2.4. *Enterococcus* spp.

The *Enterococcus* species are a commensal bacterium in the GIT of animals, while members of the genus group III are involved in neonatal piglet diarrhoea (Vancanneyt et al., 2001; Facklam et al., 2002). The pathogenesis remains unclear, but it may be linked to reduced activity of brush border enzymes and disruptions in digestion and absorption processes. Three main species are reported to be associated with neonatal piglet diarrhoea: *E. villorum*, *E. durans*, and *E. hirae* (Cheon and Chae, 1996; Vancanneyt et al., 2001). Lesions related to *E. durans* are caused by the extensive colonization of the small intestinal enterocytes by Gram-positive cocci and a few sloughed enterocytes (Cheon and Chae, 1996). Lesions related to *E. hirae* are characterized by consisted of flaccid and dilated intestines with liquid to creamy content, while numerous Gram-positive cocci were associated with villous epithelial damage and atrophy (Larsson et al., 2014; Jonach et al., 2014; Larsson et al., 2015; Jang et al., 2019). In cases of neonatal piglet diarrhoea, the amount of *Enterococcus* spp. was up to 24 times more abundant in the intestinal content of sick piglets in comparison to healthy piglets, revealing the important role of *Enterococcus* spp. has in the pathogenesis of neonatal piglet diarrhoea (Gryaznova et al., 2022).

2.5. Coronaviruses

Neonatal piglet diarrhoea, predominantly attributed to coronaviruses, stands as a significant challenge within the global swine industry (Liu et al., 2021). Presently, six coronaviruses are recognized to infect pigs, encompassing four alphacoronaviruses: transmissible gastroenteritis virus (TGEV), porcine respiratory coronavirus (PRCV), porcine epidemic diarrhoea virus (PEDV), and swine acute diarrhoea syndrome-coronavirus (SADS-CoV), one betacoronavirus - the porcine hemagglutinating encephalomyelitis virus (PHEV), and a porcine deltacoronavirus (PDCoV) (Wang et al., 2019). Among these porcine coronaviruses, TGEV and PEDV are known to trigger diarrhoeal outbreaks with notable morbidity and mortality rates, particularly in neonatal pigs (Vidal et al., 2019).

TGEV serves as the causative agent of transmissible gastroenteritis (TGE), an acute enteric disease affecting swine. Historically, this virus elicited severe gastrointestinal symptoms in pigs globally (Turlewicz-Podbielska and Pomorska-Mól, 2021). The mortality

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rate of TGEV in piglets reaches nearly 100%, leading to substantial losses in the breeding industry across Europe, Asia, America, and beyond (Doyle and Hutchings, 1946; Pritchard 1987; McGoldrick et al., 1999; Kim et al., 2000). TGEV primarily spreads through fecal-oral, respiratory, and breastfeeding routes (Casanova et al., 2009). Severity of illness post-TGEV infection inversely correlates with piglet age, with symptoms like vomiting, diarrhoea, and dehydration surfacing two weeks after exposure (Enjuanes et al., 1995). The histopathological lesions are characterized by severe villus atrophy, as well as coexisting brain lesions (diffuse gliosis in pia matter with mild congestion of the meningeal and parenchymal vessels) and neuronal degeneration (Papatsiros et al., 2019). Additionally, adult pigs may exhibit transient elevated body temperature, vomiting, diarrhoea, anorexia, and lactation cessation, with minimal mortality (Saif, 1999). The detection rate of TGEV has dwindled over time due to advancements in TGEV control measures like vaccines, resulting in low detection rates in diarrheic pigs (Zhang et al., 2019).

Porcine epidemic diarrhoea (PED) was initially identified in the United Kingdom in 1971 as a severe enteric disease marked by sporadic outbreaks during winter, causing significant economic losses in breeding farms due to high piglet mortality (Song and Park, 2012). The devastating impact of PEDV arises from the acute watery diarrhoea observed in infected pigs, leading to a high mortality rate of 50-80%, especially in neonatal piglets (Geiger and Connor, 2013). Recovery to baseline piglet production levels in sow farms post-PED outbreak can take over 12 weeks (Goede and Morrison, 2014). Since 2010, highly pathogenic variants of PEDV have swept through the global swine industry, resulting in substantial economic losses, primarily due to the virus's strong transmissibility (Li et al., 2020). Infections with PEDV in neonatal pigs typically lead to death from watery diarrhoea and dehydration, with millions of piglet deaths reported in previous studies, exhibiting death rates of 80-100% and significant economic ramifications (Sun et al., 2012). Furthermore, surviving piglets may suffer long-term performance impairments, exacerbating economic burdens associated with PEDV infections (Alvarez et al., 2015).

Enhanced biosecurity measures serve as a fundamental approach in curbing viral spread and thwarting viral incursions on swine farms, with vaccination standing out as the most potent preventive strategy against TGEV and PEDV outbreaks. Due to high piglet mortality rates, vaccinating sows to confer lactogenic immunity and shield piglets from TGEV and PEDV infections has become a common practice. Both live attenuated and inactivated virus vaccines have been developed for TGEV and PEDV, focusing on the spike protein as the primary immunogen. Various technologies, such as DNA vaccines, vectored

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vaccines, subunit vaccines, and dendritic cell-based vaccines, have been harnessed to express the spike protein effectively (Liu and Gerdts, 2021).

2.6. Rotavirus

Rotavirus (RV) is classified as A–J based on antigenic properties, while RV groups A, B, and C are most identified (Vlasova et al., 2017). RVA to RVE and RVH have been reported in pigs, and RVA to RVC may be involved in neonatal piglet diarrhoea (Morin et al., 1990; Marthaler et al., 2013; Vlasova et al., 2017; Molinari et al., 2012). The clinical symptoms observed in experimentally infected piglets (aged 2 to 28 days) encompass lethargy, loss of appetite, vomiting, severe diarrhea, and a swift decline in overall health (Woode et al., 1976; Theil et al., 1985). In naïve herds, the clinical signs in 1-2 days-old piglets are characterized by within-litter morbidity of 100%, and mortality rates of 5–10% (Morin et al., 1990). Subclinical infections are common, while the age of onset is related to the level of maternal immunity, which may suppress the development of active immunity (Woode et al., 1976; Lecce et al., 1978; Bohl et al., 1978; Vlasova et al., 2017). Lesions in the small intestine are marked by shedding of the epithelial cells, villous atrophy and fusion, malabsorption, and thin-walled intestines, occasionally resembling those caused by TGE (Woode et al., 1976; Bohl et al., 1978; Chasey et al., 1989). Co-infections of RVA, RVB, and RVC are also reported (Marthaler et al., 2013). The large genetic diversity might explain the varying clinical signs, as well as the efficacy of vaccination (Vlasova et al., 2017).

2.7. *Cystoisospora suis*

C. suis (formerly known as *Isospora suis*) is a prevalent pathogen in suckling piglets worldwide, with high prevalence reported in various countries such as Germany, Austria, Switzerland, Poland, Sweden, Canada, and China (Mundt et al., 2005; Karamon et al., 2007; Petterson et al., 2019; Aliaga-Leyton et al., 2011; Zhang et al., 2012). The economic impact of coccidiosis caused by *C. suis* is substantial, with weight reduction at weaning per animal reaching up to 1 kg, affecting both clinically and sub-clinically infected animals (Maes et al., 2007). The damage caused by *C. suis* in the intestines can also facilitate interactions with other enteropathogens, including rotavirus, transmissible gastroenteritis virus, clostridia, and *E. coli*, exacerbating clinical infections and mortality rates (Joachim and Shrestha, 2019).

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Piglets typically acquire *C. suis* infection shortly after birth by ingesting sporulated oocysts present in the environment, often originating from previously infected litters, whereby dams play a minor role in transmitting the infection to their offspring (Sotiraki et al., 2008). Upon ingestion, the sporocysts within the oocysts release sporozoites in the small intestine, where they invade the epithelial cells, predominantly in the jejunum. This invasion leads to inflammation and macroscopic reddening of the intestine, with microscopic lesions including villous atrophy, villous fusion, and crypt hyperplasia. Infected piglets may exhibit non-hemorrhagic or white/yellow pasty diarrhoea, with clinical signs emerging as early as 6 days of age (Taylor et al., 2016). Diagnosing cystoisosporosis necessitates understanding that infected piglets shed oocysts for short durations, underscoring the importance of precise timing and sensitive detection methods to ascertain the parasite's presence and extent in a herd. Additionally, repeated fecal sampling can enhance sensitivity, with autofluorescence microscopy offering superior sensitivity for oocyst detection (Joachim et al., 2018).

Effective prevention of *C. suis* infection hinges on proper husbandry practices and stringent hygiene measures that diminish oocyst buildup in the environment and minimize piglet exposure to sporulated oocysts (Straberg and Daugschies, 2007). Joints efforts in controlling the parasite involve interrupting its development cycle in the gut promptly, thereby preventing intestinal damage, clinical signs, and oocyst production. Disinfection plays a crucial role in preventing numerous intestinal pathogens on piglet farms; however, it has limited efficacy in controlling coccidiosis alone as *C. suis* oocysts exhibit resistance to common disinfectants (Hinney et al., 2020). Despite the availability of highly effective drugs like toltrazuril, coccidiosis remains prevalent in key swine-producing nations globally, leading to significant economic repercussions (Gong et al., 2021). Nonetheless, sustained use of anticoccidials for coccidiosis control in intensively managed farm animals has resulted in the emergence of resistance against these products over time (Noack et al., 2019).

3. Gut health – Antibiotics – Alternatives to Antibiotics

3.1. Importance of gut health in swine production

The concept of “Swine Gut Health” has garnered attention in recent reviews, emphasizing that gut health encompasses not just the absence of enteric diseases but also the functionality of the intestinal system, influenced by factors such as diet, the integrity of the gastrointestinal barrier, interactions with the intestinal microbiota, efficient digestion

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and nutrient absorption, and the maturation of immune functions (Celi et al., 2017; Pluske et al., 2018). A comprehensive understanding of gut health in pigs aims to explore the impact of lipid nutrition on gut health and function, focusing on how dietary fatty acids can modulate the intestinal microbiota, immune responses, and epithelial functions in piglets (Lauridsen, 2020).

The global pig production industry plays a critical economic role, with a recent report from the European Union (EU) highlighting that approximately 150 million pigs were produced in 2018, positioning pigs as the largest livestock category in the region (Augère-Granier, 2020). To meet escalating demands, strategies have been devised to enhance production efficiency, meat quality, profitability, and sustainability in pig production (Maltecca et al., 2020). However, challenges persist, including rising feed costs, suboptimal growth performance, and disease occurrences in pig herds. Researchers have uncovered the intricate relationship between the gut microbiome and pig health and nutrition, with the GIT playing a vital role in processing food into essential nutrients for pig maintenance, production, and immune function (Szabó et al., 2023). The gut microbiome is increasingly recognized as a crucial component of overall health, acting as an additional organ influencing host functions and disease development (Baquero and Nombela, 2012).

The bidirectional communication between the gut microbiome and the host underscores their profound impact on health and disease pathogenesis, with the gut immune system playing a crucial regulatory role in maintaining microbial balance and intestinal homeostasis (Brody, 2020; Lemon et al., 2012). The neonatal gut undergoes a remarkable transition from a sterile state to a populated microbial community post-birth, influencing the establishment of the gut microbiota composition. The piglet gut microbiota, rich in various bacterial genera, plays a crucial role in pig health and development, with factors like diet, antibiotics, probiotics, prebiotics, and synbiotics shaping the composition of the gut microbiota during early life (Pajarillo et al., 2014; Frese et al., 2015). In recent years, studies have delved into the relationship between piglet gut microbiota composition, growth performance, and health, focusing on key factors like diet, antibiotics, probiotics, prebiotics, and synbiotics that shape the gut microbiota. Maintaining intestinal health is crucial in animal production, yet piglets often face various stressors such as birth, weaning, heat, and transport stress, which can impact their gut health and lead to economic losses (Gill et al., 2012; Cao et al., 2018; Liu et al., 2016; Roldan-Santiago et al., 2015).

Health, as defined by the WHO, encompasses not merely the absence of diseases but a state of overall well-being. “Gut health” is characterized by physical and mental well-

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being without gastrointestinal complaints that necessitate medical consultation, absence of bowel disease indications or risks, and confirmed bowel disease. Five major criteria proposed by Bischoff (2011) form the basis of an overarching definition of gut health, including efficient digestion and absorption, absence of gastrointestinal illness, a stable microbiome, effective immune status, and overall well-being. A deeper understanding of the gut microbiome's structural and functional aspects will enhance comprehension of microbial resources and host-microbe interactions, further advancing knowledge in this field (Levin et al., 2021).

3.2. Effects of antibiotics on swine gut microbiota

The swine microbiome is influenced by various factors, such as age, host genetics, breed, gender, and castration, with microbial diversity increasing as pigs age (Xiao et al., 2016; Soler et al., 2018). A diverse microbiome is vital for pig health as it aids in pathogen exclusion, but factors like antibiotic treatment at an early age can reduce microbial diversity and lead to lasting compositional changes in the microbiome (Schokker et al., 2015; Fouhse et al., 2016). Historically, antimicrobials have been used in the swine industry to eliminate pathogenic microbes, promote growth, and restore beneficial microbial communities (Holman and Chénier, 2015).

Antibiotics are commonly administered to eradicate pathogenic microbes during acute infections, with sub-therapeutic doses like ASP50, Tylosin, and Carbadox being utilized in swine health management (Looft et al., 2012, 2014; Kim et al., 2012; Cromwell, 2002). However, antibiotics can have adverse effects on host health, such as antibiotic-associated diarrhoea and indigestion, with *C. difficile* infection being a common example of antibiotic-induced diarrhoea in pigs (Young and Schmidt, 2004; Post and Songer, 2004). Broad-spectrum antibiotics not only target pathogenic bacteria but also disrupt beneficial gut microbiota crucial for pig health (Neuman et al., 2018).

Studies have shown that antibiotic administration, such as lincomycin treatment, can lead to a loss of microbial diversity and alterations in major phyla like *Firmicutes*, *Bacteroidetes*, *Spirochetes*, and *Actinobacteria*, which are predominant in the swine gut (Jo et al., 2021; Ke et al., 2019). The disruption of the swine gut microbial environment by lincomycin administration suggests a potential impact on pig health (Jo et al., 2021). Antibiotic use can induce gut dysbiosis, characterized by alterations in gut microbiota composition and a loss of colonization resistance (Stecher et al., 2013; Wilkins et al., 2019). While some microbial populations may return to pre-treatment levels following antibiotic

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administration, others may exhibit persistent changes even months after treatment, affecting both abundant and less abundant gut microbiota in growing piglets (Jernberg et al., 2010; Kim et al., 2012). For instance, tylosin-treated piglets displayed higher abundance of certain genera compared to untreated piglets (Kim et al., 2012). In-feed administration of antibiotics like colistin sulfate and bacitracin zinc in weaned piglets also led to significant shifts in gut microbiota composition across different gut locations (Li K. et al., 2017; Soler et al., 2018).

Direct impacts of antibiotics on intestinal bacteria involve inhibiting opportunistic pathogens and decreasing competition for nutrients (Rahman et al., 2022). However, the use of antibiotics negatively affects commensal bacterial populations as well. Research by Gao et al. (2018) demonstrated that administering ceftriaxone sodium leads to a substantial decrease in *Lactobacillus* and *Bifidobacterium* spp. in the porcine ileum. Similarly, Bosi et al. (2011) found that amoxicillin treatment reduces the relative abundance of lactic acid bacteria in the jejunum of weaned piglets. Additionally, supplementing feed with chlortetracycline, sulfamethazine, and penicillin results in a decline of intestinal *Coprococcus*, *Succinivibrio*, *Streptococcus*, *Treponema*, and *Turicibacter* spp. in pigs (Allen et al., 2011). These studies also noted a consistent rise in *Escherichia* populations regardless of the antibiotic classes used. Beyond causing gut microbiota dysbiosis, excessive antibiotic usage eventually leads to the presence of resistance virulence factors in gene families unique to the swine fecal metagenome, including those with sequence homology to genes in dominant bacterial populations such as *Bacteroidetes* and Clostridia (Lamendella et al., 2011), as well as immune system dysregulation (Bosi et al., 2011; Schokker et al., 2014).

The gut microbiota of pigs is influenced by factors such as host genetics, supplements, age, and notably, oral antibiotic use, which can disrupt the delicate balance of the gut microbiota (Schmidt et al., 2018). Antibiotic-induced dysbiosis can create favorable conditions for the growth of pathogenic bacteria, potentially leading to infections in otherwise healthy hosts (Zeineldin et al., 2019). Despite the known risks, the use of antibiotics in swine production remains a common practice for efficiency. Therefore, a comprehensive understanding of the impact of in-feed antibiotics on the structure and function of the swine gut microbiome is essential for optimizing pig health and production (Jo et al., 2021).

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3.3. Effects of alternatives to antibiotics on swine gut microbiota

There is an extensive range of products, such as feed additives, and feeding/management strategies available that impact, or claim to impact, various aspects of gut health (Jayaraman and Nyachoti, 2017). Alternatives to antibiotics are sought to enhance nutrient digestion and absorption, regulate gut microbiota towards more beneficial bacterial species, and modulate immunity to boost disease resistance. These goals can be achieved through the supplementation of amino acids, phytogenics, probiotics, prebiotics, and organic acids (Yang and Liao, 2019; Draskovic et al., 2018; Markowiak and Śliżewska, 2018; Jiménez et al., 2020). The numerous feed and water additives offered to pork producers as substitutes or replacements for AGPs aim to enhance immune responses, reduce pathogen load in the pig's GIT, promote beneficial GIT microbe establishment, and stimulate digestive function (de Lange et al., 2010).

Various antibiotic alternatives, including medicinal plants (phytogenics), probiotics, prebiotics, organic acids and immunostimulants, and management practices, have been used to enhance animal health and performance.

3.3.1. Phytogenic blends (phytochemicals, phytobiotics, or simply phytogenics)

Phytogenic blends, also known as phytochemicals, phytobiotics, or simply phytogenics, are composed of various plant-derived bioactive compounds that can be used as feed additives for livestock animals. A list of more than 5,000 biologics identified from essential oil, fruits and vegetables, whole grains, herbs, and nuts, etc. (Li et al., 2021). These blends are recognized for their antioxidant, anti-inflammatory, and antimicrobial properties, which contribute to enhanced growth performance and overall positive effects on the health and well-being of farm animals (Oh et al., 2017; Taha-Abdelaziz et al., 2018; Rafiq et al., 2022; Papatsiros et al., 2024). Given their beneficial properties, phytogenic blends have recently gained attention as potential alternatives to antimicrobial growth promoters (AGPs) (Kikusato, 2021). There is an increasing preference for natural products because they are considered to have fewer undesirable side effects than synthetic products (Laudato and Capasso, 2013). The development of plant-based feed additives has become a research hotspot and is significant for the healthy development of animal husbandry and the improvement of animal product quality.

Phytogenics are heterogeneous compounds with different biological activities considered to have some similar benefits as AGPs (Rossi et al., 2020; Kuralkar and Kuralkar, 2021). Their beneficial effects on animals a include increased nutrient digestion, absorption,

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and secretion of intestinal mucus, saliva, and bile, anti-bacterial activities, anti-oxidation and immuno-modulation (Lillehoj et al., 2018; Valenzuela-Grijalva et al., 2017). Most of the phytogenic feed additives based on plant extracts have beneficial antimicrobial, antioxidative, and growth-promoting effects in swine, including sows, weaning piglets, and finishers (Papatsiros et al., 2009; Jacela et al., 2010; Papatsiros et al., 2011; Kantas et al., 2015; Tzika et al., 2017). The substances of phytogenic feed additives (spices, herbs, or extracts) as feed supplements have been reported to improve certain growth and health parameters in pigs (Burt, 2004; Papatsiros et al., 2009; Jacela et al., 2010; Kantas et al., 2015; Papakonstantinou et al., 2023). Numerous herbal products have been demonstrated to have beneficial effects and medicinal properties, including antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory activities, without adversely affecting growth and feed efficiency, and are therefore used as growth-promoting feed additives in livestock production (Kumar et al., 2014; Lillehoj et al., 2018; Kuralkar and Kuralkar, 2021).

Numerous studies have shown that the key to the beneficial effects of phytogenics on swine health and production performance lies in their ability to modulate the gut microbiota. This is crucial because the composition and activity of intestinal microbes are closely linked to intestinal morphology, the immune system, and gastrointestinal physiology (Gardiner et al., 2020; Vasquez et al., 2022; Wang et al., 2022; Popov et al., 2023). Martel et al. (2020) have recently proposed that a fraction of phytogenics that pass by the gut lumen may improve intestinal health and animal development by acting as prebiotics (Martel et al., 2020).

3.3.2. Probiotics

Probiotics can help maintain the balance of intestinal microbes, and they have an antibiotic-like impact on pathogenic microorganisms in pigs (Papatsiros et al., 2011; Kantas et al., 2015; Denkova et al., 2017). The term probiotics has been defined as live microbial cell preparations or microbial cell components feed supplements, which beneficially affect the host animal by improving its intestinal balance, such as restoring the gut microbiota homeostasis and improving the immunophysiological health of mammals (Fuller, 1992; Salminen et al., 1999; Salminen et al., 2021). According to WHO's definition, probiotics are non-pathogenic living microorganisms that provide a health advantage to the host when administered in optimum amounts (Hill et al., 2014).

Probiotics, mostly *Lactobacillus* or *Bifidobacterium* species, are derived from the intestinal microbiota of animals, humans, and dairy products (Linares et al., 2017).

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Recently, in addition to conventional probiotics based on the genera *Lactobacillus* and *Bifidobacterium*, preparations based on representatives of the genus *Bacillus* have come into the foreground. At present, 77 species belonging to the genus *Bacillus* are recognized, of which the following are used most frequently: *coagulans, subtilis*, *clausii, cereus, toyoi* (Stamati et al., 2006; Papatsiros et al., 2011; Kantas et al., 2015) Probiotics also include yeasts such as *Saccharomyces* and bacteria of the genera *Bacillus*, *Enterococcus*, and *Streptococcus* (Boyle et al., 2006). Probiotics have the potential to act against various infectious agents. Lactic acid probiotic bacteria, including *Lactobacillus*, *Streptococcus*, and *Bifidobacteria*, have been demonstrated to suppress *Helicobacter pylori*, *Salmonella*, and *E. coli* (Halder et al., 2017). Among the tested probiotics, which include lactic acid bacteria, *Bacillus* (e.g., *B. subtilis*), *Enterococcus* (e.g., *E. faecium*), *Streptococcus* (e.g., *S. infantarius*), *Pediococcus* (e.g., *P. acidilactici*), some butyrate-producing bacteria, yeast, *Aspergillus*, and *Trichoderma*, *Lactobacillus* species appear to hold the greatest promise for replacing antibiotics in swine (Patel et al., 2015).

Considering the EU ban on antibiotic usage in livestock production, probiotic supplementation has gained traction as a viable alternative to boost pig health and productivity (Barba-Vidal et al., 2019). Using probiotics to restore the optimal balance between beneficial and harmful microorganisms is especially beneficial during stress periods, when this balance can be disrupted (Fox, 1988). Probiotics function as growth promoters from the weaning stage to slaughter, as nutritional bioregulators that compete with detrimental gut flora, as stimulators of the animal's immune system (Fuller, 1989), and as enhancers of the body's resistance to infectious diseases (Tannock, 1997; Tannock, 2001). Incorporating a probiotic containing *Bacillus cereus* var. *Toyoi* in both sows and their litters, as well as in weaners, has demonstrated positive impacts on health and performance metrics (Taras et al., 2005, Link et al., 2005; Stamati et al., 2006; Papatsiros et al., 2011). Probiotics have been shown to modulate the gut microbiome and influence its metabolite levels (Kim and Isaacson, 2015). By regulating gut microbiota homeostasis and strengthening intestinal barriers, probiotics play a significant role in improving gut health in pigs (Su et al., 2022). Research indicates that supplementing piglet diets with lactic acid bacteria like *Lactobacillus* and *Enterococcus* species can increase the relative abundance of beneficial bacteria, decrease harmful bacteria like *E. coli*, and boost the production of short-chain fatty acids in the gut (Su et al., 2022; He et al., 2022; Liao and Nyachoti, 2017; Tsukahara et al., 2020; Wang et al., 2021). Specific probiotics such as *Lactobacillus salivarius* and *E. faecium* have been found to enhance gut microbiota diversity in piglets,

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highlighting their positive impact (Riboulet-Bisson et al., 2012; Pajarillo et al., 2015). Understanding the exact roles of probiotics in modulating piglet microbiota and immune response remains an area for further investigation.

The concept of prebiotics, defined by Gibson and Roberfroid as “non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of specific bacteria in the colon”, has seen further refinement to include substrates that are selectively utilized by host microorganisms, conferring benefits to the host (Gibson et al., 2017). Novel prebiotics, unlike traditional non-digestible carbohydrates, are still selectively utilized by host microbes, potentially offering host health benefits. However, additional research is needed to understand the mechanisms of action and microbial utilization of these novel prebiotics (Kiernan et al., 2023). The benefits of prebiotics position them as promising bioactive compounds that could serve as alternatives to antibiotic and antimicrobial use on pig farms. Identifying the most effective prebiotics at different developmental stages, considering the broad array of traditional and novel prebiotics, is essential for maximizing their potential benefits (Kiernan et al., 2023).

3.3.3. Prebiotics

Prebiotics are substrates that promote the growth and/or metabolism of specific groups of commensal bacteria, providing health benefits to the host (Salminen et al., 2021). For instance, the well-known prebiotic inulin can boost the population of probiotic *Lactobacillus* spp. and *Bifidobacterium* spp., stimulate the production of lactic acids and short-chain fatty acids (SCFAs), and lower intestinal pH, thereby inhibiting the growth of pathogenic bacteria (van der Aar et al., 2017). Additionally, prebiotics' proposed mechanisms of action include blocking receptor sites for bacterial adhesion and modulating the immune system, among others (Bai et al., 2022; Cunningham et al., 2021).

The most commonly used prebiotics in swine is dietary fiber fractions and non-digestible oligosaccharides derived from plants. It includes arabinoxylans, pectin, xyloglucans, resistant starch (Azad et al., 2020; Bach Knudsen et al., 2017), fructooligosaccharides, lactulose, raffinose, maltodextrin, mannitol, galactooligosaccharide and inulin (Williams et al., 2019). The administration of prebiotic supplementations has been shown to a) improve their growth performance and gut function in weaned piglets (Liu et al., 2014b), b) reduce pro-inflammatory interleukin production and increase anti-inflammatory cytokine levels in pigs (Pié et al., 2007; Trachsel et al., 2019; Wan et al., 2016; Wang et al., 2016; Yin et al., 2008), c) increase the production of antibodies in the

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GIT as well as in circulation (Naqid et al., 2015; Wang et al., 2016; Wu et al., 2017; Czech et al., 2022).

The positive impacts of a particular prebiotic are directly related to its fermentability (Jiao et al., 2021), which is influenced by the maturity of the gut microbiota. Additionally, the beneficial effects of a prebiotic are also contingent upon its structural complexity (Beukema et al., 2020).

3.3.4. Essential amino acids

Essential amino acids play a crucial role in pig nutrition, as pigs require them in their diet since they cannot synthesize them in sufficient amounts to meet their needs. Adding these essential amino acids to pig diets is essential for maintaining nitrogen balance, meeting growth requirements, and enhancing immunity (Chen et al., 2009). Various amino acids, such as threonine, lysine, methionine, tryptophan, and glutamine, have been studied for their effects on gut microbiota, showing positive outcomes by promoting beneficial microbiota growth, reducing intestinal ammonia levels, and suppressing opportunistic pathogens (Ma et al., 2022; Liao, 2021). Further exploration is needed to understand the interactions between added feed additives, including dietary fiber, and the fundamental effects of different amino acids. With the global movement to phase out in-feed antibiotics and medicinal doses of zinc oxide in swine production, investigating the specific effects of individual amino acids and their combined impact on gut microbiota composition and function in young pigs is paramount (Liao, 2021). Additional research is warranted to delve deeper into the microbial metabolism of other amino acids and their implications for pig intestinal health (Ma et al., 2021).

3.3.5. Organic acids (acidifiers)

Organic acids are defined as any organic carboxylic acids, which may include keto or hydroxyl groups but exclude amino acids, characterized by the general R-COOH structure (Khan and Iqbal, 2016). They can be categorized into three main functional groups: short-chain fatty acids (SCFA), medium-chain fatty acids (MCFA), and tricarboxylic acids (TCA) (Grilli et al., 2012). SCFAs, which are single mono-carboxylic acids with up to 5 carbon atoms like acetic, formic, propionic, and butyric acids, are produced in the lower intestine through microbial fermentation of non-digestible sugars and amino acids. MCFAs, containing a hydroxyl group and consisting of 6–12 carbon atoms, such as malic, citric, tartaric, and lactic acids, serve as significant energy sources and exhibit

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higher antimicrobial activity due to their higher pKa values. Lastly, TCAs, which include carboxylic acids with double bonds like sorbic and fumaric acids, act as intermediates in the Krebs cycle and play a role in energy metabolism (Khan and Iqbal, 2016; Tugnoli et al., 2020; Hajati, 2018). Other organic acids, including sorbic, benzoic, and lactic acids, have varied structures. These acids are prevalent in nature as normal components of plant and animal tissues and are produced either through chemical synthesis or microbial fermentation of carbohydrates in the large intestine (Hajati, 2018).

The exact mechanisms of action for common acidifiers are not yet fully understood. However, their effectiveness may be partially attributed to several factors: a) inhibiting the growth of pathogenic microbes in the GIT by lowering gut pH, b) reducing gastric emptying rates and supporting the secretion of endogenous enzymes, c) chelating minerals and stimulating intermediary metabolism, and d) aiding proper digestion through lower gastric pH and increased pepsin secretion (Rathnayake et al., 2021).

Organic acids and essential oils have been shown to enhance nutrient digestibility, growth performance, carcass characteristics, gut morphology, microflora balance, meat quality, and chemical composition in pigs (Suiryanrayna et al., 2015; Nhara et al., 2024). These compounds improve a pig's capacity to inhibit pathogenic bacteria colonization in the intestinal tract (Liu et al., 2018), stabilize gut microflora, and promote eubiosis (Lückstädt et al., 2014). They also enhance mineral utilization, serve as energy sources, stimulate endogenous enzyme secretion, and boost immunity and antioxidant stability (Liu et al., 2018). Organic acids exhibit both bacteriostatic and bacteriocidal properties, depending on their inclusion levels. They can be used effectively alongside other feed additives (Luckstadt and Mellor, 2010). These acids penetrate bacterial cell walls, disrupting the functions of certain bacteria such as *Salmonella* spp, *E. coli*, Clostridia spp, *Listeria* spp, and some coliforms, which results in a reduction of both normal intestinal and pathogenic bacteria in animals fed with organic acids. They are believed to improve growth performance (Papatsiros et al., 2011, Diao et al., 2016, Luise et al., 2017) by reducing microbial competition for nutrients, lowering the risk of subclinical infections, diminishing the intestinal immune response, and reducing the production of harmful bacterial compounds (Suiryanrayna et al., 2015; Nhara et al., 2024). The capability of organic acids and essential oils to boost pig performance and pork quality is comparable to that of AGPs and can serve as an alternative in intelligent pig production practices and the production of safe meat (Lückstädt et al., 2014; Pateiro et al., 2018; Zhai et al., 2018; Nhara et al., 2024).

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06/09/2026 21:12:45 EEST - 216.73.216.250

References

Aarestrup FM (2000) Occurrence, selection and spread of resistance to antimicrobial agents used for growth promotion for food animals in Denmark. APMIS Suppl. 101, 1-48.

Ahmad M, Khan AU (2019) Global economic impact of antibiotic resistance: A review. J Glob Antimicrob Resist 19, 313-316.

Aliaga-Leyton A, Webster E, Friendship R, Dewey C, Vilaça K, Peregrine AS (2011) An observational study on the prevalence and impact of Isospora suis in suckling piglets in southwestern Ontario, and risk factors for shedding oocysts. Can Vet J 52, 184–88.

Allen HK, Looft T, Bayles DO, Humphrey S, Levine UY, Alt D, Stanton TB (2011) Antibiotics in feed induce prophages in swine fecal microbiomes. mBio 2, e00260-11. doi: 10.1128/mbio.00260-11

Alvarez J, Sarradell J, Morrison R, Perez A (2015) Impact of Porcine Epidemic Diarrhea on Performance of Growing Pigs. PLOS ONE 10(3), e0120532.

an der Aar PJ, Molist F, van der Klis JD (2017) The central role of intestinal health on the effect of feed additives on feed intake in swine and poultry. Anim Feed Sci Technol 233, 64-75. https://doi.org/10.1016/j.anifeedsci.2016.07.019

Argudin MA, Deplano A, Meghraoui A, Dodemont M, Heinrichs A, Denis O, Nonhoff C, Roisin S (2017) Bacteria from animals as a pool of antimicrobial resistance genes. Antibiotics 6, 12.

Augère-Granier ML (2020) The EU pig meat sector. Cardiff: European Parliamentary Research Service. Report No, PE 652.044.

Azad MAK, Gao J, Ma J, Li T, Tan B, Huang X, Yin J (2020) Opportunities of prebiotics for the intestinal health of monogastric animals. Anim Nutr 6, 379-388. https://doi.org/10.1016/j.aninu.2020.08.001

Bach Knudsen KE, Nørskov NP, Bolvig AK, Hedemann MS, Lærke HN (2017) Dietary fibers and associated phytochemicals in cereals. Mol Nutr Food Res 61, 1600518.

Bai Y, Zhou X, Zhao J, Wang Z, Ye H, Pi Y, Che D, Han D, Zhang S, Wang J (2022) Sources of dietary fiber affect the SCFA production and absorption in the hindgut of growing pigs. Front Nutr 8, 719935. https://doi.org/10.3389/fnut.2021.719935

Baquero F, Nombela C (2012) The microbiome as a human organ. Clin Microbiol Infect 18, 2–4.

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Barba-Vidal E, Martín-Orúe SM, Castillejos L (2019) Practical aspects of the use of probiotics in pig production: a review. Livest Sci 223, 84–96.

Barros MM, Castro J, Araújo D, Campos AM, Oliveira R, Silva S, Outor-Monteiro D, Almeida C (2023) Swine Colibacillosis: Global Epidemiologic and Antimicrobial Scenario. Antibiotics 12(4), 682.

Begemann S, Perkins E, Van Hoyweghen I, Christley R, Watkins F (2018) How political cultures produce different antibiotic policies in agriculture: a historical comparative case study between the United Kingdom and Sweden. Soc Ruralis 58(4), 765-785.

Beukema M, Faas MM, de Vos P (2020) The effects of different dietary fiber pectin structures on the gastrointestinal immune barrier: impact via gut microbiota and direct effects on immune cells. Exp Mol Med 52, 1364-1376. https://doi.org/10.1038/s12276-020-0449-2

Bian G, Ma S, Zhu Z, Su Y, Zoetendal EG, Mackie R, Liu J, Mu C, Huang R, Smidt H, Zhu W (2016) Age, introduction of solid feed and weaning are more important determinants of gut bacterial succession in piglets than breed and nursing mother as revealed by a reciprocal cross-fostering model. Environ Microbiol 18, 1566–77.

Bilkei G, Biro O, Bolcskei A, Clavadetscher E, Orban P, Waller C (1995) Practice related management strategies on post-weaning E. coli problems in the intensive pig production. Proceedings of the 8th Symposium of International Society for Animal Hygiene. Hung Vet J 10, 776–777.

Bischoff SC (2011) 'Gut health': a new objective in medicine? BMC Med 924.

Bohl FH, Kohler EM, Saif LJ, Cross RF, Agnes AG, Theil KW (1978) Rotavirus as a cause of diarrhea in pigs. J Am Vet Med Assoc 172, 458-463

Bosi P, Merialdi G, Scandurra S, Messori S, Bardasi L, Nisi I, Russo D, Casini L, Trevisi P (2011) Feed supplemented with 3 different antibiotics improved food intake and decreased the activation of the humoral immune response in healthy weaned pigs but had differing effects on intestinal microbiota. J Anim Sci 89, 4043-4053. https://doi.org/10.2527/jas.2010-3311

Boyle RJ, Robins-Browne RM, Tang MLK (2006) Probiotic use in clinical practice: What are the risks? Am J Clin Nutr 83, 1256-1264.

Brazier JS, Borriello SP (2000) Microbiology, epidemiology, and diagnosis of Clostridium difficile infection. In: Clostridium difficile, ed. Aktories K, Wilkens TC, pp, 1–8. Springer, Berlin, Germany.

Brody H (2020) The gut microbiome. Nature 577, S5.

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Burt S (2004) Essential oils: Their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol 94, 223-253.

Callens B, Persoons D, Maes D, Laanen M, Postma M, Boyen F, Haesebrouck F, Butaye P, Catry B, Dewulf J (2012) Prophylactic and metaphylactic antimicrobial use in Belgian fattening pig herds. Prev Vet Med 106, 53-62.

Cao ST, Wang CC, Wu H, Zhang QH, Jiao LF, Hu CH (2018) Weaning disrupts intestinal antioxidant status, impairs intestinal barrier and mitochondrial function, and triggers mitophagy in piglets. J Anim Sci 96(3), 1073–83.

Casanova L, Rutala WA, Weber DJ, Sobsey MD (2009) Survival of surrogate coronaviruses in water. Water Res 43, 1893–1898.

CDC (2013) Antibiotic resistance threats in the United States; 2013. Available at: https://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf. Accessed on 24 July 2020.

CDC (2018) Antibiotic resistance: a global threat | features | CDC. Available at: https://www.cdc.gov/features/antibiotic-resistance-global/index.html. Accessed 28 February 2021.

CDC (2019) Antibiotic Resistance Threats in the United States, Atlanta, GA: U.S. Department of Health and Human Services.

Celi P, Cowieson AJ, Fru-Nji F, Steinert RE, Kluenter A-M, Verlhac V (2017) Gastrointestinal functionality in animal nutrition and health: new opportunities for sustainable animal production. Anim Feed Sci Tech 234, 88–110.

Chae JP, Pajarillo EA, Oh JK, Kim H, Kang DK (2016) Revealing the combined effects of lactulose and probiotic enterococci on the swine faecal microbiota using 454 pyrosequencing. Microb Biotechnol 9, 486–95.

Chan G, Farzan A, Soltes G, Nicholson VM, Pei Y, Friendship R, Prescott JF (2012) The epidemiology of Clostridium perfringens type A on Ontario swine farms, with special reference to cpb2-positive isolates. BMC Vet Res 8, 156.

Chang K-O, Saif LJ, Junjeong K (2012) Reoviruses (rotaviruses and Reoviruses). In: Zimmerman JJ, editor. Diseases of Swine Volume 1, 10 edn. West Sussex: Wiley. p. 621–34.

Chantziaras I, Dewulf J, Boyen F, Callens B, Butaye P (2014) Antimicrobial resistance prevalence of pathogenic and commensal Escherichia coli in food-producing animals in Belgium. Vlaams Diergeneeskd Tijdschr 83, 225-233.

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Chasey D, Higgins RJ, Jeffrey M, Banks J (1989) Atypical rotavirus and villous epithelial cell syncytia in piglets. J Comp Path 100, 217-222. doi: 10.1016/0021-9975(89)90134-5

Chen L, Li P, Wang J, Li X, Gao H, Yin Y, Hou Y, Wu G (2009) Catabolism of nutritionally essential amino acids in developing porcine enterocytes. Amino Acids. 37, 143–152. doi: 10.1007/s00726-009-0268-1

Cheon DS, Chae C (1996) Outbreak of diarrhea associated with Enterococcus durans in piglets. J Vet Diagn Investig 8, 123-124. doi: 10.1177/104063879600800123

Cromwell GL (2002) Why and how antibiotics are used in swine production. Anim Biotechnol 13, 7–27. doi: 10.1081/abio-120005767

Cunningham M, Azcarate-Peril MA, Barnard A, Benoit V, Grimaldi R, Guyonnet D, Holscher HD, Hunter K, Manurung S, Obis D, Petrova MI, Steinert RE, Swanson KS, van Sinderen D, Vulevic J, Gibson GR (2021) Shaping the future of probiotics and prebiotics. Trends Microbiol 29, 667-685. https://doi.org/10.1016/j.tim.2021.01.003

Czech A, Nowakowicz-Debek B, Łukaszewicz M, Florek M, Ossowski M, Wlazło Ł (2022) Effect of fermented rapeseed meal in the mixture for growing pigs on the gastrointestinal tract, antioxidant status, and immune response. Sci Rep 12, 15764. https://doi.org/10.1038/s41598-022-20227-2

Dall C. Global antibiotic use rises, fueled by economic growth (2018). Available at: http://www.cidrap.umn.edu/news-perspective/2018/03/global-antibiotic-use-rises-fueled-economic-growth. Accessed on 25 September 2021.

Davies R, Wales A (2019) Antimicrobial resistance on farms: a review including biosecurity and the potential role of disinfectants in resistance selection. Compr Rev Food Sci Food Saf 18, 753–74. doi: 10.1111/1541-4337.12438

DE Knegt LV, Pires SM, Hald T (2015) Attributing foodborne salmonellosis in humans to animal reservoirs in the European Union using a multi-country stochastic model. Epidemiol Infect 143(6), 1175-86. doi: 10.1017/S0950268814001903

De Lange C, Pluske J, Gong J, Nyachoti GM (2010) Strategic use of feed ingredients and feed additives to stimulate gut health and development in young pigs. Livest Sci 134(1-3). doi: 10.1016/j.livsci.2010.06.117

DebRoy C, Maddox CW (2001) Identification of virulence attributes of gastrointestinal Escherichia coli isolates of veterinary significance. CAB Int 1, 129–140.

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Denkova R, Goranov B, Teneva D, Denkova Z, Kostov G (2017) Antimicrobial activity of probiotic microorganisms: mechanisms of interaction and methods of examination. Antimicrob Res Nov Bioknowl Educ Programs 1, 201-212.

Denver S, Jensen JD, Christensen T (2021) Consumer preferences for reduced antibiotic use in Danish pig production. Prev Vet Med 189, 105310. doi: 10.1016/j.prevetmed.2021.105310

Dewey CE, Cox BD, Straw BE, Bush EJ, Hurd S (1999) Use of antimicrobials in swine feeds in the United States. Swine Health Prod 7, 19–25.

Dewulf J, Joosten P, Chantziaras I, Bernaerdt E, Vanderhaeghen W, Postma M, Maes D (2022) Antibiotic Use in European Pig Production: Less Is More. Antibiotics 11, 1493.

Diab SS (2016) Diseases Produced by Clostridium perfringens Type C. In: Uzal FA, Songer JG, Prescott JF, Popoff MR (Eds.), Clostridial Diseases of Animals. John Wiley & Sons, Inc, Hoboken, NJ, pp. 143–155. https://doi.org/10.1002/9781118728291.ch12

Diab SS, Uzal FA, Songer JG (2016) Diseases Produced by Clostridium difficile. In: Uzal FA, Songer JG, Prescott JF, Popoff MR (Eds.), Clostridial Diseases of Animals. John Wiley & Sons, Inc, Hoboken, NJ, pp. 177–195. https://doi.org/10.1002/9781118728291.ch15

Diao H, Gao Z, Yu B, Zheng P, He J, Yu J, Huang Z, Chen D, Mao X (2016) Effects of benzoic acid (VevoVitall®) on the performance and jejunal digestive physiology in young pigs. J Anim Sci Biotechnol 7, 32. doi: 10.1186/s40104-016-0091-ys.

Dou S, Gadonna-Widehem P, Rome V, Hamoudi D, Rhazi L, Lakhal L, Larcher T, Bahi-Jaber N, Pinon-Quintana A, Guyonvarch A, Huërou-Luron IL, Abdennebi-Najar L (2017) Characterisation of early-life fecal microbiota in susceptible and healthy pigs to post-weaning Diarrhoea. PLoS One 12, e0169851.

Doyle LP, Hutchings LM (1946) A transmissible gastroenteritis in pigs. J Am Vet Med Assoc 108, 257–259.

Draskovic V, Bosnjak-Neumuller J, Vasiljevic M, Petrujkic B, Aleksic N, Kukolj V, Stanimirovic Z (2018) Influence of phytogenic feed additive on Lawsonia intracellularis infection in pigs. Prev Vet Med 151, 46–51. doi: 10.1016/j.prevetmed.2018.01.002

Duarte ME, Kim SW (2022) Phytobiotics from oregano extracts enhance the intestinal health and growth performance of pigs. Antioxidants 11, 2066.

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Dubreuil JD (2021) Pig vaccination strategies based on enterotoxigenic Escherichia coli toxins. Braz J Microbiol 52(4), 2499-2509. doi: 10.1007/s42770-021-00567-3

Dubreuil JD, Isaacson RE, Schifferli DM (2016) Animal enterotoxigenic Escherichia coli. EcoSal Plus 7. doi: 10.1128/ecosalplus.ESP-0006-2016.

Economou V, Gousia P (2015) Agriculture and food animals as a source of antimicrobial-resistant bacteria. Infect Drug Resist 8, 49–61.

Enjuanes L, Smerdou C, Castilla J, Antón IM, Torres JM, Sola I, Golvano J, Sánchez JM, Pintado B (1995) Development of protection against coronavirus induced diseases. A review. Adv Exp Med Biol 380, 197–211. doi: 10.1007/978-1-4615-1899-0_34

European Food Safety Authority, European Centre for Disease Prevention and Control (2019) The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017. EFSA J 17, 5598.

European Parliament and the Council of the European Union Regulation (EC) No 1831/2003. Off. J. Eur. Union 2003, 4:29–43. Available at: http://eur-lex.europa.eu/legal-content/PT/TXT/?uri=celex:32003R1831 Accessed on 17 February 2021.

Facklam RR, Carvalho MG, Teixeira LM (2002) Enterococcus. In : Gilmore MS, Clewell DB, Courvalin P, Dunny GM, Murray BE, Rice LB, (eds) The Enterococci: Pathogenesis, Molecular Biology, and Antibiotic Resistance. ASM Press, Washington, DC, USA, pp. 1-54.

Fairbrother JM, Nadeau É. Colibacillosis. In: Zimmerman JJ, Karriker LA, Ramirez A, Schwartz KJ, Stevenson GW, Zhang J (eds). Diseases of Swine. John Wiley & Sons; Hoboken, NJ, USA: 2019. pp. 807–834.

Fleming A. Penicillin; Nobel Lecture; 11 December 1945; pp. 84–93. Available at: https://www.nobelprize.org/prizes/medicine/1945/fleming/lecture/. Accessed on 24 July 2020.

Food and Agriculture Organization of the United Nations/World Organisation for Animal Health/World Bank (2010) Good practices for biosecurity in the pig sector – Issues and options in developing and transition countries. FAO Animal Production and Health Paper No. 169. Rome, FAO.

Fouhse JM, Zijlstra RT, Willing BP (2016) The role of gut microbiota in the health and disease of pigs. Anim Front 6(3), 30–6. https://doi.org/10.2527/af.2016-0031

Founou LL, Founou RC, Essack SY (2016) Antibiotic resistance in the food chain: A developing country-perspective. Front Microbiol 7, 1881.

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Founou LL, Founou RC., Essack S. Y. (2021) Antimicrobial resistance in the farm-to-plate continuum: more than a food safety issue. Futur Sci FSO692.

Fox SM (1988) Probiotics: Intestinal inoculants for production animals. Vet Med 83, 806-810.

Frese SA, Parker K, Calvert CC, Mills DA (2015) Diet shapes the gut microbiome of pigs during nursing and weaning. Microbiome 3, 28.

Fuller R (1989) Probiotics in man and animals. J Appl Bacteriol 66, 365-378.

Fuller R (1992) History and development of probiotics. In: Fuller R (ed) Probiotics. The scientific basis. Chapman and Hall, London, pp. 1-8.

Gao K, Pi Y, Peng Y, Mu CL, Zhu WY (2018) Time-course responses of ileal and fecal microbiota and metabolite profiles to antibiotics in cannulated pigs. Appl Microbiol Biotechnol 102, 2289-2299. https://doi.org/10.1007/s00253-018-8774-2

García-Meniño I, García V, Alonso MP, Blanco JE, Blanco J, Mora A (2021) Clones of Enterotoxigenic and Shiga Toxin-Producing Escherichia coli Implicated in Swine Enteric Colibacillosis in Spain and Rates of Antibiotic Resistance. Vet Microbiol 252, 108924. doi: 10.1016/j.vetmic.2020.108924

Gardiner GE, Metzler-Zebeli BU, Lawlor PG (2020) Impact of intestinal microbiota on growth and feed efficiency in pigs: a review. Microorganisms. 8, 1886. doi: 10.3390/microorganisms8121886

Gaskins HR, Collier CT, Anderson DB (2002) Antibiotics as growth promotants: mode of action. Anim Biotechnol 13, 29–42.

Geiger JO, Connor JF (2013) Porcine epidemic diarrhea, diagnosis, and elimination. Available at: https://www.aasv.org/aasv%20website/Resources/Diseases/PED/13-05-29PEDWhitePaper.pdf Accessed on 25 September 2021.

Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, Scott K, Stanton C, Swanson KS, Cani PD, Verbeke K, Gregor R (2017) Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastroenterol Hepatol 14, 491–502.

Gibson GR, Roberfroid MB (1995) Dietary Modulation of the Human Colonic Microbiota: Introducing the Concept of Prebiotics. J Nutr. 125, 1401–1412. doi: 10.1093/jn/125.6.1401

Gill RS, Lee TF, Liu JQ, Chaudhary H, Brocks DR, Bigam DL, Cheung PY (2012) Cyclosporine treatment reduces oxygen free radical generation and oxidative stress

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

in the brain of hypoxia-reoxygenated newborn piglets. PLoS One. 7(7), e40471. doi: 10.1371/journal.pone.0040471

Goede D, Morrison R. Production impact study update. Swine Health Monitoring Project 08/01/2014. University of Minnesota. Available at: http://www.cvm.umn.edu/sdec/SwineDiseases/pedv/SHMP_14/index.htm Accessed on 17 February 2021.

Gong Q-L, Zhao W-X, Wang Y-C, Zong Y, Wang Q, Yang Y, Yang Y, Shi K, Li J-M, Leng X, Du R, Zhao Q (2021) Prevalence of coccidia in domestic pigs in China between 1980 and 2019: a systematic review and meta-analysis. Parasit Vectors 14, 248. https://doi.org/10.1186/s13071-021-04611-x

Goossens H, Ferech M, Vander Stichele R, Elseviers M, for the ESAC Working Group. (2005) Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. Lancet 365, 579–87. doi: 10.1016/S0140-6736(05)17907-0

Grilli E, Piva A (2012) Organic acids and their role in reducing foodborne pathogens in food animals. In: Callaway TR, Edrington TS, (eds) On-Farm Strategies to Control Foodborne Pathogens. Nova Science Pub. Inc., Hauppauge, NY, pp. 183-210.

Gryaznova MV, Dvoretskaya YD, Syromyatnikov MY, Shabunin SV, Parshin PA, Mikhaylov EV, Strelnikov NA, Popov VN (2022) Changes in the microbiome profile in different parts of the intestine in piglets with diarrhea. Animals 12, 320. doi: 10.3390/ani12030320

Hajati H (2018) Application of organic acids in poultry nutrition. Int J Avian Wildl Biol 3, 324-329.

Halder D, Mandal M, Chatterjee SS, Pal NK, Mandal S (2017) Indigenous probiotic Lactobacillus isolates presenting antibiotic-like activity against human pathogenic bacteria. Biomedicines 5, 31.

Han YS, Tang CH, Zhao QY, Zhan TF, Zhang K, Han YM, Zhang JM (2018) Effects of dietary supplementation with combinations of organic and medium chain fatty acids as replacements for chlortetracycline on growth performance, serum immunity, and faecal microbiota of weaned piglets. Livest Sci 216, 210-218.

He Y, Jinno C, Kim K, Wu Z, Tan B, Li X, Whelan R, Liu Y (2020) Dietary Bacillus spp. Enhanced growth and disease resistance of weaned pigs by modulating intestinal microbiota and systemic immunity. J Anim Sci Biotechnol 11, 101. doi: 10.1186/s40104-020-00498-3

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Hermann-Bank ML, Skovgaard K, Stockmarr A, Lenz Strube M, Larsen N, Kongsted H, Ingerslev HC, Mølbak L, Boye M (2015) Characterization of the bacterial gut microbiota of piglets suffering from new neonatal porcine diarrhoea. BMC Vet Res 11, 139. doi: 10.1186/s12917-015-0419-4

Herskin MS, Di Giminiani P (2017) Pain in pigs: Characterisation, mechanisms and indicators. In Advances in Pig Welfare; Špinka M., Camerlink I. (Eds.), Elsevier Ltd.: Cambridge, UK, pp. 325–355.

Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, Calder PC, Sanders ME (2014) The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 11, 506-514.

Hinney B, Cvjetkovic V, Espigares D, Vanhara J, Waehner C, Ruttkowski B, Selista R, Sperling D, Joachim A (2020) Cystoisospora suis control in Europe is not always effective. Front Vet Sci 7, 113. https://doi.org/10.3389/fvets.2020.00113

Hoberg EP, Brooks DR (2015) Evolution in action: Climate change, biodiversity dynamics and emerging infectious disease. Philosoph Trans R Soc B 370, 20130553.

Hockenhull J, Turner AE, Reyher KK, Barrett DC, Jones L , Hinchliffe S, Buller HJ (2017) Antimicrobial use in food-producing animals: a rapid evidence assessment of stakeholder practices and beliefs. Vet Rec 181(19), 510. doi: 10.1136/vr.104304

Holman DB, Chénier MR (2015) Antimicrobial use in swine production and its effect on the swine gut microbiota and antimicrobial resistance. Can J Microbiol 61, 785–798. doi: 10.1139/cjm-2015-0239

Holmer I, Salomonsen CM, Jorsal SE, Astrup LB, Jensen VF, Høg BB, Pedersen K (2019) Antibiotic resistance in porcine pathogenic bacteria and relation to antibiotic usage. BMC Vet Res 15(1), 449. doi: 10.1186/s12917-019-2162-8

Innes G, Randad PR, Korinek A, Davis MF, Price LB, So AD, Heaney CD (2020) External Societal Costs of Antimicrobial Resistance in Humans Attributable to Antimicrobial Use in Livestock Annu Rev Public Health 41, 141–157. doi:10.1146/annurev-publhealth-040218-043954

Jacela J, Derouchey JM, Tokach M, Goodband R, Nelssen J, Renter D, Dritz S (2010) Feed additives for swine: Fact sheets—Prebiotics and probiotics, and phytogenics. J Swine Health Prod 18, 132-136.

Jacobson M (2022) On the Infectious Causes of Neonatal Piglet Diarrhoea—A Review. Vet Sci 9, 422. https://doi.org/10.3390/vetsci9080422

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Jang S, Shin S, Kim S-H, Kim H, Moon C (2019) Diagnosis of Enterococcus hirae infection in association with piglet diarrhea. J Biomed Transl Res 20, 115-120. doi: 10.12729/jbtr.2019.20.4.115

Jayaraman B, Nyachoti CM (2017) Husbandry practices and gut health outcomes in weaned piglets: A review. Anim Nutr. 3(3), 205-211. doi: 10.1016/j.aninu.2017.06.002

Jernberg C, Löfmark S, Edlund C, Jansson JK (2010) Long-term impacts of antibiotic exposure on the human intestinal microbiota. Microbiology 156, 3216–3223. doi: 10.1099/mic.0.040618-0

Jiao A, Diao H, Yu B, He J, Yu J, Zheng P, Luo Y, Luo J, Wang Q, Wang H, Mao X, Chen D (2021) Infusion of short chain fatty acids in the ileum improves the carcass traits, meat quality and lipid metabolism of growing pigs. Anim Nutr 7, 94-100. https://doi.org/10.1016/j.aninu.2020.05.009

Jiménez MJ, Berrios R, Stelzhammer S, Bracarense APFRL (2020) Ingestion of organic acids and cinnamaldehyde improves tissue homeostasis of piglets exposed to enterotoxic Escherichia coli (ETEC). J Anim Sci 98, skaa012. doi: 10.1093/jas/skaa012

Jo HE, Kwon MS, Whon TW, Kim DW, Yun M, Lee J, Shin MY, Kim SH, Choi HJ (2021) Alteration of Gut Microbiota After Antibiotic Exposure in Finishing Swine. Front Microbiol 12, 596002. doi: 10.3389/fmicb.2021.596002

Joachim A, Ruttkowski B, Sperling D (2018) Detection of Cystoisospora suis in faeces of suckling piglets - when and how? A comparison of methods. Porcine Health Manag 4, 20. doi: 10.1186/s40813-018-0097-2

Joachim A, Shrestha A (2019) Coccidiosis of Pigs, in: Coccidiosis in Livestock, Poultry, Companion Animals, and Humans. CRC Press.

Jonach B, Boye M, Stockmarr A, Jensen TK (2014) Fluorescence in situ hybridization investigation of potentially pathogenic bacteria involved in neonatal porcine diarrhea. BMC Vet Res 10, 1-8. doi: 10.1186/1746-6148-10-68

Jones MA, Hunter D (1983) Isolation of Clostridium difficile from pigs. Vet Rec 112, 253–254.

Kahn LH (2016) ONE HEALTH and the politics of antimicrobial resistance. John Hopkins University Press, Baltimore.

Kantas D, Papatsiros VG, Tassis PD, Athanasiou LV, Tzika ED (2015) Effect of a natural feed additive (Macleaya cordata), containing sanguinarine, on the performance and health status of weaning pigs. Anim Sci J 86, 92-98.

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Kantas D, Papatsiros VG, Tassis PD, Giavasis I, Bouki P, Tzika ED (2015) A feed additive containing Bacillus toyonensis (Toyocerin®) protects against enteric pathogens in postweaning piglets. J Appl Microbiol 118, 727-738. https://doi.org/10.1111/jam.12729

Kaper JB, Nataro JP, Mobley HL (2004) Pathogenic Escherichia coli. Nat Rev Microbiol 2(2), 123–140. https://doi.org/10.1038/nrmicro818

Karamon J, Ziomko I, Cencek T (2007) Prevalence of Isospora suis and Eimeria spp. in suckling piglets and sows in Poland. Vet Parasitol 147, 171-175. doi: 10.1016/j.vetpar.2007.03.029

Khan SH, Iqbal J (2016) Recent advances in the role of organic acids in poultry nutrition. J Appl Anim Res 44:359-369

Kiernan DP, O'Doherty JV, Sweeney T. (2023) The Effect of Prebiotic Supplements on the Gastrointestinal Microbiota and Associated Health Parameters in Pigs. Animals. 13(19), 3012. https://doi.org/10.3390/ani13193012

Kikusato M (2021) Phytobiotics to improve health and production of broiler chickens: Functions beyond the antioxidant activity. Anim Biosci 34, 345-353.

Kim HB, Borewicz K, White BA, Singer RS, Sreevatsan S, Tu ZJ (2012) Microbial shifts in the swine distal gut in response to the treatment with antimicrobial growth promoter, tylosin. Proc Natl Acad Sci U.S.A. 109, 15485–15490. doi: 10.1073/pnas.1205147109

Kim HB, Isaacson RE (2015) The pig gut microbial diversity: understanding the pig gut microbial ecology through the next generation high throughput sequencing. Vet Microbiol 177, 242–51. doi: 10.1016/j.vetmic.2015.03.014

Kim HY, Cho A, Kim JW, Kim H, Kim B (2018) High prevalence of Clostridium difficile PCR ribotype 078 in pigs in Korea. Anaerobe 51, 42–46.

Kim KR, Owens G, Kwon SI, So KH, Lee DB, Ok YS (2010) Occurrence and environmental fate of veterinary antibiotics in the terrestrial environment. Water Air Soil Poll 214, 163–174. doi: 10.1007/s11270-010-0412-2

Kim L, Chang KO, Sestak K, Parwani A, Saif LJ (2000) Development of a reverse transcription-nested polymerase chain reaction assay for differential diagnosis of transmissible gastroenteritis virus and porcine respiratory coronavirus from feces and nasal swabs of infected pigs. J Vet Diagn Invest 12, 385–388. doi: 10.1177/104063870001200418

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Kimera, ZI, Mshana SE, Rweyemamu MM, Mboera LEG, Matee MIN (2020) Antimicrobial use and resistance in food-producing animals and the environment: an African perspective. Antimicrob Resist Infect Control 9, 37. doi:10.1186/s13756-020-0697-x

Kirchhelle C (2018) Pharming animals: a global history of antibiotics in food production (1935–2017). Palgrave Commun 4, 96.

Kongsted H, Pedersen K, Hjulsager CK, Larsen LE, Pedersen KS, Jorsal SE, Bækbo P (2018) Diarrhoea in neonatal piglets: a case control study on microbiological findings. Porcine Health Manag 4, 17.

Kumar M, Kumar V, Roy D, Kushwaha R, Vaswani S (2014) Application of herbal feed additives in animal nutrition—a review. Int J Livest Res 4, 1-8.

Kuppusamy S, Kakarlab D, Venkateswarluc K, Megharajd M, Yoone YE, Lee YB (2018) Veterinary antibiotics (VAs) contamination as a global agro-ecological issue: A critical view. Agric Ecosyst Environ 257, 47-59.

Kuralkar P, Kuralkar SV (2021) Role of herbal products in animal production—an updated review. J Ethnopharmacol 278, 114246.

Lamendella R, Santo Domingo JW, Ghosh S, Martinson J, Oerther DB (2011) Comparative fecal metagenomics unveils unique functional capacity of the swine gut. BMC Microbiol 11, 103. https://doi.org/10.1186/1471-2180-11-103

Landecker H (2016) Antibiotic resistance and the biology of history. Body Soc 22, 19–52.

Larsson J, Aspan A, Grandon R, Lindberg R, Westergren E, Jacobson M (2014) Neonatal piglet diarrhoea associated with enteroadherent Enterococcus hirae. J Comp Pathol 151, 137-147. doi: 10.1016/j.jcpa.2014.04.003

Larsson J, Aspán A, Lindberg R, Grandon R, Båverud V, Fall N, Jacobson M (2015) Pathological and bacteriological characterization of neonatal porcine diarrhoea of uncertain aetiology. J Med Microbiol 64, 916-926. doi: 10.1099/jmm.0.000108

Laudato M, Capasso R (2013) Useful plants for animal therapy. OA Altern Med 1, 1-6.

Lauridsen C (2020) Effects of dietary fatty acids on gut health and function of pigs pre- and post-weaning. J Anim Sci 98(4), skaa086. doi: 10.1093/jas/skaa086

Lecce JG, King MW, Dorsey WE (1978) Rearing regimen producing piglet diarrhea (rotavirus) and its relevance to acute infantile diarrhea. Science 199, 776-778. doi: 10.1126/science.203032

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Lekagul A, Tangcharoensathien V, Yeung S (2019) Patterns of antibiotic use in global pig production: a systematic review. Vet Anim Sci 7, 100058. doi: 10.1016/J.VAS.2019.100058

Lemon KP, Armitage GC, Relman DA, Fischbach MA (2012) Microbiota-targeted therapies: an ecological perspective. Sci Transl Med 4, 137rv5. https://doi.org/10.1126/scitranslmed.3004183

Levin D, Raab N, Pinto Y, Rothschild D, Zanir G, Godneva A, Mellul N, Futorian D, Gal D, Leviatan S, Zeevi D, Bachelet I, Segal E (2021) Diversity and functional landscapes in the microbiota of animals in the wild. Science. 372, eabb5352. https://doi.org/10.1126/science.abb5352

Levine MM (1987) Escherichia coli that cause diarrhea: Enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic, and enteroadherent. J Infect Dis 155, 377–389.

Li K, Xiao Y, Chen J, Chen J, He X, Yang H (2017) Microbial composition in different gut locations of weaning piglets receiving antibiotics. Asian Austral J Anim Sci 30, 78–84. doi: 10.5713/ajas.16.0285

Li L, Sun X, Zhao D, Dai H (2021) Pharmacological applications and action mechanisms of phytochemicals as alternatives to antibiotics in pig production. Front Immunol 12, 798553. https://doi.org/10.3389/fimmu.2021.798553

Li M, Li Z, Zhong Q, Liu J, Han G, Li Y, Li C (2022) Antibiotic resistance of fecal carriage of Escherichia coli from pig farms in China: a meta-analysis. Environ Sci Pollut Res Int 29(16), 22989-23000. doi: 10.1007/s11356-021-17339-z

Li Z, Ma Z, Li Y, Gao S, Xiao S (2020) Porcine epidemic diarrhea virus: Molecular mechanisms of attenuation and vaccines. Microb Pathog 149, 104553. doi: 10.1016/j.micpath.2020.104553

Liao SF (2021) Invited Review: Maintain or Improve Piglet Gut Health around Weanling: The Fundamental Effects of Dietary Amino Acids. Animals 11, 1110. doi: 10.3390/ani11041110

Liao SF, Nyachoti M (2017) Using probiotics to improve swine gut health and nutrient utilization. Anim Nutr 3, 331–343. doi: 10.1016/j.aninu.2017.06.007

Lillehoj H, Liu Y, Calsamiglia S, Fernandez-Miyakawa ME, Chi F, Cravens RL, Oh S, Gay CG (2018) Phytochemicals as antibiotic alternatives to promote growth and enhance host health. Vet Res 49, 76. https://doi.org/10.1186/s13567-018-0562-6

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Linares DM, Gómez C, Renes E, Fresno JM, Tornadijo ME, Ross RP, Stanton C (2017) Lactic acid bacteria and bifidobacteria with potential to design natural biofunctional health-promoting dairy foods. Front Microbiol 8, 846.

Link R, Kováč G, Pistl J (2005) A note on probiotics as an alternative for antibiotics in pigs. J Anim Feed Sci 14, 513-519. doi:10.22358/jafs/67049/200

Liu F, Cottrell JJ, Furness JB, Rivera LR, Kelly FW, Wijesiriwardana U, Pustovit RV, Fothergill LJ, Bravo DM, Celi P, Leury BJ, Gabler NK, Dunshea FR (2016) Selenium and vitamin E together improve intestinal epithelial barrier function and alleviate oxidative stress in heat-stressed pigs. Exp Physiol 101(7), 801–10. doi: 10.1113/EP085746

Liu HY, Dicksved J, Lundh T, Lindberg JE (2014) Expression of heat shock proteins 27 and 72 correlates with specific commensal microbes in different regions of porcine gastrointestinal tract. Am J Physiol Gastrointest Liver Physiol 306, G1033-G1041. https://doi.org/10.1152/ajpgi.00299.2013

Liu Q, Gerdts V (2021) Transmissible Gastroenteritis Virus of Pigs and Porcine Epidemic Diarrhea Virus (Coronaviridae). In: Encyclopedia of Virology. pp. 850–3. doi: 10.1016/B978-0-12-809633-8.20928-X

Liu Q, Wang HY (2021) Porcine enteric coronaviruses: an updated overview of the pathogenesis, prevalence, and diagnosis. Vet Res Commun 45(2-3), 75-86. doi: 10.1007/s11259-021-09808-0

Liu Y, Espinosa CD, Abelilla JJ, Casas GA, Lagos LV, Lee SA, Stein HH (2018) Non-antibiotic feed additives in diets for pigs: A review. Anim Nutr 4, 113-125.

Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu JH, Shen J (2016) Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis 16(2), 161-8. doi: 10.1016/S1473-3099(15)00424-7

Long SF, Xu YT, Pan L, Wang QQ, Wang CL, Wu JY, Wu YY, Han YM (2018) Mixed organic acids as antibiotic substitutes improve performance, serum immunity, intestinal morphology and microbiota for weaned piglets. J Anim Feed Sci 235, 23-32.

Looft T, Allen HK, Casey TA, Alt DP, Stanton TB (2014) Carbadox has both temporary and lasting effects on the swine gut microbiota. Front Microbiol 5, 276. 10.3389/fmicb.2014.00276

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Looft T, Johnson TA, Allen HK, Bayles DO, Alt DP, Stedtfeld RD, Sul WJ, Stedtfeld TM, Chai B, Cole JR, Hashsham SA, Tiedje JM, Stanton TB (2012) In-feed antibiotic effects on the swine intestinal microbiome. Proc Natl Acad Sci U S A 109, 1691–1696. 10.1073/pnas.1120238109

Luckstadt C, Mellor S (2010) Holoanalysis – the acid test in pig diets. Kraftfutter Feed Magazine 1-2, 18-21.

Lückstädt C, Wylie L, Remmer R, De Kok R, Costa HR, Brebels M, van Heusden C (2014) Organic Acids in Animal Nutrition; Anitox, Lawrenceville, GA, USA.

Luise D, Motta V, Salvarani C, Chiappelli M, Fusco L, Bertocchi M, Mazzoni M, Maiorano G, Costa LN, Milgen JV, Bosi P, Trevisi P (2017) Long-term administration of formic acid to weaners: influence on intestinal microbiota, immunity parameters and growth performance. Anim Feed Sci Technol 232, 160-168.

Luppi A, Gibellini M, Gin T, Vangroenweghe F, Vandenbroucke V, Bauerfeind R, Bonilauri P, Labarque G, Hidalgo A (2016) Prevalence of virulence factors in enterotoxigenic Escherichia coli isolated from pigs with post-weaning diarrhea in Europe. Porcine Health Manag 2, 20.

Luppi A. (2017) Swine enteric colibacillosis: diagnosis, therapy and antimicrobial resistance. Porc Health Manag 3, 16. https://doi.org/10.1186/s40813-017-0063-4

Ma Y, Han X, Fang J, Jiang H (2021) Role of dietary amino acids and microbial metabolites in the regulation of pig intestinal health. Anim Nutr 9, 1-6. doi: 10.1016/j.aninu.2021.10.004

Maes D, Vyt P, Rabaeys P, Gevaert D (2007) Effects of toltrazuril on the growth of piglets in herds without clinical isosporosis. Vet J 173, 197–199. https://doi.org/10.1016/j.tvjl.2005.07.002

Magnusson U, Lewerin SS, Eklund G, Rozstalnyy A (2019) Prudent and Efficient Use of Antimicrobials in Pigs and Poultry: A Practical Manual; FAO: Rome, Italy.

Maltecca C, Bergamaschi M, Tiezzi F (2020) The interaction between microbiome and pig efficiency: a review. J Anim Breed Genet 137, 4–13. doi: 10.1111/jbg.12443

MAPAQ. (2020) Rapport 2020: Résultats de la Surveillance Passive de l'Antibiorésistance; MAPAQ: Quebec, QC, Canada, 46pp.

Markowiak P, Śliżewska K (2018) The role of probiotics, prebiotics and synbiotics in animal nutrition. Gut Pathog 10, 21. doi: 10.1186/s13099-018-0250-0

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Martel J, Ojcius DM, Ko YF, Young JD (2020) Phytochemicals as prebiotics and biological stress inducers. Trends Biochem Sci 45, 462-471. https://doi.org/10.1016/j.tibs.2020.02.008

Marthaler D, Rossow K, Culhane M, Collins J, Goyal S, Ciarlet M, Matthijnssens J (2013) Identification, phylogenetic analysis and classification of porcine group C rotavirus VP7 sequences from the United States and Canada. Virology 446, 189-198. doi: 10.1016/j.virol.2013.08.001

McClane BA, Robertson SL, Li J (2013) Clostridium perfringens, p 465–489. In: Doyle MP, Buchanan RL (eds) Food Microbiology: Fundamentals and Frontiers, 4th ed. ASM Press, Washington, DC.

McCormack UM, Curião T, Buzoianu SG, Prieto ML, Ryan T, Varley P, Crispie F, Magowan E, Metzler-Zebeli BU, Berry D, O'Sullivan O, Cotter PD, Gardiner GE, Lawlor PG (2017) Exploring a possible link between the intestinal microbiota and feed efficiency in pigs. Appl Environ Microbiol 83, 1–16.

McGoldrick A, Lowings JP, Paton DJ (1999) Characterisation of a recent virulent transmissible gastroenteritis virus from Britain with a deleted ORF 3a. Arch Virol 144, 763–770. doi: 10.1007/s007050050541

Mellado M, Gaytán L, Macías-Cruz U, Avendaño-Reyes L, Meza-Herrera CA, Lozano E, Rodríguez A, Mellado J (2018) Effect of climate and insemination technique on reproductive performance of gilts and sows in a subtropical zone of Mexico. Austral J Vet Sci 50, 27-34. doi: 10.4067/S0719-81322018000100106

Mesonero-Escuredo S, Strutzberg-Minder K, Casanovas C, Segalés J (2018) Viral and bacterial investigations on the etiology of recurrent pig neonatal diarrhea cases in Spain. Porc Health Manag 4, 5.

Moberg GP, Mench JA (2000) The Biology of Animal Stress—Basic Principles and Implications for Animal Welfare, 1st ed.; CAB International: Wallingford, UK.

Molinari BLD, Lorenzetti E, Otonel RAA, Alfieri AF, Alfieri AA (2014) Species H rotavirus detected in piglets with diarrhea, Brazil, 2012. Emerg Inf Dis 20, 1019-1022. doi: 10.3201/eid2006.130776

Monger XC, Gilbert AA, Saucier L, Vincent AT (2021) Antibiotic Resistance: From Pig to Meat. Antibiotics 10, 1209. doi: 10.3390/antibiotics10101209

Morin M, Magar R, Robinson Y (1990) Porcine group C rotavirus as a cause of neonatal diarrhea in a Quebec swine herd. Can J Vet Res 54, 385-389

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Morin M, Turgeon D, Jolette J, Robinson Y, Phaneuf JB, Sauvageau R, Beauregard M, Teuscher E, Higgins R, Larivière S (1983) Neonatal diarrhea of pigs in Quebec: Infectious causes of significant outbreaks. Can J Comp Med 47, 11–17.

Mundt HC, Cohnen A, Daugschies A, Joachim A, Prosl H, Schmäschke R, Westphal B (2005) Occurrence of Isospora suis in Germany, Switzerland and Austria. J Vet Med B. 52, 93–97. doi: 10.1111/j.1439-0450.2005.00824.x

Murray CJ, Ikuta KS, Sharara F, Swetschinski L, Robles Aguilar G, Gray A, Han C, Bisignano C, Rao P, Wool E (2022) Global Burden of Bacterial Antimicrobial Resistance in 2019: A Systematic Analysis. Lancet 399:629–655. doi: 10.1016/S0140-6736(21)02724-0

Nadeau E, Fairbrother JM, Zentek J, Belanger L, Tremblay D, Tremblay CL, Rohe I, Vahjen W, Brunelle M, Hellmann K, Cvejic D, Brunner B, Schneider C, Bauer K, Wolf R, Hidalgo A (2017) Efficacy of a single oral dose of a live bivalent E. coli vaccine against post-weaning diarrhea due to F4 and F18-positive enterotoxigenic E. coli. Vet J 226, 32–39. doi: 10.1016/j.tvjl.2017.07.004

Nagy J, Bilkei G (2003) Neonatal piglet losses associated with Escherichia coli and Clostridium difficile infection in a Slovakian outdoor production unit. Vet J 166(1), 98–100. doi: 10.1016/s1090-0233(02)00252-6

Naqid IA, Owen JP, Maddison BC, Gardner DS, Foster N, Tchórzewska MA, La Ragione RM, Gough KC (2015) Prebiotic and probiotic agents enhance antibody-based immune responses to Salmonella Typhimurium infection in pigs. Anim Feed Sci Technol 201, 57-65. https://doi.org/10.1016/j.anifeedsci.2014.12.005

National Academies of Sciences, Engineering, and Medicine (2018) Understanding the economics of microbial threats: Proceedings of a workshop. Washington, DC: The National Academies Press. doi: 10.17226/25224

Neuman H, Forsythe P, Uzan A, Avni O, Koren O (2018) Antibiotics in early life: dysbiosis and the damage done. FEMS Microbiol Rev 42, 489–499. doi: 10.1093/femsre/fuy018

Nhara RB, Marume U, Nantapo CWT (2024) Potential of organic acids, essential oils and their blends in pig diets as alternatives to antibiotic growth promoters. Animals 14, 762. https://doi.org/10.3390/ani14050762

Noack S, David Chapman H, Selzer PM (2019) Anticoccidial drugs of the livestock industry. Parasitol Res 118, 2009–2026. doi.org/10.1007/s00436-019-06343-5

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Nowak P, Kasprowicz-Potocka M, Zaworska A, Nowak W, Stefańska B, Sip A, Grajek W, Juzwa W, Taciak M, Barszcz M, Tuśnio A, Grajek K, Foksowicz-Flaczyk J, Frankiewicz A (2017) The effect of eubiotic feed additives on the performance of growing pigs and the activity of intestinal microflora. Arch Anim Nutr 71, 455-469.

O'Neill J (2014) Antimicrobial Resistance: Tackling a Crisis to the Health and Wealth of Nations. Rev Antimicrob Resist 20, 1–16.

O'Neill J (2016) Tackling Drug-Resistant Infections Globally: Final Report and Recommendations. The Review on Antimicrobial Resistance. Available at: https://amr-review.org/sites/default/files/160525_Final Accessed on 17 February 2021

O'Neill L, Calderón Díaz JA, Rodrigues da Costa M, Oakes S, Leonard FC, Manzanilla EG (2021) Risk Factors for Antimicrobial Use on Irish Pig Farms. Animals 11, 2828. doi: 10.3390/ani11102828

Oh J, Wall EH, Bravo DM, Hristov AN (2017) Host-mediated effects of phytonutrients in ruminants: A review. J Dairy Sci 100, 5974-5983.

Pajarillo EA, Chae JP, Balolong MP, Kim HB, Kang DK (2014) Assessment of fecal bacterial diversity among healthy piglets during the weaning transition. J Gen Appl Microbiol 60, 140–6.

Pajarillo EA, Chae JP, Balolong MP, Kim HB, Seo KS, Kang DK (2014) Pyrosequencing-based analysis of fecal microbial communities in three purebred pig lines. J Microbiol 52, 646–51.

Pajarillo EAB, Chae JP, Balolong MP, Kim HB, Park C-S, Kang D-K (2015) Effects of probiotic Enterococcus faecium NCIMB 11181 administration on swine fecal microbiota diversity and composition using barcoded pyrosequencing. Anim Feed Sci Technol 201, 80–8.

Papakonstantinou GI, Meletis E, Petrotos K, Kostoulas P, Tsekouras N, Kantere MC, Voulgarakis N, Gougoulis D, Filippopoulos L, Christodoulopoulos G, Athanasiou LV, Papatsiros VG (2023) Effects of a natural polyphenolic product from olive mill wastewater on oxidative stress and post-weaning diarrhea in piglets. Agriculture 13, 1356. https://doi.org/10.3390/agriculture13071356

Papatsiros V, Tzika E, Tassis P, Kantas D, Filippopoulos L, Papaioannou D (2011) Greek experience of the use of phytogenic feed additives in organic pig farming. J Cell Anim Biol 5, 320-323.

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Papatsiros VG, Papakonstantinou GI, Katsogiannou E, Gougoulis DA, Voulgarakis N, Petrotos K, Braimaki S, Galamatis DA, El-Sayed A, Athanasiou LV (2024) Effects of a phytogenic feed additive on redox status, blood haematology, and piglet mortality in primiparous sows. Stresses 4, 293-307. https://doi.org/10.3390/stresses4020018

Papatsiros VG, Stylianaki I, Papakonstantinou G, Papaioannou N, Christodoulopoulos G (2019) Case Report of Transmissible Gastroenteritis Coronavirus Infection Associated with Small Intestine and Brain Lesions in Piglets. Viral Immunol 32, 63-67. doi: 10.1089/vim.2018.0095

Papatsiros VG, Tassis PD, Tzika ED, Papaioannou DS, Petridou E, Alexopoulos C, Kyriakis SC (2011) Effect of benzoic acid and combination of benzoic acid with a probiotic containing Bacillus cereus var. Toyoi in weaned pig nutrition. Pol J Vet Sci 14, 117-125. https://doi.org/10.2478/v10181-011-0017-8

Papatsiros VG, Tzika ED, Papaioannou DS, Kyriakis SC, Tassis PD, Kyriakis CS (2009) Effect of Origanum vulgaris and Allium sativum extracts for the control of proliferative enteropathy in weaning pigs. Pol J Vet Sci 12, 407-414.

Pateiro M, Francisco B, Domínguez J, Sant A (2018) Essential oils as natural additives to prevent oxidation reactions in meat and meat products: A review. Food Res Int 113, 156-166.

Patel S, Shukla R, Goyal A (2015) Probiotics in valorization of innate immunity across various animal models. J Funct Foods 145, 49-561. https://doi.org/10.1016/j.jff.2015.02.022

Pettersson E, Hestad S, Möttus I, Skiöldebrand E, Wallgren P (2019) Rotavirus and Cystoisospora suis in piglets during the suckling and early post weaning period, in systems with solid floors and age segregated rearing. Porc Health Manag 8, 5–7. doi: 10.1186/s40813-019-0114-0

Pié S, Awati A, Vida S, Falluel I, Williams BA, Oswald IP (2007) Effects of added fermentable carbohydrates in the diet on intestinal proinflammatory cytokine-specific mRNA content in weaning piglets. J Anim Sci 85, 673-683. https://doi.org/10.2527/jas.2006-535

Pluske JR, Turpin DL, Kim JC (2018) Gastrointestinal tract (gut) health in the young pig. Anim Nutr 4, 187–196. doi: 10.1016/j.aninu.2017.12.004

Popov IV, Einhardt Manzke N, Sost MM, Verhoeven J, Verbruggen S, Chebotareva IP, Ermakov AM, Venema K (2023) Modulation of swine gut microbiota by phytogenic

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

blends and high concentrations of casein in a validated swine large intestinal in vitro model. Vet Sci 10, 677. https://doi.org/10.3390/vetsci10120677

Post KW, Songer JG (2004) Antimicrobial susceptibility of Clostridium difficile isolated from neonatal pigs with enteritis. Anaerobe 10, 47–50. doi: 10.1016/j.anaerobe.2004.01.003

Posthaus H, Kittl S, Tarek B, Bruggisser J (2020) Clostridium perfringens type C necrotic enteritis in pigs: diagnosis, pathogenesis, and prevention. J Vet Diagn Invest 32(2), 203-212. doi: 10.1177/1040638719900180

Prestinaci F, Pezzotti P, Pantosti A (2015) Antimicrobial resistance: a global multifaceted phenomenon. Pathog Glob Health 109(7), 309. doi: 10.1179/2047773215Y.0000000030

Pritchard GC (1987) Transmissible gastroenteritis in endemically infected breeding herds of pigs in East Anglia, 1981–85. Vet Rec 120, 226–230. doi: 10.1136/vr.120.10.226.

Rafiq K, Tofazzal Hossain M, Ahmed R, Hasan MM, Islam R, Hossen MI, Shaha SN, Islam MR (2022) Role of different growth enhancers as alternative to in-feed antibiotics in poultry industry. Front Vet Sci 8, 794588.

Rahman MRT, Fliss I, Biron E (2022) Insights in the development and uses of alternatives to antibiotic growth promoters in poultry and swine production. Antibiotics 11, 766. https://doi.org/10.3390/antibiotics11060766

Rathnayake D, Mun HS, Dilawar MA, Baek KS, Yang CJ (2021) Time for a paradigm shift in animal nutrition metabolic pathway: dietary inclusion of organic acids on the production parameters, nutrient digestibility, and meat quality traits of swine and broilers. Life 11, 476.

Rhouma M, Beaudry F, Thériault W, Letellier A (2016) Colistin in pig production: chemistry, mechanism of antibacterial action, microbial resistance emergence, and one health perspectives. Front Microbiol 7, 1789. doi: 10.3389/fmicb.2016.01789

Riboulet-Bisson E, Sturme MH, Jeffery IB, O'Donnell MM, Neville BA, Forde BM, Claesson MJ, Harris H, Gardiner GE, Casey PG, Lawlor PG, O'Toole PW, Ross RP (2012) Effect of Lactobacillus salivarius bacteriocin Abp118 on the mouse and pig intestinal microbiota. PLoS One 7(2), e31113. doi: 10.1371/journal.pone.0031113

Roldan-Santiago P, Trujillo-Ortega M, Borderas-Tordesillas F, Martínez-Rodríguez R, Mora-Medina P, Flores-Peinado S, Sánchez-Hernández M, García-Herrera R, González-Lozano M, Mota-Rojas D (2015) Physiometabolic responses to road

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

transport in weaned piglets for a short period and the effects of straw bedding. Anim Sci J 86(5), 563–71. doi: 10.1111/asj.12324

Rood JI (2016) General physiological and virulence properties of the pathogenic clostridia. In: Uzal FA, Songer JG, Prescott JF, Popoff MR (Eds), Clostridial Diseases of Animals. John Wiley & Sons, Inc, Hoboken, NJ, pp. 7–12. https://doi.org/10.1002/9781118728291.ch2

Rood JI, Adams V, Lacey J, Lyras D, McClane BA, Melville SB, Moore RJ, Popoff MR, Sarker MR, Songer JG, Uzal FA, Van Immerseel F (2018) Expansion of the Clostridium perfringens toxin-based typing scheme. Anaerobe 53, 5–10. https://doi.org/10.1016/j.anaerobe.2018.05.014

Rossi B, Toschi A, Piva A, Grilli E (2020) Single components of botanicals and nature-identical compounds as a non-antibiotic strategy to ameliorate health status and improve performance in poultry and pigs. Nutr Res Rev 33, 218-234.

Saif LJ (1999) Comparative pathogenesis of enteric viral infections of swine. Adv Exp Med Biol 473, 47–59. doi: 10.1007/978-1-4615-4143-1_4

Salminen S, Collado MC, Endo A, Hill C, Lebeer S, Quigley EMM, Sanders ME, Shamir R, Swann JR, Szajewska H, Vinderola G (2021) The international scientific association of probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. Nat Rev Gastroenterol Hepatol 18, 649-667. https://doi.org/10.1038/s41575-021-00440-6

Salminen S, Ouwehand A, Benno Y, Lee YK (1999) Probiotics: how should they be defined? Trends Food Sci Tech 10, 107-110.

Sarrazin S, Joosten P, Van Gompel L, Luiken REC, Mevius DJ, Wagenaar JA, Heederik DJJ, Dewulf J (2019) Quantitative and qualitative analysis of antimicrobial usage patterns in 180 selected farrow-to-finish pig farms from nine European countries based on single batch and purchase data. J Antimicrob Chem. 74(3), 807–816. https://doi.org/10.1093/jac/dky503

Schmid SM, Genter CI, Heinemann C, Steinhoff-Wagner J (2021) Impact of tearing spermatic cords during castration in live and dead piglets and consequences on welfare. Porc Health Manag 7, 17.

Schmidt TSB, Raes J, Bork P (2018) The Human Gut Microbiome: From Association to Modulation. Cell 172, 1198–1215. doi: 10.1016/j.cell.2018.02.044

Schokker D, Zhang J, Vastenhouw SA, Heilig HGHJ, Smidt H, Rebel JMJ, Smits MA. (2015) Long-lasting effects of early-life antibiotic treatment and routine animal

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

handling on gut microbiota composition and immune system in pigs. PLoS ONE. 10(2), 1–18. doi.org/10.1371/journal.pone.0116523.

Schokker D, Zhang J, Zhang LL, Vastenhouw SA, Heilig HG, Smidt H, Rebel JM, Smits MA (2014) Early-life environmental variation affects intestinal microbiota and immune development in new-born piglets. PLoS One 9, e100040. https://doi.org/10.1371/journal.pone.0100040

Shrestha P, Cooper BS, Coast J, Oppong R, Do Thi Thuy N, Phodha T, Celhay O, Guerin PJ, Wertheim H, Lubell Y (2018) Enumerating the economic cost of antimicrobial resistance per antibiotic consumed to inform the evaluation of interventions affecting their use. Antimicrob Resist Infect Control 9, 7:98. doi: 10.1186/s13756-018-0384-3

Silva RO, Oliveira C, Guedes R, Lobato F (2015) Clostridium perfringens: A review of the disease in pigs, horses and broiler chickens. Ciência Rural 45. https://doi.org/10.1590/0103-8478cr20140927

Sjölund M, Zoric M, Wallgren P (2014) Financial impact of disease on pig production. Part III. Gastrointestinal Disorders. In: Proceedings of 6th European Symposium of Porcine Health Management; Italy, Sorrento p. 189.

Soler C, Goossens T, Bermejo A, Migura-García L, Cusco A, Francino O, Fraile L (2018) Digestive microbiota is different in pigs receiving antimicrobials or a feed additive during the nursery period. PLoS ONE 13, e197353. doi: 10.1371/journal.pone.0197353

Song D, Park B (2012) Porcine epidemic diarrhoea virus: a comprehensive review of molecular epidemiology, diagnosis, and vaccines. Virus Genes 44, 167–175.

Songer JG, Anderson MA (2006) Clostridium difficile: an important pathogen of food animals. Anaerobe 12, 1–4.

Songer JG, Post KW, Larson DJ, Jost BH, Glock RD (2000) Infection of neonatal swine with Clostridium difficile. Swine Health Prod 4, 185–189.

Sotiraki S, Roepstorf A, Nielsen JP, Maddox-Hyttel C, Enøe C, Boes J Murrell KD, Thamsborg SM (2008) Population dynamics and intra-litter transmission patterns of Isospora suis in suckling piglets under on-farm conditions. Parasitol 135(3), 395–405. https://doi.org/10.1017/S0031182007003952

Stamati S, Alexopoulos C, Siochu A, Saoulidis K, Kyriakis SC (2006) Probiosis in sows by administration of Bacillus toyoi spores during late pregnancy and lactation: effect

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

on their health status/performance and on litter characteristics. Intern J Probiotics and Prebiotics 1, 33-40.

Stecher B, Maier L, Hardt WD (2013) 'Blooming' in the gut: how dysbiosis might contribute to pathogen evolution. Nat Rev Microbiol 11, 277–284. doi: 10.1038/nrmicro2989

Stokes CR (2017) The development and role of microbial-host interactions in gut mucosal immune development. J Anim Sci Biotechnol 8, 12.

Stokstad EL, Jukes TH, Pierce J, Page AC, Franklin AL (1949) The multiple nature of the animal protein factor. JBC 180, 647–654.

Straberg E, Daugschies A (2007) Control of piglet coccidiosis by chemical disinfection with a cresol-based product (Neopredisan 135-1). Parasitol Res 101(3), 599–604.

Su W, Gong T, Jiang Z, Lu Z, Wang Y (2022) The Role of Probiotics in Alleviating Postweaning Diarrhea in Piglets From the Perspective of Intestinal Barriers. Front Cell Infect Microbiol 12, 883107. doi: 10.3389/fcimb.2022.883107

Suiryanrayna MV, Ramana J (2015) A review of the effects of dietary organic acids fed to swine. J Anim Sci Biotechnol 6, 45. https://doi.org/10.1186/s40104-015-0042-z

Sun RQ, Cai RJ, Chen YQ, Liang PS, Chen DK, Song CX (2012) Outbreak of porcine epidemic diarrhea in suckling piglets, China. Emerg Infect Dis 18, 161–163.

Swann MM, Baxter KL, Field HI (1969) Report of the Joint Committee on the Use of Antibiotics in Animal Husbandry and Veterinary Medicine. Her Majesty's Stationery Office, London.

Szabó C, Kachungwa Lugata J, Ortega ADSV (2023) Gut Health and Influencing Factors in Pigs. Animals 13(8), 1350. doi: 10.3390/ani13081350

Taha-Abdelaziz K, Hodgins DC, Lammers A, Alkie TN, Sharif S (2018) Effects of early feeding and dietary interventions on development of lymphoid organs and immune competence in neonatal chickens: A review. Vet Immunol Immunopathol 201:1-11

Tannock GW (1997) Modification of the normal microbiota by diet, stress, antimicrobial agents and probiotics. In: Mackie RI, White BA, Isaacson RE (eds) Gastrointestinal microbiology-gastrointestinal microbes and host interactions. Chapman and Hall, New York, pp 434-465.

Tannock GW (2001) Molecular assessment of intestinal microflora. Am J Clin Nutr 73, 410-414.

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Taras D, Vahjen W, Macha M, Simon O (2005) Response of performance characteristics and fecal consistency to long-lasting dietary supplementation with the probiotic strain Bacillus cereus var. toyoi to sows and piglets. Arch Anim Nutr 59, 405-417.

Taylor M, Coop R, Wall R (2016) Veterinary Parasitology 4th edn. West Sussex: WileyBlackwell.

Theil KW, Saif LJ, Moorhead PD, Whitmoyer RE (1985) Porcine rotavirus-like virus (Group B rotavirus): Characterization and pathogenicity for gnotobiotic pigs. J Clin Microbiol 21, 340-345. doi: 10.1128/jcm.21.3.340-345.1985

Thoms U (2012) Between promise and threat. NTM J Hist Sci Technol Med 20, 181–214.

Tiseo K, Huber L, Gilbert M, Robinson TP, Van Boeckel TP (2020) Global Trends in Antimicrobial Use in Food Animals from 2017 to 2030. Antibiotics 9(12), 918. doi: 10.3390/antibiotics9120918.

Trachsel J, Briggs C, Gabler NK, Allen HK, Loving CL (2019) Dietary resistant potato starch alters intestinal microbial communities and their metabolites, and markers of immune regulation and barrier function in swine. Front Immunol 10, 1381. https://doi.org/10.3389/fimmu.2019.01381

Tsukahara T, Kimura Y, Inoue R, Iwata T (2020) Preliminary investigation of the use of dietary supplementation with probiotic Bacillus subtilis strain QST713 shows that it attenuates antimicrobial-induced dysbiosis in weaned piglets. Anim Sci J 91, e13475. doi: 10.1111/asj.13475.

Tugnoli B, Giovagnoni G, Piva A, Grilli E (2020) From acidifiers to intestinal health enhancer: How organic acids can improve growth efficiency of pigs. Animals 10, 134.

Turlewicz-Podbielska H, Pomorska-Mól M (2021) Porcine Coronaviruses: Overview of the State of the Art. Virol Sin 36, 833–851. https://doi.org/10.1007/s12250-021-00364-0

Tzika ED, Tassis PD, Papatsiros VG, Pferschy-Wenzig EM, Siochu A, Bauer R, Alexopoulos C, Kyriakis SC, Franz C (2017) Evaluation of in-feed larch sawdust anti-inflammatory effect in sows. Pol J Vet Sci 20, 321-327.

Uzal FA, Navarro MA, Asin J, Boix O, Ballarà-Rodriguez I, Gibert X (2023) Clostridial diarrheas in piglets: A review. Vet Microbiol 280, 109691. doi: 10.1016/j.vetmic.2023.109691

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Uzal FA, Plattner BL, Hostetter JM (2016) Alimentary System. In: Jubb, Kennedy & Palmer's Pathology of Domestic Animals, Volume 2. Elsevier, pp. 1–257. https://doi.org/10.1016/B978-0-7020-5318-4.00007-3

Valenzuela-Grijalva NV, Pinelli-Saavedra A, Muhlia-Almazan A, Domínguez-Díaz D, González-Ríos H (2017) Dietary inclusion effects of phytochemicals as growth promoters in animal production. J Anim Sci Technol 59, 8. https://doi.org/10.1186/s40781-017-0133-9

Vancanneyt M, Snauwaert C, Cleenwerck I, Baele M, Descheemaeker P, Goossens H, Pot B, Vandamme P, Swings J, Haesebrouck F, Devriese LA (2001) Enterococcus villorum sp. nov., an enteroadherent bacterium associated with diarrhoea in piglets. Int J Syst Evol Microbiol 51, 393-400. doi: 10.1099/00207713-51-2-393

Vasquez R, Oh JK, Song JH, Kang DK (2022) Gut microbiome-produced metabolites in pigs: A review on their biological functions and the influence of probiotics. J Anim Sci Technol 64, 671-695.

Vidal A, Martín-Valls GE, Tello M, Mateu E, Martín M, Darwich L (2019) Prevalence of enteric pathogens in diarrheic and non-diarrheic samples from pig farms with neonatal diarrhea in the North East of Spain. Vet Microbiol 237, 108419. doi: 10.1016/j.vetmic.2019.108419

Vlasova AN, Amimo JO, Saif LJ (2017) Porcine rotavirus: Epidemiology, immune responses and control strategies. Viruses 9, 48. doi: 10.3390/v9030048

Waluszewski A, Baraldi C, Ciabuschi F (2018) Increase development and decrease use! Innovation controversies caused by antimicrobial resistance. In: Thomas H, Antonella L, Margunn A (eds) Controversies in Healthcare Innovation Service, Technology and Organization. Palgrave Macmillan, Basinstoke, pp. 309–340.

Wan J, Jiang F, Xu Q, Chen D, He J (2016) Alginic acid oligosaccharide accelerates weaned pig growth through regulating antioxidant capacity, immunity and intestinal development. RSC Adv 6, 87026-87035. https://doi.org/10.1039/C6RA18135J

Wang C, Wei S, Chen N, Xiang Y, Wang Y, Jin M (2022) Characteristics of gut microbiota in pigs with different breeds, growth periods and genders. Microb Biotechnol 15, 793-804

Wang H, Zhong Z, Luo Y, Cox E, Devriendt B (2019) Heat-stable enterotoxins of enterotoxigenic Escherichia coli and their impact on host immunity. Toxins 11(1). doi: 10.3390/toxins11010024.

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Wang J, Deng L, Chen M, Che Y, Li L, Zhu L, Chen G, Feng T (2024) Phytogenic feed additives as natural antibiotic alternatives in animal health and production: a review of the literature of the last decade. Anim Nutr 17, 244-264. https://doi.org/10.1016/j.aninu.2024.01.012

Wang Q, Vlasova AN, Kenney SP, Saif LJ. (2019) Emerging and re-emerging coronaviruses in pigs. Curr Opin Virol 34, 39-49. doi: 10.1016/j.coviro.2018.12.001.

Wang XL, Liu ZY, Li YH, Yang LY, Yin J, He JH, Hou DX, Liu YL, Huang XG (2021) Effects of Dietary Supplementation of Lactobacillus delbrueckii on Gut Microbiome and Intestinal Morphology in Weaned Piglets. Front Vet Sci 8, 692389. doi: 10.3389/fvets.2021.692389

Wang XX, Song PX, Wu H, Xue JX, Zhong X, Zhang LY (2016) Effects of graded levels of isomaltooligosaccharides on the performance, immune function and intestinal status of weaned pigs. Asian-Australas J Anim Sci 29, 250-256. https://doi.org/10.5713/ajas.15.0194

WHO library cataloguing-in-publication data global action plan on antimicrobial resistance; 2015. Available at: www.paprika-annecy.com. Accessed on 13 September 2020.

Wilkins LJ, Monga M, Miller AW (2019) Defining Dysbiosis for a Cluster of Chronic Diseases. Sci Rep 9, 12918. doi: 10.1038/s41598-019-49452-y

Williams BA, Mikkelsen D, Flanagan BM, Gidley MJ (2019) "Dietary fibre": moving beyond the "soluble/insoluble" classification for monogastric nutrition, with an emphasis on humans and pigs. J Anim Sci Biotechnol 10, 45. https://doi.org/10.1186/s40104-019-0350-9

Wise R (2007) An overview of the Specialist Advisory Committee on Antimicrobial Resistance (SACAR). J Antimicrob Chem 60(1), i5–i7.

Woode GN, Bridger J, Hall GA, Jones JM, Jackson G (1976) The isolation of reovirus-like agents (rotaviruses) from acute gastroenteritis of piglets. Med Microbiol 9, 203-209. doi: 10.1099/00222615-9-2-203

Woonwong Y, Tien DD, Thanawongnuwech R (2020) The future of the pig industry after the introduction of African swine fever into Asia. Anim Front 10, 30–7. doi: 10.1093/af/vfaa037

World Health Organization (2017) World Health Organization Critically Important Antimicrobials for Human Medicine, 5th Revision. Accessed on 28 February 2021.

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

World Health Organization (2020) Global Antimicrobial Resistance and Use Surveillance System (GLASS) Report—Early Implementation 2020; World Health Organization: Geneva, Switzerland, p. 134.

Wu Y, Pan L, Shang QH, Ma XK, Long SF, Xu YT, Piao XS (2017) Effects of isomalto-oligosaccharides as potential prebiotics on performance, immune function and gut microbiota in weaned pigs. Anim Feed Sci Technol 230, 126-135. https://doi.org/10.1016/j.anifeedsci.

Xiao L, Estellé J, Kiilerich P, Ramayo-Caldas Y, Xia Z, Feng Q, Liang S, Pedersen AØ, Kjeldsen NJ, Liu C, Maguin E, Doré J, Pons N, Le Chatelier E, Prifti E, Li J, Jia H, Liu X, Xu X, Ehrlich SD, Madsen L, Kristiansen K, Rogel-Gaillard C, Wang J (2016) A reference gene catalogue of the pig gut microbiome. Nat Microbiol 1(12), 1–6. doi.org/10.1038/nmicrobiol.2016.161

Yang C, Chowdhury MA, Huo Y, Gong J (2015) Phytogenic compounds as alternatives to in-feed antibiotics: potentials and challenges in application. Pathogens 4, 137-156.

Yang Z, Liao SF (2019) Physiological Effects of Dietary Amino Acids on Gut Health and Functions of Swine. Front Vet Sci 6, 169. doi: 10.3389/fvets.2019.00169

Yin YL, Tang ZR, Sun ZH, Liu ZQ, Li TJ, Huang RL, Ruan Z, Deng ZY, Gao B, Chen LX, Wu GY, Kim SW (2008) Effect of galacto-mannan-oligosaccharides or chitosan supplementation on cytoimmunity and humoral immunity in early-weaned piglets. Asian-australas J Anim Sci 21, 723-731. https://doi.org/10.5713/ajas.2008.70408

Young VB, Schmidt TM (2004) Antibiotic-associated diarrhea accompanied by large-scale alterations in the composition of the fecal microbiota. J Clin Microbiol 42, 1203–1206. doi: 10.1128/jcm.42.3.1203-1206.2004

Zeineldin M, Aldridge B, Lowe J (2019) Antimicrobial Effects on Swine Gastrointestinal Microbiota and Their Accompanying Antibiotic Resistome. Front Microbiol 10, 1035. doi: 10.3389/fmicb

Zhai H, Liu H, Wang S, Wu J, Kluenter AM (2018) Potential of essential oils for poultry and pigs. Anim Nutr 4, 179-186.

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Zhang F, Luo S, Gu J, Li Z, Li K, Yuan W, Ye Y, Li H, Ding Z, Song D, Tang Y (2019) Prevalence and phylogenetic analysis of porcine diarrhea associated viruses in southern China from 2012 to 2018. BMC Vet Res 15, 470. doi: 10.1186/s12917-019-2212-2

Zhang WJ, Xu LH, Liu YY, Xiong BQ, Zhang QL, Li FC, Song QQ, Khan MK, Zhou YQ, Hu M, Zhao J (2012) Prevalence of coccidian infection in suckling piglets in China. Vet Parasitol 190, 51-55. doi: 10.1016/j.vetpar.2012.05.015.

Zimmerman JJ, Karriker LA, Ramirez A, Schwartz KJ, Stephenson GW. Diseases of swine. 10th ed. Chichester, UK: Wiley; 2012.

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06/09/2026 21:12:45 EEST - 216.73.216.250

CHAPTER II
RESEARCH

Cross-Sectional Survey of Antibiotic Resistance in Extended Spectrum β-Lactamase Producing *Enterobacteriaceae* Isolated from Pigs in Greece

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1. Introduction

By the 1950s, antibiotics were being regularly used in industrial livestock production to secure animals' health and improve their productivity (Lekagul et al., 2019). Antibiotics are typically used in farms, not only for treatment purposes, but also for regulating the spread of infections (metaphylaxis), inhibiting infections (prophylaxis), especially in high-stress periods (weaning stage, post-vaccination period, after farrowing), and improving feed intake and growth performance (Aarestrup, 2005). Subtherapeutic doses of antimicrobials were administered to livestock for decades to prevent diseases and/or to enhance growth (Landers et al., 2012; WHO, 2017), a strategy that promoted the development and spread of antimicrobial resistant strains. Nowadays, the use of antibiotics (e.g., amoxicillin) is included at routine metaphylaxis programs. For instance, injectable amoxicillin can be administered postpartum for metaphylaxis of postpartum dysgalactia syndrome (PPDS) or in weaning feed for the prevention and metaphylaxis of post-weaning diarrhoea (PWD) (Burch and Sperling, 2018; Papatsiros et al., 2007).

Antimicrobial resistance (AMR) in food-producing animals has drawn global attention, as approximately 70% of the overall antibiotic consumption in Europe is related to the animal sector (EFSA, 2022; EFSA, 2021). A major outcome of AMR dissemination is non-effective treatments of livestock, which are further associated with decreased productivity and economic losses due to increased treatment costs (Bengtsson and Greko, 2014). Considering the estimated continuous rise in the global demand for animal derived products, consumption of antimicrobials by livestock is anticipated to increase by two-thirds over the next years (Van Boeckel et al., 2015). In fact, antimicrobial consumption is estimated to be greater in pigs in comparison to chicken and cattle production systems (Van Boeckel et al., 2015) and a greater possibility of AMR has already been reported in pigs than in chicken and other food animals, or aquaculture (Nhung et al., 2016; Pollock et al., 2020; Pholwat et al., 2020). Furthermore, AMR has been demonstrated in wild boars, a wildlife species that can act as a source of zoonotic pathogens causing human diseases, such as colibacillosis, salmonellosis, yersiniosis and listeriosis (Bertelloni et al., 2020; Cilia et al., 2021). AMR in domestic and wild animals poses a hazard for human health by introducing resistant pathogens into the food chain and by triggering horizontal transfer of resistance determinants to other bacteria (Munita, 2016).

ESBL display resistance to the commonly used beta-lactam antimicrobial agents, including third generation cephalosporins, such as ceftriaxone, ceftazidime and ceftiofur (Lee and Yeh, 2015). β-lactams, namely penicillins, carbapenems, monobactams and

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cephalosporins, constitute 60% (by weight) of all antibiotics used worldwide, and are among the most extensively prescribed antibiotic classes in human medicine (Livermore and Woodford, 2006; Peirano and Pitout, 2019). Due to excessive usage of β-lactams in both humans and animals, an increased spread of ESBL-producing bacteria has been observed, threatening personnel in the swine industry, and consequently posing a threat to human health (Bergspica et al., 2020). ESBLs are widespread in *Enterobacteriaceae*, especially in *E. coli* and *Salmonella* spp., while ESBL-producing *E. coli* has been reported in food animals worldwide (Pitout and Laupland, 2008; Smet et al., 2010). Previous reports have associated human ESBL carriage with exposure to ESBL-producing *Enterobacteriaceae* of livestock origin, raising concerns about the possible transfer of ESBL producers through the food chain, which could jeopardize public health (Ewers et al., 2012; Jouini et al., 2007; Overdevest et al., 2011; Leverstein-van Hall et al., 2011; Kola et al., 2012; Dohmen et al., 2015).

Reviewing previous literature revealed the scarcity of published data about ESBL-producing *Enterobacteriaceae* from pig farms in Greece. Thus, the objective of this study was to report, for the first time in Greece, the prevalence of ESBL producers in pig herds, to phenotypically and molecularly identify their antimicrobial resistance patterns and to investigate potential factors that could promote the development of AMR.

2. Materials and Methods

2.1. Ethics

All procedures were performed according to the ethical standards in the Helsinki Declaration of 1975, as revised in 2000, as well as the national law, and after receiving approval (number 96/19.12.2019) from the Institutional Animal Use Ethics Committee of the Faculty of Veterinary Science, University of Thessaly.

2.2. Study Design

The current cross-sectional study was conducted in different regions of Greece for two years (between 2019–2021) and included 34 pig farms. The farms had an overall population of about 14,300 sows, which represented approximately 24% of the entire capacity of the Greek swine production. Farms were in northern (n = 4), central (n = 13), western (n = 10) and southern (n = 7) Greece. Generally, central and western Greece are the regions with the highest pig density (more than 50% of total pig population of Greece). The classifications of pig farms according to their geographic origin and capacity are presented in **Table 1**.

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Table 1. Geographic origin and capacity of study's farms.

Region	Number of farms	Total capacity	Capacity per farm					Number of samples per age	
			50-100	101-250	251-450	451-700	701-2100	Suckling piglets	Weaning piglets
Northern Greece	**4**	1640	0	2	1	1	0	8	19
Central Greece	13	3710	4	2	3	4	0	24	44
Western Greece	10	5790	0	4	2	2	2	22	42
Southern Greece	7	3190	0	4	2	0	1	19	36
Total	34	14,300	4	12	8	7	3	73	141

Farmers or managers consented to participating in the study. The inclusion and exclusion criteria for farm selection are shown in **Table 2**. These criteria were met by all participating herds in order to include intensive farms that employ common practices of the Greek swine production system, while taking preventive measures for disease control.

Table 2. Criteria for farms selection.

Criteria	Farms	Sows	Weaners
Capacity	Minimum 50 sows		
Type of farm	Exclusive farrow-to-finish		
Antiparasitic treatment		Antiparasitic treatment (IM) before farrowing	
Vaccination		Aujeszky's disease virus Porcine parvovirus Atrophic rhinitis Erysipelas Porcine Reproductive and Respiratory Virus *E. coli* *Clostridium* spp.	Porcine Circovirus type 2 *Mycoplasma hyopneumoniae*

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Diet	Home-made diets (mixed corn/barley/wheat–soybean-based meal) balanced in dietary nutrients (essential amino acids, minerals and vitamins)[1]	
Toxin binders	Systematically used in the feed during gestation and lactation	Systematically used in the feed
Routine program for metaphylaxis of PPDS	Amoxicillin (IM) after farrowing	
Routine program of metaphylaxis for PWD		Amoxicillin via feed for the first 10 days after weaning

[1]According to Nutrient Requirements of Swine (NRC, 2012)

In addition, data concerning sow age and prior administration of antimicrobials for the last six months were collected. The most used classes of antibiotics were penicillins, collistin, cephalosporins, quinolones, pleuromoutilins (PLMs), macrolides, tetracyclines and trimethoprim/sulfonamides; these were used according to the manufacturers' instructions with respect to duration of therapy and dosage.

2.3. Sample Collection

A total of 214 fecal samples were collected from 73 suckling and 141 weaning piglets of 34 farms (**Table 1**). Samples were obtained directly from the rectum by using swabs with Amies transport medium (Transwab®, Amies, UK) and were transferred within a day to the Laboratory of Microbiology and Parasitology (Faculty of Veterinary Medicine, Karditsa, Greece).

2.4. Isolation and Identification of Extended Spectrum Cephalosporin Resistant (ESCR) Strains

For the detection of ESCR isolates, fecal swabs were directly streaked on ESBL selective media (CHROMID® ESBL, BioMérieux, Marcy l'Etoile, France) and the plates were incubated aerobically at 37 °C for 24–48 h. Subcultures were grown on both MacConkey agar and 5% sheep blood agar until pure cultures were obtained. Bacterial species identification was carried out using the automated Vitek-2 system (BioMérieux. Marcy l'Etoile, France), according to the manufacturer's instructions.

2.5. Isolation and Identification of Salmonella spp.

Isolation of *Salmonella* spp. was conducted, according to ISO 6579-1:2017. Initially, swabs were agitated and squeezed into sterilized tubes containing 9 mL Buffered Peptone Water (BPW). Subsequently, Modified Semisolid Rappaport-Vassiliadis (MSRV) agar, Xylose Lysine Deoxycholate (XLD) agar, and *Salmonella Shigella* (SS) agar were

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used as selective media under the recommended conditions. All presumptive *Salmonella* colonies were identified as to species using the Vitek-2 system.

2.6. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of all the obtained strains was performed by the Vitek-2 system. The AST-GN96 card was used to determine the minimum inhibitory concentration (MIC) of the following antimicrobial classes: penicillins (ampicillin-AMP, amoxicillin/clavulanic acid-AMC, ticarcillin/clavulanic acid-TCC), cephalosporins (cefalexin-CEX, cefalotin-CF, cefoperazone-CEP, ceftiofur-CEF, cefquinome-CEQ), carbapenems (imipenem-IMI), aminoglycosides (gentamicin-GEN, neomycin-NEO), quinolones (flumequine-FLU, enrofloxacin-ENR, marbofloxacin-MRX), tetracyclines (tetracycline-TET), amphenicols (florfenicol-FLO), polymyxin B-PL and sulfonamides (trimethoprim/sulfamethoxazole-SXT).

2.7. Phenotypic Confirmation of ESBL Production

All the isolates that presented resistance to 3rd (CEP, CEF)-generation cephalosporins were screened via the double disk synergy test (DDST) or a combination disk test (CDT) for ESBL production, according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2020) guidelines. In brief, antibiotic disks containing cefotaxime (CTX) (30 μg), ceftazidime (CAZ) (30 μg), cefepime (CPM) (30 μg) and AMC (20 μg/10 μg) were applied at a distance of 20 mm (center to center) on Mueller Hinton agar previously inoculated with an 0.5 McFarland inoculum of the isolate to be tested. After incubation, any enhanced zone of inhibition between cephalosporin disks and the AMC disk or a ‘’keyhole’’ formation in the direction of the disk containing clavulanic acid were considered as evidence for the presence of an ESBL-producing strain. In cases of ambiguous results, a combination disk test was also applied, using CTX and CAZ disks (30 μg each), alone and in combination with clavulanic acid (10 μg). A difference of ≥5 mm in zone diameter between the test using the disks alone and that using the disks combined with clavulanic acid antimicrobial agents was interpreted as ESBL production.

2.8. Antibacterial Resistance Genes of ESBL-Producing Enterobacteriaceae

Isolates that were found to be positive in the DDST or the CDT were characterized using the DNA microarray-based assay CarbaResist from InterArray (FZMB GmbH, Bad Langensalza, Germany). Primer and probe sequences have previously been described in detail (Braun et al., 2018). In addition, probes for the detection of the colistin resistance gene family mcr were included on the present microarray. Protocols and procedures were conducted in accordance with the manufacturer’s instructions (**https://www.inter-**

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array.com/Further-Genotyping-Kits, accessed on 10 May 2022). In brief, bacteria were grown overnight on Columbia blood agar. Bacteria were harvested and genomic DNA was extracted using the Qiagen blood and tissue kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The DNA was used in a multiplexed primer elongation incorporating biotin-16-dUTP. Amplicons were stringently hybridized to the microarray, washed and incubated with a horseradish-peroxidase-streptavidin conjugate. Hybridizations were detected by adding a precipitating dye.

2.9. Statistical Analysis

Principal component analysis (PCA) and hierarchical clustering were used to explore the patterns of antimicrobial co-resistance among the isolated *Enterobacteriaceae* species and identify clusters of co-resistance (Butiuc-Keul et al., 2021). Subsequently, the prevalence of AMR by species was estimated within a Bayesian estimation framework (Gelman et al., 1995).

Logistic regression models were used to assess whether (a) the presence of ESBL-producing strains and (b) antimicrobial resistance to a certain type of antibiotic are associated with a series of candidate variables. Candidate variables for both (a) and (b) were herd size, sow age and administration of antibiotics (penicillins, collistin, cephalosporins, quinolones, pleuromoutilins (PLMs), macrolides, tetracyclines and trimethoprim/sulfonamides) according to the manufacturers' instructions. All candidate variables were initially screened, one-by-one, with a significance level of 0.25. For the shortlisted variables, collinearity analyses were conducted to identify pairs of collinear variables. For each pair of collinear variables, one was excluded from further analyses. The variable that was retained was the one more strongly associated with the outcome. Variables with $p < 0.25$ were then tested in the final model and were subsequently reduced by backwards elimination, until only significant ($p < 0.05$) variables remained.

All analyses were performed in R program (R Core T, 2020). For PCA, we used the prcomp built-in functions, and for Bayesian prevalence estimation, the runjags package (Denwood, 2016) and figures were built with the ggplot2 package (Wickham, 2011). For logistic regression, the glmer function was used (Lee and Grimm, 2018).

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3. Results

3.1. Isolation and Identification of ESBL-Producing Enterobacteriaceae

A total of 98 ESCR strains were recovered by selective cultivation from 95 of the 214 (44.4%) swine samples tested. Additionally, five *Salmonella* spp. isolates were retrieved from an equal number of samples (2.3%).

Seventy-eight (36.5%) isolates presented resistance to 3rd generation cephalosporins and were phenotypically confirmed to produce ESBL. ESBL producers were identified as *E. coli* (*n* = 69), *K. pneumoniae spp. pneumoniae* (*n* = 3), *P. mirabilis* (*n* = 4), *E. cloacae* complex (*n* = 1) and *S. enterica subsp. diarizonae* (*n* = 1). The results are summarized in **Table 3**.

Table 3. Isolation and Identification of ESBL-producing bacteria from pig samples.

Bacterial species	Percentage of isolates (n=214)[1]	Percentage of ESBL-producing isolates (n=78)[2]
Escherichia coli	32.2% (n=69)	88.5% (n=69)
Klebsiella pneumoniae spp. *pneumoniae*	1.4% (n=3)	3.8% (n=3)
Proteus mirabilis	1.9% (n=4)	5.1% (n=4)
Enterobacter cloacae	0.5% (n=1)	1.3% (n=1)
Salmonella enterica subsp. *diarizonae*	0.5% (n=1)	1.3% (n=1)
Total	36.5% (n=78)	100% (n=78)

1 Number of fecal samples tested

2 Number of isolated ESBL-producing bacteria

3.2. Antimicrobial Resistance Phenotype and Genotype of the ESBL-Producing Enterobacteriaceae

All ESBL isolates (*n* = 78) presented resistance to AMP and to all the cephalosporins tested, apart from four *E. coli* strains, which were susceptible to CEQ, and the isolate of the *E. cloacae* complex, which was susceptible to CEP. Resistances to AMC (33.3%) and TCC (26.9%) were also detected, while diminished susceptibility to imipenem was identified only in the four *P. mirabilis* isolates (5.1%). ESBL phenotypes are illustrated as percentages in **Figure 1**. Three *E. coli* isolates could not be retrieved after storage in −80 °C, and were thus not genotypically characterized. Of the remaining 66 *E. coli* isolates,

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ESBL genes were detected in 65. In particular, *bla*$_{\text{CTX-M1/15}}$ was detected in 52 isolates (78.8%), *bla*$_{\text{CTX-M9}}$ in six (9.1%), *bla*$_{\text{CTX-M8}}$ in six (9.1%), *bla*$_{\text{SHV}}$ in three (4.5%) and *bla*$_{\text{TEM}}$ in 38 (57.6%), alone (n = 5) or in combination with other variants (n = 33). The *K. pneumoniae* isolates were found to harbor *bla*$_{\text{CTX-M1/15}}$ (n = 2), *bla*$_{\text{SHV}}$ (n = 2), *bla*$_{\text{TEM}}$ (n = 1) and *bla*$_{\text{CTX-M9}}$ (n = 1), while the *P. mirabilis* harbored *bla*$_{\text{CTX-M9}}$ (n = 3), *bla*$_{\text{CTX-M8}}$ (n = 1) and *bla*$_{\text{TEM}}$ (n = 3). Finally, the *S. enterica* harbored *bla*$_{\text{TEM}}$, whereas no ESBL genes were detected in the *E. cloacae*.

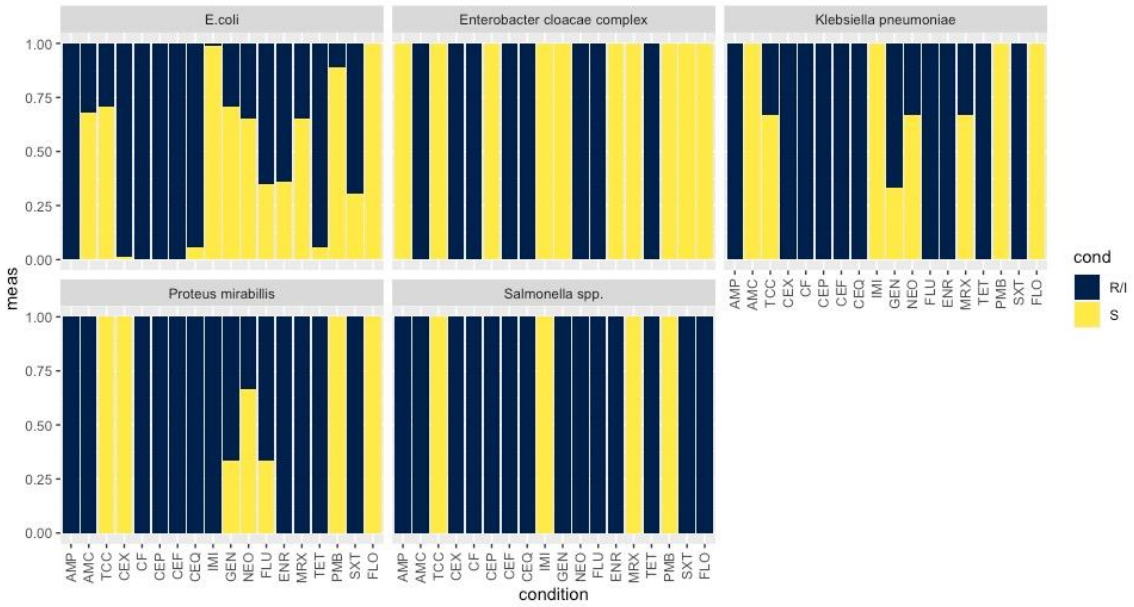

Figure 1. Bar plot showing the percentage of AMR of *E. coli*, *E. cloacae* complex, *K. pneumoniae*, *P. mirabilis* and *Salmonella* spp. to a range of antibiotics; AMP, AMC, TCC, CEX, CF, CEP, CEF, CEQ, IMI, GEN, NEO, FLU, ENR, MRX, TET, SXT, FLO. The values varied from 0.00 to 1.00; 0.00 indicates resistance and 1.00 susceptibility.

R/I: Resistance/Intermediate results

S: Susceptibility

AMP: ampicillin, AMC: amoxicillin/clavulanic acid, TCC: ticarcillin/clavulanic acid, CEX: cefalexin, CF: cefalotin, CEP: cefoperazone, CEF: ceftiofur, CEQ: cefquinome, IMI: imipenem, GEN: gentamicin, NEO: neomycin, FLU: flumequine, ENR: enrofloxacin, MRX: marbofloxacin, TET: tetracycline, FLO: florfenicol, PMB: polymyxin B, SXT: trimethoprim/sulfamethoxazole (SXT).

The AmpC gene *bla*$_{\text{ACT}}$ was detected in 18 *E. coli* (27.3%) and the *bla*$_{\text{CMY}}$ in one. Moreover, 21 isolates (31.8%) possessed *bla*$_{\text{OXA-1}}$ and one *bla*$_{\text{OXA-60}}$.

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3.3. Antimicrobial Resistance Phenotype and Genotype of ESBL-Producing Enterobacteriaceae to non β-lactam Antibiotics

All 78 ESBL-producing isolates displayed resistance to at least one class of non β-lactam antibiotics. Among the *E. coli* strains, resistances were reported for fluoroquinolones (FLU; *n* = 45, ENR; *n* = 42, MRX; *n* = 22), aminoglycosides (GEN; *n* = 19, NEO; *n* = 24), TET (*n* = 26) and SXT (*n* = 46) (Figure 1, Figure 2 and Figure 3).

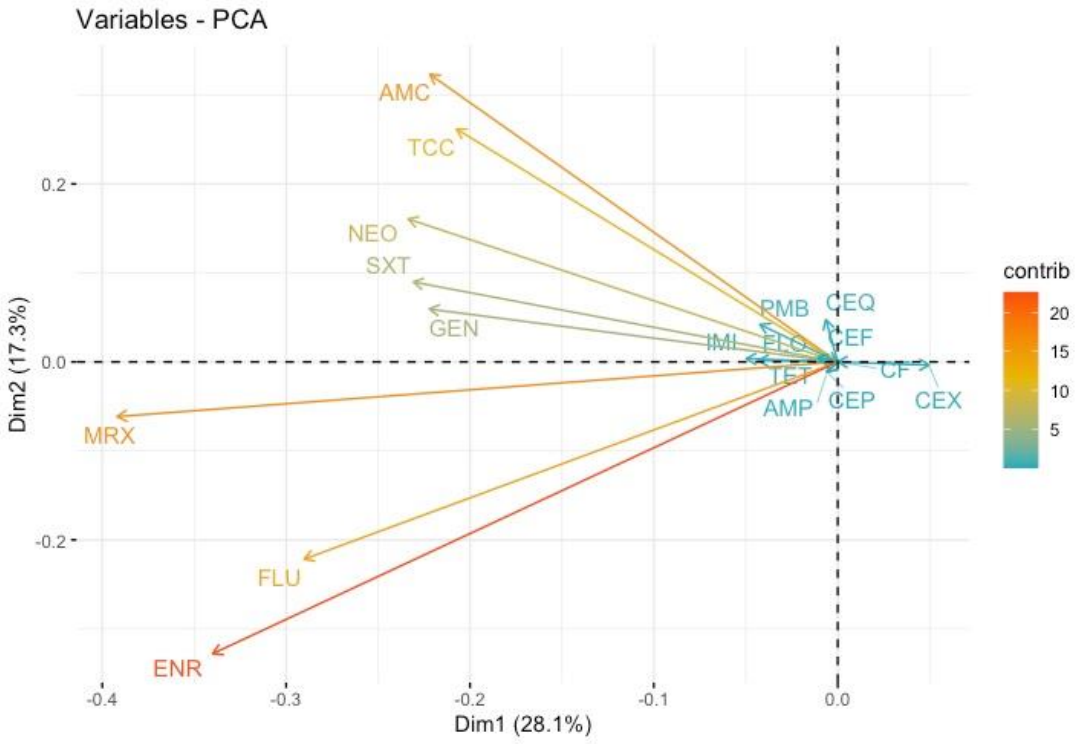

Figure 2. Principal component analysis (PCA) on co-resistances. Antibiotics are represented by vectors (arrows). Two vectors of antibiotics pointing to the same direction is an indication of positive correlation between them; when we observe AMR in one antibiotic, we are expecting development of AMR in the other one. An angle of 180 degrees between the vectors of two antibiotics is an indication of negative correlation between them; when we observe AMR in one, we are not expecting development of AMR in the other. A 90-degree angle between the vectors of two antibiotics indicates no relationship between them towards developing AMR. The longer the vectors, the greater the intensity of this relationship. AMP: ampicillin, AMC: amoxicillin/clavulanic acid, TCC: ticarcillin/clavulanic acid, CEX: cefalexin, CF: cefalotin, CEP: cefoperazone, CEF: ceftiofur, CEQ: cefquinome, IMI: imipenem, GEN: gentamicin, NEO: neomycin, FLU: flumequine, ENR: enrofloxacin, MRX: marbofloxacin, TET: tetracycline, FLO: florfenicol, PMB: polymyxin B, SXT: trimethoprim/sulfamethoxazole (SXT).

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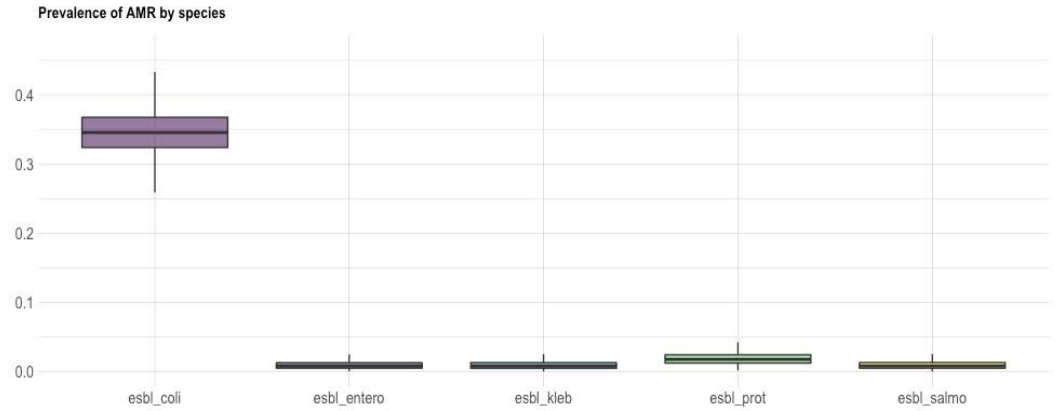

Figure 3. Box-and-whiskers plot showing the prevalence of AMR (y axis) of ESBL producing *E. coli*, *E. cloacae* complex, *K. pneumoniae*, *P. mirabilis* and *Salmonella* spp. (x axis). The bold line shows the median value, while the length of the box represents interquartile range.

Regarding *E. coli*, resistance genes were detected for fluoroquinolones in 35 isolates (*qnrA*, *qnrB*, *qnrS)*, for aminoglycosides in 59 (*aadA1*, *aadA2*, *aadA4*, *aphA*, *rmtA*, *rmtC*, *aac*(6′)-*Ib*, *aac*(3′)-*Iva*), for sulfonamides in 51 (*sul1*, *sul2, sul3)*, for trimethoprim in 58 (*dfrA1*, *dfrA12*, *dfrA13*, *dfrA14*, *dfrA15*, *dfrA17*, *dfrA19*, *dfrA5*, *dfrA7*), for macrolides in 16 (*mph*, *mrx*) and for colistin in seven (*mcr*-1/*mcr*-2, *mcr*-4, *mcr*-8). Additionally, the *intl1*, *intl2* and tnpISE*cp1* genes associated with mobile elements were identified in 20, seven and 30 *E. coli* isolates, respectively.

K. pneumoniae spp. *pneumoniae* isolates (n = 3) were resistant to fluoroquinolone (ENR; n = 3, FLU; n = 3, MRX; n = 1), TET and SXT, and two of the isolates displayed resistances to aminoglycosides (GEN; n = 2, NEO; n = 1) (**Figure 1** and **Figure 3**). All three isolates harbored genes conferring resistance to fluoroquinolones (*qnrS*), sulfonamides (*sul1*, *sul2*) and trimethoprim (*dfrA14*, *dfrA7*, *dfrA17*, *dfrA1*), while two also possessed aminoglycoside-resistance genes (*aadA1*, *aphA*). Genes encoding mobile elements (*intl1*, *intl2*, tnpISE*cp1*) as well as the oqxAB multidrug efflux pump (*oqxA*, *oqxB*) were additionally detected in the three isolates.

P. mirabilis isolates (n = 4) were resistant to fluoroquinolones (ENR; n = 4, FLU; n = 3, MRX; n = 3), TET and SXT, while a resistant phenotype for aminoglycosides (GEN; n = 3, NEO; n = 1) was observed in three of them (**Figure 1** and **Figure 3**). Antibacterial resistance genes revealed the presence of genes conferring resistance to

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sulfonamide (*sul1*, *sul2*) and trimethoprim (*dfrA1*, *dfrA5*, *dfrA17*) in all four of them, as well as of aminoglycoside-resistance genes in three (*aadA1*, *aadA2*, *aphA*, *aac*(6′)-*Ib*, *aac*(3′)-*Iva*). Furthermore, three of the strains harbored *intl2.*

The isolate of the *E. cloacae* complex presented intermediate resistance for NEO and TET, was resistant to FLU (**Figure 1** and **Figure 3**) and was only found to harbor *dfrA5*.

Finally, the *S. enterica* spp. *diarizonae* exhibited resistance to fluoroquinolones (FLU, ENR), TET, SXT, aminoglycosides (GEN, NEO) and FLO (**Figure 1** and **Figure 3**). The isolate carried resistance determinants against fluoroquinolones (*qnrS*), sulfonamides (*sul2*, *sul3*), trimethoprim (*dfrA12*, *dfrA17*) and aminoglycosides (*aadA1*, *aadA2*, *aadA4*, *aphA*), as well as the tnpISE*cp1*.

The resistance genes detected among the ESBL-producing Enterobacteriaceae are presented in **Table 4**.

Table 4. Resistance genes detected among the ESBL-producing Enterobacteriaceae.

Resistance Genes	Number of Isolates				
	E. coli	***K. pneumoniae***	***P. mirabilis***	***S. enterica* ssp. *diarizonae***	***E. cloacae* Complex**
bla$_{\text{CTX-M1/15}}$	52	2	-	-	-
bla$_{\text{CTX-M9}}$	6	1	3	-	-
bla$_{\text{CTX-M8}}$	6	-	1	-	-
bla$_{\text{SHV}}$	3	2	-	-	-
bla$_{\text{TEM}}$	38	1	3	1	-
bla$_{\text{ACT}}$	18	-	-	-	-
bla$_{\text{CMY}}$	1	-	-	-	-
bla$_{\text{OXA-1}}$	17	-	4	-	-
bla$_{\text{OXA-60}}$	1	-	-	-	-
aadA1	36	1	3	1	-
aadA2	21	-	1	1	-
aadA4	14	-	-	1	-
aphA	21	1	1	1	-
rmtA	27	-	-	-	-
rmtC	7	-	-	-	-

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Resistance Genes	Number of Isolates				
	E. coli	*K. pneumoniae*	*P. mirabilis*	*S. enterica* ssp. *diarizonae*	*E. cloacae* Complex
aac(6')-Ib	2	-	3	-	-
aac(3')-Iva	1	-	3	-	-
qnrS	29	3	-	1	-
qnrA	2	-	-	-	-
qnrB	4	-	-	-	-
sul1	18	2	3	-	-
sul2	38	3	4	1	-
sul3	17	-	-	1	-
dfrA1	13	1	3	-	-
dfrA5	42	-	2	-	1
dfrA7	4	1	-	-	-
dfrA12	12	-	-	1	-
dfrA13	2	-	-	-	-
dfrA14	12	1	-	-	-
dfrA15	1	-	-	-	-
dfrA17	15	1	1	1	-
dfrA19	4	-	-	-	-
mcr-1/mcr-2	6	-	-	1	-
mcr-4	1	-	-	-	-
mcr-8	3	-	-	1	-
Mph	12	-	-	-	-
Mrx	16	-	-	-	-
intl1	20	2	-	-	-
intl2	7	1	3	-	-
tnpISEcp1	30	3	-	1	-
oqxA	1	3	-	-	-
oqxB	1	3	-	-	-

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3.4. Logistic Regression Analysis Results

Logistic regression analysis demonstrated that the presence of ESBL-producing *E. coli* strains was negatively associated with prior administration of PMLs, and that the occurrence of ESBL-producing *P. mirabilis* was associated with herd size. Further associations concerning ESBL-producing *Enterobacteriaceae* were not recognized by the model. The results are presented in **Table 5**.

Table 5. Associations of ESBL-producing Enterobacteriaceae with herd characteristics and administration of antibiotics.

ESBL-producing	Logistic Regression			
	Parameter	Category	Estimate (95% CI)	P value
E. coli	PMLs	0	1	
		1	0.25 (0.07; 0.84)	0.0215
P. mirabilis	Size	-	85.86 (4.02; 6620.06)	0.00648

The reported antimicrobial resistances of this study were not related to the sows' ages. The development of resistances to AMP and AMC, as well as the diminished susceptibility to IMI, were positively associated with the farm size. By examining whether previous administration of antibiotics led to the development of AMR, we recognized a series of positive associations between (a) GEN resistance and previous administration of quinolones, (b) NEO resistance and previous usage of TETs, (c) MRX resistance and prior application of TETs and (d) ENR resistance and prior administration of cephalosporins. Finally, the previous application of PLMs was negatively associated with the development of resistance to both GEN and NEO. The results are described in **Table 6**.

Table 6. Association of AMR with herd characteristics and administration of antibiotics.

Antibiotic	Logistic Regression			
	Parameter	Category	Estimate (95% CI)	P value (random effect)
AMP	Size		48.72 (3.21; 1955.14)	0.0171
AMC	Size		31.38 (5.3; 290.81)	<0.005
IMI	Size		33.09 (2.16; 1454.48)	0.008
GEN	PMLs	0	1	

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		1	0.15 (0.02; 0.58)	0.016
	Quinolones	0	1	
		1	13.14 (2.46; 243.71)	0.015
NEO	PMLs	0	1	
		1	0.19 (0.03; 1.05)	0.028
	TETs	0	1	
		1	7.7 (1.62; 59.44)	0.009
MRX	TETs	0	1	
		1	14.45 (0.96; 5.59)	0.011
ENR	Cephalosporins	0	1	
		1	3.49 (1.07; 14.84)	0.045

4. Discussion

The present study aimed to describe, for the first time, the frequency of ESBL-producing *Enterobacteriaceae* from 34 pig farms located in different geographical regions of Greece, and to characterize their AMR phenotype and genotype. To that end, we collected and tested 214 fecal samples from 73 suckling and 141 weaning piglets from herds that met certain inclusion criteria. The most commonly isolated ESBL producers were *E. coli* strains that presented co-resistance to at least one class of non β-lactam antibiotics. In addition, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex and *S. enterica* subsp. *diarizonae* isolates were identified as ESBL producers. Notably, four *P. mirabilis* strains displayed diminished susceptibility to IMI.

Resistance to AMC was observed in ESBL-producing *E. coli* isolates (**Figure 3**). In the studied farms, injectable amoxicillin was used postpartum in sows as part of a routine program of metaphylaxis for PPDS, and it was also added in weaning feed for the prevention and metalphylaxis of PWD. Resistance to amoxicillin has not become a major problem to date, because it is usually combined with clavulanic acid, a highly effective ESBL inhibitor (Burch and Sperling, 2018). As this antimicrobial is routinely administered in pigs against respiratory (e.g., bacterial pneumonia), enteric (post weaning diarrhoea) and urogenital (e.g., PPDS) diseases, its unnecessary usage could further promote the selection of ESBL-producing bacteria. Therefore, laboratory diagnosis based on bacterial culture and sensitivity testing is mandatory prior to its application (Burch and Sperling, 2018). The observed AMC resistance of *E. coli* isolates retrieved from the piglets could be also associated with the administration of amoxicillin in sows postpartum, considering that

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vertical transmission of resistant bacteria from sows to piglets has been already documented (Burow et al., 2019). However, future studies, including investigation of related risk factors (e.g., farm capacity and management, previous and current treatments with antibiotics, age, and treatment groups) will help us understand the development of AMR under field conditions.

ESBL-producing *E. coli* strains presented co-resistances to fluoroquinolones (n = 49), aminoglycosides (n = 31), TETs (n = 26) and SXT (n = 46), confirming previously reported antimicrobial resistance patterns of ESBL-producing *E. coli* (Liu et al., 2018; Aguire et al., 2020; Tamta et al., 2020; Lee et al., 2021). In fact, the occurrence of strains resistant to AMP, AMC, SXT and TET (Valiakos et al., 2016; Papadopoulos et al., 2021), and the occurrence of ESBL producers resistant to at least one more class of non β-lactam antibiotics, have been formerly described in Greece (Athanasakopoulou et al., 2021). We also observed increased resistance levels with regard to fluoroquinolones, which are widely used in swine clinical practice as first-choice agents for individual injectable treatment for respiratory, enteric and urogenital disease. This finding could be attributed to the extensive use of this agent at farm level and should be considered by veterinarians and farmers, especially in acute clinical cases that demand rapid treatment processes.

The occurrence of four *P. mirabilis* ESBL-producing isolates was reported and all strains presented reduced susceptibility to IMI. At first, this was an alarming finding, considering the prohibition of the use of IMI in livestock (Poirel et al., 2014) and the reported carbapenemase resistance in humans (EFSA, 2022; Yang et al., 2014). As these isolates were not found to harbor a carbapenemase gene, the reported IMI resistance could be explained by pore mutations or mutations affecting a penicillin binding protein (Villar and Danel, 1997). Furthermore, *P. mirabilis* isolates were also resistant to fluoroquinolones, TETs and SXT, while three of them had a resistant phenotype for aminoglycosides. This bacterial species is considered to be the most common etiological agent of PPDS in sows, causing severe economic losses in swine industry, whereas fluoroquinolones, TET and SXT are recommended as first-choice antimicrobial treatments (Papatsiros et al., 2007). Our results reveal a potential risk for treatment failure in field cases of PPDS, further emphasizing the importance of etiological diagnosis and sensitivity testing prior to the use of antibiotics by swine practitioners.

An ESBL-producing *S. enterica* subsp. *diarizonae* isolate was also noticed, presenting co-resistance to aminoglycosides, fluoroquinolones (FLU, ENR), TETs, SXT and FLO. Serovars of ESBL-producing *S. enterica* of poultry origin, co-resistant to

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aminoglycosides, TETs and SXT have previously been reported in Greece (Politi et al., 2005). Herein, we identified a multidrug-resistant serovar in pigs that is usually isolated from humans (Rubira et al., 2021), and thus, our results underline that AMR in livestock could pose, through the food chain, a serious threat to public health.

Molecular characterization of the ESBL isolates revealed the predominance of CTX-M type genes, mainly those of group 1, which is in accordance with preexisting literature about pigs (Bernreiter-Hofer et al., 2021; Balazs et al., 2021; Giufre er al., 2021). Interestingly, various other genes conferring resistance to fluoroquinolones, aminoglycosides, sulfonamides, trimethoprim, macrolides and colistin were detected in the present study. This finding underlines the wide dissemination of AMR determinants among animals farmed for human consumption, which may have been facilitated by the detected mobile genetic elements (Madec et al., 2017). The co-occurrence of *mcr* variants is especially noteworthy given the importance of colistin as a last-resort therapeutic option against multidrug-resistant strains in clinical settings. Colistin is commonly used in pigs to prevent and control the clinical outcomes of *E. coli* infection, including neonatal diarrhoea, post-weaning diarrhoea and edema disease (Lekagul et al., 2020). Gene *mcr*-1, one of the most common colistin-resistance genes around the world, was detected among *E. coli* isolates from Chinese pigs at slaughter and retail meats, and its occurrence was speculated to be a result of colistin usage (Liu et al., 2016). In Greece, multidrug-resistant *mcr*-1-positive ESBL-producing *E. coli* has previously been recovered on a dairy farm and was the etiological agent of mastitis (Filiousis et al., 2020).

Logistic regression analysis demonstrated a positive association between the herd size and the development of antimicrobial resistance to AMP, AMC and IMI, further confirming previous reports (van der Fels-Klerx et al., 2011; Strom et al., 2017). Specifically, it was more likely for AMR to appear in larger farms. A potential explanation for the observed AMP and AMC resistance could be provided by the routine administration of these antimicrobials, as infections in large herds are a more common phenomenon by reason of the higher swine density and the increased rates of gilt replacement from external sources (Lekagul et al., 2020). On the contrary, diminished susceptibility to IMI cannot be ascribed to the usage of carbapenems, since this class of antibiotics is banned for swine due to the risk of compromising human treatments. In *P. mirabilis*, IMI resistance can be considered random, and it could be speculated that exposure to other beta-lactams might have been the driver (as the mechanism is unspecific).

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At a national level, the usage of specific antimicrobials is strongly associated with the development of resistance towards these agents in commensal *E. coli* isolates in pigs, poultry and cattle (Chantziaras et al., 2014). We report a positive association between previous administration of TETs and the development of resistance to NEO and MRX, while increased GEN resistance was observed following quinolone administration. It is well-known that the use of different classes of antibiotics depends on the type of infections to be treated, and varies according to the pigs' ages (Lekagul et al., 2019). For instance, the gut microbiota is more exposed to orally administered tetracycline than to injected tetracycline, and thus an increase in AMR bacteria in the intestinal microbiome could be promoted by oral administration (Ricker et al., 2020). It is critical to expand our knowledge on the impact of the administration of different antibiotics in pigs, as increased resistance in bacteria may impair treatment efficacy and potentially lead to therapeutic failure.

Recent studies in Greece and worldwide have described the AMR profile of ESBL-producing *E. coli* strains isolated from livestock, including pigs (Bengtsson and Greko, 2014); Athanasakopoulou et al., 2021; Giufre et al., 2021). Currently, as a wide dissemination of multidrug resistant bacteria in diverse ecosystems is observed, the establishment of an integrated antimicrobial surveillance system under the One Health approach is crucial for the protection of public health. The AMR profile of the ESBL-producing *Enterobacteriaceae* isolates presented in this study demonstrates the urgent need for the application of a monitoring program targeting preferentially multiple species.

5. Conclusions

We report a high percentage of ESBL-producing bacteria in the Greek swine industry, which is triggering alarm for veterinary practitioners and farmers as well as for public health authorities. The 78 ESBL producers (36.5%) were identified as *E. coli* (n = 69), *K. pneumoniae* (n = 3), *P. mirabilis* (n = 4), *E. cloacae* complex (n = 1) and *S. enterica* subsp. *diarizonae* (n = 1), and presented resistance to at least one class of non β-lactam antibiotics. CTX-M-1/15 enzymes were the most frequently observed ESBLs, followed by TEM and SHV types, and accompanied by several other resistance determinants for fluoroquinolones, aminoglycosides, sulfonamides, trimethoprim, macrolides, colistin and mobile genetic elements. As AMR increases the cost of animal production, antimicrobial usage must be prudent and based on laboratory diagnosis and antimicrobial sensitivity testing, in order to keep antibiotics as a therapeutic weapon in experts' hands. The integration of a surveillance system for AMR monitoring under the One

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Health approach will contribute not only to the rational use of antibiotics, but also to the protection of public health.

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Supplementary Files

S1: Genes detected by the CarbaResist DNA microarray-based assay

Target gene	Gene function	Accession number
gad	glutamate decarboxylase of *Escherichia coli*	AE014075.1
ihfA	integration host factor subunit alpha	U00096.3
dnaE	DNA polymerase III subunit alpha	U00096.3
basC	acinetobactin biosynthesis protein of *Acinetobacter baumannii*	AY571146.1
efp	elongation factor P of *Acinetobacter baumannii*	CP001172.1
pld	phospholipase D of *Acinetobacter baumannii*	CP000521.1
cfa	colicin five activity protein of *Citrobacter freundii* and *Citrobacter braakii*	U09771.1
ecfX	extracytoplasmic function sigma factor of *Pseudomonas aeruginosa* [LavenirR-JocktaneD-2007]	DQ996558.1
invA	invasin A, highly specific for genus *Salmonella*	CP000026.1
ipaH9.8	invasion plasmid antigen	AF047365.1
khe	klebsolysin of *Klebsiella pneumoniae*	AF293352.1
lacY	lactose permease; the *lacY* gene is missing in all *Shigella spp*.	U00096.2
rrs	16S rRNA	U00096.3
*bla*BIC	carbapenemase, class A beta-lactamase	GQ260093.1
*bla*DIM	carbapenemase, class B metallo beta-lactamase	KC004136.2
*bla*GES	carbapenemase, class A beta-lactamase	AY219651.1
*bla*GIM	carbapenemase, class B metallo beta-lactamase	consensus
*bla*GOB	carbapenemase, class B metallo beta-lactamase	consensus
*bla*IMI-3 (NmcA)	carbapenemase, class A beta-lactamase associated with imipenem resistance	AY780889.1
*bla*IMI-R	regulator of *bla*IMI-3 (NMC-A)	Z21956.1
*bla*IMP	carbapenemase, class B metallo beta-lactamase	consensus
*bla*IMP25 (*bla*SIM-1)	carbapenemase, class B metallo beta-lactamase (synonym: *bla*SIM)	EU686387.1
*bla*IMP35	carbapenemase, class B metallo beta-lactamase	JQ432564.1
*bla*IND	carbapenemase, class B metallo beta-lactamase of Chryseobacterium	consensus
*bla*KHM	carbapenemase, class B metallo beta-lactamase	consensus
*bla*KPC	carbepenemase, class A serin beta-lactamase	consensus
*bla*NDM	carbapenemase, class B metallo beta-lactamase (New Dehli metallo beta-lactamase)	consensus
*bla*PAM-1	carbapenemase, subclass B3 metallo beta-lactamase (Pseudomonas alcaligenes metallo-betalactamase)	AB858498.1
*bla*SFH-1	carbapenemase, class B metallo beta-lactamase	AF197943.1
*bla*SMB-1	carbapenemase, class B metallo beta-lactamase	AB636283.1
*bla*SME	carbapenemase, class A beta-lactamase	consensus
*bla*SPM-1	carbapenemase, class B metallo beta-lactamase	AY341249.1
*bla*VIM	carbapenemase, class B metallo beta-lactamase	consensus
*bla*VIM-2	carbapenemase, class B metallo beta-lactamase	AF191564.1
*bla*VIM-7	carbapenemase, class B metallo beta-lactamase	AJ536835.1
*bla*OXA-23-like	carbapenemase, class D beta-lactamase	AJ132105.1
*bla*OXA-40-like	carbapenemase, class D beta-lactamase	AF509241.1
*bla*OXA-48-like	carbapenemase, class D beta-lactamase	AY236073.2
*bla*OXA-51-like	carbapenemase, class D beta-lactamase	CP000863.1
ISABa1 to *bla*OXA-51	Insertion sequence ABa1 is adjacent to *bla*OXA-51-like gene. This combination mediated carbapenem resistant in Acinetobacter baumannii isolates	CP001921.1
no ISABa1 to *bla*OXA-51	Insertion sequence ABa1 is not adjacent to *bla*OXA-51-like gene. This combination does not mediate carbapenem resistant in Acinetobacter baumannii isolates	CU459141.1
*bla*OXA-54	carbapenemase, class D beta-lactamase	AY500137.1
*bla*OXA-55	carbapenemase, class D beta-lactamase	AY343493.1

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Target gene	Gene function	Accession number
*bla*OXA-58	carbapenemase, class D beta-lactamase	AY665723.1
*bla*OXA-134/235/284	carbapenemase *bla*OXA-134 or -235 or -284, class D beta-lactamase	AYHO01000005.1
*bla*OXA-143/182/253/255	carbapenemase *bla*OXA-40-like, class D beta-lactamase	GQ861437.1
*bla*OXA-181/232	carbapenemase *bla*OXA-48-like, class D beta-lactamase	CP000469.1
*bla*OXA-214	carbapenemase, class D beta-lactamase	JN861783.1
*bla*OXA-279	carbapenemase, class D beta-lactamase	APOK01000044.1
*bla*OXA-292	carbapenemase, class D beta-lactamase	APRH01000012.1
*bla*CME	extended spectrum beta-lactamase, class A	AF033200.1
*bla*CTX-M1/15	extended spectrum beta-lactamase, class A	X92506.1, HQ202266.1
*bla*CTX-M2	extended spectrum beta-lactamase, class A	AF286192.1
*bla*CTX-M8	extended spectrum beta-lactamase, class A	AY750914.2
*bla*CTX-M9	extended spectrum beta-lactamase, class A	FQ482074.1
*bla*MOX-CMY9	extended-spectrum beta-lactamase precursor, class C	AF381617.1
*bla*PER-1	extended-spectrum beta-lactamase, class A beta-lactamase PER-1	Z21957.1
*bla*PER-2	extended-spectrum beta-lactamase, class A beta-lactamase PER-2	X93314.1
*bla*SHV	class A beta-lactamase - consensus sequence for *bla*SHV genes, including extended-spectrum beta-lactamases	consensus
*bla*TEM	class A beta-lactamase - consensus sequence for *bla*TEM genes, including extended-spectrum beta-lactamases	consensus
*bla*VEB	extended-spectrum beta-lactamase, class A	consensus
*bla*OXA-18	extended spectrum beta-lactamase, class D	EU503121.1
*bla*OXA-45	extended spectrum beta-lactamase, class D	AJ519683.1
*bla*OXA-1	narrow spectrum beta-lactamase, class D	AY458016.1
*bla*OXA-9	narrow spectrum beta-lactamase, class D	M55547.1
*bla*OXA-2	consensus probe for extended and narrow spectrum class D beta-lactamases belonging to group *bla*OXA-2	consensus
*bla*OXA-10	consensus probe for extended and narrow spectrum class D beta-lactamases belonging to group *bla*OXA-10	consensus
*bla*OXA-60	narrow spectrum beta-lactamase, class D	AF525303.2
*bla*MIR	extended spectrum beta-lactamase, class C beta-lactamase	M37839.2
*bla*ACC	AmpC beta-lactamase	EF554600.1
*bla*ACT	AmpC beta-lactamase	U58495.2
*bla*CMY	AmpC beta-lactamase, consensus sequence	consensus
*bla*DHA	AmpC beta-lactamase	EF406115.1
*bla*FOX	AmpC beta-lactamase	consensus
*bla*MOX	AmpC beta-lactamase	consensus
aac(3')	3-N-aminoglycoside acetyltransferase; associated with resistance to astromicin; gentamicin; sisomicin	consensus
aac(3')-Ia	3-N-aminoglycoside acetyltransferase; associated with resistance to astromicin; gentamicin; sisomicin	U90945.1
aac(3')-Ib	3-N-aminoglycoside acetyltransferase; associated with resistance to astromicin; gentamicin; sisomicin	KJ679408.1
aac(3')-Ic	3-N-aminoglycoside acetyltransferase; associated with resistance to astromicin; gentamicin; sisomicin	AJ511268.1
aac(3')-Ie	3-N-aminoglycoside acetyltransferase; associated with resistance to astromicin; gentamicin; sisomicin	AY458224.1
aac(3')-IVa	3-N-aminoglycoside acetyltransferase; associated with resistance to apramycin; dibekacin; gentamicin; netilmicin; sisomicin; tobramycin	EU784152.1
aac(6')	aminoglycoside 6'-N-acetyltransferase, associated with resistance to amikacin; dibekacin; isepamicin; netilmicin; sisomicin; tobramycin	consensus
aac(6')-31	aminoglycoside 6'-N-acetyltransferase; associated with resistance to streptomycin, spectinomycin	AJ640197.1
aac(6')-Ib	aminoglycoside 6'-N-acetyltransferase; associated with resistance to streptomycin, spectinomycin	M21682.1
aac(6')-Il	aminoglycoside 6'-N-acetyltransferase; associated with resistance to streptomycin, spectinomycin	EF127959.1
aac(6')-Ila	aminoglycoside 6'-N-acetyltransferase; associated with resistance to streptomycin, spectinomycin	EU912537.1
aac(6')-IIc	aminoglycoside 6'-N-acetyltransferase; associated with resistance to streptomycin, spectinomycin	EU855788.1
aac-aph	6'-aminoglycoside-N-acetyltransferase/2''-aminoglycoside phosphotransferase; associated with resistance to gentamycin	AE017171.1
aadA1	aminoglycoside adenyltransferase; associated with resistance to streptomycin, spectinomycin	EU704128.1

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Target gene	Gene function	Accession number
aadA2	aminoglycoside adenyltransferase; associated with resistance to streptomycin, spectinomycin	EU704128.1
aadA4	aminoglycoside adenyltransferase; associated with resistance to streptomycin, spectinomycin	Z50802.3
aadB	2''-aminoglycoside nucleotidyltransferase	L06418.4
ant2	aminoglycoside (2") adenylyltransferase; associated with resistance to dibekacin; gentamicin; kanamycin; sisomicin; tobramycin	L06418.4
aphA	aminoglycoside 3'-phosphotransferase; kanamycin resistance protein	AY260546.3
armA	16S rRNA methylase, associated with aminoglycoside resistance	AB117519.1
grm	16S rRNA methylase, associated with gentamicin resistance	M55521.1
npmA	16S rRNA methylase, associated with aminoglycoside resistance	AB261016.1
rmtA	16S rRNA methylase, associated with aminoglycoside resistance	AB083212.2
rmtB	16S rRNA methylase, associated with aminoglycoside resistance	DQ345788.1
rmtC	16S rRNA methylase, associated with aminoglycoside resistance	AB194779.2
rmtD	16S rRNA methylase, associated with aminoglycoside resistance	DQ914960.2
mph	macrolide 2'-phosphotransferase	consensus
mrx	member of macrolide inactivation gene cluster *mphA – mrx - mphR*	consensus
qepA	QepA - fluoroquinolone/quinolone efflux pump	AM886293.1
qnrA1	quinolone or fluoroquinolone resistance protein	AY931018.1
qnrB	quinolone or fluoroquinolone resistance protein	AB281054.1
qnrC	quinolone or fluoroquinolone resistance protein	EU917444.1
qnrD	quinolone or fluoroquinolone resistance protein	FJ228229.1
qnrS	quinolone or fluoroquinolone resistance protein	AM234722.1
sul1	dihydropteroate synthetase type 1	AJ698325.1
sul2	dihydropteroate synthetase type 2	DQ464881.1
sul3	dihydropteroate synthetase type 3	AJ459418.2
dfrA1	dihydrofolate reductase type 1	AJ884723.1
dfrA5	dihydrofolate reductase type 5	AB188269.1
dfrA7	dihydrofolate reductase type 7	AB161450.1, AM237806.1
dfrA12	dihydrofolate reductase type 12	AB154407.1
dfrA13	dihydrofolate reductase type 13 (synonym A21)	Z50802.3
dfrA14	dihydrofolate reductase type 14	AJ313522.1
dfrA15	dihydrofolate reductase type 15	Z83311.1
dfrA17	dihydrofolate reductase type 17	AF169041.1
dfrA19	dihydrofolate reductase type 19	AJ310778.1
intI1	class 1 integron integrase	AY260546.3
intI2	class 2 integron integrase	AY183453.1
intI3	class 3 integron integrase	EF469602.1
tnpISEcp1	transposase for the transposon ISEcp1	AB543698.1
oqxA	OqxA - membran fusion protein, component of RND-type multidrug efflux pump, associated with olaquindox resistance	EU370913.1
oqxB	OqxB - integral membrane protein, component of RND-type multidrug efflux pump, associated with olaquindox resistance	EU370913.1
higA	higA is the antitoxin of the translation-dependent mRNA interferase toxin higB	U43847.1
higB	Ectopic expression of higB causes inhibition of cell growth which is alleviated by co-expression of higA	U43847.1
splA	splA is the antitoxin of the translation-dependent mRNA interferase toxin splT	EU294228.1
splT	Ectopic expression of splT causes inhibition of cell growth which is alleviated by co-expression of splA	EU294228.1
mcr-1 / mcr-2	mcr-1 and mcr-2, phosphoethanolamine transferase associated with resistance to colistin and polymyxin-type antibiotics	KP347127.1
mcr-3	mcr-3, phosphoethanolamine transferase associated with resistance to colistin and polymyxin-type antibiotics	KY924928.1
mcr-4	mcr-4, phosphoethanolamine transferase associated with resistance to colistin and polymyxin-type antibiotics	MF543359.1.1
mcr-5	mcr-5, phosphoethanolamine transferase associated with resistance to colistin and polymyxin-type antibiotics	NG_055658.1

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Target gene	Gene function	Accession number
mcr-6	mcr-6, phosphoethanolamine transferase associated with resistance to colistin and polymyxin-type antibiotics	NG_055781.1
mcr-7	mcr-7, phosphoethanolamine transferase associated with resistance to colistin and polymyxin-type antibiotics	NG_056413.1
mcr-8	mcr-8, phosphoethanolamine transferase associated with resistance to colistin and polymyxin-type antibiotics	NG_061399.1
mcr-9	mcr-9, phosphoethanolamine transferase associated with resistance to colistin and polymyxin-type antibiotics	MK070339.1

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| Lab ID | IDENTIFICATION RESULT | MIC Ampicillin | | Ampicillin | MIC Amoxicillin/ Clavulanic Acid | | Amoxicillin/ Clavulanic Acid | MIC Ticarcillin/Clavulanic acid | | Ticarcillin/Clavulanic acid | MIC Cefalexin | | MIC Cefadroxil | | MIC Cefaloridin | | MIC Cefalotin | | MIC Cefalothin | | MIC Cefazolin | | MIC Cefoperazone | | MIC Cefoxitin | | MIC Ceftiofur | | MIC Ceftiofur sodium | | MIC Cefquinome | | MIC Cefotaxime | | MIC Ceftriaxone | | MIC Imipenem | | Imipenem | MIC Gentamicin | | Gentamicin | MIC Neomycin | | Neomycin | MIC Plumequine | | Plumequine | MIC Enrofloxacin | | Enrofloxacin | MIC Marbofloxacin | | MarboFloxacin | MIC Tetracycline | | Tetracycline | MIC Trimethoprim/ Sulfamethoxazole | | Trimethoprim/ Sulfamethoxazole | MIC Florfenicol | | Florfenicol | ESBL genes | Carbapenemase genes | AmpC genes | Other β-lactamases genes | Aminoglycoside resistance genes | PMQR | Sulfonamide resistance genes | Trimethoprim resistance genes | Colistin resistance genes | Macrolide resistance genes | Genes associated with mobile genetic elements | Genes associated with a Multidrug Efflux Pump |
|---|---|---

References

Aarestrup FM (2005) Veterinary Drug Usage and Antimicrobial Resistance in Bacteria of Animal Origin. Basic Clin Pharmacol Toxicol 96:271–281

Aguirre L, Vidal A, Seminati C, Tello M, Redondo N, Darwich L, Martín M (2020) Antimicrobial Resistance Profile and Prevalence of Extended-Spectrum Beta-Lactamases (ESBL), AmpC Beta-Lactamases and Colistin Resistance (Mcr) Genes in Escherichia Coli from Swine between 1999 and 2018. Porc Health Manag 6:8

Athanasakopoulou Z, Reinicke M, Diezel C, Sofia M, Chatzopoulos D.C, Braun S.D, Reissig A, Spyrou V, Monecke S, Ehricht R, Tsilipounidaki K, Giannakopoulos A, Petinaki E, Billinis C (2021) Antimicrobial Resistance Genes in ESBL-Producing Escherichia Coli Isolates from Animals in Greece. Antibiotics 10:389

Balázs B, Nagy JB, Tóth Z, Nagy F, Károlyi S, Turcsányi I, Bistyák A, Kálmán A, Sárközi R, Kardos G (2021) Occurrence of Escherichia Coli Producing Extended Spectrum β-Lactamases in Food-Producing Animals. Acta Vet Hung 69:211–215

Bengtsson B, Greko C (2014) Antibiotic Resistance—Consequences for Animal Health, Welfare, and Food Production. Ups J Med Sci 119:96–102

Bergšpica I, Kaprou G, Alexa EA, Prieto M, Alvarez-Ordóñez A (2020) Extended Spectrum β-Lactamase (ESBL) Producing Escherichia Coli in Pigs and Pork Meat in the European Union. Antibiotics 9:678

Bernreiter-Hofer T, Schwarz L, Müller E, Cabal-Rosel A, Korus M, Misic D, Frankenfeld K, Abraham K, Grünzweil O, Weiss A, Feßler AT, Allerberger F, Schwarz S, Szostak MP, Ruppitsch W, Ladinig A, Spergser J, Braun SD, Monecke S, Ehricht R, Loncaric I (2021) The Pheno- and Genotypic Characterization of Porcine Escherichia Coli Isolates. Microorganisms 9:1676

Bertelloni F, Cilia G, Bogi S, Ebani VV, Turini L, Nuvoloni R, Cerri D, Fratini F, Turchi B (2020) Pathotypes and Antimicrobial Susceptibility of Escherichia Coli Isolated from Wild Boar (Sus Scrofa) in Tuscany. Anim 10:744

Braun SD, Jamil B, Syed MA, Abbasi SA, Weiß D, Slickers P, Monecke S, Engelmann I, Ehricht R (2018) Prevalence of Carbapenemase-Producing Organisms at the Kidney Center of Rawalpindi (Pakistan) and Evaluation of an Advanced Molecular Microarray-Based Carbapenemase Assay. Future Microbiol 13:1225–1246

Breakpoint Tables for Interpretation of MICs and Zone Diameters, Version 10.0. The European Committee on Antimicrobial Susceptibility Testing, Växjö, Sweden, 2020; 77

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Burch DGS, Sperling D (2018) Amoxicillin—Current Use in Swine Medicine. J Vet Pharmacol Ther 41:356–368

Burow E, Rostalski A, Harlizius J, Gangl A, Simoneit C, Grobbel M, Kollas C, Tenhagen B-A, Käsbohrer A (2019) Antibiotic Resistance in Escherichia Coli from Pigs from Birth to Slaughter and Its Association with Antibiotic Treatment. Prev Vet Med 165:52–62

Butiuc-Keul A, Carpa R, Podar D, Szekeres E, Muntean V, Iordache D, Farkas A (2021) Antibiotic Resistance in Pseudomonas spp. Through the Urban Water Cycle. Curr Microbiol 78:1227–1237

Chantziaras I, Boyen F, Callens B, Dewulf J (2014) Correlation between Veterinary Antimicrobial Use and Antimicrobial Resistance in Food-Producing Animals: A Report on Seven Countries. J Antimicrob Chemother 69:827–834

Cilia G, Turchi B, Fratini F, Bilei S, Bossù T, De Marchis ML, Cerri D, Pacini MI, Bertelloni F (2021) Prevalence, Virulence and Antimicrobial Susceptibility of Salmonella Spp., Yersinia Enterocolitica and Listeria Monocytogenes in European Wild Boar (Sus Scrofa) Hunted in Tuscany (Central Italy). Pathogens 10:93

Denwood MJ (2016) Runjags: An R Package Providing Interface Utilities, Model Templates, Parallel Computing Methods and Additional Distributions for MCMC Models in JAGS. J Stat Softw 71:1–25

Dohmen W, Bonten MJM, Bos MEH, van Marm S, Scharringa J, Wagenaar JA, Heederik DJJ (2015) Carriage of Extended-Spectrum β-Lactamases in Pig Farmers Is Associated with Occurrence in Pigs. Clin Microbiol Infect 21:917–923

ECDC/EFSA/EMA Second Joint Report on the Integrated Analysis of the Consumption of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Humans and Food-Producing Animals. Available online: https://www.efsa.europa.eu/en/efsajournal/pub/4872 (accessed on 4 May 2022)

European Food Safety Authority, European Centre for Disease Prevention and Control (2021) The European Union Summary Report on Antimicrobial Resistance in Zoonotic and Indicator Bacteria from Humans, Animals and Food in 2018/2019. EFSA J 19:e06490

Ewers C, Bethe A, Semmler T, Guenther S, Wieler LH (2012) Extended-Spectrum β-Lactamase-Producing and AmpC-Producing Escherichia Coli from Livestock and

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Companion Animals, and Their Putative Impact on Public Health: A Global Perspective. Clin Microbiol Infect 18:646–655

Filioussis G, Kachrimanidou M, Christodoulopoulos G, Kyritsi M, Hadjichristodoulou C, Adamopoulou M, Tzivara A, Kritas SK, Grinberg A (2020) Short communication: Bovine mastitis caused by a multidrug-resistant, mcr-1-positive (colistin-resistant), extended-spectrum β-lactamase-producing Escherichia coli clone on a Greek dairy farm. J Dairy Sci 103:852–857

Gelman A, Carlin JB, Stern HS, Rubin DB (1995) Bayesian Data Analysis. Chapman and Hall/CRC, New York, NY, USA. ISBN 978-0-429-25841-1

Giufrè M, Mazzolini E, Cerquetti M, Brusaferro S, CCM2015 One-Health ESBL-producing Escherichia coli Study Group (2021) Extended-Spectrum β-Lactamase-Producing Escherichia Coli from Extraintestinal Infections in Humans and from Food-Producing Animals in Italy: A 'One Health' Study. Int J Antimicrob Agents 58:106433

Jouini A, Vinué L, Slama KB, Sáenz Y, Klibi N, Hammami S, Boudabous A, Torres C (2007) Characterization of CTX-M and SHV Extended-Spectrum β-Lactamases and Associated Resistance Genes in Escherichia Coli Strains of Food Samples in Tunisia. J Antimicrob Chemother 60:1137–1141

Kola A, Kohler C, Pfeifer Y, Schwab F, Kühn K, Schulz K, Balau V, Breitbach K, Bast A, Witte W, Gastmeier P, Steinmetz I (2012) High Prevalence of Extended-Spectrum-β-Lactamase-Producing Enterobacteriaceae in Organic and Conventional Retail Chicken Meat, Germany. J Antimicrob Chemother 67:2631–2634

Landers TF, Cohen B, Wittum TE, Larson EL (2012) A Review of Antibiotic Use in Food Animals: Perspective, Policy, and Potential. Public Health Rep 127:4–22

Lee S, An J-U, Guk J-H, Song H, Yi S, Kim W-H, Cho S (2021) Prevalence, Characteristics and Clonal Distribution of Extended-Spectrum β-Lactamase- and AmpC β-Lactamase-Producing Escherichia Coli Following the Swine Production Stages, and Potential Risks to Humans. Front Microbiol 12:710747

Lee W, Grimm KJ (2018) Generalized Linear Mixed-Effects Modeling Programs in R for Binary Outcomes. Struct Equ Modeling A Multidiscip J 25:824–828

Lee WC, Yeh KS (2017) Characteristics of Extended-Spectrum β-Lactamase–Producing Escherichia Coli Isolated from Fecal Samples of Piglets with Diarrhea in Central and Southern Taiwan in 2015. BMC Vet Res 13:66

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Lekagul A, Tangcharoensathien V, Mills A, Rushton J, Yeung S (2020) How antibiotics are used in pig farming: A mixed-methods study of pig farmers, feed mills and veterinarians in Thailand. BMJ Glob Health 5:e001918

Lekagul A, Tangcharoensathien V, Yeung S (2019) Patterns of Antibiotic Use in Global Pig Production: A Systematic Review. Vet Anim Sci 7:100058

Leverstein-van Hall MA, Dierikx CM, Cohen Stuart J, Voets GM, van den Munckhof MP, van Essen-Zandbergen A, Platteel T, Fluit AC, van de Sande-Bruinsma N, Scharinga J, Bonten MJ, Mevius DJ (2011) Dutch Patients, Retail Chicken Meat and Poultry Share the Same ESBL Genes, Plasmids and Strains. Clin Microbiol Infect 17:873–880

Liu X, Liu H, Wang L, Peng Q, Li Y, Zhou H, Li Q (2018) Molecular Characterization of Extended-Spectrum β-Lactamase-Producing Multidrug Resistant Escherichia Coli from Swine in Northwest China. Front Microbiol 9:1756

Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu JH, Shen J (2016) Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: A microbiological and molecular biological study. Lancet Infect Dis 16:161–168

Livermore DM, Woodford N (2006) The β-Lactamase Threat in Enterobacteriaceae, Pseudomonas and Acinetobacter. Trends Microbiol 14:413–420

Madec JY, Haenni M, Nordmann P, Poirel L (2017) Extended-Spectrum β-Lactamase/AmpC- and Carbapenemase-Producing Enterobacteriaceae in Animals: A Threat for Humans? Clin Microbiol Infect 23:826–833

Munita JM, Arias CA (2016) Mechanisms of Antibiotic Resistance. Microbiol Spectr 4:2–4

National Research Council (2012) Nutrient Requirements of Swine (NRC): Eleventh Revised Edition. The National Academies Press (NAP), Washington, DC, USA

Nhung NT, Cuong NV, Thwaites G, Carrique-Mas J (2016) Antimicrobial Usage and Antimicrobial Resistance in Animal Production in Southeast Asia: A Review. Antibiotics 5:37

Overdevest I, Willemsen I, Rijnsburger M, Eustace A, Xu L, Hawkey P, Heck M, Savelkoul P, Vandenbroucke-Grauls C, van der Zwaluw K, Huijsdens X, Kluytmans J (2011) Extended-Spectrum β-Lactamase Genes of Escherichia Coli in Chicken Meat and Humans, The Netherlands. Emerg Infect Dis 17:1216–1222

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Papadopoulos D, Papadopoulos T, Papageorgiou K, Sergelidis D, Adamopoulou M, Kritas S.K, Petridou E (2021) Antimicrobial Resistance Rates in Commensal Escherichia Coli Isolates from Healthy Pigs in Greek Swine Farms. J Hell Vet Med Soc 72:2909–2916

Papatsiros VG, Alexopoulos C, Kyriakis SC (2007) Latest Information in Relation to Postpartum Dysgalactia Syndrome of Sows. J Hell Vet Med Soc 58:61–75

Peirano G, Pitout JDD (2019) Extended-Spectrum β-Lactamase-Producing Enterobacteriaceae: Update on Molecular Epidemiology and Treatment Options. Drugs 79:1529–1541

Pholwat S, Pongpan T, Chinli R, Rogawski McQuade ET, Thaipisuttikul I, Ratanakorn P, Liu J, Taniuchi M, Houpt ER, Foongladda S (2020) Antimicrobial Resistance in Swine Fecal Specimens Across Different Farm Management Systems. Front Microbiol 11:1238

Pitout JD, Laupland KB (2008) Extended-Spectrum β-Lactamase-Producing Enterobacteriaceae: An Emerging Public-Health Concern. Lancet Infect Dis 8:159–166

Poirel L, Stephan R, Perreten V, Nordmann P (2014) The Carbapenemase Threat in the Animal World: The Wrong Culprit. J Antimicrob Chemother 69:2007–2008

Politi L, Tassios PT, Lambiri M, Kansouzidou A, Pasiotou M, Vatopoulos AC, Mellou K, Legakis NJ, Tzouvelekis LS (2005) Repeated Occurrence of Diverse Extended-Spectrum β-Lactamases in Minor Serotypes of Food-Borne Salmonella Enterica Subsp. Enterica. J Clin Microbiol 43:3453–3456

Pollock J, Muwonge A, Hutchings MR, Mainda G, Bronsvoort BM, Gally DL, Corbishley A (2020) Resistance to Change: AMR Gene Dynamics on a Commercial Pig Farm with High Antimicrobial Usage. Sci Rep 10:1708

R Core Team (2020) European Environment Agency. Available online: https://www.eea.europa.eu/data-and-maps/indicators/oxygen-consuming-substances-in-rivers/r-development-core-team-2006 (accessed on 4 May 2022)

Ricker N, Trachsel J, Colgan P, Jones J, Choi J, Lee J, Coetzee JF, Howe A, Brockmeier SL, Loving CL, Allen HK (2020) Toward Antibiotic Stewardship: Route of Antibiotic Administration Impacts the Microbiota and Resistance Gene Diversity in Swine Feces. Front Vet Sci 7:255

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Rubira I, Figueras LP, Jiménez JC, de Arcaute MR, Ruiz H, Ventura JA, Lacasta D (2021) Salmonella Enterica Subsp. Diarizonae Serotype 61:K:1:5:(7) a Host Adapted to Sheep. IntechOpen, London, UK. ISBN 978-1-83969-018-1

Scientific Opinion on Carbapenem Resistance in Food Animal Ecosystems|EFSA. Available online: https://www.efsa.europa.eu/en/efsajournal/pub/3501 (accessed on 4 May 2022)

Smet A, Martel A, Persoons D, Dewulf J, Heyndrickx M, Herman L, Haesebrouck F, Butaye P (2010) Broad-Spectrum β-Lactamases among Enterobacteriaceae of Animal Origin: Molecular Aspects, Mobility and Impact on Public Health. FEMS Microbiol Rev 34:295–316

Ström G, Halje M, Karlsson D, Jiwakanon J, Pringle M, Fernström L-L, Magnusson U (2017) Antimicrobial Use and Antimicrobial Susceptibility in Escherichia Coli on Small- and Medium-Scale Pig Farms in North-Eastern Thailand. Antimicrob Resist Infect Control 6:75

Tamta S, Kumar ORV, Singh SV, Pruthvishree BS, Karthikeyan R, Rupner R, Sinha DK, Singh BR (2020) Antimicrobial Resistance Pattern of Extended-Spectrum β-Lactamase-Producing Escherichia Coli Isolated from Fecal Samples of Piglets and Pig Farm Workers of Selected Organized Farms of India. Vet World 13:360–363

Valiakos G, Vontas A, Tsokana C.N, Giannakopoulos A, Chatzopoulos D, Billinis C (2016) Resistance in Escherichia Coli Strains Isolated from Pig Faecal Samples and Pig Farm Workers, Greece. Am J Anim Vet Sci 11:142–144

Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP, Teillant A, Laxminarayan R (2015) Global Trends in Antimicrobial Use in Food Animals. Proc Natl Acad Sci USA 112:5649–5654

van der Fels-Klerx HJ, Puister-Jansen LF, van Asselt ED, Burgers SLGE (2011) Farm Factors Associated with the Use of Antibiotics in Pig Production. J Anim Sci 89:1922–1929

Villar HE, Danel F, Livermore DM (1997) Permeability to Carbapenems of Proteus Mirabilis Mutants Selected for Resistance to Imipenem or Other Beta-Lactams. J Antimicrob Chemother 40:365–370

Wickham H (2011) Ggplot2. WIREs Comput Stat 3:180–185.

World Health Organization (2017) WHO Guidelines on Use of Medically Important Antimicrobials in Food-Producing Animals. Geneva, Switzerland

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Yang P, Chen Y, Jiang S, Shen P, Lu X, Xiao Y (2018) Association between Antibiotic Consumption and the Rate of Carbapenem-Resistant Gram-Negative Bacteria from China Based on 153 Tertiary Hospitals Data in 2014. Antimicrob Resist Infect Control 7:137

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CHAPTER III
RESEARCH

Detection of Enterotoxigenic *Escherichia coli* and Clostridia in the Aetiology of Neonatal Piglet Diarrhoea: Important Factors for Their Prevention

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1. Introduction

Neonatal piglet diarrhoea is a major economic and welfare issue in the swine industry worldwide due to increased pre-weaning mortality and therapeutic cost, as well as decreased growth rates in piglets (Chan et al., 2013; Sjolund et al., 20140. To this day, it has proven to be challenging to identify the agents responsible for neonatal diarrhoea clinical signs in pig herds (Kongsted et al., 2018). Several previous studies have reported various common pathogens as etiological agents, including *Escherichia coli* (*E. coli*), *Clostridium perfringens* (*C. perfringens*), *Clostridioides difficile* (*C. difficile*), *Cystoisospora suis*, rotavirus, and *Cryptosporidium parvum* (Dubreuil et al., 2016; Luppi et al., 2016; Ruiz et al., 2016; Uzal and Songer, 2019; Vidal et al., 2019). In many field cases, combinations of pathogens have been isolated (Vidal et al., 2019).

Neonatal diarrhoea is one of the most frequent clinical signs in newborn piglets, significantly increasing pre-weaning mortality and the number of weaning piglets with a lower body weight than the farm's target (Holland, 1990). Even though enteric diseases in newborn piglets are often endemic, outbreaks characterized by high morbidity and mortality have been reported (Morin et al., 1983; Svensmark et al., 1988). For example, in Sweden and Denmark, diarrhoea represented 5–24% of the overall pre-weaning mortality cases (Svendsen et al., 1975) and is responsible for a reduction in average daily gain (8–14 g/day) during the first week of life (Kongsted et al., 2014; Johansen et al., 2004). The cost of neonatal diarrhoea in affected pig herds was recently estimated to be EUR 134 per sow per year (Sjolund et al., 2014). Commercial pig farms use vaccinations against *E. coli* and clostridial diseases, antibiotics, and alternatives to antibiotics to prevent or reduce economic losses due to neonatal diarrhoea.

E. coli is a Gram-negative bacterium that normally lives in the intestine, but imbalances in the gut microbiota can potentially cause diarrhoea in pigs (Dubreuil, 2008). Neonatal piglets affected by *E. coli* suffer from severe diarrhoea and have a high mortality rate (Moon et al., 1986). Enteropathogenic *E. coli* strains (EPEC) and enterotoxigenic *E. coli* strains (ETEC) are classified by their virulence factors, with EPEC being characterized by the production of intimin and ETEC strains by their principal virulence factors, enterotoxins (STa, STb, and LT) and fimbriae adhesins (Toledo et al., 2012). ETEC are a significant and common cause of diarrhoea among suckling and weaned piglets (Zajacova et al., 2012). ETEC colonize the mucosal surface of the small intestine via surface proteins (fimbriae or pilli), producing the following enterotoxins: heat-stable (Sta and STb) and heat-labile (LT) ones, or both (Nagy and Fekete, 1999). The reported porcine fimbriae are

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F4 (K88), F5 (K99), F6 (987P), F18, and F41 (Vu-Khac et al., 2007). Neonatal diarrhoea due to ETEC infection is characterized by high morbidity rates of suckling piglets per litter and is associated with increased economic losses for the swine farms due to an increased mortality rate, reduced growth performance, and increased veterinary cost for treatments and preventive strategies (e.g., antimicrobials, vaccines, alternatives to antibiotics, and biosecurity measures) (Fairbrother and Gyles, 2012; Luppi, 2017).

Clostridia are large, rod-shaped bacteria that also form spores that persist in the environment for long periods. *C. perfringens* and *C. difficile* are classified as the main swine enteric clostridial pathogens (Songer and Uzal, 2005). *C. perfringens* type C causes severe and foetal necrotic enteritis in neonatal piglets due to the production of α-toxin and β-toxin (Songer and Uzal, 2005; Petit et al., 1999; Songer, 1996; Rood et al., 2018). Diarrhoea can spread rapidly amongst the herd, inducing high mortality (up to 100%) in affected piglets of non-vaccinated herds (Songer and Uzal, 2005). The peracute and acute forms of diarrhoea disease affect piglets mainly at 0–3 days of age (Songer and Uzal, 2005).

C. difficile is a Gram-positive spore-forming bacterium that causes the enteric disease in humans and pigs, among many mammals (Gould and Limbago, 2010; Keel and Songer, 2006; Keessen et al., 2011). *C. difficile* is widespread in the environment and is a common part of the gastrointestinal microbiota of mammals (Britton and Young, 2012; Lim et al., 2020). Previous studies have revealed *C. difficile* to be an emerging pathogen (Chan et al., 2013), which has been involved in cases of uncontrolled enteritis outbreaks affecting neonatal piglets in the USA and Europe (Keel and Songer, 2006; Songer, 2004; Debast et al., 2009). Previous studies also reported *C. difficile* strains usually isolated from piglets that have infected humans in North America and Europe (Goorhuis et al., 2008) and play a serious role in human diarrhoea (Mylonakis et al., 2001).

The fundamental tools to tackling neonatal diarrhoea are a good knowledge of epidemiology, an efficient diagnostic approach, as well as appropriate control or preventive strategies (Luppi, 2017). For example, to control enteric colibacillosis in neonatal piglets, it is crucial to better comprehend the pathotypes and virotypes of *E. coli* and the predisposing factors that allow the bacterium to cause diseases, as well as to apply rapid diagnostic methods and efficient control or preventive tools (Luppi, 2017). For a correct diagnosis of neonatal piglet diarrhoea, detecting the presence of pathogenicity factors is fundamental, as many agents are included in bacterial flora and bacterial isolation is not proof that these agents cause diarrhoea. Multiplex polymerase chain reaction (PCR) is a very effective technique to detect virulence factors, such as the presence of fimbriae F4, F5,

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F6, and LT of *E. coli*, and the β-toxin of *C. perfringens* type C (Songer and Uzal, 2005). Multiplex PCR approaches enable the amplification of several genes simultaneously, reducing the cost and time needed (DebRoy et al., 2011; Bai et al., 2012; Baker et al., 2016; Baker et al., 2016b). FTA (Flinders Technology Associates) cards provide important advantages, such as sample storage, transport, and extraction, leading to a decrease in the cost and time needed for molecular diagnosis. FTA cards have been widely used to extract and stabilize DNA from samples of human and swine clinical cases (Salvador and De Ungria, 2003; Muthukrishnan et al., 2008; Linhares et al., 2012; Shalaby et al., 2020; Stringer et al., 2021; Rajendram et al., 2006). The method of FTA cards is reported to be a valid diagnostic tool, and it can be used for a short period (24 h) for the storage and transport of live bacteria, specifically Gram-positive types (Shalaby et al., 2020).

Multidrug resistance genes among ESBL-producing *E. coli* strains retrieved from feces of pigs were reported, underlining the issue of antimicrobial resistance (Li, 2017; Pamer, 2016; Laird et al., 2021; Athanasakopoulou et al., 2021; Tsekouras et al., 2022). Various non-antibiotic strategies, including feed additives such as acidifiers or probiotics, have been proposed in swine herd health management strategies as other optional prophylactic or therapeutic protocols (Liu et al., 2018; Papatsiros and Billinis, 2012; Ji et al., 2020; Wellison et al., 2022; Kim et al., 2005; Jacela et al., 2009; Roth and Kirchgessner, 1998; Papatsiros et al., 2013; Kantas et al., 2015; Sayan et al., 2018; Yue et al., 2020; Alexopoulos et al., 2000; Bohmer et al., 2006; Kantas et al., 2015; Hayakawa et al., 2016; Menegat et al., 2018; Kritas et al., 2015; Papatsiros et al., 2011; Papatsiros et al., 2012; Pearlin et al., 2020). Probiotic supplementation in feed is beneficial for improving the immune response and growth performance, maintaining gut health, and controlling enteric infections due to its antimicrobial effects on several enteric pathogens by stimulating the growth of beneficial microorganisms in the gut (Ji et al., 2020; Wellison et al., 2022 Yue et al., 2020; Alexopoulos et al., 2000; Bohmer et al., 2006; Kantas et al., 2015; Hayakawa et al., 2016; Menegat et al., 2018; Kritas et al., 2015; Papatsiros et al., 2011). Previous studies have reported that dietary acidifiers provide prophylactic effects such as antibiotics do, mainly by limiting the growth of enteric pathogens and simultaneously providing the opportunity for the proliferation of beneficial organisms (Kim et al., 2005; Jacela et al., 2009; Roth and Kirchgessner, 1998; Papatsiros et al., 2013; Papatsiros et al., 2011; Papatsiros et al., 2012; Pearlin et al., 2020).

There are limited published data about the aetiology of neonatal piglet diarrhoea in the Greek swine industry, especially on the prevalence of *C. difficile*. The objective of this

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study was to investigate the detection of ETEC and *C. difficile* or *C. perfringens* type C pathogens in neonatal piglet diarrhoea, as well as the factors contributing to their prevention in commercial pig farms.

2. Materials and Methods

2.1. Study Design

2.1.1. Description of Farms, Criteria for Inclusion, and Study Groups

The present study was carried out from January 2020 to October 2021 in Greece. A total of 26 commercial farrow-to-finish pig farms, with an overall population of 12,380 sows comprising approximately 24.5% of the Greek swine industry, representing different regions with high pig density, as well as with different capacities, were included in the study (**Table 1**). Inclusion criteria for the participated commercial pig farms were the ability to vaccinate sows against *E. coli* (F4ab, F4ac, F5, F6, and LT) and *C. perfringens* type C (beta toxoid) using commercial vaccines (intramuscularly, 2 mL per animal) in a routine program 15–20 days before farrowing. The pig farmers participated voluntarily and provided data about the possible use of acidifiers or/and probiotics in gestation and lactation feed. Moreover, the administration or not of injectable antibiotics to sows on the first day of farrowing was recorded. The injectable antibiotic that was used in sows of selected farms was amoxicillin LA at the dose of 15 mg/kg of body weight (BW) after cultivation and antibiogram in samples of former clinical cases to prevent postpartum dysgalactia syndrome (PDS). Moreover, some of the selected farms used, according to the manufacturer's guidelines, acidifiers based on commercial products containing a combination of formic acid, propionic acid, phosphoric acid, lactic acid, and acetic acid and/or probiotics based on commercial products with *Bacillus licheniformis* and *Bacillus subtilis*. The study groups of the trial are presented in **Table 2**.

Table 1. Overview of the 26 commercial pig farms included in the present study: number per geographic region, the capacity of sows, and the number of samples.

Region of Greece	Number of farms	Capacity of sows per farm				Number of faecal pool samples
		<100	101-300	301-500	>500	
Central Greece	10	2	3	1	4	30
North Greece	7	0	4	1	2	21
West Greece	5	0	2	0	3	15

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South Greece	4	1	1	1	1	12
Total	26	3	10	3	10	78

Table 2. Overview of the study groups according to the use of antibiotics, probiotics, or acidifiers.

Groups of the trial farms based on their routine program in sows							
None use of AB*, PR, AC*** **	**Injectable AB at 1st day of farrowing**	**Use in pre-farrowing feed**			**Combination of injectable AB and PR or/and AC in pre-farrowing feed**		
Group None	**Group AB**	**Group PR**	**Group AC**	**Group PR+AC**	**Group PR+AB**	**Group AC+AB**	**Group AB+PR+AC**
6	8	2	1	4	1	1	3

**AB: antibiotics*

*** PR: probiotics*

**** AC: acidifiers*

2.1.2. Sampling and Laboratory Examinations

In total, 78 pooled faecal samples were collected from 234 suckling piglets (1–4 days of age) with diarrhoea (**Figure 1**). No medication or treatment was given to the selected piglets. Concerning diarrhoea, the sampling time was random. The collected samples were initially screened for the presence of *E. coli* and *C. difficile* or *C. perfringens* via cultivation on MacConkey and anaerobic blood agar, respectively (Goldstein et al., 2012). A modified scoring system created by Pedersen et al. (2011) based on faecal consistency was applied for each selected piglet for faecal sampling, as follows: scores 1 (soft faeces), 2 (mild diarrhoea), or 3 (severe diarrhoea). Three rectal swab samples per litter (1 pool) were collected randomly from suckling piglets with symptoms of diarrhoea for three litters per farm (3 piglets × 3 litters × 26 farms = 234 piglets = 78 faecal pool samples). Subsequently, the samples were pooled on ELUTE cards (FTA-like) according to the manufacturer's instructions (Enterocheck®, Hipra, Spain). The DNA extraction process, as well as the one-step multiplex PCR technique to detect genes that codify the adhesion factors F4, F5, F6, and the LT toxin of enterotoxigenic *E. coli* (ETEC), β-toxin of *C. perfringens type C*, and toxins A and B of *C. difficile*, were carried out using specific probes according to laboratory guidelines (Laboratorios Hipra, Amer, Girona, Spain) (Quilitis et al., 2013; Kitchodok et al., 2018; Kitchodok et al., 2019). The results

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were determined as negative (−) based on the Cycle threshold (Ct) values (>38.5 Ct value). Positive samples were categorized into three categories: pos (+): a low quantity of genetic material of the tested pathogens was detected (35–38.5 Ct value); pos (++): a moderate quantity of genetic material of the tested pathogens was detected (30–35 Ct value); pos (+++): a large quantity of genetic material of the tested pathogens was detected (<30 Ct value).

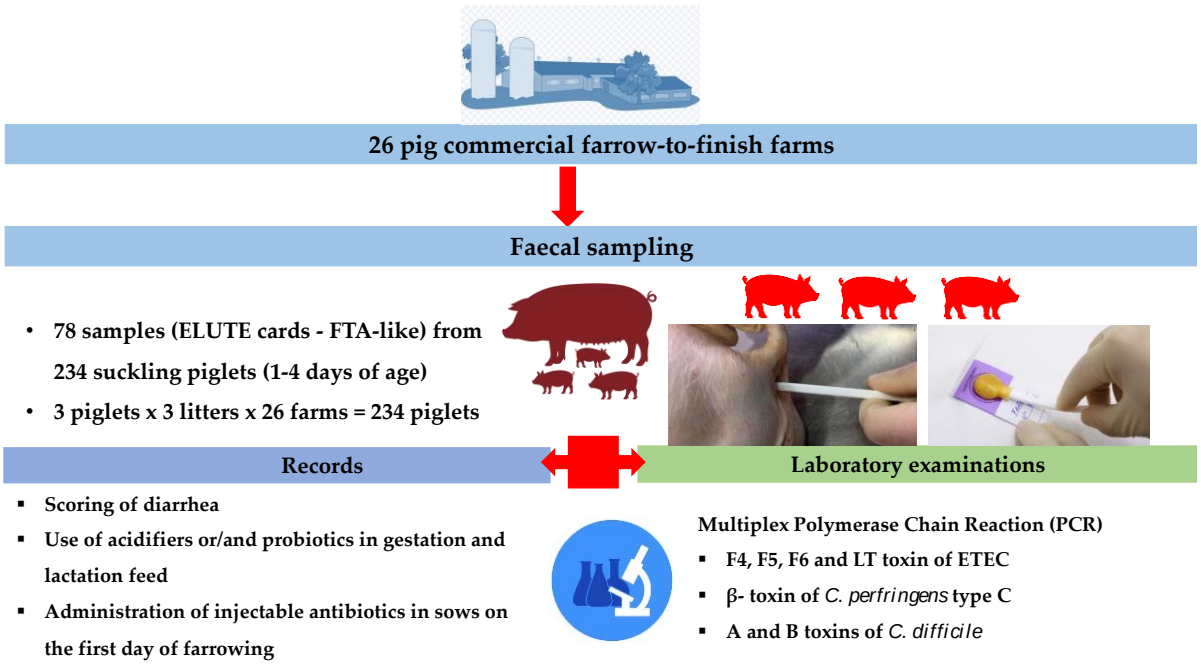

Figure 1. A flowchart of study design (animals, sampling, laboratory exams).

2.2. Statistical Analysis

The results were statistically analysed using R programming language (R Core Team, 2021), and all graphs were created with Seaborn, a Python data visualization library based on matplotlib (Levene, 1960). The results of the detection of the genes F4, F5, and F6 of ETEC, *E. coli* enterotoxin LT, β-toxin of *C. perfringens*, and toxins A and B of *C. difficile* in faeces of piglets were analysed statistically three times based on the following groups: None, AB, PR, AC, PR + AC, PR + AB, AC + AB, and AB + PR + AC. The results were analysed using the Kruskal–Wallis test (Kruskal and Wallis, 1952). All comparisons were performed at a significant level of $p < 0.05$. Power analysis with G-Power software (version 3.1.) was performed to estimate the study power and the minimum sample size. The actual power of this study was greater than 95% for a total sample size of 78 samples.

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3. Results

The percentages (proportions) of 26 farms when at least one sample was positive and of 78 samples where ETEC F4, ETEC F5, ETEC F6, enterotoxin LT, and β-toxin of *C. perfringens* type C and toxins A and B of *C. difficile* were detected are presented in **Table 3**.

Table 3. Percentage (%) and number (no) of positive pig farms (when at least one sample was positive) and positive samples in the detection, by PCR of ETEC F4, F5 and F6 genes, enterotoxin LT, β toxin of *C. perfringens* and toxins A and B of *C. difficile.*

Analytical target	% positive farms (No farms)	% positive samples (No pool samples)
F4 gene	69.23% (18)	53.85% (42)
F5 gene	30.77% (8)	24.36% (19)
F6 gene	61.54% (16)	55.13% (43)
Enterotoxin LT	57.69% (15)	44.88% (35)
F4 gene + Enterotoxin LT	42.31% (11)	25.64% (20)
F5 gene + Enterotoxin LT	19.23% (5)	16.67% (13)
F6 gene + Enterotoxin LT	42.31% (11)	32.05% (25)
β-toxin - *C. perfringens* type C	0.00% (0)	0.00% (0)
Toxin A - *C. difficile*	84.62% (22)	65.38% (51)
Toxin B - *C. difficile*	88.46% (23)	61.54% (48)

Regarding the detection of F4 gen, the quantity was significantly higher in group None in comparison to those in groups AB ($p = 0.018$), AB + PR + AC ($p = 0.014$), AC + AB ($p = 0.0117$), and PR + AC ($p = 0.04$). A statistical difference was found between group AB + PR + AC in comparison and groups AC ($p = 0.02$), PR ($p = 0.02$), and PR + AB ($p = 0.02$). Lastly, groups AC + AB and PR were found to be statistically different ($p = 0.03$). Groups AB, AB + PR + AC, AC + AB had the lowest median (higher quantity of genetic material), while the highest median (lower quantity of genetic material) was observed in groups AC, PR, PR + AB, and None (**Figure 2**a).

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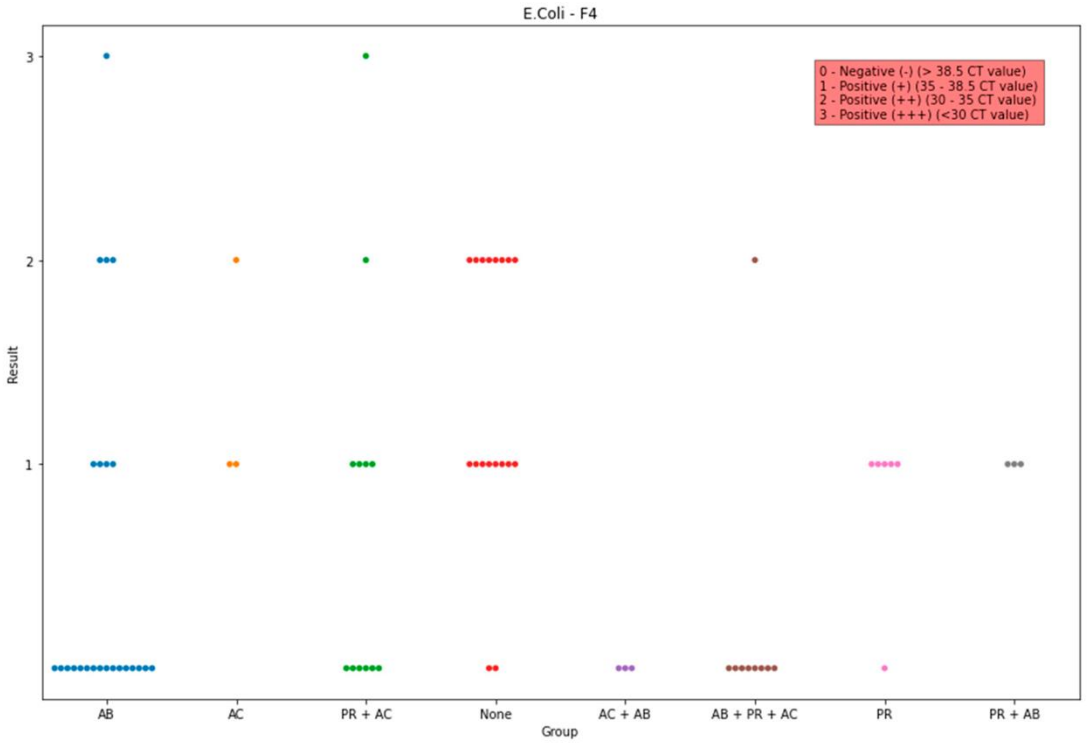
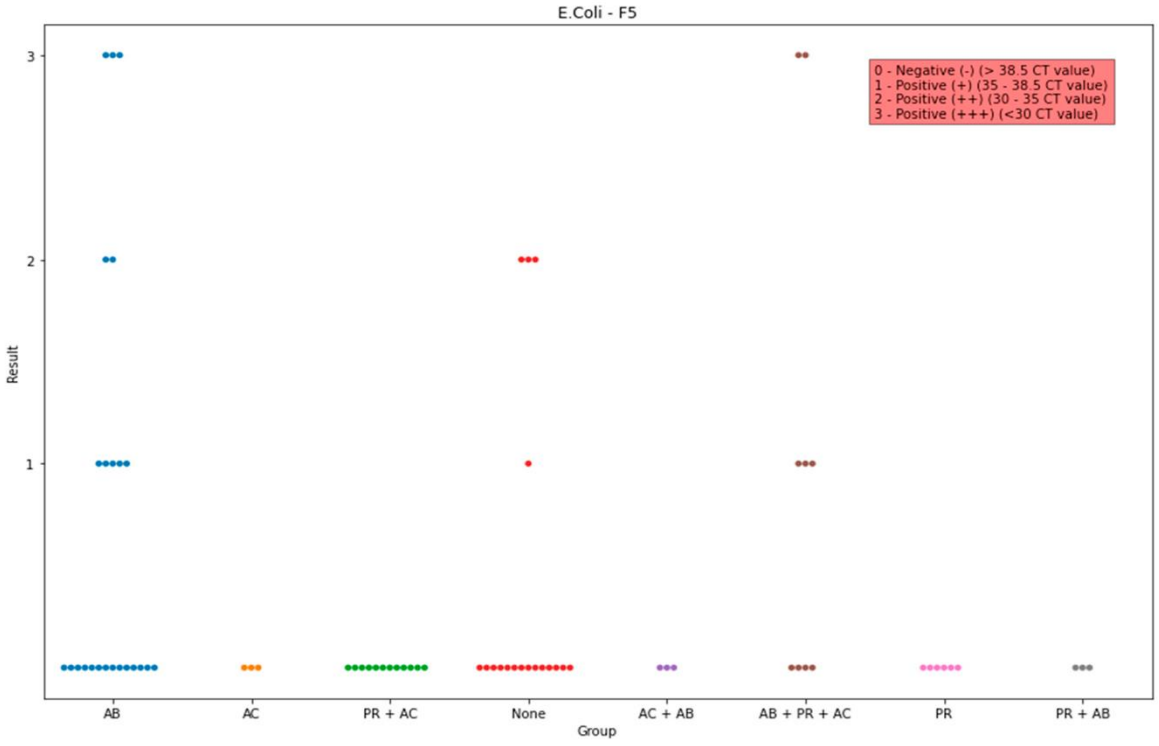


(**a**)



(**b**)

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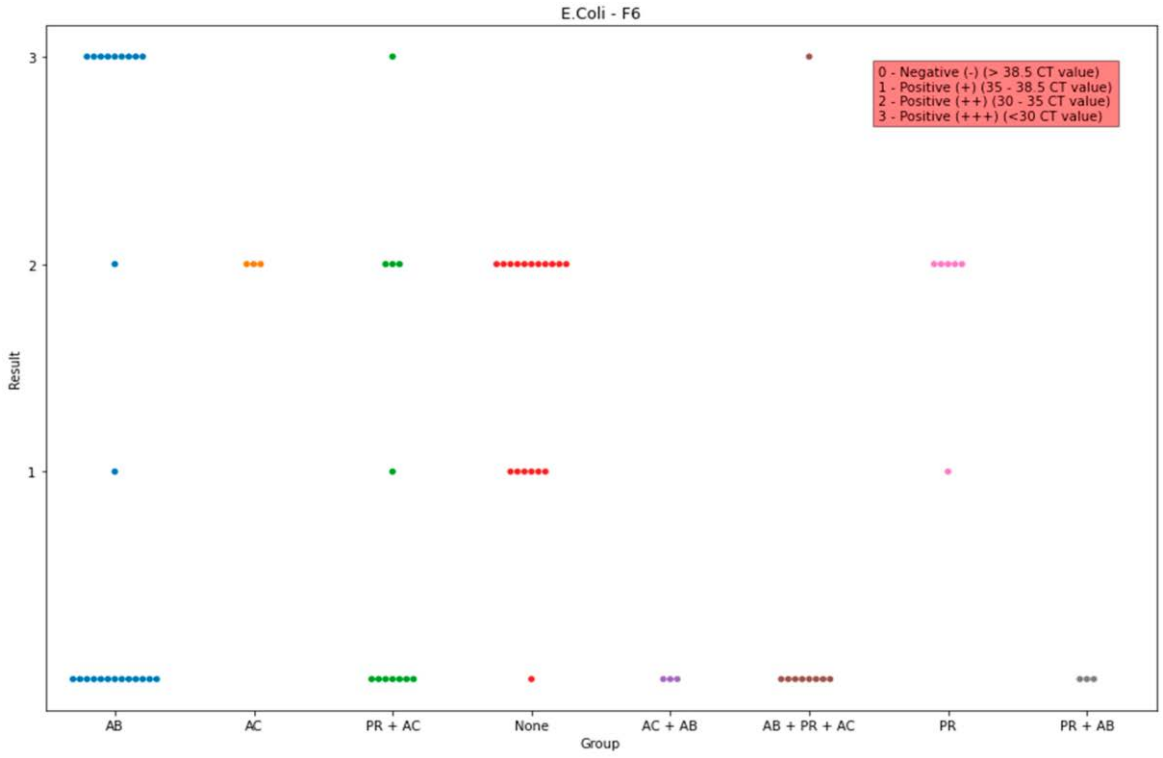

(**c**)

Figure 2. (**a**) Observed data points of the scores of the PCR detected ETEC F4 in all groups. The x-axis indicates the administration group, and y-axis indicates the amount of the detected genes. (**b**) Observed data points of the scores of the PCR detected ETEC F5 in all groups. The x-axis indicates the administration group, and the y-axis indicates the amount of the detected genes. (**c**) Observed data points of the scores of the PCR detected ETEC F6 in all groups. The x-axis indicates the administration group, and the y-axis indicates the amount of the detected genes.

Statistical analysis of ETEC F5 detection in the faeces of piglets showed that the proportion was significantly lower in group PR in comparison to that in group AB + PR + AC ($p = 0.04$), and it was lower in group PR + AC compared to those in groups AB ($p = 0.01$) and AB + PR + AC ($p = 0.004$). Group AB + PR + AC had the highest median (**Figure 2**b).

Regarding the detection of ETEC F6 in the faeces of piglets, the proportion was significantly lower in group PR + AB in comparison to those in groups AC ($p = 0.04$), None ($p = 0.005$), and PR ($p = 0.01$). Similarly, group AB + PR + AC had a significant difference in comparison to groups PR ($p = 0.001$) and PR ($p = 0.007$). Lastly, there was a statistical difference between groups AC + AB and PR ($p = 0.001$). The highest median was in groups AC, PR, and None (**Figure 2**c).

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Statistical analysis of the detection of enterotoxin LT showed that the proportion was significantly higher in group PR in comparison to those in groups AC ($p = 0.007$), AC + AB ($p = 0.007$), and None ($p = 0.003$). Statistical differences were also found between groups AB and B + PR + AC ($p = 0.01$), AB and None ($p = 0.03$), PR and PR + AB ($p = 0.007$), and PR + AC and AB ($p = 0.02$) and PR ($p = 0.003$). The highest medians were in groups PR and AB (**Figure 3**).

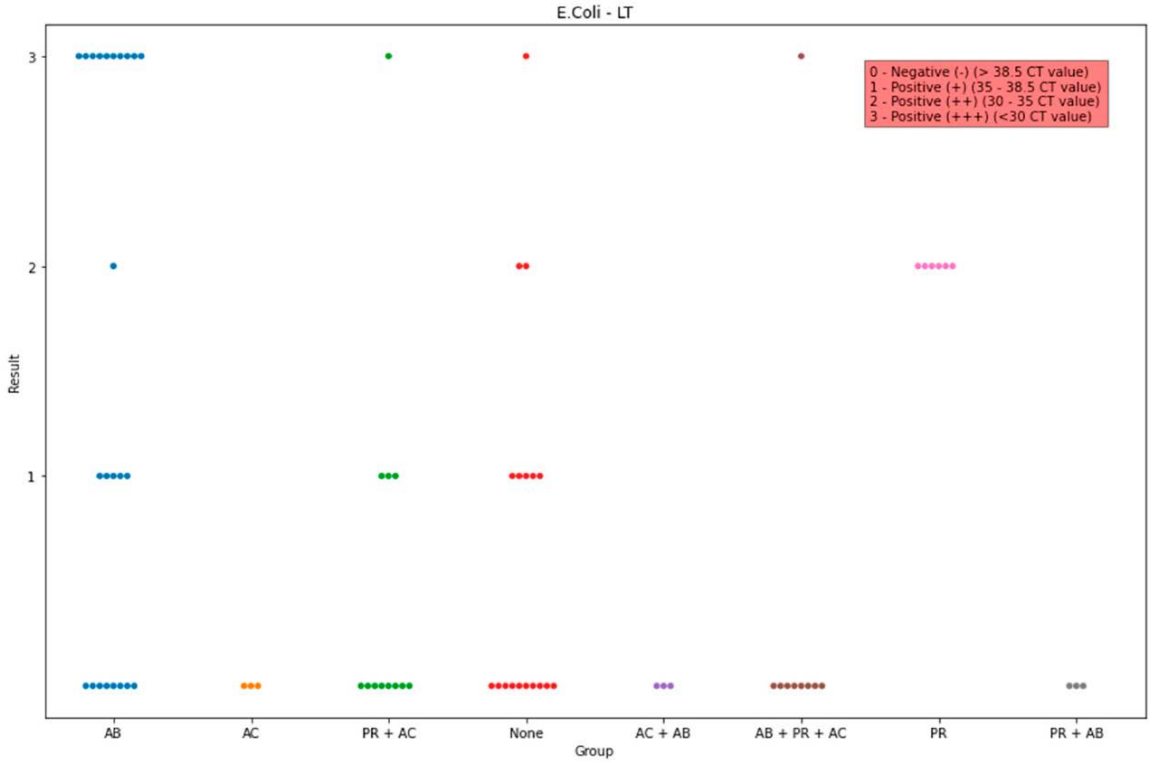

Figure 3. Observed data points of the scores of the PCR detected *E. coli* toxin LT in all groups. The x-axis indicates the administration group, and the y-axis indicates the amount of the detected genes.

Concerning the detection of toxin A of *C. difficile*, the proportion was found significantly higher in group AB in comparison to those in groups AB + PR + AC ($p < 0.001$), AC + AB ($p = 0.009$), PR ($p = 0.01$), and PR + AB ($p = 0.01$), and it was lower in group AB + PR + AC in comparison to group None ($p = 0.01$). Group AB had the highest median, while groups AB + PR + AC, AC + AB, and PR + AB had the lowest median (**Figure 4**).

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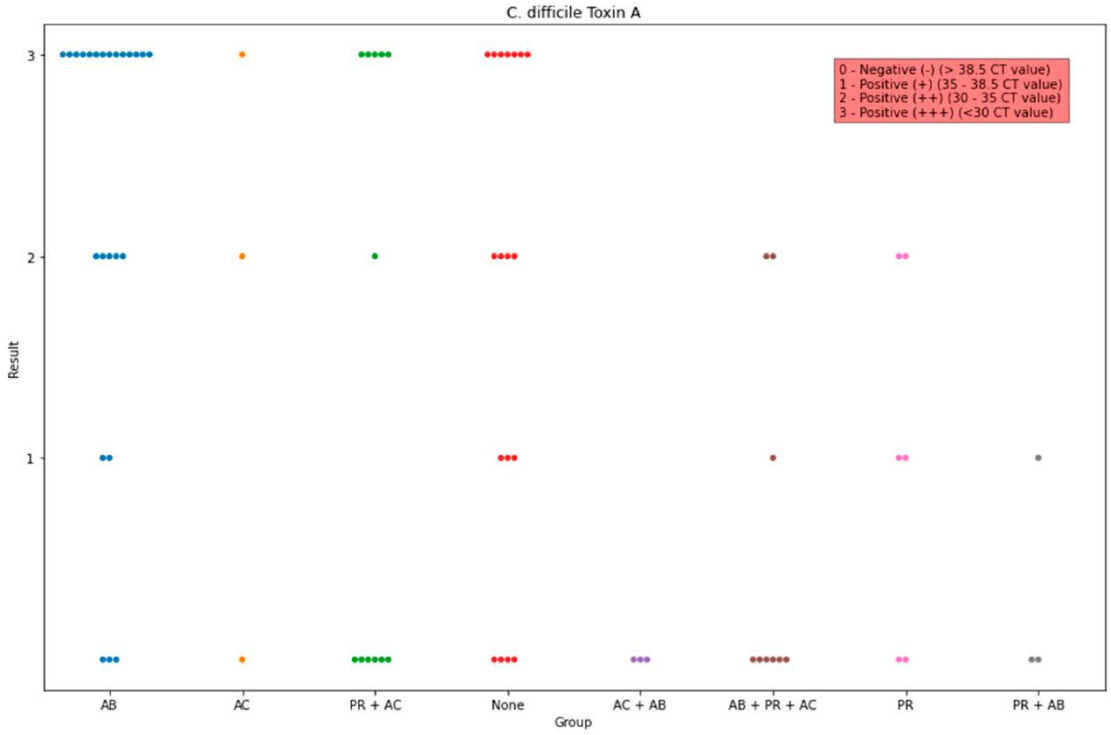

Figure 4. Observed data points of the scores of the PCR detected toxin A of *C. difficile* in all groups. The x-axis indicates the administration group, and the y-axis indicates the amount of the detected genes.

Moreover, statistical analysis of the detected toxin B of *C. difficile* showed that the proportion is significantly higher in group AB in comparison to those in groups AB + PR + AC ($p < 0.001$), AC + AB ($p = 0.0078$, None ($p = 0.03$), PR ($p = 0.003$), and PR + AB ($p = 0.01$). Lastly, a significant difference between groups AB + PR + AC and None ($p = 0.165$) was found. For the detection of toxin B of *C. difficile*, the lowest median was in groups AC + AB, None, and AB + PR + AC (**Figure 5**).

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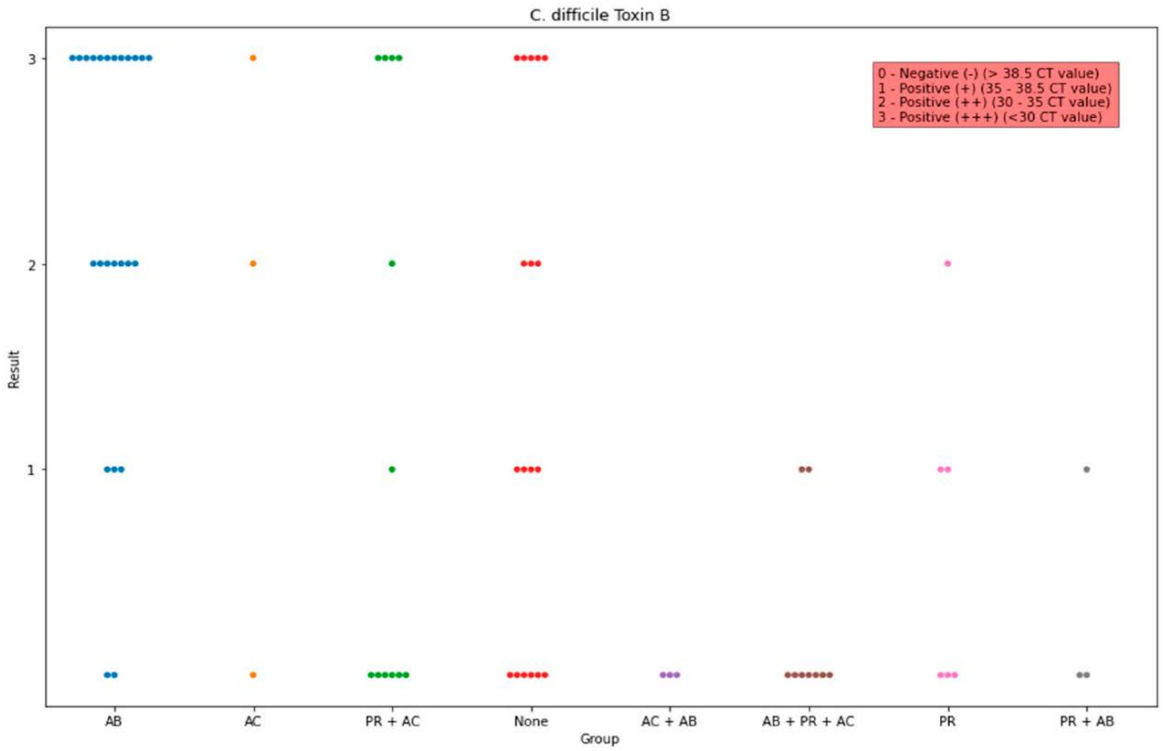

Figure 5. Observed data points of the scores of the PCR detected toxin B of *C. difficile* in all groups. The x-axis indicates the administration group, and the y-axis indicates the amount of the detected genes.

Concerning the score of piglet diarrhoea, it was found significantly higher in group AB in comparison to those in groups AB + PR + AC ($p = 0.001$), AC + AB ($p = 0.015$), None ($p = 0.04$), and PR ($p = 0.01$). The highest median was in group AB, while the lowest one was in groups AB + PR + AC, AC + AB, and PR (**Figure 6**).

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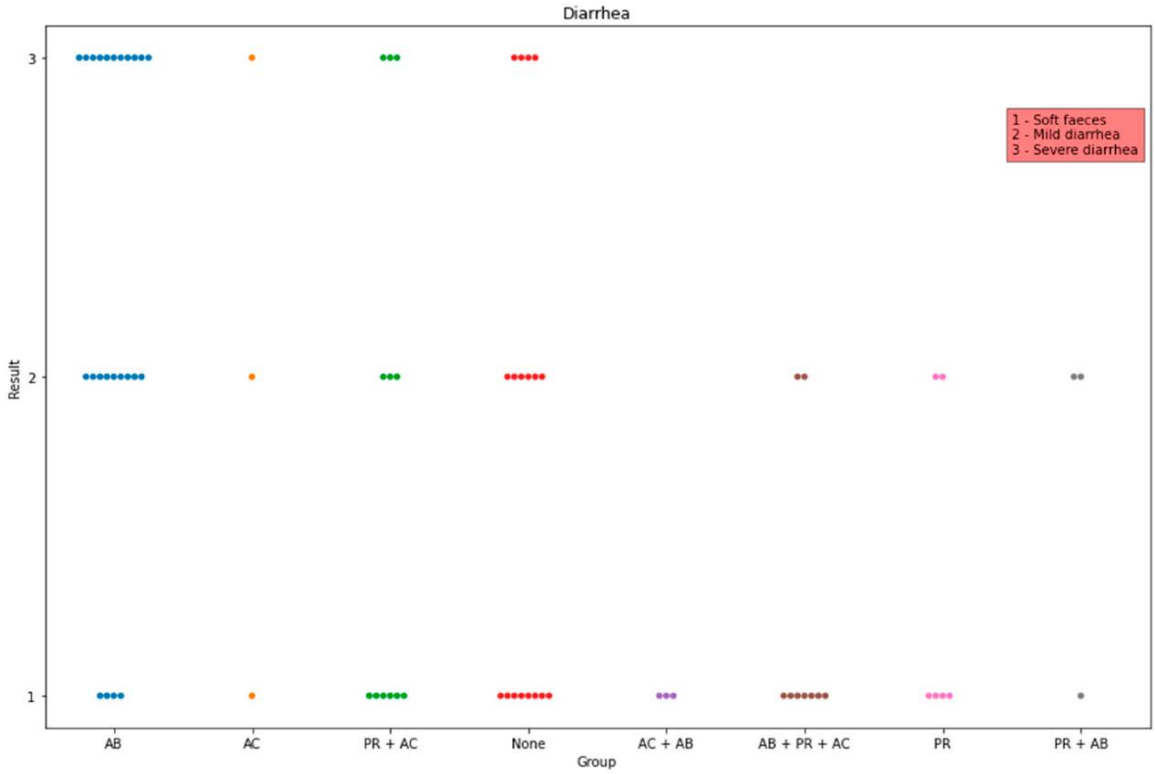

Figure 6. Observed data points of diarrhoea scores in all groups. The x-axis indicates the administration group, and the y-axis indicates the amount of the detected scores.

4. Discussion

Diarrhoea in neonatal piglets up to 4 days of age is a common issue in many sow herds worldwide, and its diagnosis remains an ongoing challenge for swine practitioners. Among the causal agents of neonatal diarrhoea, *E. coli* is present in swine herds around the world (Chan et al., 2013; Kongsted et al., 2014). The results of our study confirmed that *E. coli* is an enteric pathogen of concern for Greek swine farms, even if vaccinations against *E. coli* are given to sows. However, in contrast to previous studies, we noticed a higher prevalence of F4 and F6, as well as LT toxins, in comparison to that of F5 (Wang et al., 2020). Based on our results, 69.23% (18/26) of those in the farms were F4-positive, 30.77% (8/26) were F5-positive, 61.54% (16/26) were F6-positive, 42.31% (11/26) were F4- and enterotoxin LT-positive, 19.23% (5/26) were F5- and LT-positive, 42.31% (11/26) were F6- and LT-positive, and in 57.69% (15/26) of those in the farms, *E. coli* enterotoxin LT was found. However, our study did not investigate heat-stable (Sta and STb) *E. coli* enterotoxins due to the absence of this option in the ELUTE cards used according to the manufacturer's instructions (Enterocheck®, Hipra, Madrid, Spain) (Quilitis et al., 2013; Kitchodok et al., 2018; Kitchodok et al., 2019).

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In recent years, the focus of vaccination strategies against neonatal and post-weaning diarrhoea has been on anti-adhesin strategies, as this is the initial step of ETEC pathogenesis. However, while these vaccines have provided some protection against ETEC infections, there is still no universally effective ETEC vaccine that is commercially available (Dubreuil, 2021). A comprehensive approach that includes an appropriate vaccination program for the sow herd, the adequate intake of piglet colostrum and milk, and effective preventive strategies could help reduce the incidence of infectious diarrhoea in piglets. Several risk factors associated with clinical forms of neonatal diarrhoea have been reported to enhance protection against enteric pathogens, including passive immunity transferred by colostrum and milk (Mesonero-Escuredo et al., 2018; Le Dividich and Noblet, 1981), environmental conditions (e.g., temperature and humidity) (Fairbrother et al., 2012; Pereira et al., 2016), herd health management (Pedersen et al., 2013), the inadequate intake of colostrum, parity of the sows, and infection pressure by specific pathogens among the herd (Pedersen et al., 2013; Martineau et al., 1995; Muirhead and Alexander, 1997).

Various studies suggest that further investigations of *C. difficile* as an etiological agent in neonatal diarrhoea are needed (Chan et al., 2013; Haesebrouck et al., 2004; Yaeger et al., 2007). Previous studies reported that *C. difficile* could be an etiological agent of enteritis in neonatal piglets (Cruz et al., 2013; Steele et al., 2010; Arruda et al., 2013). While it is not considered to be a primary diarrheic pathogen in pigs based on epidemiological studies (McElroy et al., 2016; Silva et al., 2011; Jonach et al., 2014; Alvarez-Perez et al., 2009), other studies indicate that *C. difficile* is an emerging pathogen in neonatal diarrhoea (Chan et al., 2013; Larsson et al., 2015; Yaeger et al., 2002). These findings highlight the need for farmers to use recently developed commercial vaccines that provide immunization against *C. difficile* to sows to prevent neonatal diarrhoea via passive immunity. A new commercial vaccine for the immunization of sows with *C. difficile* (toxins A and B) and *C. perfringens* type A was recently registered in Europe, resulting in a significant reduction in diarrhoea and productive losses caused by *C. difficile* and *C. perfringens* type A (Farzan et al., 2013).

While a previous study reported that pathogenic *E. coli* was only found in combination with other pathogens in Spanish swine farms (Mesonero-Escuredo et al., 2018), our study found that *E. coli* is present in combination with *C. difficile*, but no detection of *C. perfringens* type C was noticed among the 26 pig farms studied. Even if *C. difficile* causes disease in piglets worldwide, it is considered to be much less important than

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other enteric pathogens are from a global perspective (Gibert et al., 2021). For example, a low prevalence of *C. perfringens* type C (1,4%) in herds was reported in Poland (Dors et al., 2016). Our results regarding the absence of *C. perfringens* type C in faecal samples from newborn piglets with diarrhoea are consistent with those in previous studies, which included a larger number of herds and samples (Kongsted et al., 2018). These findings may be explained by the sufficient protection of routine vaccinations for sows against *C. perfringens* type C (Chan et al., 2013; Kongsted et al., 2018). However, in contrast to our results, these studies did not find a relationship between *C. difficile* and diarrhoeal status.

Our study found that administering antibiotics alone to sows did not reduce the detection of ETEC antigens. However, combining antibiotics with probiotics or acidifiers or both resulted in better outcomes. This is supported by several studies that have reported the beneficial effects of probiotics as a feed additive in the health and performance of piglets and sows (Estienne et al., 2005; Szabo et al., 2009; Liao and Nyachoti, 2017). The supplementation of probiotics in the feed of gestating and lactating sows has been shown to improve their health status and reproductive performance, as well as increase the production of immunoglobulins in colostrum and milk, resulting in a decrease in the incidence of neonatal diarrhoea (Satora et al., 2020; Betancur et al., 2021; Satora et al., 2021; Laskowska et al., 2019; Tsukahara et al., 2018). Additionally, the administration of acidifiers in pig diets can enhance their growth performance and modulate the intestinal microbiota, reducing gastric pH and delaying the multiplication of enterotoxigenic *E. coli* (Partanen and Morz, 1999; Thompson and Lawrence, 1981). The supplementation of acidifiers in sow feed could influence the mother's microbiota and affects those of piglets (Tanaka et al., 2010). Our results could support the findings of previous trials relating to the administration of acidifiers in sow gestation feed, which reported beneficial effects on the sows' performance during lactation (Ferronato and Prandini, 2020; Sampath et al., 2022) and their gut microbiome, reducing the *E. coli* counts during farrowing and weaning (Devi et al., 2016).

Feed additives, including probiotics and acidifiers, have been explored as antibiotic alternatives, but their effectiveness varies (Turner et al., 2001). Our study found that administering antibiotics alone did not reduce the detection of pathogen toxins (such as enterotoxin LT and toxin A of *C. difficile*) but combining antibiotics with probiotics or acidifiers decreased the toxin levels. Feeding sows probiotics or acidifiers also resulted in lower neonatal piglet diarrhoea scores compared to those who received nothing or only injectable antibiotics. In contrast to our results, Greeff et al. (2020) reported that oral

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amoxicillin administration during the last week of gestation to sows can modulate the gut development of their piglets for a period of at least 5 weeks after the last antibiotic administration.

It is worth noting that in the case of *C. difficile*, the highest median toxin detection was found in the group that received antibiotics alone, while a lower detection rate was observed in groups that received alternatives to antibiotics, such as probiotics and acidifiers, either alone or in combination with antibiotics. In humans, *C. difficile* infection (CDI) is becoming increasingly difficult to treat due to severe antibiotic resistance, and for this reason, there are very limited treatment options (Lessa et al., 2012; Kociolek and Gerdink, 2016). Currently, only three antibiotics (metronidazole, vancomycin, and fidaxomicin) are available for CDI treatment (Nale et al., 2018). These antibiotics are forbidden for use in swine. Fry et al. (2012) reported that significant proportions of *C. difficile* in swine are toxigenic and are often associated with antimicrobial resistance genes, although they are not resistant to drugs that are used to treat CDI. A connection between the presence of *C. difficile* and diarrhoea or antibiotic treatments in piglets has not been proven thus far (Alvarez-Perez et al., 2009; Avbersek et al., 2009; Susick et al., 2012). Moreover, a previous study by Schneeberg et al. (2013) reported that emerging human-pathogenic *C. difficile* PCR ribotypes were the predominant PCR ribotypes in piglets in Germany. However, they did not find a link between the presence of *C. difficile* in piglets and antibiotic treatment or diarrhoea in piglets.

Our findings highlight the potential benefits of using alternatives to antibiotics, such as probiotics and acidifiers, in swine feed to improve the health and performance of both sows and their piglets. In addition, our results suggest that further research is needed to investigate the potential use of these alternatives in the treatment of CDI in swine and humans, either alone or in combination with antibiotics.

5. Conclusions

In our research study, despite routine vaccinations of sows against *E. coli*, a high prevalence of positive samples was found, revealing that *E. coli* remains a main pathogen of great importance in neonatal pig diarrhoea. Additionally, our study reports, for the first time in Greece, the involvement of *C. difficile* in most clinical cases of neonatal pig diarrhoea in commercial pig farms. Therefore, the need for herd immunization against *C. difficile* may be necessary. However, these findings reveal the necessity of extended future studies on the epidemiology of *C. difficile* in pigs and the need for research on this pathogen in the future, as well as the need for herd immunization against *C. difficile*. Maternal

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interventions with antibiotics in combination with probiotics or acidifiers appear to have beneficial effects in terms of neonatal pig diarrhoea, reducing the detection of ETEC antigens and the enterotoxin LT of *E. coli*.

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Supplementary file

Farm no	Group	Commercial products	Composition / Dose
Farm 1	Group None	-	
Farm 2	Group AB	Citramox® LA (Laboratorios Karizoo S.A.)	Amoxicillin 150.00 mg/ml (15 mg of amoxicillin/kg BW)
Farm 3	Group AB	Vetrimoxin® LA (Ceva Animal Health)	Amoxicillin 150.00 mg/ml (15 mg of amoxicillin/kg BW)
Farm 4	Group None	-	
Farm 5	Group AC	Acid LAC® XT dry (Kemin)	Lactic acid, fumaric acid, citric acid and formic acid (4 Kg/t feed)
Farm 6	Group None	-	
Farm 7	Group AB	Betamox® LA (Norbrook Laboratories)	Amoxicillin 150.00 mg/ml (15 mg of amoxicillin/kg BW)
Farm 8	Group AC +AB	Vetrimoxin® LA (Ceva Animal Health) + Biotronic® (BIOMIN GmbH, DSM)	Organic acid blend (formic, propionic and acetic acid), cinnamaldehyde and a permeabilising substance (3 kg/t feed) + Amoxicillin 150.00 mg/ml (15 mg of amoxicillin/kg BW)
Farm 9	Group PR + AC	Bioplus YC (Biochem) + Arma (O3 Benelux GmbH)	*Bacillus licheniformis* (DSM 5749) and *Bacillus subtilis* (DSM 5750) in a 1:1 ratio (1.6 x 10^9 CFU + 1.6 x 109 CFU per g product, 400 g/t feed) + Formic acid, propionic acid, phosphoric acid, lactic acid, and acetic acid (3 Kg/t feed)
Farm 10	Group PR + AC	Bioplus YC (Biochem) + Arma (O3 Benelux GmbH)	*Bacillus licheniformis* (DSM 5749) and *Bacillus subtilis* (DSM 5750) in a 1:1 ratio (1.6 x 10^9 CFU + 1.6 x 109 CFU per g product, 400 g/t feed) + Formic acid, propionic acid, phosphoric acid, lactic acid, and acetic acid (3 Kg/t feed)
Farm 11	Group None	-	
Farm 12	Group AB + PR + AC	Vetrimoxin® LA (Ceva Animal Health) + Bioplus YC (Biochem) + Acid LAC® XT dry (Kemin)	Amoxicillin 150.00 mg/ml (15 mg of amoxicillin per kg BW) + *Bacillus licheniformis* (DSM 5749) and *Bacillus subtilis* (DSM 5750) in a 1:1 ratio (1.6 x 10^9 CFU + 1.6 x 109 CFU per g product, 400 g/t feed) + Formic acid, propionic acid, phosphoric acid, lactic acid, and acetic acid (3 Kg/t feed)

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Farm 13	Group AB	Vetrimoxin ® LA (Ceva Animal Health)	Amoxicillin 150.00 mg/ml (15 mg of amoxicillin/kg BW)
Farm 14	Group PR + AB	Citramox® LA (Laboratorios Karizoo S.A.) + Bioplus YC (Biochem)	*Bacillus licheniformis* (DSM 5749) and *Bacillus subtilis* (DSM 5750) in a 1:1 ratio (1.6 x 10^9 CFU + 1.6 x 109 CFU per g product, 400 g/t feed) + Amoxicillin 150.00 mg/ml (15 mg of amoxicillin/kg BW)
Farm 15	Group AB	Citramox® LA (Laboratorios Karizoo S.A.)	Amoxicillin 150.00 mg/ml (15 mg of amoxicillin/kg BW)
Farm 16	Group PR + AC	Bioplus YC (Biochem) + Acid LAC® XT dry (Kemin)	*Bacillus licheniformis* (DSM 5749) and *Bacillus subtilis* (DSM 5750) in a 1:1 ratio (1.6 x 10^9 CFU + 1.6 x 109 CFU per g product, 400 g/t feed) + Lactic acid, fumaric acid, citric acid and formic acid (4 Kg/t feed)
Farm 17	Group AB + PR + AC	Betamox® LA (Norbrook Laboratories) + BioPlus® YC (Biochem) + Arma (O3 Benelux GmbH)	Amoxicillin 150.00 mg/ml (15 mg of amoxicillin/kg BW) + *Bacillus licheniformis* (DSM 5749) and *Bacillus subtilis* (DSM 5750) in a 1:1 ratio (1.6 x 10^9 CFU + 1.6 x 109 CFU per g product, 400 g/t feed) + Formic acid, propionic acid, phosphoric acid, lactic acid, and acetic acid (3 Kg/t feed)
Farm 18	Group AB	Betamox® LA (Norbrook Laboratories)	Amoxicillin 150.00 mg/ml (15 mg of amoxicillin/kg BW)
Farm 19	Group AB	Betamox® LA (Norbrook Laboratories)	Amoxicillin 150.00 mg/ml (15 mg of amoxicillin/kg BW)
Farm 20	Group None	-	
Farm 21	Group PR	BioPlus® YC (Biochem)	*Bacillus licheniformis* (DSM 5749) and *Bacillus subtilis* (DSM 5750) in a 1:1 ratio (1.6 x 10^9 CFU + 1.6 x 109 CFU per g product, 400 g/t feed)
Farm 22	Group PR + AC	BioPlus® YC (Biochem) + Acid LAC® XT dry (Kemin)	*Bacillus licheniformis* (DSM 5749) and *Bacillus subtilis* (DSM 5750) in a 1:1 ratio (1.6 x 10^9 CFU + 1.6 x 109 CFU per g product, 400 g/t feed) + Lactic acid, fumaric acid, citric acid and formic acid (4 Kg/t feed)
Farm 23	Group AB	Betamox® LA (Norbrook Laboratories)	Amoxicillin 150.00 mg/ml (15 mg of amoxicillin/kg BW)
Farm 24	Group AB + PR + AC	Citramox® LA (Laboratorios Karizoo S.A.) +	Amoxicillin 150.00 mg/ml (15 mg of amoxicillin/kg BW) +

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		Bioplus YC (Biochem) + Acid LAC® XT dry (Kemin)	*Bacillus licheniformis* (DSM 5749) and *Bacillus subtilis* (DSM 5750) in a 1:1 ratio (1.6 x 10^9 CFU + 1.6 x 109 CFU per g product, 400 g/t feed) + Lactic acid, fumaric acid, citric acid and formic acid (4 Kg/t feed)
Farm 25	Group None	-	
Farm 26	Group PR	Bioplus YC (Biochem)	*Bacillus licheniformis* (DSM 5749) and *Bacillus subtilis* (DSM 5750) in a 1:1 ratio (1.6 x 10^9 CFU + 1.6 x 109 CFU per g product, 400 g/t feed)

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References

Alexopoulos C, Georgoulakis IE, Tzivara A, Kritas SK, Siochu A, Kyriakis SC (2000) Field evaluation of the efficacy of a probiotic containing Bacillus licheniformis and Bacillus subtilis spores, on the health status and performance of sows and their litters. J Anim Physiol Anim Nutr 88:381–392.

Alvarez-Perez S, Alba P, Blanco JL, Garcia ME (2009) Detection of toxigenic Clostridium difficile in pig feces by PCR. Vet Med 54:360-366.

Alvarez-Perez S, Blanco JL, Bouza E, Alba P, Gibert X, Maldonado J, Garcia ME (2009) Prevalence of Clostridium difficile in diarrhoeic and non-diarrhoeic piglets. Vet Microbiol 137:302-305.

Arruda PHE, Madson DM, Ramirez A, Rowe E, Lizer JT, Songer JG (2013) Effect of age, dose and antibiotic therapy on the development of Clostridium difficile infection in neonatal piglets. Anaerobe 22:104-110.

Athanasakopoulou Z, Reinicke M, Diezel C, Sofia M, Chatzopoulos DC, Braun SD, Reissig A, Spyrou V, Monecke S, Ehricht R, Tsilipounidaki K, Giannakopoulos A, Petinaki E, Billinis C (2021) Antimicrobial Resistance Genes in ESBL-Producing Escherichia Coli Isolates from Animals in Greece. Antibiotics 10:389

Avbersek J, Janezic S, Pate M, Rupnik M, Zidaric V, Logar K, Vengust M, Zemljic M, Pirs T, Ocepek M (2009) Diversity of Clostridium difficile in pigs and other animals in Slovenia. Anaerobe 15:252-255.

Bai J, Paddock ZD, Shi X, Li S, An B, Nagaraja TG (2012) Applicability of a multiplex PCR to detect the seven major Shiga toxin–producing Escherichia coli based on genes that code for serogroup-specific O-antigens and major virulence factors in cattle feces. Foodborne Pathog Dis 9:541–548

Baker CA, Rubinelli PM, Park SH, Carbonero F, Ricke SC (2016) Shiga toxin-producing Escherichia coli in food: Incidence, ecology, and detection strategies. Food Control 59:407–419

Baker CA, Rubinelli PM, Park SH, Ricke SC (2016) Immuno-based detection of Shiga toxin-producing pathogenic Escherichia coli in food—A review on current approaches and potential strategies for optimization. Crit Rev Microbiol 42:656–675

Betancur C, Martínez Y, Tellez-Isaias G, Castillo R, Ding X (2021) Effect of oral administration with Lactobacillus plantarum CAM6 strain on sows during gestation-

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

lactation and the derived impact on their progeny performance. Med Inflamm 2021:6615960.

Böhmer BM, Kramer W, Roth-Maier DA (2006) Dietary probiotic supplementation and resulting effects on performance, health status, and microbial characteristics of primiparous sows. J Anim Physiol Anim Nutr 90:309–315.

Britton RA, Young VB (2012) Interaction between the intestinal microbiota and host in Clostridium difficile colonization resistance. Trends Microbiol 20:313–319

Chan G, Farzan A, DeLay J, McEwen B, Prescott JF, Friendship RM (2013) A retrospective study on the etiological diagnoses of diarrhea in neonatal piglets in Ontario, Canada, between 2001 and 2010. Can J Vet Res 77:254–260

Cruz EC Jr, Salvarani FM, Silva ROS, Silva MX, Lobato FCF, Guedes RMC (2013) A surveillance of enteropathogens in piglets from birth to seven days of age in Brazil. Pesqui Vet Bras 33:963-969.

Debast SB, van Leengoed LAMG, Goorhuis A, Harmanus C, Kuijper EJ, Bergwerff AA (2009) Clostridium difficile PCR ribotype 078 toxinotype V found in diarrhoeal pigs identical to isolates from affected humans. Environ Microbiol 11:505–511

DebRoy C, Roberts E, Valadez AM, Dudley EG, Cutter CN (2011) Detection of Shiga toxin–producing Escherichia coli O26, O45, O103, O111, O113, O121, O145, and O157 serogroups by multiplex polymerase chain reaction of the wzx gene of the O-antigen gene cluster. Foodborne Pathog Dis 8:651–652

Devi SM, Lee KY, Kim IH (2016) Analysis of the effect of dietary protected organic acid blend on lactating sows and their piglets. Rev Bras Zootech 45:39-47.

Dors A, Czyżewska-Dors E, Wasyl D, Pomorska-Mól M (2016) Prevalence and factors associated with the occurrence of bacterial enteropathogens in suckling piglets in farrow-to-finish herds. Vet Rec 179:598.

Dubreuil JD (2008) Escherichia coli STb toxin and colibacillosis: Knowing is half the battle. FEMS Microbiol Lett 78:137–145

Dubreuil JD (2021) Pig vaccination strategies based on enterotoxigenic Escherichia coli toxins. Braz J Microbiol 52:2499-2509.

Dubreuil JD, Isaacson RE, Schifferli DM (2016) Animal enterotoxigenic Escherichia coli. EcoSal Plus 7

Estienne MJ, Hartsock TG, Harper AF (2005) Effects of antibiotics and probiotics on suckling pig and weaned pig performance. Int J Appl Res Vet Med 4:303-308.

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Fairbrother JM, Gyles CL (2012) Colibacillosis. In Diseases of Swine, 10th ed.; Zimmerman J, Karriker LA, Ramirez A, Schwartz KJ, Stevenson GW, Eds.; WileyBlackwell: Chichester, UK, pp. 723–749

Farzan A, Kircanki J, DeLay J, Soltes G, Songer JG, Friendship R, Prescott JF (2013) An investigation into the association between cpb2-encoding C. perfringens type A and diarrhea in neonatal piglets. Can J Vet Res 77:45-53.

Ferronato G, Prandini A (2020) Dietary Supplementation of Inorganic, Organic, and Fatty Acids in Pig: A Review. Animals 10:1740.

Fry PR, Thakur S, Abley M, Gebreyes WA (2012) Antimicrobial resistance, toxinotype, and genotypic profiling of Clostridium difficile isolates of swine origin. J Clin Microbiol 50:2366-2372.

Gibert X, Puig A, Sabaté D, Vidal-Mas J, March R (2021) Effects of a new vaccine against Clostridioides Difficile and Clostridium Perfigens Type A on the incidence of diarrhoea and antibiotic treatments under field conditions. In: Proceedings of the European Symposium of Porcine Health Management (ESPHM 2021), Bern, Switzerland, 14-16 April 2021.

Goldstein MR, Kruth SA, Bersenas AM, Holowaychuk MK, Weese JS (2012) Detection and characterization of Clostridium perfringens in the feces of healthy and diarrheic dogs. Can J Vet Res 76:161-165.

Goorhuis A, Bakker D, Corver J, Debast SB, Harmanus C, Notermans DW, Bergwerff AA, Dekker FW, Kuijper EJ (2008) Emergence of Clostridium difficile infection due to a new hypervirulent strain, polymerase chain reaction ribotype 078. Clin Infect Dis 47:1162–1170

Goorhuis A, Debast SB, van Leengoed LAMG, Harmanus C, Notermans DW, Bergwerff AA, Kuijper EJ (2008) Clostridium difficile PCR ribotype 078: An emerging strain in humans and in pigs? J Clin Microbiol 46:1157

Gould LH, Limbago B (2010) Clostridium difficile in food and domestic animals: A new foodborne pathogen? Clin Infect Dis 51:577–582

Greeff A, Schokker D, Roubos-van den Hil P, Ramaekers P, Vastenhouw SA, Harders F, Bossers A, Smits MA, Rebel JMJ (2020) The effect of maternal antibiotic use in sows on intestinal development in offspring. J Anim Sci 98:skaa181.

Haesebrouck F, Pasmans F, Chiers K, Maes D, Ducatelle R, Decostere A (2004) Efficacy of vaccines against bacterial diseases in swine: What can we expect? Vet Microbiol 100:255-268.

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Hayakawa T, Masuda T, Kurosawa D, Tsukahara T (2016) Dietary administration of probiotics to sows and/or their neonates improves the reproductive performance, incidence of post-weaning diarrhea, and histopathological parameters in the intestine of weaned piglets. Anim Sci J 87:1501-1510.

Holland RE (1990) Some infectious causes of diarrhea in young farm animals. Clin Microbiol Rev 3:345–375

Jacela JY, DeRouchey JM, Tokach MD, Goodband RD, Nelssen JL, Renter DG, Dritz SS (2009) Feed additives for swine: Fact sheets—Acidifiers and antibiotics. J Swine Health Prod 17:270–275.

Ji P, Li X, Liu Y (2020) Dietary Intervention to Reduce E. coli Infectious Diarrhea in Young Pigs. In: E. Coli Infections—Importance of Early Diagnosis and Efficient Treatment (Internet); Rodrigo L, Ed.; IntechOpen: London, UK. Available online: https://www.intechopen.com/chapters/71010 (accessed on 24 July 2022).

Johansen M, Alban L, Kjærsgård HD, Bækbo P (2004) Factors associated with suckling piglet average daily gain. Prev Vet Med 63:91–102

Jonach B, Boye M, Stockmarr A, Jensen T (2014) Fluorescence in situ hybridization investigation of potentially pathogenic bacteria involved in neonatal porcine diarrhea. BMC Vet Res 10:68.

Kantas D, Papatsiros VG, Tassis PD, Athanasiou LV, Tzika ED (2015) Effect of a natural feed additive (Macleaya cordata), containing sanguinarine, on the performance and health status of weaning pigs. Anim Sci J 86:92–98.

Kantas D, Papatsiros VG, Tassis PD, Giavasis I, Bouki P, Tzika ED (2015) A feed additive containing Bacillus toyonensis (Toyocerin®) protects against enteric pathogens in postweaning piglets. J Appl Microbiol 118:727–738.

Keel MK, Songer JG (2006) The comparative pathology of Clostridium difficile-associated disease. Vet Pathol 43:225–240

Keessen EC, van den Berkt AJ, Haasjes NH, Hermanus C, Kuijper EJ, Lipman LJA (2011) The relation between farm specific factors and prevalence of Clostridium difficile in slaughter pigs. Vet Microbiol 154:130–134

Kim YY, Kil D, Oh HK, Han I (2005) Acidifier as an Alternative Material to Antibiotics in Animal Feed. Asian Australas J Anim Sci 18:1048–1060.

Kitchodok R, Ananratanakul C, Kongthong T (2018) Efficacy and safety of Suiseng in prevention of neonatal diarrhea according to enterotoxigenic E. coli under a mixed infection with PRRSV involved from the field. Thai J Vet Med 48:169-170.

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Kitchodok R, Triyarach S, Sutheerakul K, Serod C, Chompupun D (2019) Prevalence of Genotypic Fimbrial Antigens of Enterotoxigenic E. Coli Isolated in Thai Pig Herds. In Proceedings of the 20th Khon Kaen Veterinary Annual International Conference, Khon Kaen, Thailand, 21-22 March.

Kociolek LK, Gerding DN (2016) Breakthroughs in the treatment and prevention of Clostridium difficile infection. Nat Rev Gastroenterol Hepatol 13:150-160.

Kongsted H, Pedersen K, Hjulsager CK, Larsen LE, Pedersen KS, Jorsal SE, Bækbo P (2018) Diarrhoea in neonatal piglets: A case control study on microbiological findings. Porc Health Manag 4:17

Kongsted H, Stege H, Toft N, Nielsen JP (2014) The effect of new neonatal porcine Diarrhoea syndrome (NNPDS) on average daily gain and mortality in 4 Danish pig herds. BMC Vet Res 10:90

Kritas SK, Marubashi T, Filioussis G, Petridou E, Christodoulopoulos G, Burriel AR, Tzivara A, Theodoridis A, Pískoriková M (2015) Reproductive performance of sows was improved by administration of a sporing bacillary probiotic (Bacillus subtilis C-3102). J Anim Sci 93:405-413.

Kruskal WH, Wallis WA (1952) Use of ranks in one-criterion variance analysis. J Am Stat Assoc 47:583-621, Erratum in J Am Stat Assoc 48:907-911.

Laird TJ, Abraham S, Jordan D, Pluske JR, Hampson DJ, Trott DJ, O'Dea M (2021) Porcine Enterotoxigenic Escherichia Coli: Antimicrobial Resistance and Development of Microbial-Based Alternative Control Strategies. Vet Microbiol 258:109117

Larsson J, Aspan A, Lindberg R, Grandon R, Baverud V, Fall N, Jacobson M (2015) Pathological and bacteriological characterization of neonatal porcine diarrhoea of uncertain aetiology. J Med Microbiol 64:916-926.

Laskowska E, Jarosz Ł, Grądzki Z (2019) Effect of multi-microbial probiotic formulation bokashi on pro- and anti-inflammatory cytokines profile in the serum, colostrum and milk of sows, and in a culture of polymorphonuclear cells isolated from colostrum. Probiotics Antimicrob Proteins 11:220-232.

Le Dividich J, Noblet J (1981) Colostrum intake and thermoregulation in the neonatal pig in relation to environmental temperature. Biol Neonate 40:167-174.

Lessa FC, Gould CV, McDonald LC (2012) Current status of Clostridium difficile infection epidemiology. Clin Infect Dis 55:S65-S70.

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Levene H (1960) Contributions to Probability and Statistics: Essays in Honor of Harold Hotelling; Olkin I, Ghurye SG, Hoeffding W, Madow WG, Mann HB, Eds.; Stanford University Press: Redwood City, CA, USA. pp. 278-292.

Li JY (2017) Current Status and Prospects for in-Feed Antibiotics in the Different Stages of Pork Production—A Review. Asian Austral J Anim 30:1667–1673

Liao SF, Nyachoti M (2017) Using probiotics to improve swine gut health and nutrient utilization. Anim Nutr 3:331-343.

Lim SC, Knight DR, Riley TV (2020) Clostridium difficile and One Health. Clin Microbiol Infect 26:857–863

Linhares DC, Rovira A, Torremorell M (2012) Evaluation of Flinders Technology Associates cards for collection and transport of samples for detection of Porcine reproductive and respiratory syndrome virus by reverse transcription polymerase chain reaction. J Vet Diagn Investig 24:328–332

Liu Y, Espinosa CD, Abelilla JJ, Casas GA, Lagos LV, Lee SA, Kwon WB, Mathai JK, Navarro DMDL, Jaworski NW, Stein HH (2018) Non-antibiotic feed additives in diets for pigs: A review. Anim Nutr 4:113–125.

Luppi A (2017) Swine enteric colibacillosis: Diagnosis, therapy and antimicrobial resistance. Porc Health Manag 3:16

Luppi A, Gibellini M, Gin T, Vangroenweghe F, Vandenbroucke V, Bauerfeind R, Bonilauri P, Labarque G, Hidalgo A (2016) Prevalence of virulence factors in enterotoxigenic Escherichia coli isolated from pigs with post-weaning diarrhea in Europe. Porc Health Manag 2:20

Martineau GP, Vaillancourt JP, Broes A (1995) Principal neonatal diseases. In: The Neonatal Pig Development and Survival; Varley MA, Ed.; CAB International: Wallingford, UK. pp. 239-264.

McElroy MC, Hill M, Moloney G, MacAogain M, McGettrick S (2016) Typhlocolitis associated with Clostridium difficile ribotypes 078 and 110 in neonatal piglets from a commercial Irish pig herd. Ir Vet J 69:10.

Menegat MB, Gourley KM, Braun MB, DeRouchey JM, Woodworth JC, Bryte J, Tokach MD, Dritz SS, Goodband RD (2018) Effects of a Bacillus-Based Probiotic on Sow Performance and on Progeny Growth Performance, Fecal Consistency, and Fecal Microflora. Kans Agric Exp Stn Res Rep 4(9).

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Mesonero-Escuredo S, Strutzberg-Minder K, Casanovas C, Segalés J (2018) Viral and bacterial investigations on the aetiology of recurrent pig neonatal diarrhoea cases in Spain. Porc Health Manag 4:5.

Moon HW, Schineider RA, Mosely SL (1986) Comparative prevalence of four enterotoxin genes among Escherichia coli isolates from swine. Am J Vet Res 47:210–212

Morin M, Turgeon D, Jolette J, Robinson Y, Phaneuf JB, Sauvageau R, Beauregard M, Teuscher E, Higgins R, Lariviere S (1983) Neonatal diarrhea of pigs in Quebec: Infectious causes of significant outbreaks. Can J Comp Med 47:11–17

Muirhead MR, Alexander TL (1997) Managing Pig Health and the Treatment of Disease: A Reference for the Farm; 5M Enterprises: Sheffield, UK.

Muthukrishnan M, Singanallur NB, Ralla K, Villuppanoor SA (2008) Evaluation of FTA® cards as a laboratory and field sampling device for the detection of foot-and-mouth disease virus and serotyping by RT-PCR and real-time RT-PCR. J Virol Methods 151:311–316

Mylonakis E, Ryan ET, Calderwood SB (2001) Clostridium difficile–associated diarrhea: A review. Arch Intern Med 161:525–533

Nagy B, Fekete PZ (1999) Enterotoxigenic E. coli (ETEC) in farm animals. Vet Res 30:259–284

Nale JY, Redgwell TA, Millard A, Clokie MRJ (2018) Efficacy of an Optimised Bacteriophage Cocktail to Clear Clostridium difficile in a Batch Fermentation Model. Antibiotics 7:13.

Pamer EG (2016) Resurrecting the Intestinal Microbiota to Combat Antibiotic-Resistant Pathogens. Science 352:535–538

Papatsiros V, Christodouloupoulos G, Filippopoulos LC (2012) The use of organic acids in monogastric animals (swine and rabbits). JCAB 6:154-159.

Papatsiros VG, Billinis C (2012) The prophylactic use of acidifiers as antibacterial agents in swine. In: Antimicrobial Agents; Bobbarala V, Ed.; InTech: Rijeka, Croatia, pp. 295–310.

Papatsiros VG, Katsoulos PD, Koutoulis KC, Karatzia M, Dedousi A, Christodoulopoulos G (2013) Alternatives to antibiotics for farm animals. CAB Rev Perspect Agric Vet Sci Nutr Nat Resour 8:1–15.

Papatsiros VG, Tassis PD, Tzika ED, Papaioannou DS, Petridou E, Alexopoulos C, Kyriakis SC (2011) Effect of benzoic acid and combination of benzoic acid with a probiotic

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

containing Bacillus cereus var. Toyoi in weaned pig nutrition. Pol J Vet Sci 14:117-125.

Partanen KH, Morz Z (1999) Organic acids for performance enhancement in pig diets. Nutr Res Rev 12:117-145.

Pearlin BV, Muthuvel S, Govidasamy P, Villavan M, Alagawany M, Farag MR, Dhama K, Gopi M (2020) Role of acidifiers in livestock nutrition and health: A review. J Anim Physiol Anim Nutr 104:558-569.

Pedersen KS, Holyoake P, Stege H, Nielsen JP (2011) Observations of variable inter-observer agreement for clinical evaluation of fecal consistency in pigs. Prev Vet Med 98:284-287.

Pedersen LJ, Malmkvist J, Kammersgaard T, Jorgensen E (2013) Avoiding hypothermia in neonatal pigs: Effect of duration of floor heating at different room temperatures. J Anim Sci 91:425-432.

Pereira DA, Vidotto MC, Nascimento KA, Santos ACR, Mechler ML, Oliveira LG (2016) Virulence factors of Escherichia coli in relation to the importance of vaccination in pigs. Cienc Rural 46:8.

Petit L, Gibert M, Popoff MR (1999) Clostridium perfringens: Toxinotype and genotype. Trends Microbiol 7:104–110

Quilitis M, Lumabiang J, Camprodon A, Torres M, Magcalas J, Bautista C, Nuestro F, Vergel de Dios R, Santos R, Manuel R (2013) Control of pre-weaning mortality associated with Escherichia coli using Suiseng® in two Philippine commercial swine farms. In Proceedings of the 6th Asian Pig Veterinary Society Congress, Ho Chi Minh City, Vietnam, 23-25 September.

R Core Team. R: A language and Environment for Statistical Computing; R Foundation for Statistical Computing: Vienna, Austria, 2021; Available online: https://www.R-project.org/ (accessed on 18 February 2023).

Rajendram D, Ayenza R, Holder FM, Moran B, Long T, Shah HN (2006) Long-term storage and safe retrieval of DNA from microorganisms for molecular analysis using FTA matrix cards. J Microbiol Methods 67:582–592

Rood JI, Adams V, Lacey J, Lyras D, McClane BA, Melville SB, Moore RJ, Popoff MR, Sarker MR, Songer JG, Uzal FA, Van Immerseel F (2018) Expansion of the Clostridium perfringens toxin-based typing scheme. Anaerobe 53:5–10

Roth FX, Kirchgessner M (1998) Organic acids as feed additives for young pigs: Nutritional and gastrointestinal effects. J Anim Feed Sci 7:25–33.

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Ruiz VLA, Bersano JG, Carvalho AF, Catroxo MHB, Chiebao DP, Gregori F, Miyashiro S, Nassar AFC, Oliveira TMFS, Ogata RA, Scarcelli EP, Tonietti PO (2016) Case–control study of pathogens involved in piglet diarrhea. BMC Res Notes 9:22

Salvador JM, De Ungria MCA (2003) Isolation of DNA from saliva of betel quid chewers using treated cards. J Forensic Sci 48:794–797

Sampath V, Park JH, Pineda L, Han Y, Kim IH (2022) Impact of synergistic blend of organic acids on the performance of late gestating sows and their offspring. Ital J Anim Sci 21:1334-1342.

Satora M, Magdziarz M, Rząsa A, Rypuła K, Płoneczka-Janeczko K (2020) Insight into the intestinal microbiome of farrowing sows following the administration of garlic (Allium sativum) extract and probiotic bacteria cultures under farming conditions. BMC Vet Res 16:442.

Satora M, Rząsa A, Rypuła K, Płoneczka-Janeczko K (2021) Field evaluation of the influence of garlic extract and probiotic cultures on sows and growing pigs. Med Weter 77:21-29.

Sayan H, Assavacheep P, Angkanaporn K, Assavacheep A (2018) Effect of Lactobacillus salivarius on growth performance, diarrhea incidence, fecal bacterial population and intestinal morphology of suckling pigs challenged with F4+ enterotoxigenic Escherichia coli. Asian Australas J Anim Sci 31:1308–1314.

Schneeberg A, Neubauer H, Schmoock G, Baier S, Harlizius J, Nienhoff H, Brase K, Zimmermann S, Seyboldt C (2013) Clostridium difficile genotypes in piglet populations in Germany. J Clin Microbiol 51:3796-3803.

Shalaby AG, Bakry NR, Mohamed A, Khalil AA (2020) Evaluating Flinders Technology Associates card for transporting bacterial isolates and retrieval of bacterial DNA after various storage conditions. Vet World 13:2243–2251

Silva ROS, Salvarani FM, Cruz ECC Jr, Pires PS, Santos RLR, Antunes de Assis R, Guedes RMC, Lobato FCF (2011) Detection of enterotoxin a and cytotoxin B, and isolation of Clostridium difficile in piglets in Minas Gerais, Brazil. Cienc Rural 41:1430-1435.

Sjölund M, Zoric M, Wallgren P (2014) Financial impact of disease on pig production. Part III. Gastrointestinal disorders. In Proceedings of the 6th European Symposium of Porcine Health Management, Sorrento, Italy, 7–9 May 2014, p. 189.

Songer JG (1996) Clostridial enteric diseases of domestic animals. Clin Microbiol Rev 9:216–234

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Songer JG (2004) The emergence of Clostridium difficile as a pathogen of food animals. Anim Health Res Rev 5:321–326

Songer JG, Uzal FA (2005) Clostridial Enteric Infections in Pigs. J Vet Diagn 17:528–536

Steele J, Feng H, Parry N, Tzipori S (2010) Piglet models of acute or chronic Clostridium difficile illness. J Infect Dis 201:428-434.

Stringer OW, Bossé JT, Lacouture S, Gottschalk M, Fodor L, Angen Ø, Velazquez E, Penny P, Lei L, Langford PR, Li Y (2021) Rapid Detection and Typing of Actinobacillus pleuropneumoniae Serovars Directly From Clinical Samples: Combining FTA® Card Technology With Multiplex PCR. Front Vet Sci 8:728660

Susick EK, Putnam M, Bermudez DM, Thakur S (2012) Longitudinal study comparing the dynamics of Clostridium difficile in conventional and antimicrobial free pigs at farm and slaughter. Vet Microbiol 157:172-178.

Svendsen J, Bille N, Nielsen N, Larsen J, Riising H (1975) Preweaning mortality in pigs. Diseases of the gastrointestinal tract in pigs. Nordisk Veterinär 27:85–101

Svensmark B, Jorsal S, Nielsen K, Willeberg P (1988) Epidemiological studies of piglet diarrhoea in intensively managed Danish sow herds. I. Pre-weaning diarrhoea. Acta Vet Scand 30:43–53

Szabó I, Wieler LH, Tedin K, Scharek-Tedin L, Taras D, Hensel A, Appel B, Nöckler K (2009) Influence of a probiotic strain of Enterococcus faecium on Salmonella enterica serovar Typhimurium DT104 infection in a porcine animal infection model. Appl Environ Microbiol 96:219-233.

Tanaka T, Imai Y, Kumagae N, Sato S (2010) The effect of feeding lactic acid to Salmonella typhimurium experimentally infected swine. J Vet Med Sci 72:827-831.

Thompson JL, Lawrence TLJ (1981) Dietary manipulation of gastric pH in the profilaxis of enteric disease in weaned pigs. Some field observations. Vet Rec 109:120-122.

Toledo A, Gómez D, Cruz C, Carreón R, López J, Giono S, Castro AM (2012) Prevalence of virulence genes in Escherichia coli strains isolated from piglets in the suckling and weaning period in Mexico. J Med Virol 61:148–156

Tsekouras N, Athanasakopoulou Z, Diezel C, Kostoulas P, Braun SD, Sofia M, Monecke S, Ehricht R, Chatzopoulos DC, Gary D, Krähmer D, Spyrou V, Christodoulopoulos G, Billinis C, Papatsiros VG (2022) Cross-Sectional Survey of Antibiotic Resistance in Extended Spectrum β-Lactamase-Producing Enterobacteriaceae Isolated from Pigs in Greece. Animals 12:1560

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

Tsukahara T, Inatomi T, Otomaru K, Amatatsu M, Romero-Pérez GA, Inoue R (2018) Probiotic supplementation improves reproductive performance of unvaccinated farmed sows infected with porcine epidemic diarrhea virus. Anim Sci J 89:1144-1151.

Turner JL, Dritz S, Minton JE (2001) Alternatives to Conventional Antimicrobials in Swine Diets. PAS 17:4.

Uzal FA, Songer JG (2019) Clostridial diseases. In Diseases of Swine, 11th ed.; Zimmerman J, Karriker LA, Ramirez A, Schwartz KJ, Stevenson GW, Eds.; John Wiley & Sons Inc.: Hoboken, NJ, USA, pp. 792–806

Vidal A, Martín-Valls GE, Tello M, Mateu E, Martín M, Darwich L (2019) Prevalence of enteric pathogens in diarrheic and non-diarrheic samples from pig farms with neonatal diarrhea in the North East of Spain. Vet Microbiol 237:108419

Vu-Khac H, Holoda E, Pilipcinec E, Blanco M, Blanco JE, Dahbi G, Mora A, López C, González EA, Blanco J (2007) Serotypes, virulence genes, intimin types and PFGE profiles of Escherichia coli isolated from piglets with diarrhoea in Slovakia. Vet J 174:176–187

Wang H, Sanz Garcia R, Cox E, Devriendt B (2020) Porcine Enterotoxigenic Escherichia coli Strains Differ in Their Capacity To Secrete Enterotoxins through Varying YghG Levels. Appl Environ Microbiol 86:e00523-20.

Wellison AP, Franco SM, Reis IL, Mendonça CMN, Piazentin ACM, Azevedo POS, Tse MLP, De Martinis ECP, Gierus M, Oliveira RPS (2022) Beneficial effects of probiotics on the pig production cycle: An overview of clinical impacts and performance. Vet Microbiol 269:109431.

Yaeger M, Funk N, Hoffman L (2002) A survey of agents associated with neonatal diarrhea in Iowa swine including Clostridium difficile and porcine reproductive and respiratory syndrome virus. J Vet Diag 14:281-287.

Yaeger MJ, Kinyon JM, Songer JG (2007) A prospective, case-control study evaluating the association between Clostridium difficile toxins in the colon of neonatal swine and gross and microscopic lesions. J Vet Diagn Investig 19:52-59.

Yue S, Li Z, Hu F, Picimbon JF (2020) Curing piglets from diarrhea and preparation of a healthy microbiome with Bacillus treatment for industrial animal breeding. Sci Rep 10:19476.

Zajacova ZS, Konstantinová L, Alexa P (2012) Detection of virulence factors of Escherichia coli focused on prevalence of EAST1 toxin in the stool of diarrheic and

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non-diarrheic piglets and presence of adhesion involving virulence factors in astA positive strains. Vet Microbiol 154:369–375

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GENERAL CONCLUSIONS

The studies carried out during this PhD thesis led to the following conclusions:

- This study described, for the first time, the frequency of Extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* in the Greek swine industry and characterized their Antimicrobial Resistance (AMR) phenotype and genotype.
- Increased levels of AMR were found in Greek pig farms, raising concern about treatment effectiveness and public health.
- Seventy-eight isolates (36.5%) showed resistance to 3rd generation cephalosporins and were confirmed to produce ESBL.
- ESBL producers were identified as *Escherichia coli (E. coli)* (69), *Klebsiella pneumoniae* spp. *pneumoniae (K. Pneumoniae)* (3), *Proteus mirabilis (P. mirabilis)* (4), *Enterobacter cloacae (E. cloacae)* complex (1) and *Salmonella enterica* subsp. *diarizonae (S. enterica)* (1).
- ESBL-producing isolates were resistant to at least one class of non-β-lactam antibiotics.
- Four *P. mirabilis* strains showed decreased susceptibility to Imipenem (IMI), which is concerning due to the banning of IMI use in livestock and reported carbapenemase resistance in humans.
- Statistical analysis linked increased AMR to various risk factors such as past inappropriate antibiotic use and herd size.

Chapter II general conclusions

- Correlations were found between previous antimicrobial usage and resistance to different antibiotics, including Gentamycin (GEN) resistance with quinolones, Neomycin (NEO) resistance with tetracyclines (TETs), marbofloxacin (MRX) resistance with TETs, and enrofloxacin (ENR) resistance with cephalosporins.
- Future studies are needed to investigate the correlation of various risk factors (e.g., farm capacity, management, previous and current antibiotic treatments, age, and treatment groups) to understand better AMR development under field conditions.
- The implementation of a surveillance system for AMR monitoring, under the One Health approach, deemed necessary in order to promote the rational use of antibiotics and also protect public health.

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Chapter III general conclusions

- According to our results *E. Coli,* despite of vaccinations in sows, remains an enteric pathogen of concern for Greek pig industry farms. Our study revealed high prevalence of F4 and F6, as well as LT toxins.
- From study's farm 69.23% (18/26) were F4-positive, 30.77% (8/26) were F5-positive, 61.54% (16/26) were F6-positive, 42.31% (11/26) were F4- and enterotoxin LT-positive, 19.23% (5/26) were F5- and LT-positive, 42.31% (11/26) were F6- and LT-positive and in 57.69% (15/26) *E. coli* enterotoxin LT was found.
- *Clostridium perfringens (C. perfringens)* type C was absent in faecal samples from newborn piglets with diarrhoea probably due to sufficient protection of routine vaccinations in sows against *C. perfringens* type C.
- Toxin A of *Clostridioides difficile (C. difficile)* was found in 84.62% and Toxin B in 88.46% of the farms.
- This research revealed also that administering antibiotics alone to sows did not reduce the detection of Enterotoxigenic (ETEC) antigens in neonatal piglets but the combination of antibiotics and probiotics or acidifiers or both gave better results in the detection of ETEC antigens and *E. coli* LT enterotoxin.
- Probiotics or acidifiers in feed seemed to result in lower neonatal piglet diarrhoea scores compared to those who received nothing or only injectable antibiotics.

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SCHOOL OF HEALTH SCIENCES

FACULTY OF VETERINARY MEDICINE

CLINIC OF MEDICINE

ADVANCING THE UNDERSTANDING OF ANTIBIOTIC RESISTANCE AND NEONATAL PIGLET DIARRHOEA

Doctoral Thesis by

Nikolaos A. Tsekouras

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Summary

This doctoral thesis examines the antimicrobial resistance and the neonatal piglet diarrhoea in Greek farms. Due to the imprudent use of antimicrobials in livestock, the emerging phenomenon of antibiotic resistance is increasing, reducing the efficacy of treatments, increasing the treatments' cost and posing a threat to public health. Meanwhile, neonatal diarrhoea remains a problem for pig farms, reducing production and increasing mortality in combination with increases in production costs. The dissertation is divided into three parts: a review of the literature and two studies. The literature review focuses on the use of antimicrobials in pig farming, neonatal diarrhoea, and swine gut health.

The first study investigates the presence of Extended-spectrum beta-lactamase (ESBL)-positive *Enterobacteriaceae* in Greek farms. Antimicrobial resistance in livestock animals has globally caused concern for public health, as nearly 70% of the total consumption of antimicrobial drugs in Europe is related to animal production. Bacteria producing extended-spectrum beta-lactamases show resistance to commonly used beta-lactam antimicrobials, including third-generation cephalosporins such as ceftriaxone, ceftazidime, and ceftiofur. This cross-sectional study was conducted over two years across various regions of Greece (2019-2021) and included 34 pig farms with a total population of 14,300 sows, representing about 24% of Greek pig farming. The antimicrobial sensitivity testing of the isolated strains was conducted using the Vitek-2 system. The 78 ESBL-positive strains isolated, accounting for 36.5% of all samples, were identified as follows: *Escherichia coli (E. coli)* (69 strains), *Klebsiella pneumoniae (K. pneumoniae)* (3 strains), *Proteus mirabilis (P. mirabilis)* (4 strains), *Enterobacter cloacae (E. cloacae)* complex (1 strain), and *Salmonella enterica (S. enterica)* subsp. *diarizonae* (1 strain) which presented resistance to at least one class of non-beta-lactam antimicrobials. The present study found high rates of ESBL-positive bacteria, which is a significant concern and responsibility for veterinarians, farmers and public health authorities. The increase in antibiotic resistance is also associated with increased production costs. The use of antimicrobials must be prudent and based on laboratory investigation and antimicrobial sensitivity testing of bacteria to ensure the effectiveness of antimicrobial agents. Implementing a surveillance system for antibiotic resistance in the context of One Health will contribute not only to the rational use of antimicrobials but also to the protection of public health.

The second study examines neonatal piglet diarrhoea, which remains a severe cause of production losses in pig farming. Among other pathogens, *E. coli* and Clostridia bacteria

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are frequently isolated. This study was carried out from January 2020 to October 2021 in Greece across 26 farms with a total population of 12,380 sows. In all selected farms, sows were vaccinated against *E. coli* and Clostridia 15-20 days before farrowing. The study aimed to investigate the presence of ETEC *E. coli* strains and Clostridia as causes of neonatal diarrhoea in piglets, as well as the effectiveness of preventive measures.

A total of 78 fecal samples were collected from 234 nursing piglets aged 1-4 days. Three fecal samples were collected using cotton swabs from three piglets in each farrowing group, for three farrowing groups per farm. Among the samples examined, 69.23% (18/26) of the farms were found positive for the ETEC F4 strain, 30.77% (8/26) for ETEC F5, 61.54% (16/26) for ETEC F6, while the LT enterotoxin was detected in 57.69% (15/26) of the farms. In a significant number of diarrhoea cases, the *C. difficile* bacterium was isolated, indicating it might be among the etiological factors of neonatal diarrhoea. Specifically, *C. difficile* toxin A was detected in 84.62% (22/26) and toxin B in 88.46% (23/26) of the farms. Therapeutic and preventive interventions, such as administering injectable antimicrobials combined with the use of probiotics or acidifiers in the feed of sows before farrowing, contribute to reducing the detection of antigens of ETEC strains and the LT enterotoxin of *E. coli*. This comprehensive examination underscores the importance of responsible antimicrobial use and effective preventive measures in maintaining not only animal health but also public health.

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ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΙΑΣ

ΣΧΟΛΗ ΕΠΙΣΤΗΜΩΝ ΥΓΕΙΑΣ

ΤΜΗΜΑ ΚΤΗΝΙΑΤΡΙΚΗΣ

ΠΑΘΟΛΟΓΙΚΗ ΚΛΙΝΙΚΗ

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Διδακτορική Διατριβή του

Νικολάου Α. Τσεκούρα

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Περίληψη

Η παρούσα διδακτορική διατριβή μελετά την αντιβιοαντοχή και τη νεογνική διάρροια των χοιριδίων στις ελληνικές εκτροφές. Εξαιτίας της αλόγιστης χρήσης των αντιμικροβιακών στην κτηνοτροφία παρατηρείται, βαίνοντας εντονότερο, το φαινόμενο της αντιβιοαντοχής μειώνοντας την αποτελεσματικότητα των θεραπειών, αυξάνοντας το κόστος θεραπείας αλλά και απειλώντας τη Δημόσια Υγεία ενώ η νεογνική διάρροια παραμένει ένα πρόβλημα για τις εκτροφές των χοίρων μειώνοντας τις αποδόσεις και αυξάνοντας τη θνησιμότητα μαζί με το κόστος παραγωγής. Η διατριβή χωρίζεται σε τρία μέρη: την ανασκόπηση της βιβλιογραφίας και τις δύο μελέτες. Η ανασκόπηση της βιβλιογραφίας εστιάζει στη χρήση των αντιμικροβιακών στη χοιροτροφία, στη νεογνική διάρροια και στην υγεία του εντέρου των χοίρων.

Στην πρώτη μελέτη ερευνάται η ύπαρξη εντεροβακτηριοειδών θετικών στην παραγωγή ευρέως φάσματος β-λακταμασών (ESBL) στις ελληνικές εκτροφές. Η αντιβιοαντοχή στα παραγωγικά ζώα εν γένει, έχει προκαλέσει παγκόσμια ανησυχία για τη δημόσια υγεία καθώς σχεδόν το 70% της συνολικής κατανάλωσης αντιμικροβιακών φαρμάκων στην Ευρώπη σχετίζεται με τη ζωική παραγωγή. Τα βακτήρια που παράγουν ευρέως φάσματος β-λακταμάσες παρουσιάζουν αντοχή στα συχνά χρησιμοποιούμενα β-λακταμικά αντιμικροβιακά, συμπεριλαμβανομένων των κεφαλοσπορινών τρίτης γενιάς, όπως η κεφτριαξόνη, η κεφταζιδίμη και η κεφτιοφούρη. Η παρούσα συγχρονική μελέτη διενεργήθηκε για δύο χρόνια στις διάφορες περιφέρειες της Ελλάδας (μεταξύ 2019-2021) και περιλάμβανε 34 εκτροφές χοίρων με συνολικό πληθυσμό 14.300 χοιρομητέρων, αριθμός που αντιστοιχεί στο 24% περίπου της ελληνικής χοιροτροφίας. Ο έλεγχος της αντιμικροβιακής ευαισθησίας των στελεχών που απομονώθηκαν, διενεργήθηκε με το σύστημα Vitek-2. Τα 78 ESBL-θετικά στελέχη που απομονώθηκαν, 36,5% επί του συνόλου των δειγμάτων, ταυτοποιήθηκαν ως εξής: *Escherichia coli (E. coli)* (69 στελέχη), *Klebsiella pneumoniae (K. pneumoniae)* (3 στελέχη), *Proteus mirabilis (P. mirabilis)* (4 στελέχη), *Enterobacter cloacae (E. cloacae)* complex (1 στέλεχος), και *Salmonella enterica (S. enterica)* subsp. *diarizonae* (1 στέλεχος) και παρουσίασαν αντιβιοαντοχή σε τουλάχιστον μία κλάση μη β-λακταμικών αντιμικροβιακών. Στην παρούσα μελέτη διαπιστώθηκαν υψηλά ποσοστά ESBL-θετικών βακτηρίων και αποτελεί εύρημα αυξημένης ανησυχίας και ευθύνης για τους κτηνιάτρους, τους κτηνοτρόφους καθώς και τις κρατικές αρχές δημόσιας υγείας. Η αύξηση της αντιβιοαντοχής, μεταξύ άλλων, συνδέεται

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και με αυξημένο κόστος παραγωγής. Η χρήση αντιμικροβιακών επιβάλλεται να είναι συνετή και να βασίζεται στην εργαστηριακή διερεύνηση και στον έλεγχο της αντιμικροβιακής ευαισθησίας των βακτηρίων, προκειμένου η χρήση των αντιμικροβιακών παραγόντων να παραμείνει αποτελεσματική. Η ολοκλήρωση ενός συστήματος επιτήρησης της αντιβιοαντοχής στο πλαίσιο της Ενιαίας Υγείας, θα συνεισφέρει όχι μόνο στη ορθολογική χρήση των αντιμικροβιακών αλλά και στην προστασία της δημόσιας υγείας.

Στη δεύτερη μελέτη εξετάζεται η νεογνική διάρροια των χοιριδίων, η οποία παραμένει μια σοβαρή αιτία απωλειών παραγωγής στη χοιροτροφία. Μεταξύ άλλων παθογόνων, συχνά απομονώνονται το βακτήριο *E. coli* και τα κλωστρήδια. Η παρούσα μελέτη διενεργήθηκε από τον Ιανουάριο του 2020 μέχρι τον Οκτώβριο του 2021 στην Ελλάδα σε 26 εκτροφές με συνολικό πληθυσμό 12.380 χοιρομητέρων. Σε όλες τις εκτροφές που επιλέχθηκαν, εφαρμοζόταν εμβολιασμός των χοιρομητέρων 15-20 ημέρες προ τοκετού κατά του *E. coli* και κλωστρηδίων. Σκοπός της μελέτης ήταν η διερεύνηση της παρουσίας των εντεροτοξινογόνων στελεχών του *E. coli* (ETEC) και των κλωστρηδίων σαν αίτια νεογνικής διάρροιας των χοιριδίων, καθώς και η αποτελεσματικότητα των μέτρων πρόληψης. Συνολικά 78 δείγματα κοπράνων συλλέχθηκαν από 234 θηλάζοντα χοιρίδια ηλικίας 1-4 ημερών. Τρία δείγματα κοπράνων συλλέχθηκαν μέσω βαμβακοφόρου στειλεού από τρία χοιρίδια κάθε τοκετοομάδας, για τρεις τοκετοομάδες κάθε εκτροφής. Από τα δείγματα που εξετάστηκαν το 69,23% (18/26) των εκτροφών διαπιστώθηκαν θετικές στο στέλεχος ETEC F4, το 30,77% (8/26) στο ETEC F5, το 61,54% (16/26) στο ETEC F6 ενώ στο 57,69% (15/26) των εκτροφών ανιχνεύθηκε η εντεροτοξίνη LT. Σε σημαντικό αριθμό περιστατικών διάρροιας απομονώθηκε το βακτήριο *Clostridioides (C. difficile)*, υποδεικνύοντας ότι πιθανόν συμπεριλαμβάνεται στους αιτιολογικούς παράγοντες νεογνικής διάρροιας. Συγκεκριμένα η τοξίνη Α του *C. difficile* ανιχνεύθηκε στο 84,62% (22/26) και η τοξίνη Β στο 88,46% (23/26) των εκτροφών. Οι θεραπευτικές και προληπτικές παρεμβάσεις, όπως η χορήγηση ενέσιμων αντιμικροβιακών, σε συνδυασμό με τη χρήση προβιοτικών ή οξινιστών στην τροφή των χοιρομητέρων προ τοκετού, συμβάλλουν στη μείωση της ανίχνευσης αντιγόνων των ETEC στελεχών και της εντεροτοξίνης LT του *E. coli*.

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ANNEX I

Curriculum Vitae

Nikolaos A. Tsekouras

DVM, PhD(c)

1. EDUCATION

Undergraduate studies

- Doctor of Veterinary Medicine, DVM. Faculty of Veterinary Medicine, School of Health Sciences, University of Thessaly, Greece (July 2018).

Postgraduate studies

- Master of Science: "Animals: Ethics, Law, Welfare", Department of Philosophy, National and Kapodistrian University of Athens (October 2023-present).
- PhD candidate. Clinic of Farm Animal Medicine (Porcine Medicine), Faculty of Veterinary Medicine, School of Health Sciences, University of Thessaly (November 2019- present): "Advancing the understanding of antibiotic resistance and neonatal piglet diarrhoea".
- Internship in Clinic of Farm Animal Medicine, Faculty of Veterinary Medicine, School of Health Sciences, University of Thessaly, Greece (November 2018-November 2019).

2. PROFESSIONAL ACTIVITIES

Work experience

- 17/7/2019-3/12/2019: Veterinary officer in Department of Animal Health, Directorate General of Veterinary Services, Region of Thessaly, Greece.
- 4/12/2019-28/4/2021: Scholar in "Novel technologies for surveillance and characterization of Extended-spectrum β-lactamase and Carbapenemase producing Enterobacteriaceae, in humans and animals–CARBATECH, 2018-2021-co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call German- Greek Research and Innovation Program, NSRF 2014-2020".

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- 1/3/2021-30/4/2024: Veterinary Supervisor in West Thessaly's Cow Farmers and Sheep Farmers' Cooperative (clinical services to sheep and cow farmers as veterinary practitioner, herd health and reproduction management).
- 1/5/2024-present: Key Account Sales and Technical Manager – Small Ruminants (Greece and Cyprus), MSD Animal Health Greece.

Undergraduate teaching

- November 2019-Present: Farm Animal Medicine, Faculty of Veterinary Medicine, School of Health Sciences, University of Thessaly, Greece.
- Lectures: Farm Animal Diseases, Clinical Examination and Diagnosis, Treatment and Prevention, Metaphylaxis and Prophylaxis strategies.
- Practical training: On farm disease cases – Field visits of high-/low-health swine, cattle and sheep farms-Discussion of clinical cases-Diagnostic techniques and Nursing of animals in Clinic of Medicine.

3. RESEARCH

Research interests

- Farm Animal Medicine (diseases, diagnosis, treatment, prevention, metaphylaxis, prophylaxis).
- Herd Health Management, including animal husbandry (genetics, housing, nutrition, production systems), internal medicine (infectious and other diseases), animal welfare, pathogenesis, diagnosis, therapy, prevention, and control of diseases.
- Clinical and field trials in veterinary medicine and animal production (vaccines, veterinary pharmaceuticals, antibiotics, probiotics, phytogenics, feed additives).

Associate Investigator in the following research projects:

- "Porcine Reproductive & Respiratory Syndrome (PRRS): Epidemiological and pathological investigation and comparative molecular characterization of vaccinal and field viral strains, in Greece''. Faculty of Veterinary Medicine, University of Thessaly, (2012-2015). Granted by "Pfizer Hellas, Greece".
- "Novel technologies for surveillance and characterization of Extended- spectrum β-lactamase and Carbapenemase producing Enterobacteriaceae, in humans and animals–CARBATECH, 2018-2021". Co-financed by the European Union and Greek national funds through the Operational Program Competitiveness,

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Entrepreneurship, and Innovation, under the call German- Greek Research and Innovation Program, NSRF 2014-2020).

- "LIFE ARCPROM: Improving human-bear coexistence in 4 National Parks of South Europe" as field practitioner in collaboration with the Department of Microbiology and Parasitology, Faculty of Veterinary Science, University of Thessaly.
- "Development of mechanism for West Nile Virus monitoring in Prefecture of Peloponnese for 2019-2020" as field practitioner in collaboration with the Department of Microbiology and Parasitology, Faculty of Veterinary Science, University of Thessaly.
- "Development of novel technologies based on alternative products (phytogenic), for sheep and goats health and reproduction improvement" (Clinic of Farm Animal Medicine, Faculty of Veterinary Medicine, School of Health Sciences, University of Thessaly, September 2023-May 2024).

4. DISTINCTIONS

Invited speaker - trainer

- Seminar in Swine Medicine with subject: "Swine pleuropneumonia (*Actinobacillus pleuropneumoniae*): High-risk disease in Greek pig farms: treatment and biosecurity measures" (Drama, Greece, 29/03/2019-organizer: Hellafarm S.A. / Bayer Animal Health).
- 15th Panhellenic Veterinary Conference: "Prevalence of fimbrial antigens of enterotoxigenic *Escherichia coli* and toxins of clostridia in neonatal piglets with diarrhoea" (Athens, Greece, 4-6/11/2022-organizer: Hellenic Veterinary Society).
- 15th Panhellenic Veterinary Conference: "Cross-Sectional Survey of Antibiotic Resistance in Extended Spectrum β-Lactamase-Producing Enterobacteriaceae Isolated from Pigs in Greece" (Athens, Greece, 4-6/11/2022-organizer: Hellenic Veterinary Society).

5. PUBLICATIONS

Papers in peer reviewed journals

1. Papatsiros VG, Athanasiou LV, Spanou VM, Stylianaki I, Papakonstantinou G, Letsios M, Villioti CS, **Tsekouras N**, Maragkakis G, Papaioannou N, Christodoulopoulos G. First case of *Serratia liquefaciens* isolated from urinary tract infection in sows and

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associated clinicopathological and pathological findings. Lett Appl Microbiol. 2020;70(4):259-262.

2. Papatsiros V, Stylianaki I, Papakonstantinou G, **Tsekouras N**, Bitchava D, Christodoulopoulos G, Papaioannou N. Histopathological Lesions Accompanied with First-Time Isolation of a PRRSV-2 Strain in Greece. Viral Immunol. 2020;33(8):565-570.
3. Papatsiros VG, Stylianaki I, **Tsekouras N**, Papakonstantinou G, Gómez-Nicolau NS, Letsios M, Papaioannou N. Exposure Biomarkers and Histopathological Analysis in Pig Liver After Exposure to Mycotoxins Under Field Conditions: Special Report on Fumonisin B1. Foodborne Pathog Dis. 2021;18(5):315-321.
4. Papatsiros VG, Papakonstantinou G, Meletis E, **Tsekouras N**, Maragkakis G, Bitchava D, Kostoulas P. Occurrence and Associated Risk Factors of Porcine Reproductive and Respiratory Syndrome Virus and Porcine Circovirus Type 2 Infections in Greece. Viral Immunol. 2022;35(3):200-211. doi: 10.1089/vim.2021.0124.
5. Papatsiros V, Argyris G, Papakonstantinou G, Meletis E, **Tsekouras N**, Kantas D, Kostoulas P. Evaluation of an on-farm method to assess colostrum IgG content in hyperprolific sows. Anim Rep Sci. 2022; 239:106958. doi: 10.1016/j.anireprosci.2022.106958.
6. **Tsekouras N**, Athanasakopoulou Z, Diezel C, Kostoulas P, Braun SD, Sofia M, Monecke S, Ehricht R, Chatzopoulos DC, Gary D, Krähmer D, Spyrou V, Christodoulopoulos G, Billinis C, Papatsiros VG. Cross-Sectional Survey of Antibiotic Resistance in Extended Spectrum β-Lactamase-Producing Enterobacteriaceae Isolated from Pigs in Greece. Animals. 2022; 12(12):1560. https://doi.org/10.3390/ani12121560.
7. **Tsekouras N,** Meletis E, Kostoulas P, Labronikou G, Athanasakopoulou Z, Christodoulopoulos G, Billinis C, Papatsiros VG. Detection of enterotoxigenic *Escherichia coli* and Clostridia in the aetiology of neonatal piglet diarrhea: important factors for their prevention. Life. 2023; 13:1092. https://doi.org/10.3390/life13051092.
8. Papakonstantinou GI, Meletis E, Petrotos K, Kostoulas P, **Tsekouras N**, Kantere MC, Voulgarakis N, Gougoulis D, Filippopoulos L, Christodoulopoulos G, Athanasiou LV, Papatsiros VG. Effects of a Natural Polyphenolic Product from Olive Mill Wastewater on Oxidative Stress and Post-Weaning Diarrhea in Piglets. Agriculture. 2023; 13(7):1356. https://doi.org/10.3390/agriculture13071356.

Proceedings in international congresses

1. Tsilipounidaki K, Trikalinou C, Kartalidis P, Athanasakopoulou Z, Spyrou V,

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Giannakopoulos A, Sofia M, Chatzopoulos D, Valiakos G, Karakousis J, **Tsekouras N**, Papatsiros V, Billinis Ch, Petinaki E. Low prevalence of multi-drug resistant bacteria (ESKAPE) in animals in Greece. *Proceedings of 30th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Paris, France, 18 - 21 April 2020.*

2. Papatsiros VG, Athanasiou LV, Spanou VM, Stylianaki I, Papakonstantinou G, Letsios M, Villioti CS, **Tsekouras N**, Maragkakis G, Papaioannou N, Christodoulopoulos G. First case of *Serratia liquefaciens* isolated from urinary tract infection in sows. *Proceedings of the 1st International Conference of the European College of Veterinary Microbiology, Athens, Greece, 26-27 September 2019.*

3. **N Tsekouras**, Z Athanasakopoulou, C Diezel, P Kostoulas, SD. Braun, M Sofia, S Monecke, R Ehricht, D Chatzopoulos, D Gary, D Krähmer, V Spyrou, G Christodoulopoulos, Ch Billinis, V Papatsiros**.** Cross-sectional survey of antibiotic resistance in extended spectrum B-Lactamase-producing Enterobacteriaceae isolated from pigs in Greece. *Proceedings of 15th Panhellenic Veterinary Congress (Athens 4-7 November 2022)*: 6.

4. **N Tsekouras**, E Katsogiannou, L Athanasiou, G Labronikou, Z Athanasakopoulou, G Christodoulopoulos, Ch Billinis, V Papatsiros**.** Prevalence of fimbrial antigens of enterotoxigenic Escherichia Coli and toxins of Clostridia in neonatal piglets with diarrhoea. *Proceedings of 15th Panhellenic Veterinary Congress (Athens 4-7 November 2022)*: 8.

6. LANGUAGES

- **English:** Michigan B2
- **French:** Delf B2

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ANNEX II

Publications from PhD Dissertations

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1. "Cross-Sectional Survey of Antibiotic Resistance in Extended Spectrum β-Lactamase-Producing *Enterobacteriaceae* Isolated from Pigs in Greece"

Published

Tsekouras N, Athanasakopoulou Z, Diezel C, Kostoulas P, Braun SD, Sofia M, Monecke S, Ehricht R, Chatzopoulos DC, Gary D, Krähmer D, Spyrou V, Christodoulopoulos G, Billinis C, Papatsiros VG (2022) Cross-Sectional Survey of Antibiotic Resistance in Extended Spectrum β-Lactamase-Producing *Enterobacteriaceae* Isolated from Pigs in Greece. Animals 12(12), 1560. https://doi.org/10.3390/ani12121560.

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Article

Cross-Sectional Survey of Antibiotic Resistance in Extended Spectrum β-Lactamase-Producing *Enterobacteriaceae* Isolated from Pigs in Greece

Nikolaos Tsekouras [1], Zoi Athanasakopoulou [2], Celia Diezel [3,4], Polychronis Kostoulas [5], Sascha D. Braun [3,4], Marina Sofia [2], Stefan Monecke [3,4], Ralf Ehricht [3,4], Dimitris C. Chatzopoulos [5], Dominik Gary [6], Domenique Krähmer [6], Vassiliki Spyrou [7], Georgios Christodoulopoulos [1], Charalambos Billinis [2,5] and Vasileios G. Papatsiros [1,*]

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Simple Summary: The objective of this study was to investigate, for the first time in Greece, the prevalence of ESBL producers in swine populations and to correlate their occurrence with risk factors. A total of 214 fecal samples were collected from the farms from December 2019 to April 2021. A subset of 78 (78/214, 36.5%) ESBL producers were identified as *Escherichia coli* (*E. coli*, 88.5%), *Klebsiella pneumoniae* spp. *pneumoniae* (*K. pneumoniae*, 3.8%), *Proteus mirabilis* (*P. mirabilis*, 5.1%), *Enterobacter cloacae* complex (*E. cloacae* complex, 1.3%) and *Salmonella enterica* spp. *diarizonae* (*S. enterica* spp. *diarizonae*, 1.3%). CTX-M, SHV and TEM genes were detected along with genes conferring resistance to fluoroquinolones, aminoglycosides, sulfonamides, trimethoprim, macrolides and colistin. This study displayed high antimicrobial resistance rates in the Greek swine industry, and our results are alarming for both human and animal health.

Abstract: This study aimed to estimate the prevalence of extended-spectrum β-lactamase-producing (ESBL) bacteria in swine. Thus, 214 fecal samples were collected from suckling and weaned piglets from 34 farms in Greece (out of an overall population of about 14,300 sows). A subset of 78 (36.5%) ESBL producers were identified as *E. coli* (69/78, 88.5%), *K. pneumoniae* spp. *pneumoniae* (3.8%), *P. mirabilis* (5.1%), *E. cloacae* complex (1.3%) and *S. enterica* spp. *diarizonae* (1.3%). Resistance to at least one class of non-β-lactam antibiotics was detected in 78 isolates. Among the *E. coli* strains, resistance was identified with regard to aminoglycosides (n = 31), fluoroquinolones (n = 49), tetracycline (n = 26) and trimethoprim/sulfamethoxazole (n = 46). Of the three *K. pneumoniae* spp. pneumoniae, two displayed resistances to aminoglycosides and all were resistant to fluoroquinolones, tetracyclines and trimethoprim/sulfamethoxazole. As for the four *P. mirabilis* isolates, three had a resistant phenotype for aminoglycosides and all were resistant to imipenem, fluoroquinolones, tetracyclines and trimethoprim/sulfamethoxazole. Molecular characterization of the isolates revealed the presence of CTX-M, SHV and TEM genes, as well as of genes conferring resistance to fluoroquinolones, aminoglycosides, sulfonamides, trimethoprim, macrolides and colistin. High levels of antimicrobial resistance (AMR) were demonstrated in Greek swine herds posing a concern for the efficacy of treatments at the farm level as well as for public health.

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Citation: Tsekouras, N.; Athanasakopoulou, Z.; Diezel, C.; Kostoulas, P.; Braun, S.D.; Sofia, M.; Monecke, S.; Ehricht, R.; Chatzopoulos, D.C.; Gary, D.; et al. Cross-Sectional Survey of Antibiotic Resistance in Extended Spectrum β-Lactamase-Producing *Enterobacteriaceae* Isolated from Pigs in Greece. *Animals* **2022**, *12*, 1560. https://doi.org/10.3390/ani12121560

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Keywords: antimicrobial resistance; pigs; ESBL-producing *Enterobacteriaceae*; multidrug resistance genes; Greece

1. Introduction

By the 1950s, antibiotics were being regularly used in industrial livestock production to secure animals' health and improve their productivity [1]. Antibiotics are typically used in farms, not only for treatment purposes, but also for regulating the spread of infections (metaphylaxis), inhibiting infections (prophylaxis), especially in high-stress periods (weaning stage, post-vaccination period, after farrowing), and improving feed intake and growth performance [2]. Subtherapeutic doses of antimicrobials were administered to livestock for decades to prevent diseases and/or to enhance growth [3,4], a strategy that promoted the development and spread of antimicrobial resistant strains. Nowadays, the use of antibiotics (e.g., amoxicillin) is included at routine metaphylaxis programs. For instance, injectable amoxicillin can be administered postpartum for metaphylaxis of postpartum dysgalactia syndrome (PPDS) or in weaning feed for the prevention and metaphylaxis of post-weaning diarrhea (PWD) [5,6].

Antimicrobial resistance (AMR) in food-producing animals has drawn global attention, as approximately 70% of the overall antibiotic consumption in Europe is related to the animal sector [7,8]. A major outcome of AMR dissemination is non-effective treatments of livestock, which are further associated with decreased productivity and economic losses due to increased treatment costs [9]. Considering the estimated continuous rise in the global demand for animal derived products, consumption of antimicrobials by livestock is anticipated to increase by two-thirds over the next years [10]. In fact, antimicrobial consumption is estimated to be greater in pigs in comparison to chicken and cattle production systems [10] and a greater possibility of AMR has already been reported in pigs than in chicken and other food animals, or aquaculture [11–13]. Furthermore, AMR has been demonstrated in wild boars, a wildlife species that can act as a source of zoonotic pathogens causing human diseases, such as colibacillosis, salmonellosis, yersiniosis and listeriosis [14,15]. AMR in domestic and wild animals poses a hazard for human health by introducing resistant pathogens into the food chain and by triggering horizontal transfer of resistance determinants to other bacteria [16].

Extended spectrum β-lactamase-producing bacteria (ESBL) display resistance to the commonly used beta-lactam antimicrobial agents, including third generation cephalosporins, such as ceftriaxone, ceftazidime and ceftiofur [17]. β-lactams, namely penicillins, carbapenems, monobactams and cephalosporins, constitute 60% (by weight) of all antibiotics used worldwide, and are among the most extensively prescribed antibiotic classes in human medicine [18,19]. Due to excessive usage of β-lactams in both humans and animals, an increased spread of ESBL-producing bacteria has been observed, threatening personnel in the swine industry, and consequently posing a threat to human health [20]. ESBLs are widespread in *Enterobacteriaceae*, especially in *E. coli* and *Salmonella* spp., while ESBL-producing *E. coli* has been reported in food animals worldwide [21,22]. Previous reports have associated human ESBL carriage with exposure to ESBL-producing *Enterobacteriaceae* of livestock origin, raising concerns about the possible transfer of ESBL producers through the food chain, which could jeopardize public health [23–28].

Reviewing previous literature revealed the scarcity of published data about ESBL-producing *Enterobacteriaceae* from pig farms in Greece. Thus, the objective of this study was to report, for the first time in Greece, the prevalence of ESBL producers in pig herds, to phenotypically and molecularly identify their antimicrobial resistance patterns and to investigate potential factors that could promote the development of AMR.

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2. Materials and Methods

2.1. Ethics

All procedures were performed according to the ethical standards in the Helsinki Declaration of 1975, as revised in 2000, as well as the national law, and after receiving approval (number 96/19.12.2019) from the Institutional Animal Use Ethics Committee of the Faculty of Veterinary Science, University of Thessaly.

2.2. Study Design

The current cross-sectional study was conducted in different regions of Greece for two years (between 2019–2021) and included 34 pig farms. The farms had an overall population of about 14,300 sows, which represented approximately 24% of the entire capacity of the Greek swine production. Farms were in northern (n = 4), central (n = 13), western (n = 10) and southern (n = 7) Greece. Generally, central and western Greece are the regions with the highest pig density (more than 50% of total pig population of Greece). The classifications of pig farms according to their geographic origin and capacity are presented in Table 1.

Table 1. Geographic origin and capacity of study's farms.

Region	Number of Farms	Total Capacity	Capacity per Farm					Number of Samples per Age	
			50–100	101–250	251–450	451–700	701–2100	Suckling Piglets	Weaning Piglets
Northern Greece	4	1640	0	2	1	1	0	8	19
Central Greece	13	3710	4	2	3	4	0	24	44
Western Greece	10	5790	0	4	2	2	2	22	42
Southern Greece	7	3190	0	4	2	0	1	19	36
Total	34	14,300	4	12	8	7	3	73	141

Farmers or managers consented to participating in the study. The inclusion and exclusion criteria for farm selection are shown in Table 2. These criteria were met by all participating herds in order to include intensive farms that employ common practices of the Greek swine production system, while taking preventive measures for disease control.

Table 2. Criteria for farms selection.

Criteria	Farms	Sows	Weaners
Capacity	Minimum 50 sows		
Type of farm	Exclusive farrow-to-finish		
Antiparasitic treatment		Antiparasitic treatment (IM) before farrowing	
Vaccination		Aujeszky's disease virus Porcine parvovirus Atrophic rhinitis Erysipelas Porcine Reproductive and Respiratory Virus *E. coli* *Clostridium* spp.	Porcine Circovirus type 2 *Mycoplasma hyopneumoniae*
Diet		Home-made diets (mixed corn/barley/wheat–soybean-based meal) balanced in dietary nutrients (essential amino acids, minerals and vitamins) [1]	
Toxin binders		Systematically used in the feed during gestation and lactation	Systematically used in the feed
Routine program for metaphylaxis of PPDS		Amoxicillin (IM) after farrowing	
Routine program of metaphylaxis for PWD			Amoxicillin via feed for the first 10 days after weaning

[1] According to Nutrient Requirements of Swine (NRC) [29].

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In addition, data concerning sow age and prior administration of antimicrobials for the last six months were collected. The most used classes of antibiotics were penicillins, collistin, cephalosporins, quinolones, pleuromoutilins (PLMs), macrolides, tetracyclines and trimethoprim/sulfonamides; these were used according to the manufacturers' instructions with respect to duration of therapy and dosage.

2.3. Sample Collection

A total of 214 fecal samples were collected from 73 suckling and 141 weaning piglets of 34 farms (Table 1). Samples were obtained directly from the rectum by using swabs with Amies transport medium (Transwab®, Amies, UK) and were transferred within a day to the Laboratory of Microbiology and Parasitology (Faculty of Veterinary Medicine, Karditsa, Greece).

2.4. Isolation and Identification of Extended Spectrum Cephalosporin Resistant (ESCR) Strains

For the detection of ESCR isolates, fecal swabs were directly streaked on ESBL selective media (CHROMID® ESBL, BioMérieux, Marcy l'Etoile, France) and the plates were incubated aerobically at 37 °C for 24–48 h. Subcultures were grown on both MacConkey agar and 5% sheep blood agar until pure cultures were obtained. Bacterial species identification was carried out using the automated Vitek-2 system (BioMérieux. Marcy l'Etoile, France), according to the manufacturer's instructions.

2.5. Isolation and Identification of Salmonella spp.

Isolation of *Salmonella* spp. was conducted, according to ISO 6579-1:2017. Initially, swabs were agitated and squeezed into sterilized tubes containing 9 mL Buffered Peptone Water (BPW). Subsequently, Modified Semisolid Rappaport-Vassiliadis (MSRV) agar, Xylose Lysine Deoxycholate (XLD) agar, and *Salmonella Shigella* (SS) agar were used as selective media under the recommended conditions. All presumptive *Salmonella* colonies were identified as to species using the Vitek-2 system.

2.6. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of all the obtained strains was performed by the Vitek-2 system. The AST-GN96 card was used to determine the minimum inhibitory concentration (MIC) of the following antimicrobial classes: penicillins (ampicillin-AMP, amoxicillin/clavulanic acid-AMC, ticarcillin/clavulanic acid-TCC), cephalosporins (cefalexin-CEX, cefalotin-CF, cefoperazone-CEP, ceftiofur-CEF, cefquinome-CEQ), carbapenems (imipenem-IMI), aminoglycosides (gentamicin-GEN, neomycin-NEO), quinolones (flumequine-FLU, enrofloxacin-ENR, marbofloxacin-MRX), tetracyclines (tetracycline-TET), amphenicols (florfenicol-FLO), polymyxin B-PL and sulfonamides (trimethoprim/sulfamethoxazole-SXT).

2.7. Phenotypic Confirmation of ESBL Production

All the isolates that presented resistance to 3rd (CEP, CEF)-generation cephalosporins were screened via the double disk synergy test (DDST) or a combination disk test (CDT) for ESBL production, according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [30]. In brief, antibiotic disks containing cefotaxime (CTX) (30 μg), ceftazidime (CAZ) (30 μg), cefepime (CPM) (30 μg) and AMC (20 μg/10 μg) were applied at a distance of 20 mm (center to center) on Mueller Hinton agar previously inoculated with an 0.5 McFarland inoculum of the isolate to be tested. After incubation, any enhanced zone of inhibition between cephalosporin disks and the AMC disk or a ''keyhole" formation in the direction of the disk containing clavulanic acid were considered as evidence for the presence of an ESBL-producing strain. In cases of ambiguous results, a combination disk test was also applied, using CTX and CAZ disks (30 μg each), alone and in combination with clavulanic acid (10 μg). A difference of $\geq$5 mm in zone diameter

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between the test using the disks alone and that using the disks combined with clavulanic acid antimicrobial agents was interpreted as ESBL production.

2.8. Antibacterial Resistance Genes of ESBL-Producing Enterobacteriaceae

Isolates that were found to be positive in the DDST or the CDT were characterized using the DNA microarray-based assay CarbaResist from InterArray (FZMB GmbH, Bad Langensalza, Germany). Primer and probe sequences have previously been described in detail [31]. In addition, probes for the detection of the colistin resistance gene family mcr were included on the present microarray (see Supplementary File S1). Protocols and procedures were conducted in accordance with the manufacturer's instructions (https://www.inter-array.com/Further-Genotyping-Kits, accessed on 10 May 2022). In brief, bacteria were grown overnight on Columbia blood agar. Bacteria were harvested and genomic DNA was extracted using the Qiagen blood and tissue kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The DNA was used in a multiplexed primer elongation incorporating biotin-16-dUTP. Amplicons were stringently hybridized to the microarray, washed and incubated with a horseradish-peroxidase-streptavidin conjugate. Hybridizations were detected by adding a precipitating dye.

2.9. Statistical Analysis

Principal component analysis (PCA) and hierarchical clustering were used to explore the patterns of antimicrobial co-resistance among the isolated *Enterobacteriaceae* species and identify clusters of co-resistance [32]. Subsequently, the prevalence of AMR by species was estimated within a Bayesian estimation framework [33].

Logistic regression models were used to assess whether (a) the presence of ESBL-producing strains and (b) antimicrobial resistance to a certain type of antibiotic are associated with a series of candidate variables. Candidate variables for both (a) and (b) were herd size, sow age and administration of antibiotics (penicillins, collistin, cephalosporins, quinolones, pleuromoutilins (PLMs), macrolides, tetracyclines and trimethoprim/sulfonamides) according to the manufacturers' instructions. All candidate variables were initially screened, one-by-one, with a significance level of 0.25. For the shortlisted variables, collinearity analyses were conducted to identify pairs of collinear variables. For each pair of collinear variables, one was excluded from further analyses. The variable that was retained was the one more strongly associated with the outcome. Variables with $p < 0.25$ were then tested in the final model and were subsequently reduced by backwards elimination, until only significant ($p < 0.05$) variables remained.

All analyses were performed in R program [34]. For PCA, we used the prcomp built-in functions, and for Bayesian prevalence estimation, the runjags package [35] and figures were built with the ggplot2 package [36]. For logistic regression, the glmer function was used [37].

3. Results

3.1. Isolation and Identification of ESBL-Producing Enterobacteriaceae

A total of 98 ESCR strains were recovered by selective cultivation from 95 of the 214 (44.4%) swine samples tested. Additionally, five *Salmonella* spp. isolates were retrieved from an equal number of samples (2.3%).

Seventy-eight (36.5%) isolates presented resistance to 3rd generation cephalosporins and were phenotypically confirmed to produce ESBL. ESBL producers were identified as *E. coli* ($n = 69$), *K. pneumoniae spp. pneumoniae* ($n = 3$), *P. mirabilis* ($n = 4$), *E. cloacae* complex ($n = 1$) and *S. enterica subsp. diarizonae* ($n = 1$). The results are summarized in Table 3.

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Table 3. Isolation and identification of ESBL-producing bacteria from pig samples.

Bacterial Species	Percentage of Isolates (n = 214) [1]	Percentage of ESBL-Producing Isolates (n = 78) [2]
Escherichia coli	32.2 (n = 69)	88.5 (n = 69)
Klebsiella pneumoniae spp. *pneumoniae*	1.4 (n = 3)	3.8 (n = 3)
Proteus mirabilis	1.9 (n = 4)	5.1 (n = 4)
Enterobacter cloacae	0.5 (n = 1)	1.3 (n = 1)
Salmonella enterica subsp. *diarizonae*	0.5 (n = 1)	1.3 (n = 1)
Total	36.5 (n = 78)	100 (n = 78)

[1] Number of fecal samples tested; [2] Number of isolated ESBL-producing bacteria.

3.2. Antimicrobial Resistance Phenotype and Genotype of the ESBL-Producing Enterobacteriaceae

All ESBL isolates (n = 78) presented resistance to AMP and to all the cephalosporins tested, apart from four *E. coli* strains, which were susceptible to CEQ, and the isolate of the *E. cloacae* complex, which was susceptible to CEP. Resistances to AMC (33.3%) and TCC (26.9%) were also detected, while diminished susceptibility to imipenem was identified only in the four *P. mirabilis* isolates (5.1%). ESBL phenotypes are illustrated as percentages in Figure 1. Three *E. coli* isolates could not be retrieved after storage in −80 °C, and were thus not genotypically characterized. Of the remaining 66 *E. coli* isolates, ESBL genes were detected in 65. In particular, $bla_{\text{CTX-M1/15}}$ was detected in 52 isolates (78.8%), $bla_{\text{CTX-M9}}$ in six (9.1%), $bla_{\text{CTX-M8}}$ in six (9.1%), bla_{SHV} in three (4.5%) and bla_{TEM} in 38 (57.6%), alone (n = 5) or in combination with other variants (n = 33). The *K. pneumoniae* isolates were found to harbor $bla_{\text{CTX-M1/15}}$ (n = 2), bla_{SHV} (n = 2), bla_{TEM} (n = 1) and $bla_{\text{CTX-M9}}$ (n = 1), while the *P. mirabilis* harbored $bla_{\text{CTX-M9}}$ (n = 3), $bla_{\text{CTX-M8}}$ (n = 1) and bla_{TEM} (n = 3). Finally, the *S. enterica* harbored bla_{TEM}, whereas no ESBL genes were detected in the *E. cloacae*.

The AmpC gene bla_{ACT} was detected in 18 *E. coli* (27.3%) and the bla_{CMY} in one. Moreover, 21 isolates (31.8%) possessed $bla_{\text{OXA-1}}$ and one $bla_{\text{OXA-60}}$.

The detailed antimicrobial resistance phenotype and genotype of the ESBL isolates is reported in Supplementary File S1.

3.3. Antimicrobial Resistance Phenotype and Genotype of ESBL-Producing Enterobacteriaceae to non β-lactam Antibiotics

All 78 ESBL-producing isolates displayed resistance to at least one class of non β-lactam antibiotics. Among the *E. coli* strains, resistances were reported for fluoroquinolones (FLU; n = 45, ENR; n = 42, MRX; n = 22), aminoglycosides (GEN; n = 19, NEO; n = 24), TET (n = 26) and SXT (n = 46) (Figures 1–3).

Regarding *E. coli*, resistance genes were detected for fluoroquinolones in 35 isolates (*qnrA*, *qnrB*, *qnrS)*, for aminoglycosides in 59 (*aadA1*, *aadA2*, *aadA4*, *aphA*, *rmtA*, *rmtC*, *aac*(6′)-*Ib*, *aac*(3′)-*Iva*), for sulfonamides in 51 (*sul1*, *sul2*, *sul3)*, for trimethoprim in 58 (*dfrA1*, *dfrA12*, *dfrA13*, *dfrA14*, *dfrA15*, *dfrA17*, *dfrA19*, *dfrA5*, *dfrA7*), for macrolides in 16 (*mph*, *mrx*) and for colistin in seven (*mcr*-1/*mcr*-2, *mcr*-4, *mcr*-8). Additionally, the *intl1*, *intl2* and tnpISE*cp1* genes associated with mobile elements were identified in 20, seven and 30 *E. coli* isolates, respectively.

K. pneumoniae spp. *pneumoniae* isolates (n = 3) were resistant to fluoroquinolone (ENR; n = 3, FLU; n = 3, MRX; n = 1), TET and SXT, and two of the isolates displayed resistances to aminoglycosides (GEN; n = 2, NEO; n = 1) (Figures 1 and 3). All three isolates harbored genes conferring resistance to fluoroquinolones (*qnrS*), sulfonamides (*sul1*, *sul2*) and trimethoprim (*dfrA14*, *dfrA7*, *dfrA17*, *dfrA1*), while two also possessed aminoglycoside-resistance genes (*aadA1*, *aphA*). Genes encoding mobile elements (*intl1*, *intl2*, tnpISE*cp1*) as well as the oqxAB multidrug efflux pump (*oqxA*, *oqxB*) were additionally detected in the three isolates.

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	16	-	-	-	-
1	20	2	-	-	-
2	7	1	3	-	-
Ecp1	30	3	-	1	-
A	1	3	-	-	-
3	1	3	-	-	-

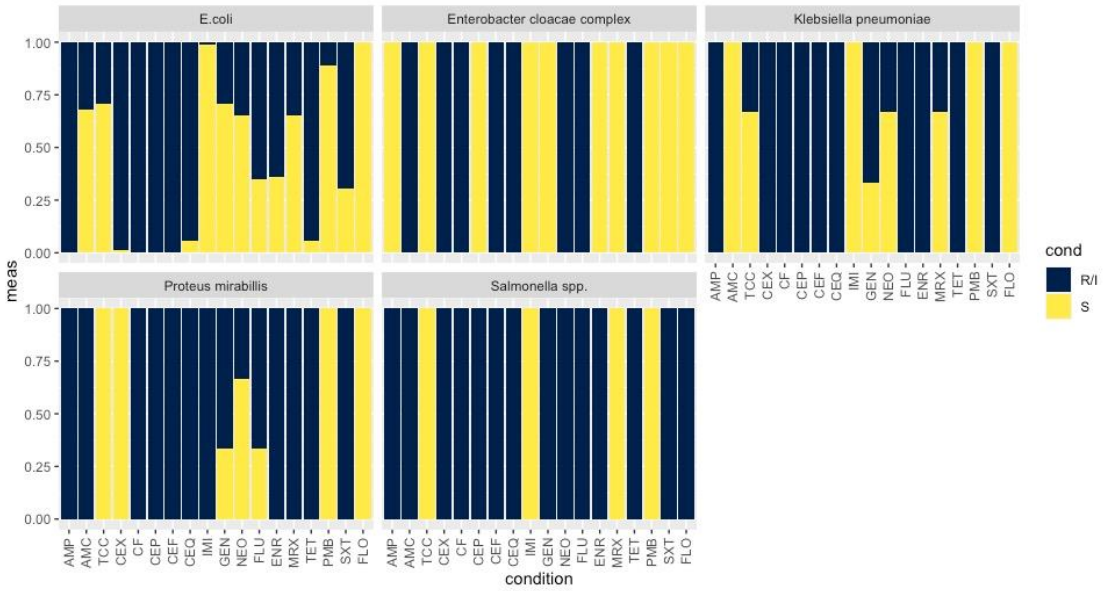

Figure 1. Bar plot showing the percentage of AMR of *E. coli*, *E. cloacae* complex, *K. pneumoniae*, *P. mirabilis* and *Salmonella* spp. to a range of antibiotics. AMP, AMC, TCC, CEX, CF, CEP, CEF, CEQ, IMI, GEN, NEO, FLU, ENR, MRX, TET, SXT and FLO. The values varied from 0.00 to 1.00; 0.00 indicates resistance and 1.00 susceptibility. R/I: Resistance/Intermediate results; S: Susceptibility; AMP: ampicillin, AMC: amoxicillin/clavulanic acid, TCC: ticarcillin/clavulanic acid, CEX: cefalexin, CF: cefalotin, CEP: cefoperazone, CEF: ceftiofur, CEQ: cefquinome, IMI: imipenem, GEN: gentamicin, NEO: neomycin, FLU: flumequine, ENR: enrofloxacin, MRX: marbofloxacin, TET: tetracycline, FLO: florfenicol, PMB: polymyxin B, SXT: trimethoprim/sulfamethoxazole (SXT).

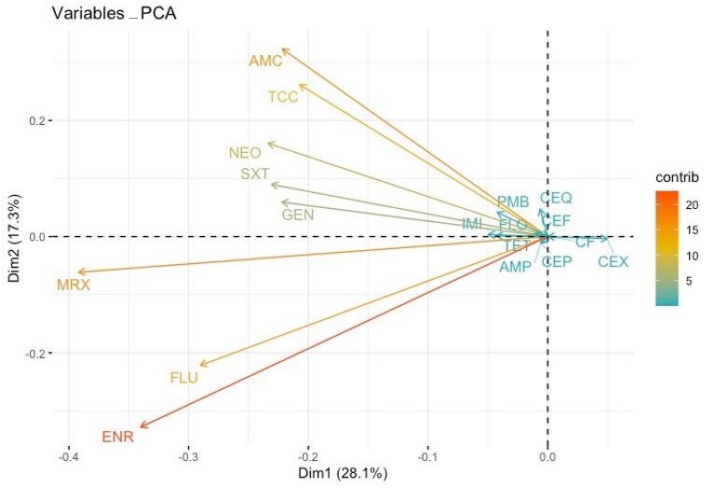

Figure 2. Principal component analysis (PCA) on co-resistances. Antibiotics are represented by vectors (arrows). Two vectors of antibiotics pointing in the same direction is an indication of a positive correlation between them; when we observe AMR in one antibiotic, we are expecting the development of AMR in the other one. An angle of 180 degrees between the vectors of two antibiotics is an indication of a negative correlation between them; when we observe AMR in one, we are not expecting the development of AMR in the other. A 90-degree angle between the vectors of two antibiotics indicates no relationship between them toward the developing AMR. The longer the vectors, the greater the intensity of this relationship. AMP: ampicillin, AMC: amoxicillin/clavulanic acid, TCC: ticarcillin/clavulanic acid, CEX: cefalexin, CF: cefalotin, CEP: cefoperazone, CEF: ceftiofur, CEQ: cefquinome, IMI: imipenem, GEN: gentamicin, NEO: neomycin, FLU: flumequine, ENR: enrofloxacin, MRX: marbofloxacin, TET: tetracycline, FLO: florfenicol, PMB: polymyxin B, SXT: trimethoprim/sulfamethoxazole (SXT).

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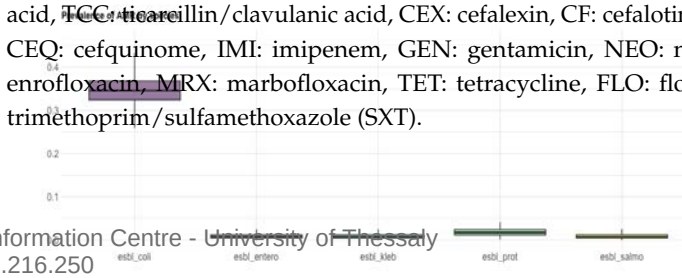

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otics indicates no relationship between them toward the developing AMR. The longer the vectors, the greater the intensity of this relationship. AMP: ampicillin, AMC: amoxicillin/clavulanic acid, TCC: ticarcillin/clavulanic acid, CEX: cefalexin, CF: cefalotin, CEP: cefoperazone, CEF: ceftiofur, CEQ: cefquinome, IMI: imipenem, GEN: gentamicin, NEO: neomycin, FLU: flumequine, ENR: enrofloxacin, MRX: marbofloxacin, TET: tetracycline, FLO: florfenicol, PMB: polymyxin B, SXT: trimethoprim/sulfamethoxazole (SXT).

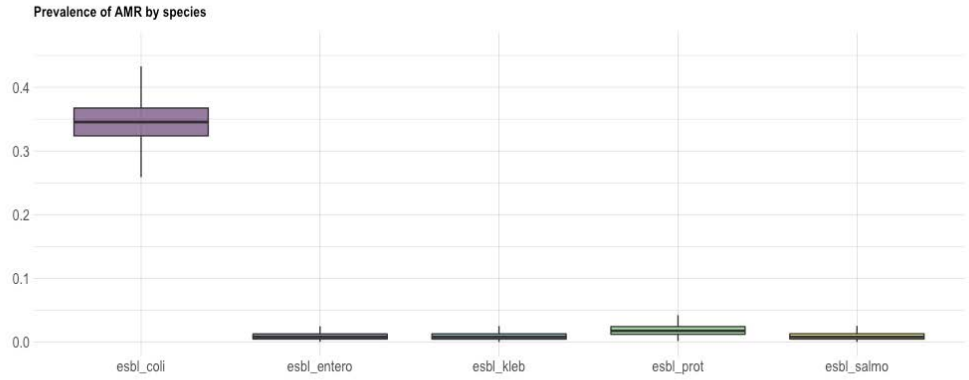

Figure 3. Box and whisker plot showing the prevalence of AMR (*y* axis) of ESBL-producing *E. coli*, *Enterobacter* complex, *K. pneumoniae*, *P. mirabilis* and *Salmonella* spp. (*x* axis). The bold line shows the median value, while the length of the box represents the interquartile range.

Figure 3. Box and whisker plot showing the prevalence of AMR (*y* axis) of ESBL-producing *E. coli*, *E. cloacae* complex, *K. pneumoniae*, *P. mirabilis* and *Salmonella* spp. (*x* axis). The bold line shows the median value, while the length of the box represents the interquartile range.

P. mirabilis isolates (n = 4) were resistant to fluoroquinolones (ENR; n = 4, FLU; n = 3, MRX; n = 3), TET and SXT, while a resistant phenotype for aminoglycosides (GEN; n = 3, NEO; n = 1) was observed in three of them (Figures 1 and 3). Antibacterial resistance genes revealed the presence of genes conferring resistance to sulfonamide (*sul1*, *sul2*) and trimethoprim (*dfrA1*, *dfrA5*, *dfrA17*) in all four of them, as well as of aminoglycoside-resistance genes in three (*aadA1*, *aadA2*, *aphA*, *aac*(6′)-*Ib*, *aac*(3′)-*Iva*). Furthermore, three of the strains harbored *intl2*.

The isolate of the *E. cloacae* complex presented intermediate resistance for NEO and TET, was resistant to FLU (Figures 1 and 3) and was only found to harbor *dfrA5*.

Finally, the *S. enterica* spp. *diarizonae* exhibited resistance to fluoroquinolones (FLU, ENR), TET, SXT, aminoglycosides (GEN, NEO) and FLO (Figures 1 and 3). The isolate carried resistance determinants against fluoroquinolones (*qnrS*), sulfonamides (*sul2*, *sul3*), trimethoprim (*dfrA12*, *dfrA17*) and aminoglycosides (*aadA1*, *aadA2*, *aadA4*, *aphA*), as well as the tnpISE*cp1*.

The antimicrobial resistance phenotype and genotype of the ESBL isolates are detailed in Supplementary File S2. The resistance genes detected among the ESBL-producing Enterobacteriaceae are presented in Table 4.

Table 4. Resistance genes detected among the ESBL-producing Enterobacteriaceae.

Resistance Genes	Number of Isolates				
	E. coli	*K. pneumoniae*	*P. mirabilis*	*S. enterica* ssp. *diarizonae*	*E. cloacae* Complex
$bla_{\text{CTX-M1/15}}$	52	2	-	-	-
$bla_{\text{CTX-M9}}$	6	1	3	-	-
$bla_{\text{CTX-M8}}$	6	-	1	-	-
bla_{SHV}	3	2	-	-	-
bla_{TEM}	38	1	3	1	-
bla_{ACT}	18	-	-	-	-
bla_{CMY}	1	-	-	-	-
$bla_{\text{OXA-1}}$	17	-	4	-	-
$bla_{\text{OXA-60}}$	1	-	-	-	-
aadA1	36	1	3	1	-
aadA2	21	-	1	1	-
aadA4	14	-	-	1	-
aphA	21	1	1	1	-
rmtA	27	-	-	-	-

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Table 4. *Cont.*

Resistance Genes	Number of Isolates				
	E. coli	***K. pneumoniae***	***P. mirabilis***	***S. enterica* ssp. *diarizonae***	***E. cloacae* Complex**
rmtC	7	-	-	-	-
aac(6′)-Ib	2	-	3	-	-
aac(3′)-Iva	1	-	3	-	-
qnrS	29	3	-	1	-
qnrA	2	-	-	-	-
qnrB	4	-	-	-	-
sul1	18	2	3	-	-
sul2	38	3	4	1	-
sul3	17	-	-	1	-
dfrA1	13	1	3	-	-
dfrA5	42	-	2	-	1
dfrA7	4	1	-	-	-
dfrA12	12	-	-	1	-
dfrA13	2	-	-	-	-
dfrA14	12	1	-	-	-
dfrA15	1	-	-	-	-
dfrA17	15	1	1	1	-
dfrA19	4	-	-	-	-
mcr-1/mcr-2	6	-	-	1	-
mcr-4	1	-	-	-	-
mcr-8	3	-	-	1	-
mph	12	-	-	-	-
mrx	16	-	-	-	-
intl1	20	2	-	-	-
intl2	7	1	3	-	-
tnpISEcp1	30	3	-	1	-
oqxA	1	3	-	-	-
oqxB	1	3	-	-	-

3.4. Logistic Regression Analysis Results

Logistic regression analysis demonstrated that the presence of ESBL-producing *E. coli* strains was negatively associated with prior administration of PMLs, and that the occurrence of ESBL-producing *P. mirabilis* was associated with herd size. Further associations concerning ESBL-producing *Enterobacteriaceae* were not recognized by the model. The results are presented in Table 5.

Table 5. Associations of ESBL-producing Enterobacteriaceae with herd characteristics and administration of antibiotics.

ESBL-Producing	Logistic Regression			
	Parameter	**Category**	**Estimate (95% CI)**	***p* Value**
E. coli	PMLs	0	1	
		1	0.25 (0.07; 0.84)	0.0215
P. mirabilis	Size	-	85.86 (4.02; 6620.06)	0.00648

The reported antimicrobial resistances of this study were not related to the sows' ages. The development of resistances to AMP and AMC, as well as the diminished susceptibility

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to IMI, were positively associated with the farm size. By examining whether previous administration of antibiotics led to the development of AMR, we recognized a series of positive associations between (a) GEN resistance and previous administration of quinolones, (b) NEO resistance and previous usage of TETs, (c) MRX resistance and prior application of TETs and (d) ENR resistance and prior administration of cephalosporins. Finally, the previous application of PLMs was negatively associated with the development of resistance to both GEN and NEO. The results are described in Table 6.

Table 6. Association of AMR with herd characteristics and administration of antibiotics.

Antibiotic	Logistic Regression			
	Parameter	Category	Estimate (95% CI)	*p* Value (Random Effect)
AMP	Size		48.72 (3.21; 1955.14)	0.0171
AMC	Size		31.38 (5.3; 290.81)	<0.005
IMI	Size		33.09 (2.16; 1454.48)	0.008
GEN	PMLs	0	1	
		1	0.15 (0.02; 0.58)	0.016
	Quinolones	0	1	
		1	13.14 (2.46; 243.71)	0.015
NEO	PMLs	0	1	
		1	0.19 (0.03; 1.05)	0.028
	TETs	0	1	
		1	7.7 (1.62; 59.44)	0.009
MRX	TETs	0	1	
		1	14.45 (0.96; 5.59)	0.011
ENR	Cephalosporins	0	1	
		1	3.49 (1.07; 14.84)	0.045

4. Discussion

The present study aimed to describe, for the first time, the frequency of ESBL-producing *Enterobacteriaceae* from 34 pig farms located in different geographical regions of Greece, and to characterize their AMR phenotype and genotype. To that end, we collected and tested 214 fecal samples from 73 suckling and 141 weaning piglets from herds that met certain inclusion criteria. The most commonly isolated ESBL producers were *E. coli* strains that presented co-resistance to at least one class of non β-lactam antibiotics. In addition, *K. pneumoniae*, *P. mirabilis*, *E. cloacae* complex and *S. enterica* subsp. *diarizonae* isolates were identified as ESBL producers. Notably, four *P. mirabilis* strains displayed diminished susceptibility to IMI.

Resistance to AMC was observed in ESBL-producing *E. coli* isolates (Figure 3). In the studied farms, injectable amoxicillin was used postpartum in sows as part of a routine program of metaphylaxis for PPDS, and it was also added in weaning feed for the prevention and metalphylaxis of PWD. Resistance to amoxicillin has not become a major problem to date, because it is usually combined with clavulanic acid, a highly effective ESBL inhibitor [5]. As this antimicrobial is routinely administered in pigs against respiratory (e.g., bacterial pneumonia), enteric (post weaning diarrhea) and urogenital (e.g., PPDS) diseases, its unnecessary usage could further promote the selection of ESBL-producing bacteria. Therefore, laboratory diagnosis based on bacterial culture and sensitivity testing is mandatory prior to its application [5]. The observed AMC resistance of *E. coli* isolates retrieved from the piglets could be also associated with the administration of amoxicillin in sows postpartum, considering that vertical transmission of resistant bacteria from sows to piglets has been already documented [38]. However, future studies, including investigation of related risk factors (e.g., farm capacity and management, previous and current treatments

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with antibiotics, age, and treatment groups) will help us understand the development of AMR under field conditions.

ESBL-producing *E. coli* strains presented co-resistances to fluoroquinolones (n = 49), aminoglycosides (n = 31), TETs (n = 26) and SXT (n = 46), confirming previously reported antimicrobial resistance patterns of ESBL-producing *E. coli* [39–42]. In fact, the occurrence of strains resistant to AMP, AMC, SXT and TET [43,44], and the occurrence of ESBL producers resistant to at least one more class of non β-lactam antibiotics, have been formerly described in Greece [45]. We also observed increased resistance levels with regard to fluoroquinolones, which are widely used in swine clinical practice as first-choice agents for individual injectable treatment for respiratory, enteric and urogenital disease. This finding could be attributed to the extensive use of this agent at farm level and should be considered by veterinarians and farmers, especially in acute clinical cases that demand rapid treatment processes.

The occurrence of four *P. mirabilis* ESBL-producing isolates was reported and all strains presented reduced susceptibility to IMI. At first, this was an alarming finding, considering the prohibition of the use of IMI in livestock [46] and the reported carbapenemase resistance in humans [47,48]. As these isolates were not found to harbor a carbapenemase gene, the reported IMI resistance could be explained by pore mutations or mutations affecting a penicillin binding protein [49]. Furthermore, *P. mirabilis* isolates were also resistant to fluoroquinolones, TETs and SXT, while three of them had a resistant phenotype for aminoglycosides. This bacterial species is considered to be the most common etiological agent of PPDS in sows, causing severe economic losses in swine industry, whereas fluoroquinolones, TET and SXT are recommended as first-choice antimicrobial treatments [6]. Our results reveal a potential risk for treatment failure in field cases of PPDS, further emphasizing the importance of etiological diagnosis and sensitivity testing prior to the use of antibiotics by swine practitioners.

An ESBL-producing *S. enterica* subsp. *diarizonae* isolate was also noticed, presenting co-resistance to aminoglycosides, fluoroquinolones (FLU, ENR), TETs, SXT and FLO. Serovars of ESBL-producing *S. enterica* of poultry origin, co-resistant to aminoglycosides, TETs and SXT have previously been reported in Greece [50]. Herein, we identified a multidrug-resistant serovar in pigs that is usually isolated from humans [51], and thus, our results underline that AMR in livestock could pose, through the food chain, a serious threat to public health.

Molecular characterization of the ESBL isolates revealed the predominance of CTX-M type genes, mainly those of group 1, which is in accordance with preexisting literature about pigs [52–54]. Interestingly, various other genes conferring resistance to fluoroquinolones, aminoglycosides, sulfonamides, trimethoprim, macrolides and colistin were detected in the present study. This finding underlines the wide dissemination of AMR determinants among animals farmed for human consumption, which may have been facilitated by the detected mobile genetic elements [55]. The co-occurrence of *mcr* variants is especially noteworthy given the importance of colistin as a last-resort therapeutic option against multidrug-resistant strains in clinical settings. Colistin is commonly used in pigs to prevent and control the clinical outcomes of *E. coli* infection, including neonatal diarrhea, post-weaning diarrhea and edema disease [56]. Gene *mcr*-1, one of the most common colistin-resistance genes around the world, was detected among *E. coli* isolates from Chinese pigs at slaughter and retail meats, and its occurrence was speculated to be a result of colistin usage [57]. In Greece, multidrug-resistant *mcr*-1-positive ESBL-producing *E. coli* has previously been recovered on a dairy farm and was the etiological agent of mastitis [58].

Logistic regression analysis demonstrated a positive association between the herd size and the development of antimicrobial resistance to AMP, AMC and IMI, further confirming previous reports [59,60]. Specifically, it was more likely for AMR to appear in larger farms. A potential explanation for the observed AMP and AMC resistance could be provided by the routine administration of these antimicrobials, as infections in large herds are a more common phenomenon by reason of the higher swine density and the increased rates of gilt

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replacement from external sources [56]. On the contrary, diminished susceptibility to IMI cannot be ascribed to the usage of carbapenems, since this class of antibiotics is banned for swine due to the risk of compromising human treatments. In *P. mirabilis*, IMI resistance can be considered random, and it could be speculated that exposure to other beta-lactams might have been the driver (as the mechanism is unspecific).

At a national level, the usage of specific antimicrobials is strongly associated with the development of resistance towards these agents in commensal *E. coli* isolates in pigs, poultry and cattle [61]. We report a positive association between previous administration of TETs and the development of resistance to NEO and MRX, while increased GEN resistance was observed following quinolone administration. It is well-known that the use of different classes of antibiotics depends on the type of infections to be treated, and varies according to the pigs' ages [1]. For instance, the gut microbiota is more exposed to orally administered tetracycline than to injected tetracycline, and thus an increase in AMR bacteria in the intestinal microbiome could be promoted by oral administration [62]. It is critical to expand our knowledge on the impact of the administration of different antibiotics in pigs, as increased resistance in bacteria may impair treatment efficacy and potentially lead to therapeutic failure.

Recent studies in Greece and worldwide have described the AMR profile of ESBL-producing *E. coli* strains isolated from livestock, including pigs [9,45,54]. Currently, as a wide dissemination of multidrug resistant bacteria in diverse ecosystems is observed, the establishment of an integrated antimicrobial surveillance system under the One Health approach is crucial for the protection of public health. The AMR profile of the ESBL-producing *Enterobacteriaceae* isolates presented in this study demonstrates the urgent need for the application of a monitoring program targeting preferentially multiple species.

5. Conclusions

We report a high percentage of ESBL-producing bacteria in the Greek swine industry, which is triggering alarm for veterinary practitioners and farmers as well as for public health authorities. The 78 ESBL producers (36.5%) were identified as *E. coli* (n = 69), *K. pneumoniae* (n = 3), *P. mirabilis* (n = 4), *E. cloacae* complex (n = 1) and *S. enterica* subsp. *diarizonae* (n = 1), and presented resistance to at least one class of non β-lactam antibiotics. CTX-M-1/15 enzymes were the most frequently observed ESBLs, followed by TEM and SHV types, and accompanied by several other resistance determinants for fluoroquinolones, aminoglycosides, sulfonamides, trimethoprim, macrolides, colistin and mobile genetic elements. As AMR increases the cost of animal production, antimicrobial usage must be prudent and based on laboratory diagnosis and antimicrobial sensitivity testing, in order to keep antibiotics as a therapeutic weapon in experts' hands. The integration of a surveillance system for AMR monitoring under the One Health approach will contribute not only to the rational use of antibiotics, but also to the protection of public health.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ani12121560/s1, Supplementary File S1: Genes detected by the CarbaResist DNA microarray-based assay; Supplementary File S2: Phenotype and Genotype of the ESBL isolates.

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Institutional Review Board Statement: All samples were obtained by noninvasive rectal swabs and no research on animals, as defined in the EU Ethics for Researchers document (European Commission, 2013, Ethics for Researchers-Facilitating Research Excellence in FP7, Luxembourg: Office for Official Publications of the European Communities, ISBN 978-92-79-28854-8), was carried out for this study. Handling and sampling of the animals complied with European and national legislation and the study protocol was approved by the academic board of the Veterinary Faculty of the University of Thessaly, Meeting 96/19.12.2019.

Data Availability Statement: All data generated for this study are presented within the manuscript and the supplementary files.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Lekagul, A.; Tangcharoensathien, V.; Yeung, S. Patterns of Antibiotic Use in Global Pig Production: A Systematic Review. *Vet. Anim. Sci.* **2019**, *7*, 100058. [CrossRef]
2. Aarestrup, F.M. Veterinary Drug Usage and Antimicrobial Resistance in Bacteria of Animal Origin. *Basic Clin. Pharmacol. Toxicol.* **2005**, *96*, 271–281. [CrossRef]
3. Landers, T.F.; Cohen, B.; Wittum, T.E.; Larson, E.L. A Review of Antibiotic Use in Food Animals: Perspective, Policy, and Potential. *Public Health Rep.* **2012**, *127*, 4–22. [CrossRef]
4. World Health Organization. *WHO Guidelines on Use of Medically Important Antimicrobials in Food-Producing Animals*; World Health Organization: Geneva, Switzerland, 2017.
5. Burch, D.G.S.; Sperling, D. Amoxicillin—Current Use in Swine Medicine. *J. Vet. Pharmacol. Ther.* **2018**, *41*, 356–368. [CrossRef]
6. Papatsiros, V.G.; Alexopoulos, C.; Kyriakis, S.C. Latest Information in Relation to Postpartum Dysgalactia Syndrome of Sows. *J. Hell. Vet. Med. Soc.* **2007**, *58*, 61–75. [CrossRef]
7. ECDC/EFSA/EMA Second Joint Report on the Integrated Analysis of the Consumption of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Humans and Food-Producing Animals | EFSA. Available online: https://www.efsa.europa.eu/en/efsajournal/pub/4872 (accessed on 4 May 2022).
8. European Food Safety Authority; European Centre for Disease Prevention and Control. The European Union Summary Report on Antimicrobial Resistance in Zoonotic and Indicator Bacteria from Humans, Animals and Food in 2018/2019. *EFSA J.* **2021**, *19*, e06490. [CrossRef]
9. Bengtsson, B.; Greko, C. Antibiotic Resistance—Consequences for Animal Health, Welfare, and Food Production. *Ups. J. Med. Sci.* **2014**, *119*, 96–102. [CrossRef]
10. Van Boeckel, T.P.; Brower, C.; Gilbert, M.; Grenfell, B.T.; Levin, S.A.; Robinson, T.P.; Teillant, A.; Laxminarayan, R. Global Trends in Antimicrobial Use in Food Animals. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 5649–5654. [CrossRef]
11. Nhung, N.T.; Cuong, N.V.; Thwaites, G.; Carrique-Mas, J. Antimicrobial Usage and Antimicrobial Resistance in Animal Production in Southeast Asia: A Review. *Antibiotics* **2016**, *5*, 37. [CrossRef]
12. Pollock, J.; Muwonge, A.; Hutchings, M.R.; Mainda, G.; Bronsvoort, B.M.; Gally, D.L.; Corbishley, A. Resistance to Change: AMR Gene Dynamics on a Commercial Pig Farm with High Antimicrobial Usage. *Sci. Rep.* **2020**, *10*, 1708. [CrossRef]
13. Pholwat, S.; Pongpan, T.; Chinli, R.; Rogawski McQuade, E.T.; Thaipisuttikul, I.; Ratanakorn, P.; Liu, J.; Taniuchi, M.; Houpt, E.R.; Foongladda, S. Antimicrobial Resistance in Swine Fecal Specimens Across Different Farm Management Systems. *Front. Microbiol.* **2020**, *11*, 1238. [CrossRef]
14. Bertelloni, F.; Cilia, G.; Bogi, S.; Ebani, V.V.; Turini, L.; Nuvoloni, R.; Cerri, D.; Fratini, F.; Turchi, B. Pathotypes and Antimicrobial Susceptibility of Escherichia Coli Isolated from Wild Boar (Sus Scrofa) in Tuscany. *Animals* **2020**, *10*, 744. [CrossRef]
15. Cilia, G.; Turchi, B.; Fratini, F.; Bilei, S.; Bossù, T.; De Marchis, M.L.; Cerri, D.; Pacini, M.I.; Bertelloni, F. Prevalence, Virulence and Antimicrobial Susceptibility of Salmonella Spp., Yersinia Enterocolitica and Listeria Monocytogenes in European Wild Boar (Sus Scrofa) Hunted in Tuscany (Central Italy). *Pathogens* **2021**, *10*, 93. [CrossRef]
16. Munita, J.M.; Arias, C.A. Mechanisms of Antibiotic Resistance. *Microbiol. Spectr.* **2016**, *4*, 2–4. [CrossRef]
17. Lee, W.-C.; Yeh, K.-S. Characteristics of Extended-Spectrum β-Lactamase–Producing Escherichia Coli Isolated from Fecal Samples of Piglets with Diarrhea in Central and Southern Taiwan in 2015. *BMC Vet. Res.* **2017**, *13*, 66. [CrossRef]
18. Livermore, D.M.; Woodford, N. The β-Lactamase Threat in Enterobacteriaceae, Pseudomonas and Acinetobacter. *Trends Microbiol.* **2006**, *14*, 413–420. [CrossRef]

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06/09/2026 21:12:45 EEST - 216.73.216.250

19. Peirano, G.; Pitout, J.D.D. Extended-Spectrum β-Lactamase-Producing Enterobacteriaceae: Update on Molecular Epidemiology and Treatment Options. *Drugs* **2019**, *79*, 1529–1541. [CrossRef]
20. Bergšpica, I.; Kaprou, G.; Alexa, E.A.; Prieto, M.; Alvarez-Ordóñez, A. Extended Spectrum β-Lactamase (ESBL) Producing Escherichia Coli in Pigs and Pork Meat in the European Union. *Antibiotics* **2020**, *9*, 678. [CrossRef]
21. Pitout, J.D.; Laupland, K.B. Extended-Spectrum β-Lactamase-Producing Enterobacteriaceae: An Emerging Public-Health Concern. *Lancet Infect. Dis.* **2008**, *8*, 159–166. [CrossRef]
22. Smet, A.; Martel, A.; Persoons, D.; Dewulf, J.; Heyndrickx, M.; Herman, L.; Haesebrouck, F.; Butaye, P. Broad-Spectrum β-Lactamases among Enterobacteriaceae of Animal Origin: Molecular Aspects, Mobility and Impact on Public Health. *FEMS Microbiol. Rev.* **2010**, *34*, 295–316. [CrossRef]
23. Ewers, C.; Bethe, A.; Semmler, T.; Guenther, S.; Wieler, L.H. Extended-Spectrum β-Lactamase-Producing and AmpC-Producing Escherichia Coli from Livestock and Companion Animals, and Their Putative Impact on Public Health: A Global Perspective. *Clin. Microbiol. Infect.* **2012**, *18*, 646–655. [CrossRef]
24. Jouini, A.; Vinué, L.; Slama, K.B.; Sáenz, Y.; Klibi, N.; Hammami, S.; Boudabous, A.; Torres, C. Characterization of CTX-M and SHV Extended-Spectrum β-Lactamases and Associated Resistance Genes in Escherichia Coli Strains of Food Samples in Tunisia. *J. Antimicrob. Chemother.* **2007**, *60*, 1137–1141. [CrossRef]
25. Overdevest, I.; Willemsen, I.; Rijnsburger, M.; Eustace, A.; Xu, L.; Hawkey, P.; Heck, M.; Savelkoul, P.; Vandenbroucke-Grauls, C.; van der Zwaluw, K.; et al. Extended-Spectrum β-Lactamase Genes of Escherichia Coli in Chicken Meat and Humans, The Netherlands. *Emerg. Infect. Dis.* **2011**, *17*, 1216–1222. [CrossRef]
26. Leverstein-van Hall, M.A.; Dierikx, C.M.; Cohen Stuart, J.; Voets, G.M.; van den Munckhof, M.P.; van Essen-Zandbergen, A.; Platteel, T.; Fluit, A.C.; van de Sande-Bruinsma, N.; Scharinga, J.; et al. Dutch Patients, Retail Chicken Meat and Poultry Share the Same ESBL Genes, Plasmids and Strains. *Clin. Microbiol. Infect.* **2011**, *17*, 873–880. [CrossRef]
27. Kola, A.; Kohler, C.; Pfeifer, Y.; Schwab, F.; Kühn, K.; Schulz, K.; Balau, V.; Breitbach, K.; Bast, A.; Witte, W.; et al. High Prevalence of Extended-Spectrum-β-Lactamase-Producing Enterobacteriaceae in Organic and Conventional Retail Chicken Meat, Germany. *J. Antimicrob. Chemother.* **2012**, *67*, 2631–2634. [CrossRef]
28. Dohmen, W.; Bonten, M.J.M.; Bos, M.E.H.; van Marm, S.; Scharringa, J.; Wagenaar, J.A.; Heederik, D.J.J. Carriage of Extended-Spectrum β-Lactamases in Pig Farmers Is Associated with Occurrence in Pigs. *Clin. Microbiol. Infect.* **2015**, *21*, 917–923. [CrossRef]
29. National Research Council. *Nutrient Requirements of Swine (NRC): Eleventh Revised Edition*; The National Academies Press (NAP): Washington, DC, USA, 2012.
30. *Breakpoint Tables for Interpretation of MICs and Zone Diameters*, Version 10.0; The European Committee on Antimicrobial Susceptibility Testing: Växjö, Sweden, 2020; 77.
31. Braun, S.D.; Jamil, B.; Syed, M.A.; Abbasi, S.A.; Weiß, D.; Slickers, P.; Monecke, S.; Engelmann, I.; Ehricht, R. Prevalence of Carbapenemase-Producing Organisms at the Kidney Center of Rawalpindi (Pakistan) and Evaluation of an Advanced Molecular Microarray-Based Carbapenemase Assay. *Future Microbiol.* **2018**, *13*, 1225–1246. [CrossRef]
32. Butiuc-Keul, A.; Carpa, R.; Podar, D.; Szekeres, E.; Muntean, V.; Iordache, D.; Farkas, A. Antibiotic Resistance in *Pseudomonas* spp. Through the Urban Water Cycle. *Curr. Microbiol.* **2021**, *78*, 1227–1237. [CrossRef]
33. Gelman, A.; Carlin, J.B.; Stern, H.S.; Rubin, D.B. *Bayesian Data Analysis*; Chapman and Hall/CRC: New York, NY, USA, 1995; ISBN 978-0-429-25841-1.
34. R Core Team (2020). European Environment Agency. Available online: https://www.eea.europa.eu/data-and-maps/indicators/oxygen-consuming-substances-in-rivers/r-development-core-team-2006 (accessed on 4 May 2022).
35. Denwood, M.J. Runjags: An R Package Providing Interface Utilities, Model Templates, Parallel Computing Methods and Additional Distributions for MCMC Models in JAGS. *J. Stat. Softw.* **2016**, *71*, 1–25. [CrossRef]
36. Wickham, H. Ggplot2. *WIREs Comput. Stat.* **2011**, *3*, 180–185. [CrossRef]
37. Lee, W.; Grimm, K.J. Generalized Linear Mixed-Effects Modeling Programs in R for Binary Outcomes. *Struct. Equ. Modeling A Multidiscip. J.* **2018**, *25*, 824–828. [CrossRef]
38. Burow, E.; Rostalski, A.; Harlizius, J.; Gangl, A.; Simoneit, C.; Grobbel, M.; Kollas, C.; Tenhagen, B.-A.; Käsbohrer, A. Antibiotic Resistance in Escherichia Coli from Pigs from Birth to Slaughter and Its Association with Antibiotic Treatment. *Prev. Vet. Med.* **2019**, *165*, 52–62. [CrossRef] [PubMed]
39. Liu, X.; Liu, H.; Wang, L.; Peng, Q.; Li, Y.; Zhou, H.; Li, Q. Molecular Characterization of Extended-Spectrum β-Lactamase-Producing Multidrug Resistant Escherichia Coli from Swine in Northwest China. *Front. Microbiol.* **2018**, *9*, 1756. [CrossRef]
40. Aguirre, L.; Vidal, A.; Seminati, C.; Tello, M.; Redondo, N.; Darwich, L.; Martín, M. Antimicrobial Resistance Profile and Prevalence of Extended-Spectrum Beta-Lactamases (ESBL), AmpC Beta-Lactamases and Colistin Resistance (Mcr) Genes in Escherichia Coli from Swine between 1999 and 2018. *Porc. Health Manag.* **2020**, *6*, 8. [CrossRef]
41. Tamta, S.; Kumar, O.R.V.; Singh, S.V.; Pruthvishree, B.S.; Karthikeyan, R.; Rupner, R.; Sinha, D.K.; Singh, B.R. Antimicrobial Resistance Pattern of Extended-Spectrum β-Lactamase-Producing Escherichia Coli Isolated from Fecal Samples of Piglets and Pig Farm Workers of Selected Organized Farms of India. *Vet. World* **2020**, *13*, 360–363. [CrossRef]
42. Lee, S.; An, J.-U.; Guk, J.-H.; Song, H.; Yi, S.; Kim, W.-H.; Cho, S. Prevalence, Characteristics and Clonal Distribution of Extended-Spectrum β-Lactamase- and AmpC β-Lactamase-Producing Escherichia Coli Following the Swine Production Stages, and Potential Risks to Humans. *Front. Microbiol.* **2021**, *12*, 710747. [CrossRef]

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06/09/2026 21:12:45 EEST - 216.73.216.250

43. Valiakos, G.; Vontas, A.; Tsokana, C.N.; Giannakopoulos, A.; Chatzopoulos, D.; Billinis, C. Resistance in *Escherichia Coli* Strains Isolated from Pig Faecal Samples and Pig Farm Workers, Greece. *Am. J. Anim. Vet. Sci.* **2016**, *11*, 142–144. [CrossRef]
44. Papadopoulos, D.; Papadopoulos, T.; Papageorgiou, K.; Sergelidis, D.; Adamopoulou, M.; Kritas, S.K.; Petridou, E. Antimicrobial Resistance Rates in Commensal Escherichia Coli Isolates from Healthy Pigs in Greek Swine Farms. *J. Hell. Vet. Med. Soc.* **2021**, *72*, 2909–2916. [CrossRef]
45. Athanasakopoulou, Z.; Reinicke, M.; Diezel, C.; Sofia, M.; Chatzopoulos, D.C.; Braun, S.D.; Reissig, A.; Spyrou, V.; Monecke, S.; Ehricht, R.; et al. Antimicrobial Resistance Genes in ESBL-Producing Escherichia Coli Isolates from Animals in Greece. *Antibiotics* **2021**, *10*, 389. [CrossRef]
46. Poirel, L.; Stephan, R.; Perreten, V.; Nordmann, P. The Carbapenemase Threat in the Animal World: The Wrong Culprit. *J. Antimicrob. Chemother.* **2014**, *69*, 2007–2008. [CrossRef]
47. Scientific Opinion on Carbapenem Resistance in Food Animal Ecosystems | EFSA. Available online: https://www.efsa.europa.eu/en/efsajournal/pub/3501 (accessed on 4 May 2022).
48. Yang, P.; Chen, Y.; Jiang, S.; Shen, P.; Lu, X.; Xiao, Y. Association between Antibiotic Consumption and the Rate of Carbapenem-Resistant Gram-Negative Bacteria from China Based on 153 Tertiary Hospitals Data in 2014. *Antimicrob. Resist. Infect. Control.* **2018**, *7*, 137. [CrossRef]
49. Villar, H.E.; Danel, F.; Livermore, D.M. Permeability to Carbapenems of Proteus Mirabilis Mutants Selected for Resistance to Imipenem or Other Beta-Lactams. *J. Antimicrob. Chemother.* **1997**, *40*, 365–370. [CrossRef]
50. Politi, L.; Tassios, P.T.; Lambiri, M.; Kansouzidou, A.; Pasiotou, M.; Vatopoulos, A.C.; Mellou, K.; Legakis, N.J.; Tzouvelekis, L.S. Repeated Occurrence of Diverse Extended-Spectrum β-Lactamases in Minor Serotypes of Food-Borne Salmonella Enterica Subsp. Enterica. *J. Clin. Microbiol.* **2005**, *43*, 3453–3456. [CrossRef]
51. Rubira, I.; Figueras, L.P.; Jiménez, J.C.; de Arcaute, M.R.; Ruiz, H.; Ventura, J.A.; Lacasta, D. *Salmonella Enterica Subsp. Diarizonae Serotype 61:K:1:5:(7) a Host Adapted to Sheep*; IntechOpen: London, UK, 2021; ISBN ISBN 978-1-83969-018-1.
52. Bernreiter-Hofer, T.; Schwarz, L.; Müller, E.; Cabal-Rosel, A.; Korus, M.; Misic, D.; Frankenfeld, K.; Abraham, K.; Grünzweil, O.; Weiss, A.; et al. The Pheno- and Genotypic Characterization of Porcine Escherichia Coli Isolates. *Microorganisms* **2021**, *9*, 1676. [CrossRef]
53. Balázs, B.; Nagy, J.B.; Tóth, Z.; Nagy, F.; Károlyi, S.; Turcsányi, I.; Bistyák, A.; Kálmán, A.; Sárközi, R.; Kardos, G. Occurrence of Escherichia Coli Producing Extended Spectrum β-Lactamases in Food-Producing Animals. *Acta Vet. Hung.* **2021**, *69*, 211–215. [CrossRef]
54. Giufrè, M.; Mazzolini, E.; Cerquetti, M.; Brusaferro, S.; Accogli, M.; Agnoletti, F.; Agodi, A.; Alborali, G.L.; Arghittu, M.; Auxilia, F.; et al. Extended-Spectrum β-Lactamase-Producing Escherichia Coli from Extraintestinal Infections in Humans and from Food-Producing Animals in Italy: A 'One Health' Study. *Int. J. Antimicrob. Agents* **2021**, *58*, 106433. [CrossRef]
55. Madec, J.Y.; Haenni, M.; Nordmann, P.; Poirel, L. Extended-Spectrum β-Lactamase/AmpC- and Carbapenemase-Producing Enterobacteriaceae in Animals: A Threat for Humans? *Clin. Microbiol. Infect.* **2017**, *23*, 826–833. [CrossRef]
56. Lekagul, A.; Tangcharoensathien, V.; Mills, A.; Rushton, J.; Yeung, S. How antibiotics are used in pig farming: A mixed-methods study of pig farmers, feed mills and veterinarians in Thailand. *BMJ Glob. Health* **2020**, *5*, e001918. [CrossRef]
57. Liu, Y.Y.; Wang, Y.; Walsh, T.R.; Yi, L.X.; Zhang, R.; Spencer, J.; Doi, Y.; Tian, G.; Dong, B.; Huang, X.; et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: A microbiological and molecular biological study. *Lancet Infect. Dis.* **2016**, *16*, 161–168. [CrossRef]
58. Filioussis, G.; Kachrimanidou, M.; Christodoulopoulos, G.; Kyritsi, M.; Hadjichristodoulou, C.; Adamopoulou, M.; Tzivara, A.; Kritas, S.K.; Grinberg, A. Short communication: Bovine mastitis caused by a multidrug-resistant, mcr-1-positive (colistin-resistant), extended-spectrum β-lactamase-producing Escherichia coli clone on a Greek dairy farm. *J. Dairy Sci.* **2020**, *103*, 852–857. [CrossRef]
59. van der Fels-Klerx, H.J.; Puister-Jansen, L.F.; van Asselt, E.D.; Burgers, S.L.G.E. Farm Factors Associated with the Use of Antibiotics in Pig Production. *J. Anim. Sci.* **2011**, *89*, 1922–1929. [CrossRef]
60. Ström, G.; Halje, M.; Karlsson, D.; Jiwakanon, J.; Pringle, M.; Fernström, L.-L.; Magnusson, U. Antimicrobial Use and Antimicrobial Susceptibility in Escherichia Coli on Small- and Medium-Scale Pig Farms in North-Eastern Thailand. *Antimicrob. Resist. Infect. Control.* **2017**, *6*, 75. [CrossRef]
61. Chantziaras, I.; Boyen, F.; Callens, B.; Dewulf, J. Correlation between Veterinary Antimicrobial Use and Antimicrobial Resistance in Food-Producing Animals: A Report on Seven Countries. *J. Antimicrob. Chemother.* **2014**, *69*, 827–834. [CrossRef]
62. Ricker, N.; Trachsel, J.; Colgan, P.; Jones, J.; Choi, J.; Lee, J.; Coetzee, J.F.; Howe, A.; Brockmeier, S.L.; Loving, C.L.; et al. Toward Antibiotic Stewardship: Route of Antibiotic Administration Impacts the Microbiota and Resistance Gene Diversity in Swine Feces. *Front. Vet. Sci.* **2020**, *7*, 255. [CrossRef]

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2. "Detection of enterotoxigenic *Escherichia coli* and Clostridia in the aetiology of neonatal piglet diarrhea: important factors for their prevention"

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Detection of Enterotoxigenic *Escherichia coli* and Clostridia in the Aetiology of Neonatal Piglet Diarrhoea: Important Factors for Their Prevention

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Abstract: This study aimed to research the involvement of enterotoxigenic *E. coli* (ETEC) and *C. difficile* or *C. perfringens* type C in the aetiology of neonatal piglet diarrhoea in Greece and to identify preventive factors for them. A total of 78 pooled faecal samples were collected randomly from 234 suckling piglets (1–4 days of age) with diarrhoea from 26 pig farms (3 piglets × 3 litters × 26 farms = 234 piglets = 78 faecal pool samples). The collected samples were initially screened for the presence of *E. coli* and *C. difficile* or *C. perfringens* via cultivation on MacConkey and anaerobic blood agar, respectively. Subsequently, the samples were pooled on ELUTE cards. From samples tested, 69.23% of those in the farms were ETEC F4-positive, 30.77% were ETEC F5-positive, 61.54% ETEC were F6-positive, 42.31% were ETEC F4- and *E. coli* enterotoxin LT-positive, 19.23% were ETEC F5- and LT-positive, 42.31% were ETEC F6- and LT-positive, while LT was found in 57.69% of those in the farms. *C. difficile* was involved in many cases and identified as an emerging neonatal diarrhoea etiological agent. Specifically, Toxin A of *C. difficile* was found in 84.62% and Toxin B in 88.46% of those in the farms. Antibiotic administration to sows in combination with probiotics or acidifiers was revealed to reduce the detection of antigens of ETEC and the enterotoxin LT of *E. coli*.

Keywords: *E. coli*; ETEC; *C. difficile*; neonatal diarrhoea; piglets

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1. Introduction

Neonatal piglet diarrhoea is a major economic and welfare issue in the swine industry worldwide due to increased pre-weaning mortality and therapeutic cost, as well as decreased growth rates in piglets [1,2]. To this day, it has proven to be challenging to identify the agents responsible for neonatal diarrhoea clinical signs in pig herds [3]. Several previous studies have reported various common pathogens as etiological agents, including *Escherichia coli* (*E. coli*), *Clostridium perfringens* (*C. perfringens*), *Clostridioides difficile* (*C. difficile*), *Cystoisospora suis*, rotavirus, and *Cryptosporidium parvum* [4–8]. In many field cases, combinations of pathogens have been isolated [8].

Neonatal diarrhoea is one of the most frequent clinical signs in newborn piglets, significantly increasing pre-weaning mortality and the number of weaning piglets with a lower body weight than the farm's target [9]. Even though enteric diseases in newborn piglets are often endemic, outbreaks characterized by high morbidity and mortality have been reported [10,11]. For example, in Sweden and Denmark, diarrhoea represented 5–24% of the overall pre-weaning mortality cases [12] and is responsible for a reduction in average

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daily gain (8–14 g/day) during the first week of life [13,14]. The cost of neonatal diarrhoea in affected pig herds was recently estimated to be EUR 134 per sow per year [2]. Commercial pig farms use vaccinations against *E. coli* and clostridial diseases, antibiotics, and alternatives to antibiotics to prevent or reduce economic losses due to neonatal diarrhoea.

E. coli is a Gram-negative bacterium that normally lives in the intestine, but imbalances in the gut microbiota can potentially cause diarrhoea in pigs [15]. Neonatal piglets affected by *E. coli* suffer from severe diarrhoea and have a high mortality rate [16]. Enteropathogenic *E. coli* strains (EPEC) and enterotoxigenic *E. coli* strains (ETEC) are classified by their virulence factors, with EPEC being characterized by the production of intimin and ETEC strains by their principal virulence factors, enterotoxins (STa, STb, and LT) and fimbriae adhesins [17]. ETEC are a significant and common cause of diarrhoea among suckling and weaned piglets [18]. ETEC colonize the mucosal surface of the small intestine via surface proteins (fimbriae or pilli), producing the following enterotoxins: heat-stable (Sta and STb) and heat-labile (LT) ones, or both [19]. The reported porcine fimbriae are F4 (K88), F5 (K99), F6 (987P), F18, and F41 [20]. Neonatal diarrhoea due to ETEC infection is characterized by high morbidity rates of suckling piglets per litter and is associated with increased economic losses for the swine farms due to an increased mortality rate, reduced growth performance, and increased veterinary cost for treatments and preventive strategies (e.g., antimicrobials, vaccines, alternatives to antibiotics, and biosecurity measures) [21,22].

Clostridia are large, rod-shaped bacteria that also form spores that persist in the environment for long periods. *C. perfringens* and *C. difficile* are classified as the main swine enteric clostridial pathogens [23]. *C. perfringens* type C causes severe and foetal necrotic enteritis in neonatal piglets due to the production of α-toxin and β-toxin [23–26]. Diarrhoea can spread rapidly amongst the herd, inducing high mortality (up to 100%) in affected piglets of non-vaccinated herds [23]. The peracute and acute forms of diarrhoea disease affect piglets mainly at 0–3 days of age [23].

C. difficile is a Gram-positive spore-forming bacterium that causes the enteric disease in humans and pigs, among many mammals [27–29]. *C. difficile* is widespread in the environment and is a common part of the gastrointestinal microbiota of mammals [30,31]. Previous studies have revealed *C. difficile* to be an emerging pathogen [1], which has been involved in cases of uncontrolled enteritis outbreaks affecting neonatal piglets in the USA and Europe [28,32,33]. Previous studies also reported *C. difficile* strains usually isolated from piglets that have infected humans in North America and Europe [34,35] and play a serious role in human diarrhoea [36].

The fundamental tools to tackling neonatal diarrhoea are a good knowledge of epidemiology, an efficient diagnostic approach, as well as appropriate control or preventive strategies [22]. For example, to control enteric colibacillosis in neonatal piglets, it is crucial to better comprehend the pathotypes and virotypes of *E. coli* and the predisposing factors that allow the bacterium to cause diseases, as well as to apply rapid diagnostic methods and efficient control or preventive tools [22]. For a correct diagnosis of neonatal piglet diarrhoea, detecting the presence of pathogenicity factors is fundamental, as many agents are included in bacterial flora and bacterial isolation is not proof that these agents cause diarrhoea. Multiplex polymerase chain reaction (PCR) is a very effective technique to detect virulence factors, such as the presence of fimbriae F4, F5, F6, and LT of *E. coli*, and the β-toxin of *C. perfringens* type C [23]. Multiplex PCR approaches enable the amplification of several genes simultaneously, reducing the cost and time needed [37–40]. FTA (Flinders Technology Associates) cards provide important advantages, such as sample storage, transport, and extraction, leading to a decrease in the cost and time needed for molecular diagnosis. FTA cards have been widely used to extract and stabilize DNA from samples of human and swine clinical cases [41–46]. The method of FTA cards is reported to be a valid diagnostic tool, and it can be used for a short period (24 h) for the storage and transport of live bacteria, specifically Gram-positive types [44].

Multidrug resistance genes among ESBL-producing *E. coli* strains retrieved from feces of pigs were reported, underlining the issue of antimicrobial resistance [47–51]. Various

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non-antibiotic strategies, including feed additives such as acidifiers or probiotics, have been proposed in swine herd health management strategies as other optional prophylactic or therapeutic protocols [52–71]. Probiotic supplementation in feed is beneficial for improving the immune response and growth performance, maintaining gut health, and controlling enteric infections due to its antimicrobial effects on several enteric pathogens by stimulating the growth of beneficial microorganisms in the gut [54,55,62–69]. Previous studies have reported that dietary acidifiers provide prophylactic effects such as antibiotics do, mainly by limiting the growth of enteric pathogens and simultaneously providing the opportunity for the proliferation of beneficial organisms [56–59,69–71].

There are limited published data about the aetiology of neonatal piglet diarrhoea in the Greek swine industry, especially on the prevalence of *C. difficile*. The objective of this study was to investigate the detection of ETEC and *C. difficile* or *C. perfringens* type C pathogens in neonatal piglet diarrhoea, as well as the factors contributing to their prevention in commercial pig farms.

2. Materials and Methods

2.1. Study Design

2.1.1. Description of Farms, Criteria for Inclusion, and Study Groups

The present study was carried out from January 2020 to October 2021 in Greece. A total of 26 commercial farrow-to-finish pig farms, with an overall population of 12,380 sows comprising approximately 24.5% of the Greek swine industry, representing different regions with high pig density, as well as with different capacities, were included in the study (Table 1). Inclusion criteria for the participated commercial pig farms were the ability to vaccinate sows against *E. coli* (F4ab, F4ac, F5, F6, and LT) and *C. perfringens* type C (beta toxoid) using commercial vaccines (intramuscularly, 2 mL per animal) in a routine program 15–20 days before farrowing. The pig farmers participated voluntarily and provided data about the possible use of acidifiers or/and probiotics in gestation and lactation feed. Moreover, the administration or not of injectable antibiotics to sows on the first day of farrowing was recorded. The injectable antibiotic that was used in sows of selected farms was amoxicillin LA at the dose of 15 mg/kg of body weight (BW) after cultivation and antibiogram in samples of former clinical cases to prevent postpartum dysgalactia syndrome (PDS). Moreover, some of the selected farms used, according to the manufacturer's guidelines, acidifiers based on commercial products containing a combination of formic acid, propionic acid, phosphoric acid, lactic acid, and acetic acid and/or probiotics based on commercial products with *Bacillus licheniformis* and *Bacillus subtilis* (the used commercial products are analytically presented in Supplementary File S1). The study groups of the trial are presented in Table 2.

Table 1. Overview of the 26 commercial pig farms included in the present study: number per geographic region, the capacity of sows, and the number of samples.

Region of Greece	Number of Farms	Capacity of Sows per Farm				Number of Faecal Pool Samples
		<100	101–300	301–500	>500	
Central Greece	10	2	3	1	4	30
North Greece	7	0	4	1	2	21
West Greece	5	0	2	0	3	15
South Greece	4	1	1	1	1	12
Total	26	3	10	3	10	78

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Table 2. Overview of the study groups according to the use of antibiotics, probiotics, or acidifiers.

Groups of the Trial Farms Based on Their Routine Program in Sows								
Non-Use of AB *, PR **, and AC *** in Pre-Farrowing Feed and Non-Use of AB at 1st Day of Farrowing	Injectable AB at 1st Day of Farrowing	Use in Pre-Farrowing Feed				Combination of Injectable AB and PR or/and AC in Pre-Farrowing Feed		
Group None	Group AB	Group AB	Group PR	Group AC	Group PR + AC	Group PR + AB	Group AC + AB	Group AB + PR + AC
6	8	2	1	4	1	1	1	3

* AB: antibiotics. ** PR: probiotics. *** AC: acidifiers.

2.1.2. Sampling and Laboratory Examinations

In total, 78 pooled faecal samples were collected from 234 suckling piglets (1–4 days of age) with diarrhoea (Figure 1). No medication or treatment was given to the selected piglets. Concerning diarrhoea, the sampling time was random. The collected samples were initially screened for the presence of *E. coli* and *C. difficile* or *C. perfringens* via cultivation on MacConkey and anaerobic blood agar, respectively [72]. A modified scoring system created by Pedersen et al. [73] based on faecal consistency was applied for each selected piglet for faecal sampling, as follows: scores 1 (soft faeces), 2 (mild diarrhoea), or 3 (severe diarrhoea). Three rectal swab samples per litter (1 pool) were collected randomly from suckling piglets with symptoms of diarrhoea for three litters per farm (3 piglets × 3 litters × 26 farms = 234 piglets = 78 faecal pool samples). Subsequently, the samples were pooled on ELUTE cards (FTA-like) according to the manufacturer's instructions (Enterocheck®, Hipra, Spain). The DNA extraction process, as well as the one-step multiplex PCR technique to detect genes that codify the adhesion factors F4, F5, F6, and the LT toxin of enterotoxigenic *E. coli* (ETEC), β-toxin of *C. perfringens* type C, and toxins A and B of *C. difficile*, were carried out using specific probes according to laboratory guidelines (Laboratorios Hipra, Amer, Girona, Spain) [74–78]. The results were determined as negative (−) based on the Cycle threshold (Ct) values (>38.5 Ct value). Positive samples were categorized into three categories: pos (+): a low quantity of genetic material of the tested pathogens was detected (35–38.5 Ct value), pos (++): a moderate quantity of genetic material of the tested pathogens was detected (30–35 Ct value), pos (+++): a large quantity of genetic material of the tested pathogens was detected (<30 Ct value).

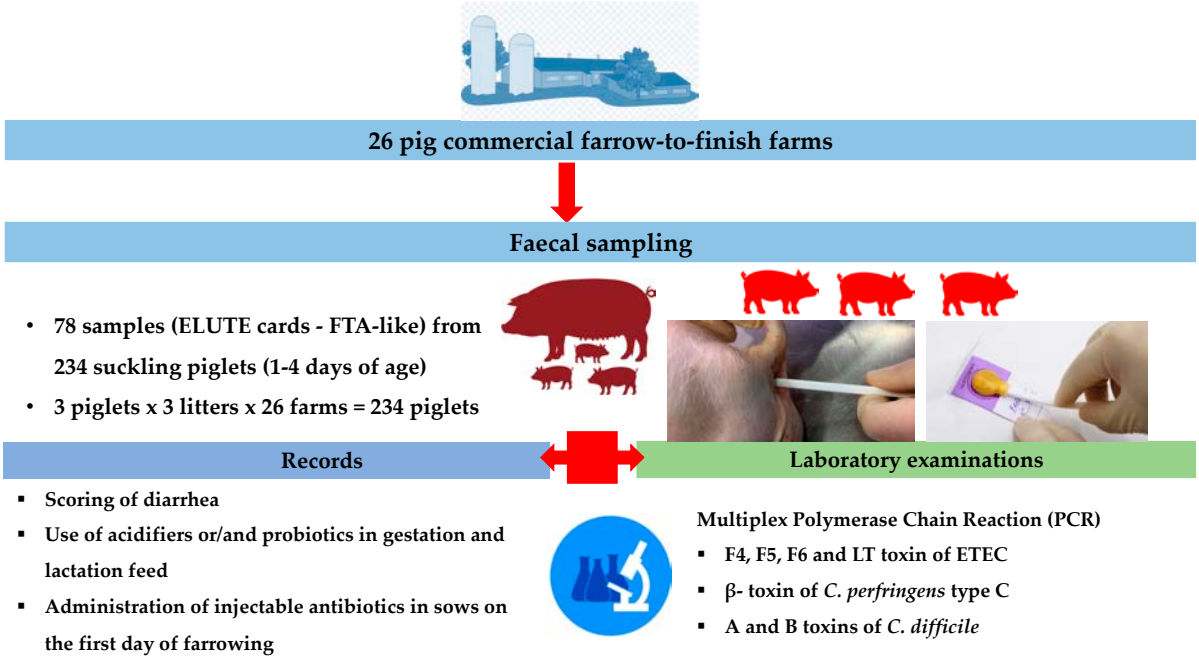

Figure 1. A flowchart of study design (animals, sampling, and laboratory exams).

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2.2. Statistical Analysis

The results were statistically analysed using R programming language [77], and all graphs were created with Seaborn, a Python data visualization library based on matplotlib [78]. The results of the detection of the genes F4, F5, and F6 of ETEC, *E. coli* enterotoxin LT, β-toxin of *C. perfringens*, and toxins A and B of *C. difficile* in faeces of piglets were analysed statistically three times based on the following groups: None, AB, PR, AC, PR + AC, PR + AB, AC + AB, and AB + PR + AC. The results were analysed using the Kruskal–Wallis test [79]. All comparisons were performed at a significant level of $p < 0.05$. Power analysis with G-Power software (version 3.1.) was performed to estimate the study power and the minimum sample size. The actual power of this study was greater than 95% for a total sample size of 78 samples.

3. Results

The percentages (proportions) of 26 farms when at least one sample was positive and of 78 samples where ETEC F4, ETEC F5, ETEC F6, enterotoxin LT, and β-toxin of *C. perfringens* type C and toxins A and B of *C. difficile* were detected are presented in Table 3.

Table 3. Percentage (%) and number (no) of positive pig farms (when at least one sample was positive) and positive samples in the detection, by PCR of ETEC F4, F5, and F6 genes, enterotoxin LT, β toxin of *C. perfringens*, and toxins A and B of *C. difficile*.

Analytical Target	% Positive Farms (No Farms)	% Positive Samples (No Pool Samples)
F4 gene	69.23% (18)	53.85% (42)
F5 gene	30.77% (8)	24.36% (19)
F6 gene	61.54% (16)	55.13% (43)
Enterotoxin LT	57.69% (15)	44.88% (35)
F4 gene + Enterotoxin LT	42.31% (11)	25.64% (20)
F5 gene + Enterotoxin LT	19.23% (5)	16.67% (13)
F6 gene + Enterotoxin LT	42.31% (11)	32.05% (25)
β-toxin—*C. perfringens* type C	0.00% (0)	0.00% (0)
Toxin A—*C. difficile*	84.62% (22)	65.38% (51)
Toxin B—*C. difficile*	88.46% (23)	61.54% (48)

Regarding the detection of F4 gen, the quantity was significantly higher in group None in comparison to those in groups AB ($p = 0.018$), AB + PR + AC ($p = 0.014$), AC + AB ($p = 0.0117$), and PR + AC ($p = 0.04$). A statistical difference was found between group AB + PR + AC in comparison and groups AC ($p = 0.02$), PR ($p = 0.02$), and PR + AB ($p = 0.02$). Lastly, groups AC + AB and PR were found to be statistically different ($p = 0.03$). Groups AB, AB + PR + AC, AC + AB had the lowest median (higher quantity of genetic material), while the highest median (lower quantity of genetic material) was observed in groups AC, PR, PR + AB, and None (Figure 2a).

Statistical analysis of ETEC F5 detection in the faeces of piglets showed that the proportion was significantly lower in group PR in comparison to that in group AB + PR + AC ($p = 0.04$), and it was lower in group PR + AC compared to those in groups AB ($p = 0.01$) and AB + PR + AC ($p = 0.004$). Group AB + PR + AC had the highest median (Figure 2b).

Regarding the detection of ETEC F6 in the faeces of piglets, the proportion was significantly lower in group PR + AB in comparison to those in groups AC ($p = 0.04$), None ($p = 0.005$), and PR ($p = 0.01$). Similarly, group AB + PR + AC had a significant difference in comparison to groups PR ($p = 0.001$) and PR ($p = 0.007$). Lastly, there was a statistical difference between groups AC + AB and PR ($p = 0.001$). The highest median was in groups AC, PR, and None (Figure 2c).

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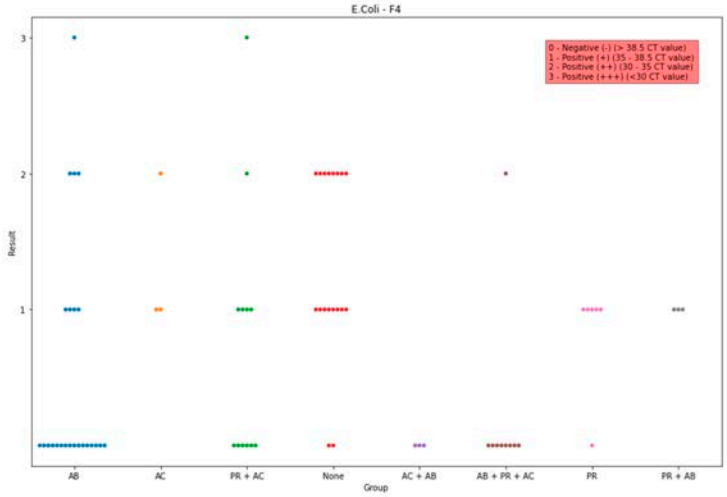
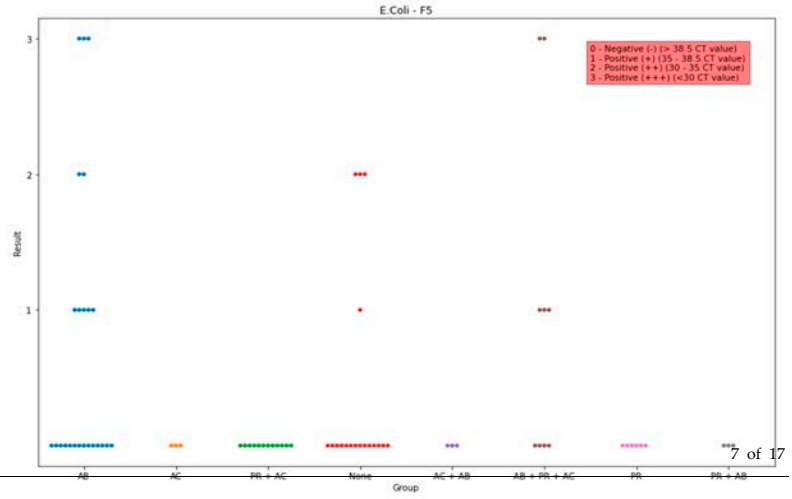
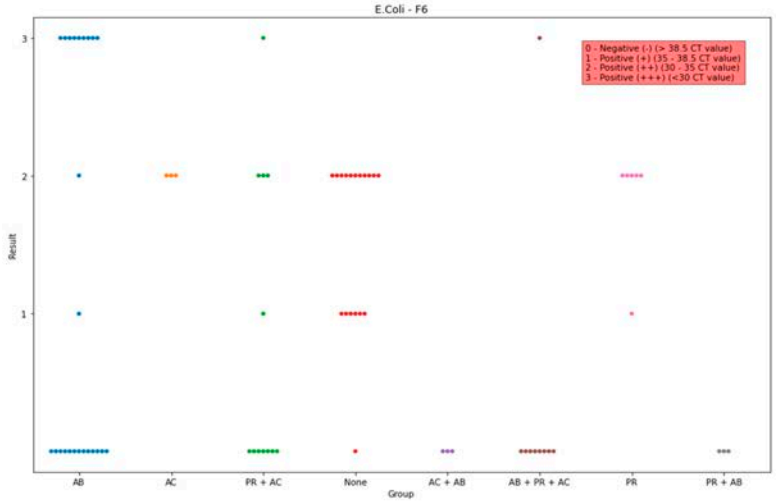


(**a**)



(**b**)



(**c**)

Figure 2. (**a**) Observed data points of the scores of the PCR detected ETEC F4 in all groups. The x-axis indicates the administration group, and y-axis indicates the amount of the detected genes. (**b**) Observed data points of the scores of the PCR detected ETEC F5 in all groups. The x-axis indicates the administration group, and the y-axis indicates the amount of the detected genes. (**c**) Observed data points of the scores of the PCR detected ETEC F6 in all groups. The x-axis indicates the administration group, and the y-axis indicates the amount of the detected genes.

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Statistical analysis of ETEC F5 detection in the faeces of piglets showed that the proportion was significantly lower in group PR in comparison to that in group AB + PR + AC ($p = 0.04$), and it was lower in group PR + AC compared to those in groups AB ($p = 0.01$) and AB + PR + AC ($p = 0.004$). Group AB + PR + AC had the highest median (Figure 2b).

Regarding the detection of ETEC F6 in the faeces of piglets, the proportion was significantly lower in group PR + AB in comparison to those in groups AC ($p = 0.04$), None ($p = 0.005$), and PR ($p = 0.01$). Similarly, group AB + PR + AC had a significant difference in comparison to groups PR ($p = 0.001$) and PR ($p = 0.007$). Lastly, there was a statistical difference between groups AC + AB and PR ($p = 0.001$). The highest median was in groups

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Statistical analysis of the detection of enterotoxin LT showed that the proportion was significantly higher in group PR in comparison to those in groups AC (p = 0.007), AC + AB (p = 0.007), and None (p = 0.003). Statistical differences were also found between groups AB and B + PR + AC (p = 0.01), AB and None (p = 0.03), PR and PR + AB (p = 0.007), and PR + AC and AB (p = 0.02) and PR (p = 0.003). The highest medians were in groups PR and AB (Figure 3).

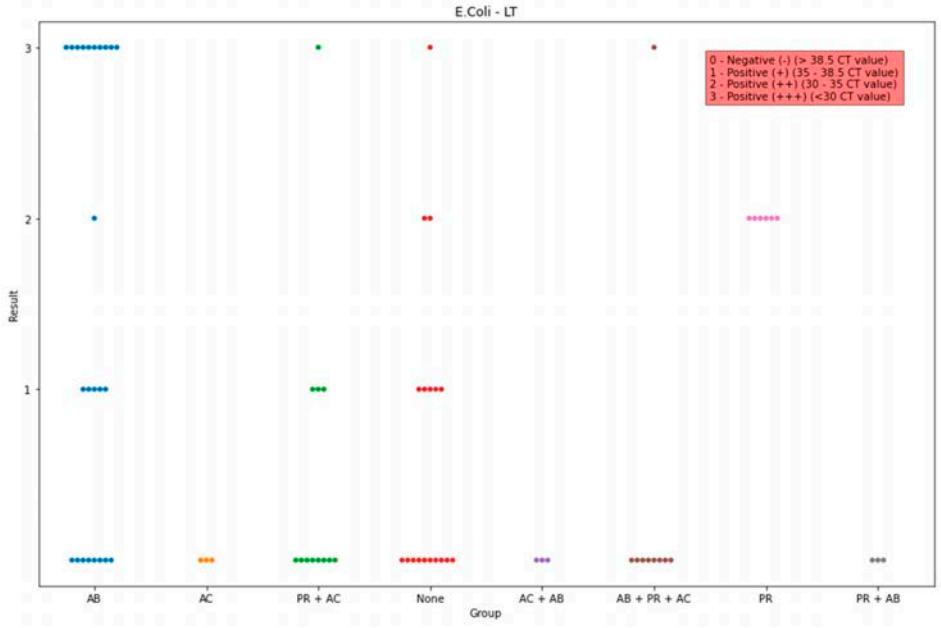

Figure 3. Observed data points of the scores of the PCR detected *E. coli* toxin LT in all groups. The x-axis indicates the administration group, and the y-axis indicates the amount of the detected genes.

Concerning the detection of toxin A of *C. difficile*, the proportion was found significantly higher in group AB in comparison to those in groups AB + PR + AC (p < 0.001), AC + AB (p = 0.002), PR (p = 0.01), and PR + AB (p = 0.01), and it was lower in group AB + PR + AC in comparison to group None (p = 0.01). Group AB had the highest median, while groups AB + PR + AC, AC + AB, and PR + AB had the lowest median (Figure 4).

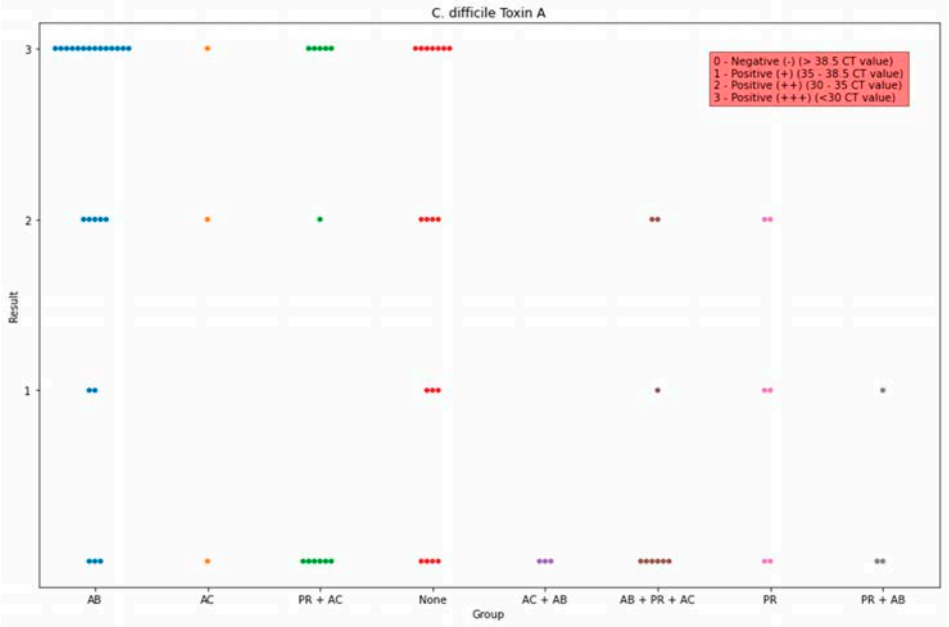

Figure 4. Observed data points of the scores of the PCR detected toxin A of *C. difficile* in all groups. The x-axis indicates the administration group, and the y-axis indicates the amount of the detected genes.

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Moreover, statistical analysis of the detected toxin B of *C. difficile* showed that the proportion is significantly higher in group AB in comparison to those in groups AB + PR + AC ($p < 0.001$), AC ($p = 0.028$), None ($p = 0.03$), PR ($p = 0.003$), and PR + AB ($p = 0.01$). Lastly, a significant difference between groups AB + PR + AC and None ($p = 0.165$) was found. For the detection of toxin B of *C. difficile*, the lowest median was in groups AC + AB, None, and AB + PR + AC (Figure 5).

Moreover, statistical analysis of the detected toxin B of *C. difficile* showed that the proportion is significantly higher in group AB in comparison to those in groups AB + PR + AC ($p < 0.001$), AC ($p = 0.028$), None ($p = 0.03$), PR ($p = 0.003$), and PR + AB ($p = 0.01$). Lastly, a significant difference between groups AB + PR + AC and None ($p = 0.165$) was found. For the detection of toxin B of *C. difficile*, the lowest median was in groups AC + AB, None, and AB + PR + AC (Figure 5).

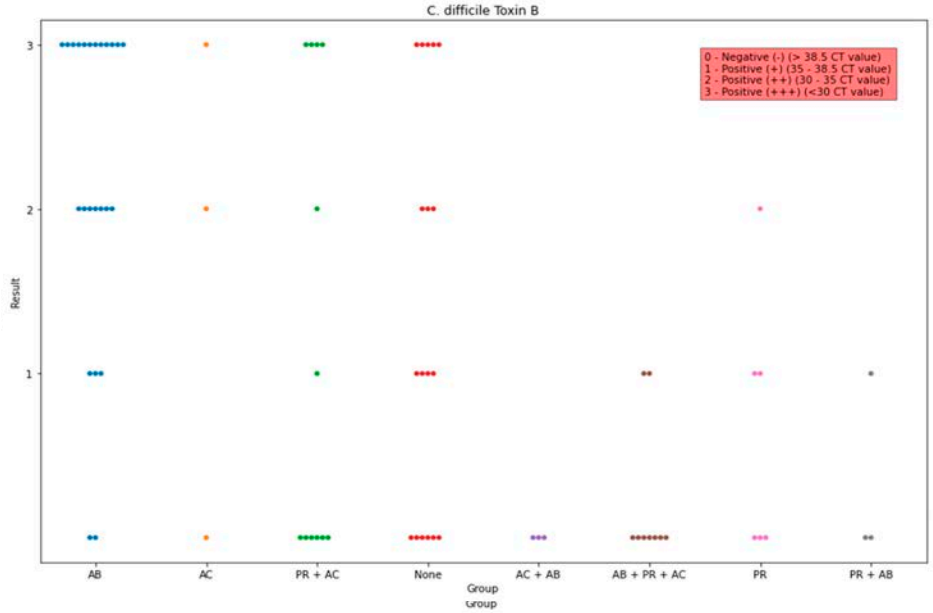

Figure 5. Observed data points of the scores of the PCR detected toxin B of *C. difficile* in all groups. The x-axis indicates the administration group, and the y-axis indicates the amount of the detected genes.

Figure 5. Observed data points of the scores of the PCR detected toxin B of *C. difficile* in all groups. The x-axis indicates the administration group, and the y-axis indicates the amount of the detected genes.

Concerning the score of piglet diarrhoea, it was found significantly higher in group AB in comparison to those in groups AB + PR + AC ($p = 0.001$), AC + AB ($p = 0.015$), None ($p = 0.04$), and PR ($p = 0.01$). The highest median was in group AB, while the lowest one was in groups AB + PR + AC, AC + AB, and PR (Figure 6).

Concerning the score of piglet diarrhoea, it was found significantly higher in group AB in comparison to those in groups AB + PR + AC ($p = 0.001$), AC + AB ($p = 0.015$), None ($p = 0.04$), and PR ($p = 0.01$). The highest median was in group AB, while the lowest one was in groups AB + PR + AC, AC + AB, and PR (Figure 6).

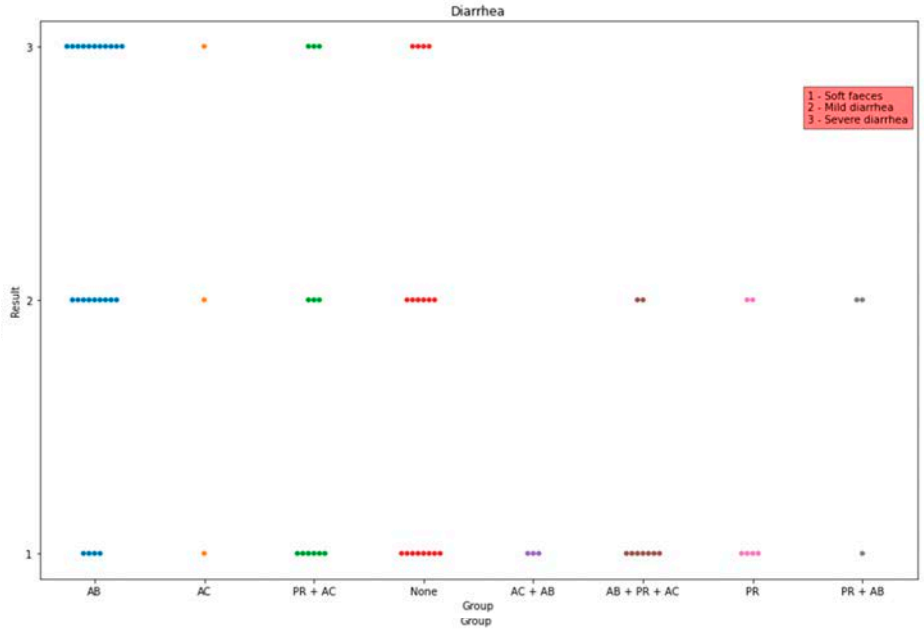

Figure 6. Observed data points of diarrhoea scores in all groups. The x-axis indicates the administration group, and the y-axis indicates the amount of the detected scores.

Figure 6. Observed data points of diarrhoea scores in all groups. The x-axis indicates the administration group, and the y-axis indicates the amount of the detected scores.

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4. Discussion

Diarrhoea in neonatal piglets up to 4 days of age is a common issue in many sow herds worldwide, and its diagnosis remains an ongoing challenge for swine practitioners. Among the causal agents of neonatal diarrhoea, *E. coli* is present in swine herds around the world [1,13]. The results of our study confirmed that *E. coli* is an enteric pathogen of concern for Greek swine farms, even if vaccinations against *E. coli* are given to sows. However, in contrast to previous studies, we noticed a higher prevalence of F4 and F6, as well as LT toxins, in comparison to that of F5 [80]. Based on our results, 69.23% (18/26) of those in the farms were F4-positive, 30.77% (8/26) were F5-positive, 61.54% (16/26) were F6-positive, 42.31% (11/26) were F4- and enterotoxin LT-positive, 19.23% (5/26) were F5- and LT-positive, 42.31% (11/26) were F6- and LT-positive, and in 57.69% (15/26) of those in the farms, *E. coli* enterotoxin LT was found. However, our study did not investigate heat-stable (Sta and STb) *E. coli* enterotoxins due to the absence of this option in the ELUTE cards used according to the manufacturer's instructions (Enterocheck®, Hipra, Madrid, Spain) [74–76].

In recent years, the focus of vaccination strategies against neonatal and post-weaning diarrhoea has been on anti-adhesin strategies, as this is the initial step of ETEC pathogenesis. However, while these vaccines have provided some protection against ETEC infections, there is still no universally effective ETEC vaccine that is commercially available [81]. A comprehensive approach that includes an appropriate vaccination program for the sow herd, the adequate intake of piglet colostrum and milk, and effective preventive strategies could help reduce the incidence of infectious diarrhoea in piglets. Several risk factors associated with clinical forms of neonatal diarrhoea have been reported to enhance protection against enteric pathogens, including passive immunity transferred by colostrum and milk [82,83], environmental conditions (e.g., temperature and humidity) [21,84], herd health management [85], the inadequate intake of colostrum, parity of the sows, and infection pressure by specific pathogens among the herd [85–87].

Various studies suggest that further investigations of *C. difficile* as an etiological agent in neonatal diarrhoea are needed [1,88,89]. Previous studies reported that *C. difficile* could be an etiological agent of enteritis in neonatal piglets [90–92]. While it is not considered to be a primary diarrheic pathogen in pigs based on epidemiological studies [93–96], other studies indicate that *C. difficile* is an emerging pathogen in neonatal diarrhoea [1,97,98]. These findings highlight the need for farmers to use recently developed commercial vaccines that provide immunization against *C. difficile* to sows to prevent neonatal diarrhoea via passive immunity. A new commercial vaccine for the immunization of sows with *C. difficile* (toxins A and B) and *C. perfringens* type A was recently registered in Europe, resulting in a significant reduction in diarrhoea and productive losses caused by *C. difficile* and *C. perfringens* type A [99].

While a previous study reported that pathogenic *E. coli* was only found in combination with other pathogens in Spanish swine farms [82], our study found that *E. coli* is present in combination with *C. difficile*, but no detection of *C. perfringens* type C was noticed among the 26 pig farms studied. Even if *C. difficile* causes disease in piglets worldwide, it is considered to be much less important than other enteric pathogens are from a global perspective [100]. For example, a low prevalence of *C. perfringens* type C (1,4%) in herds was reported in Poland [101]. Our results regarding the absence of *C. perfringens* type C in faecal samples from newborn piglets with diarrhoea are consistent with those in previous studies, which included a larger number of herds and samples [3]. These findings may be explained by the sufficient protection of routine vaccinations for sows against *C. perfringens* type C [1,3]. However, in contrast to our results, these studies did not find a relationship between *C. difficile* and diarrhoeal status.

Our study found that administering antibiotics alone to sows did not reduce the detection of ETEC antigens. However, combining antibiotics with probiotics or acidifiers or both resulted in better outcomes. This is supported by several studies that have reported the beneficial effects of probiotics as a feed additive in the health and performance of piglets

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and sows [102–104]. The supplementation of probiotics in the feed of gestating and lactating sows has been shown to improve their health status and reproductive performance, as well as increase the production of immunoglobulins in colostrum and milk, resulting in a decrease in the incidence of neonatal diarrhoea [105–109]. Additionally, the administration of acidifiers in pig diets can enhance their growth performance and modulate the intestinal microbiota, reducing gastric pH and delaying the multiplication of enterotoxigenic *E. coli* [110,111]. The supplementation of acidifiers in sow feed could influence the mother's microbiota and affects those of piglets [112]. Our results could support the findings of previous trials relating to the administration of acidifiers in sow gestation feed, which reported beneficial effects on the sows' performance during lactation [113,114] and their gut microbiome, reducing the *E. coli* counts during farrowing and weaning [115].

Feed additives, including probiotics and acidifiers, have been explored as antibiotic alternatives, but their effectiveness varies [116]. Our study found that administering antibiotics alone did not reduce the detection of pathogen toxins (such as enterotoxin LT and toxin A of *C. difficile*), but combining antibiotics with probiotics or acidifiers decreased the toxin levels. Feeding sows probiotics or acidifiers also resulted in lower neonatal piglet diarrhoea scores compared to those who received nothing or only injectable antibiotics. In contrast to our results, Greeff et al. [117] reported that oral amoxicillin administration during the last week of gestation to sows can modulate the gut development of their piglets for a period of at least 5 weeks after the last antibiotic administration.

It is worth noting that in the case of *C. difficile*, the highest median toxin detection was found in the group that received antibiotics alone, while a lower detection rate was observed in groups that received alternatives to antibiotics, such as probiotics and acidifiers, either alone or in combination with antibiotics. In humans, *C. difficile* infection (CDI) is becoming increasingly difficult to treat due to severe antibiotic resistance, and for this reason, there are very limited treatment options [118,119]. Currently, only three antibiotics (metronidazole, vancomycin, and fidaxomicin) are available for CDI treatment [120]. These antibiotics are forbidden for use in swine. Fry et al. [121] reported that significant proportions of *C. difficile* in swine are toxigenic and are often associated with antimicrobial resistance genes, although they are not resistant to drugs that are used to treat CDI. A connection between the presence of *C. difficile* and diarrhoea or antibiotic treatments in piglets has not been proven thus far [122–124]. Moreover, a previous study by Schneeberg et al. [125] reported that emerging human-pathogenic *C. difficile* PCR ribotypes were the predominant PCR ribotypes in piglets in Germany. However, they did not find a link between the presence of *C. difficile* in piglets and antibiotic treatment or diarrhoea in piglets.

Our findings highlight the potential benefits of using alternatives to antibiotics, such as probiotics and acidifiers, in swine feed to improve the health and performance of both sows and their piglets. In addition, our results suggest that further research is needed to investigate the potential use of these alternatives in the treatment of CDI in swine and humans, either alone or in combination with antibiotics.

5. Conclusions

In our research study, despite routine vaccinations of sows against *E. coli*, a high prevalence of positive samples was found, revealing that *E. coli* remains a main pathogen of great importance in neonatal pig diarrhoea. Additionally, our study reports, for the first time in Greece, the involvement of *C. difficile* in most clinical cases of neonatal pig diarrhoea in commercial pig farms. Therefore, the need for herd immunization against *C. difficile* may be necessary. However, these findings reveal the necessity of extended future studies on the epidemiology of *C. difficile* in pigs and the need for research on this pathogen in the future, as well as the need for herd immunization against *C. difficile*. Maternal interventions with antibiotics in combination with probiotics or acidifiers appear to have beneficial effects in terms of neonatal pig diarrhoea, reducing the detection of ETEC antigens and the enterotoxin LT of *E. coli*.

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Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/life13051092/s1, Supplementary File S1: used commercial products in farms.

Author Contributions: Conceptualization, V.G.P., N.T., G.C. and C.B.; methodology, N.T., V.G.P., Z.A., G.L., G.C. and C.B.; software, E.M. and P.K.; validation, N.T., V.G.P., Z.A., G.L, G.C. and C.B.; formal analysis, N.T. and V.G.P.; investigation, N.T., Z.A., V.G.P. and G.L.; resources, N.T.; data curation, N.T., E.M. and P.K.; writing—original draft preparation, N.T., V.G.P. and E.M.; writing—review and editing, V.G.P., G.C. and C.B.; visualization, E.M. and P.K. supervision, V.G.P., G.C. and C.B. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study received approval (permission number 96/19.12.2019) from our Institutional Animal Use Ethics Committee of the Faculty of Veterinary Science, University of Thessaly.

Informed Consent Statement: The farm owners involved in the research were informed and provided consent to use the animals in the present study.

Data Availability Statement: Not applicable.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Chan, G.; Farzan, A.; DeLay, J.; McEwen, B.; Prescott, J.F.; Friendship, R.M. A retrospective study on the etiological diagnoses of diarrhea in neonatal piglets in Ontario, Canada, between 2001 and 2010. *Can. J. Vet. Res.* **2013**, *77*, 254–260. [PubMed]
2. Sjölund, M.; Zoric, M.; Wallgren, P. Financial impact of disease on pig production. Part III. Gastrointestinal disorders. In Proceedings of the 6th European Symposium of Porcine Health Management, Sorrento, Italy, 7–9 May 2014; p. 189.
3. Kongsted, H.; Pedersen, K.; Hjulsager, C.K.; Larsen, L.E.; Pedersen, K.S.; Jorsal, S.E.; Bækbo, P. Diarrhoea in neonatal piglets: A case control study on microbiological findings. *Porc. Health Manag.* **2018**, *4*, 17. [CrossRef] [PubMed]
4. Dubreuil, J.D.; Isaacson, R.E.; Schifferli, D.M. Animal enterotoxigenic *Escherichia coli*. *EcoSal Plus.* **2016**, 7. [CrossRef]
5. Luppi, A.; Gibellini, M.; Gin, T.; Vangroenweghe, F.; Vandenbroucke, V.; Bauerfeind, R.; Bonilauri, P.; Labarque, G.; Hidalgo, A. Prevalence of virulence factors in enterotoxigenic *Escherichia coli* isolated from pigs with post-weaning diarrhea in Europe. *Porc. Health Manag.* **2016**, *2*, 20. [CrossRef]
6. Ruiz, V.L.A.; Bersano, J.G.; Carvalho, A.F.; Catroxo, M.H.B.; Chiebao, D.P.; Gregori, F.; Miyashiro, S.; Nassar, A.F.C.; Oliveira, T.M.F.S.; Ogata, R.A.; et al. Case–control study of pathogens involved in piglet diarrhea. *BMC Res. Notes* **2016**, *9*, 22. [CrossRef] [PubMed]
7. Uzal, F.A.; Songer, J.G. Clostridial diseases. In *Diseases of Swine*, 11th ed.; Zimmerman, J., Karriker, L.A., Ramirez, A., Schwartz, K.J., Stevenson, G.W., Eds.; John Wiley & Sons Inc.: Hoboken, NJ, USA, 2019; pp. 792–806.
8. Vidal, A.; Martín-Valls, G.E.; Tello, M.; Mateu, E.; Martín, M.; Darwich, L. Prevalence of enteric pathogens in diarrheic and non-diarrheic samples from pig farms with neonatal diarrhea in the North East of Spain. *Vet. Microbiol.* **2019**, *237*, 108419. [CrossRef] [PubMed]
9. Holland, R.E. Some infectious causes of diarrhea in young farm animals. *Clin. Microbiol. Rev.* **1990**, *3*, 345–375. [CrossRef]
10. Morin, M.; Turgeon, D.; Jolette, J.; Robinson, Y.; Phaneuf, J.B.; Sauvageau, R.; Beauregard, M.; Teuscher, E.; Higgins, R.; Lariviere, S. Neonatal diarrhea of pigs in Quebec: Infectious causes of significant outbreaks. *Can. J. Comp. Med.* **1983**, *47*, 11–17.
11. Svensmark, B.; Jorsal, S.; Nielsen, K.; Willeberg, P. Epidemiological studies of piglet diarrhoea in intensively managed Danish sow herds. I. Pre-weaning diarrhoea. *Acta Vet. Scand.* **1988**, *30*, 43–53. [CrossRef]
12. Svendsen, J.; Bille, N.; Nielsen, N.; Larsen, J.; Riising, H. Preweaning mortality in pigs. Diseases of the gastrointestinal tract in pigs. *Nordisk Veterinär.* **1975**, *27*, 85–101.
13. Kongsted, H.; Stege, H.; Toft, N.; Nielsen, J.P. The effect of new neonatal porcine Diarrhoea syndrome (NNPDS) on average daily gain and mortality in 4 Danish pig herds. *BMC Vet. Res.* **2014**, *10*, 90. [CrossRef] [PubMed]
14. Johansen, M.; Alban, L.; Kjærsgård, H.D.; Bækbo, P. Factors associated with suckling piglet average daily gain. *Prev. Vet. Med.* **2004**, *63*, 91–102. [CrossRef] [PubMed]
15. Dubreuil, J.D. *Escherichia coli* STb toxin and colibacillosis: Knowing is half the battle. *FEMS Microbiol. Lett.* **2008**, *78*, 137–145. [CrossRef] [PubMed]
16. Moon, H.W.; Schineider, R.A.; Mosely, S.L. Comparative prevalence of four enterotoxin genes among *Escherichia coli* isolates from swine. *Am. J. Vet. Res.* **1986**, *47*, 210–212. [PubMed]

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06/09/2026 21:12:45 EEST - 216.73.216.250

17. Toledo, A.; Gómez, D.; Cruz, C.; Carreón, R.; López, J.; Giono, S.; Castro, A.M. Prevalence of virulence genes in *Escherichia coli* strains isolated from piglets in the suckling and weaning period in Mexico. *J. Med. Virol.* **2012**, *61*, 148–156. [CrossRef] [PubMed]
18. Zajacova, Z.S.; Konstantinová, L.; Alexa, P. Detection of virulence factors of *Escherichia coli* focused on prevalence of EAST1 toxin in the stool of diarrheic and non-diarrheic piglets and presence of adhesion involving virulence factors in astA positive strains. *Vet. Microbiol.* **2012**, *154*, 369–375. [CrossRef] [PubMed]
19. Nagy, B.; Fekete, P.Z. Enterotoxigenic *E. coli* (ETEC) in farm animals. *Vet. Res.* **1999**, *30*, 259–284.
20. Vu-Khac, H.; Holoda, E.; Pilipcinec, E.; Blanco, M.; Blanco, J.E.; Dahbi, G.; Mora, A.; López, C.; González, E.A.; Blanco, J. Serotypes, virulence genes, intimin types and PFGE profiles of *Escherichia coli* isolated from piglets with diarrhoea in Slovakia. *Vet. J.* **2007**, *174*, 176–187. [CrossRef]
21. Fairbrother, J.M.; Gyles, C.L. Colibacillosis. In *Diseases of Swine*, 10th ed.; Zimmerman, J., Karriker, L.A., Ramirez, A., Schwartz, K.J., Stevenson, G.W., Eds.; WileyBlackwell: Chichester, UK, 2012; pp. 723–749.
22. Luppi, A. Swine enteric colibacillosis: Diagnosis, therapy and antimicrobial resistance. *Porc. Health Manag.* **2017**, *3*, 16. [CrossRef]
23. Songer, J.G.; Uzal, F.A. Clostridial Enteric Infections in Pigs. *J. Vet. Diagn.* **2005**, *17*, 528–536. [CrossRef]
24. Petit, L.; Gibert, M.; Popoff, M.R. *Clostridium perfringens*: Toxinotype and genotype. *Trends Microbiol.* **1999**, *7*, 104–110. [CrossRef] [PubMed]
25. Songer, J.G. Clostridial enteric diseases of domestic animals. *Clin. Microbiol. Rev.* **1996**, *9*, 216–234. [CrossRef] [PubMed]
26. Rood, J.I.; Adams, V.; Lacey, J.; Lyras, D.; McClane, B.A.; Melville, S.B.; Moore, R.J.; Popoff, M.R.; Sarker, M.R.; Songer, J.G.; et al. Expansion of the *Clostridium perfringens* toxin-based typing scheme. *Anaerobe* **2018**, *53*, 5–10. [CrossRef] [PubMed]
27. Gould, L.H.; Limbago, B. *Clostridium difficile* in food and domestic animals: A new foodborne pathogen? *Clin. Infect. Dis.* **2010**, *51*, 577–582. [CrossRef] [PubMed]
28. Keel, M.K.; Songer, J.G. The comparative pathology of *Clostridium difficile*-associated disease. *Vet. Pathol.* **2006**, *43*, 225–240. [CrossRef] [PubMed]
29. Keessen, E.C.; van den Berkt, A.J.; Haasjes, N.H.; Hermanus, C.; Kuijper, E.J.; Lipman, L.J.A. The relation between farm specific factors and prevalence of *Clostridium difficile* in slaughter pigs. *Vet. Microbiol.* **2011**, *154*, 130–134. [CrossRef]
30. Britton, R.A.; Young, V.B. Interaction between the intestinal microbiota and host in *Clostridium difficile* colonization resistance. *Trends Microbiol.* **2012**, *20*, 313–319. [CrossRef] [PubMed]
31. Lim, S.C.; Knight, D.R.; Riley, T.V. *Clostridium difficile* and One Health. *Clin. Microbiol. Infect.* **2020**, *26*, 857–863. [CrossRef]
32. Songer, J.G. The emergence of *Clostridium difficile* as a pathogen of food animals. *Anim. Health Res. Rev.* **2004**, *5*, 321–326. [CrossRef]
33. Debast, S.B.; van Leengoed, L.A.M.G.; Goorhuis, A.; Harmanus, C.; Kuijper, E.J.; Bergwerff, A.A. *Clostridium difficile* PCR ribotype 078 toxinotype V found in diarrhoeal pigs identical to isolates from affected humans. *Environ. Microbiol.* **2009**, *11*, 505–511. [CrossRef]
34. Goorhuis, A.; Bakker, D.; Corver, J.; Debast, S.B.; Harmanus, C.; Notermans, D.W.; Bergwerff, A.A.; Dekker, F.W.; Kuijper, E.J. Emergence of *Clostridium difficile* infection due to a new hypervirulent strain, polymerase chain reaction ribotype 078. *Clin. Infect. Dis.* **2008**, *47*, 1162–1170. [CrossRef]
35. Goorhuis, A.; Debast, S.B.; van Leengoed, L.A.M.G.; Harmanus, C.; Notermans, D.W.; Bergwerff, A.A.; Kuijper, E.J. *Clostridium difficile* PCR ribotype 078: An emerging strain in humans and in pigs? *J. Clin Microbiol.* **2008**, *46*, 1157. [CrossRef]
36. Mylonakis, E.; Ryan, E.T.; Calderwood, S.B. *Clostridium difficile*–associated diarrhea: A review. *Arch. Intern. Med.* **2001**, *161*, 525–533. [CrossRef] [PubMed]
37. DebRoy, C.; Roberts, E.; Valadez, A.M.; Dudley, E.G.; Cutter, C.N. Detection of Shiga toxin–producing *Escherichia coli* O26, O45, O103, O111, O113, O121, O145, and O157 serogroups by multiplex polymerase chain reaction of the wzx gene of the O-antigen gene cluster. *Foodborne Pathog. Dis.* **2011**, *8*, 651–652. [CrossRef]
38. Bai, J.; Paddock, Z.D.; Shi, X.; Li, S.; An, B.; Nagaraja, T.G. Applicability of a multiplex PCR to detect the seven major Shiga toxin–producing *Escherichia coli* based on genes that code for serogroup-specific O-antigens and major virulence factors in cattle feces. *Foodborne Pathog. Dis.* **2012**, *9*, 541–548. [CrossRef] [PubMed]
39. Baker, C.A.; Rubinelli, P.M.; Park, S.H.; Carbonero, F.; Ricke, S.C. Shiga toxin-producing *Escherichia coli* in food: Incidence, ecology, and detection strategies. *Food Control* **2016**, *59*, 407–419. [CrossRef]
40. Baker, C.A.; Rubinelli, P.M.; Park, S.H.; Ricke, S.C. Immuno-based detection of Shiga toxin-producing pathogenic *Escherichia coli* in food—A review on current approaches and potential strategies for optimization. *Crit. Rev. Microbiol.* **2016**, *42*, 656–675. [CrossRef] [PubMed]
41. Salvador, J.M.; De Ungria, M.C.A. Isolation of DNA from saliva of betel quid chewers using treated cards. *J. Forensic Sci.* **2003**, *48*, 794–797. [CrossRef]
42. Muthukrishnan, M.; Singanallur, N.B.; Ralla, K.; Villuppanoor, S.A. Evaluation of FTA® cards as a laboratory and field sampling device for the detection of foot-and-mouth disease virus and serotyping by RT-PCR and real-time RT-PCR. *J. Virol. Methods* **2008**, *151*, 311–316. [CrossRef]
43. Linhares, D.C.; Rovira, A.; Torremorell, M. Evaluation of Flinders Technology Associates cards for collection and transport of samples for detection of Porcine reproductive and respiratory syndrome virus by reverse transcription polymerase chain reaction. *J. Vet. Diagn. Investig.* **2012**, *24*, 328–332. [CrossRef]

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06/09/2026 21:12:45 EEST - 216.73.216.250

44. Shalaby, A.G.; Bakry, N.R.; Mohamed, A.; Khalil, A.A. Evaluating Flinders Technology Associates card for transporting bacterial isolates and retrieval of bacterial DNA after various storage conditions. *Vet. World.* **2020**, *13*, 2243–2251. [CrossRef]
45. Stringer, O.W.; Bossé, J.T.; Lacouture, S.; Gottschalk, M.; Fodor, L.; Angen, Ø.; Velazquez, E.; Penny, P.; Lei, L.; Langford, P.R.; et al. Rapid Detection and Typing of *Actinobacillus pleuropneumoniae* Serovars Directly From Clinical Samples: Combining FTA® Card Technology With Multiplex PCR. *Front. Vet. Sci.* **2021**, *8*, 728660. [CrossRef]
46. Rajendram, D.; Ayenza, R.; Holder, F.M.; Moran, B.; Long, T.; Shah, H.N. Long-term storage and safe retrieval of DNA from microorganisms for molecular analysis using FTA matrix cards. *J. Microbiol. Methods.* **2006**, *67*, 582–592. [CrossRef] [PubMed]
47. Li, J.Y. Current Status and Prospects for in-Feed Antibiotics in the Different Stages of Pork Production—A Review. *Asian Austral. J. Anim.* **2017**, *30*, 1667–1673. [CrossRef] [PubMed]
48. Pamer, E.G. Resurrecting the Intestinal Microbiota to Combat Antibiotic-Resistant Pathogens. *Science* **2016**, *352*, 535–538. [CrossRef] [PubMed]
49. Laird, T.J.; Abraham, S.; Jordan, D.; Pluske, J.R.; Hampson, D.J.; Trott, D.J.; O'Dea, M. Porcine Enterotoxigenic *Escherichia Coli*: Antimicrobial Resistance and Development of Microbial-Based Alternative Control Strategies. *Vet. Microbiol.* **2021**, *258*, 109117. [CrossRef]
50. Athanasakopoulou, Z.; Reinicke, M.; Diezel, C.; Sofia, M.; Chatzopoulos, D.C.; Braun, S.D.; Reissig, A.; Spyrou, V.; Monecke, S.; Ehricht, R.; et al. Antimicrobial Resistance Genes in ESBL-Producing *Escherichia Coli* Isolates from Animals in Greece. *Antibiotics* **2021**, *10*, 389. [CrossRef]
51. Tsekouras, N.; Athanasakopoulou, Z.; Diezel, C.; Kostoulas, P.; Braun, S.D.; Sofia, M.; Monecke, S.; Ehricht, R.; Chatzopoulos, D.C.; Gary, D.; et al. Cross-Sectional Survey of Antibiotic Resistance in Extended Spectrum β-Lactamase-Producing *Enterobacteriaceae* Isolated from Pigs in Greece. *Animals* **2022**, *12*, 1560. [CrossRef]
52. Liu, Y.; Espinosa, C.D.; Abelilla, J.J.; Casas, G.A.; Lagos, L.V.; Lee, S.A.; Kwon, W.B.; Mathai, J.K.; Navarro, D.M.D.L.; Jaworski, N.W.; et al. Non-antibiotic feed additives in diets for pigs: A review. *Anim Nutr.* **2018**, *4*, 113–125. [CrossRef]
53. Papatsiros, V.G.; Billinis, C. The prophylactic use of acidifiers as antibacterial agents in swine. In *Antimicrobial Agents*; Bobbarala, V., Ed.; InTech: Rijeka, Croatia, 2012; pp. 295–310.
54. Ji, P.; Li, X.; Liu, Y. Dietary Intervention to Reduce *E. coli* Infectious Diarrhea in Young Pigs. In *E. Coli Infections—Importance of Early Diagnosis and Efficient Treatment (Internet)*; Rodrigo, L., Ed.; IntechOpen: London, UK, 2020. Available online: https://www.intechopen.com/chapters/71010 (accessed on 24 July 2022). [CrossRef]
55. Wellison, A.P.; Franco, S.M.; Reis, I.L.; Mendonça, C.M.N.; Piazentin, A.C.M.; Azevedo, P.O.S.; Tse, M.L.P.; De Martinis, E.C.P.; Gierus, M.; Oliveira, R.P.S. Beneficial effects of probiotics on the pig production cycle: An overview of clinical impacts and performance. *Vet. Microbiol.* **2022**, *269*, 109431. [CrossRef]
56. Kim, Y.Y.; Kil, D.; Oh, H.K.; Han, I. Acidifier as an Alternative Material to Antibiotics in Animal Feed. *Asian Australas J. Anim. Sci.* **2005**, *18*, 1048–1060. [CrossRef]
57. Jacela, J.Y.; DeRouchey, J.M.; Tokach, M.D.; Goodband, R.D.; Nelssen, J.L.; Renter, D.G.; Dritz, S.S. Feed additives for swine: Fact sheets—Acidifi ers and antibiotics. *J. Swine Health Prod.* **2009**, *17*, 270–275. [CrossRef]
58. Roth, F.X.; Kirchgessner, M. Organic acids as feed additives for young pigs: Nutritional and gastrointestinal effects. *J. Anim. Feed Sci.* **1998**, *7*, 25–33. [CrossRef]
59. Papatsiros, V.G.; Katsoulos, P.D.; Koutoulis, K.C.; Karatzia, M.; Dedousi, A.; Christodoulopoulos, G. Alternatives to antibiotics for farm animals. *CAB Rev. Perspect. Agric. Vet. Sci. Nutr. Nat. Resour.* **2013**, *8*, 1–15. [CrossRef]
60. Kantas, D.; Papatsiros, V.G.; Tassis, P.D.; Athanasiou, L.V.; Tzika, E.D. Effect of a natural feed additive (*Macleaya cordata*), containing sanguinarine, on the performance and health status of weaning pigs. *Anim. Sci. J.* **2015**, *86*, 92–98. [CrossRef]
61. Sayan, H.; Assavacheep, P.; Angkanaporn, K.; Assavacheep, A. Effect of *Lactobacillus salivarius* on growth performance, diarrhea incidence, fecal bacterial population and intestinal morphology of suckling pigs challenged with F4+ enterotoxigenic *Escherichia coli*. *Asian Australas J. Anim. Sci.* **2018**, *31*, 1308–1314. [CrossRef]
62. Yue, S.; Li, Z.; Hu, F.; Picimbon, J.F. Curing piglets from diarrhea and preparation of a healthy microbiome with *Bacillus* treatment for industrial animal breeding. *Sci. Rep.* **2020**, *10*, 19476. [CrossRef]
63. Alexopoulos, C.; Georgoulakis, I.E.; Tzivara, A.; Kritas, S.K.; Siochu, A.; Kyriakis, S.C. Field evaluation of the efficacy of a probiotic containing *Bacillus licheniformis* and *Bacillus subtilis* spores, on the health status and performance of sows and their litters. *J. Anim. Physiol. Anim. Nutr.* **2000**, *88*, 381–392. [CrossRef]
64. Böhmer, B.M.; Kramer, W.; Roth-Maier, D.A. Dietary probiotic supplementation and resulting effects on performance, health status, and microbial characteristics of primiparous sows. *J. Anim. Physiol. Anim. Nutr.* **2006**, *90*, 309–315. [CrossRef]
65. Kantas, D.; Papatsiros, V.G.; Tassis, P.D.; Giavasis, I.; Bouki, P.; Tzika, E.D. A feed additive containing *Bacillus toyonensis* (Toyocerin(®)) protects against enteric pathogens in postweaning piglets. *J. Appl. Microbiol.* **2015**, *118*, 727–738. [CrossRef]
66. Hayakawa, T.; Masuda, T.; Kurosawa, D.; Tsukahara, T. Dietary administration of probiotics to sows and/or their neonates improves the reproductive performance, incidence of post-weaning diarrhea and histopathological parameters in the intestine of weaned piglets. *Anim. Sci. J.* **2016**, *87*, 1501–1510. [CrossRef] [PubMed]
67. Menegat, M.B.; Gourley, K.M.; Braun, M.B.; DeRouchey, J.M.; Woodworth, J.C.; Bryte, J.; Tokach, M.D.; Dritz, S.S.; Goodband, R.D. Effects of a Bacillus-Based Probiotic on Sow Performance and on Progeny Growth Performance, Fecal Consistency, and Fecal Microflora. *Kans. Agric. Exp. Stn. Res. Rep.* **2018**, *4*, 9. [CrossRef]

Institutional Repository - Library & Information Centre - University of Thessaly
06/09/2026 21:12:45 EEST - 216.73.216.250

68. Kritas, S.K.; Marubashi, T.; Filioussis, G.; Petridou, E.; Christodoulopoulos, G.; Burriel, A.R.; Tzivara, A.; Theodoridis, A.; Pískoriková, M. Reproductive performance of sows was improved by administration of a sporing bacillary probiotic (*Bacillus subtilis* C-3102). *J. Anim. Sci.* **2015**, *93*, 405–413. [CrossRef] [PubMed]
69. Papatsiros, V.G.; Tassis, P.D.; Tzika, E.D.; Papaioannou, D.S.; Petridou, E.; Alexopoulos, C.; Kyriakis, S.C. Effect of benzoic acid and combination of benzoic acid with a probiotic containing *Bacillus cereus* var. *Toyoi* in weaned pig nutrition. *Pol. J. Vet. Sci.* **2011**, *14*, 117–125. [CrossRef] [PubMed]
70. Papatsiros, V.; Christodouloupoulos, G.; Filippopoulos, L.C. The use of organic acids in monogastric animals (swine and rabbits). *JCAB* **2012**, *6*, 154–159. [CrossRef]
71. Pearlin, B.V.; Muthuvel, S.; Govidasamy, P.; Villavan, M.; Alagawany, M.; Farag, M.R.; Dhama, K.; Gopi, M. Role of acidifiers in livestock nutrition and health: A review. *J. Anim. Physiol. Anim. Nutr.* **2020**, *104*, 558–569. [CrossRef]
72. Goldstein, M.R.; Kruth, S.A.; Bersenas, A.M.; Holowaychuk, M.K.; Weese, J.S. Detection and characterization of *Clostridium perfringens* in the feces of healthy and diarrheic dogs. *Can. J. Vet. Res.* **2012**, *76*, 161–165.
73. Pedersen, K.S.; Holyoake, P.; Stege, H.; Nielsen, J.P. Observations of variable inter-observer agreement for clinical evaluation of faecal consistency in pigs. *Prev. Vet. Med.* **2011**, *98*, 284–287. [CrossRef]
74. Quilitis, M.; Lumabiang, J.; Camprodon, A.; Torres, M.; Magcalas, J.; Bautista, C.; Nuestro, F.; Vergel de Dios, R.; Santos, R.; Manuel, R. Control of pre-weaning mortality associated with *Escherichia coli* using Suiseng® in two Philippine commercial swine farms. In Proceedings of the 6th Asian Pig Veterinary Society Congress, Ho Chi Minh City, Vietnam, 23–25 September 2013.
75. Kitchodok, R.; Ananratanakul, C.; Kongthong, T. Efficacy and safety of Suiseng in prevention of neonatal diarrhea according to enterotoxigenic *E. coli* under a mixed infection with PRRSV involved from the field. *Thai J. Vet. Med.* **2018**, *48*, 169–170.
76. Kitchodok, R.; Triyarach, S.; Sutheerakul, K.; Serod, C.; Chompupun, D. Prevalence of Genotypic Fimbrial Antigens of Enterotoxigenic *E. Coli* Isolated in Thai Pig Herds. In Proceedings of the 20th Khon Kaen Veterinary Annual International Conference, Khon kaen, Thailand, 21–22 March 2019.
77. R Core Team. *R: A language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2021. Available online: https://www.R-project.org/ (accessed on 18 February 2023).
78. Levene, H. *Contributions to Probability and Statistics: Essays in Honor of Harold Hotelling*; Olkin, I., Ghurye, S.G., Hoeffding, W., Madow, W.G., Mann, H.B., Eds.; Stanford University Press: Redwood City, CA, USA, 1960; pp. 278–292.
79. Kruskal, W.H.; Wallis, W.A. Use of ranks in one-criterion variance analysis. *J. Am. Stat. Assoc.* **1952**, *47*, 583–621, Erratum in *J. Am. Stat. Assoc.* **1952**, *48*, 907–911. [CrossRef]
80. Wang, H.; Sanz Garcia, R.; Cox, E.; Devriendt, B. Porcine Enterotoxigenic *Escherichia coli* Strains Differ in Their Capacity To Secrete Enterotoxins through Varying YghG Levels. *Appl. Environ. Microbiol.* **2020**, *86*, e00523-20. [CrossRef] [PubMed]
81. Dubreuil, J.D. Pig vaccination strategies based on enterotoxigenic *Escherichia coli* toxins. *Braz. J. Microbiol.* **2021**, *52*, 2499–2509. [CrossRef] [PubMed]
82. Mesonero-Escuredo, S.; Strutzberg-Minder, K.; Casanovas, C.; Segalés, J. Viral and bacterial investigations on the aetiology of recurrent pig neonatal diarrhoea cases in Spain. *Porc. Health Manag.* **2018**, *4*, 5. [CrossRef] [PubMed]
83. Le Dividich, J.; Noblet, J. Colostrum intake and thermoregulation in the neonatal pig in relation to environmental temperature. *Biol. Neonate.* **1981**, *40*, 167–174. [CrossRef]
84. Pereira, D.A.; Vidotto, M.C.; Nascimento, K.A.; Santos, A.C.R.; Mechler, M.L.; Oliveira, L.G. Virulence factors of *Escherichia coli* in relation to the importance of vaccination in pigs. *Cienc. Rural.* **2016**, *46*, 8. [CrossRef]
85. Pedersen, L.J.; Malmkvist, J.; Kammersgaard, T.; Jorgensen, E. Avoiding hypothermia in neonatal pigs: Effect of duration of floor heating at different room temperatures. *J. Anim. Sci.* **2013**, *91*, 425–432. [CrossRef]
86. Martineau, G.P.; Vaillancourt, J.P.; Broes, A. Principal neonatal diseases. In *The Neonatal Pig Development and Survival*; Varley, M.A., Ed.; CAB International: Wallingford, UK, 1995; pp. 239–264.
87. Muirhead, M.R.; Alexander, T.L. *Managing Pig Health and the Treatment of Disease: A Reference for the Farm*; 5M Enterprises: Sheffield, UK, 1997.
88. Haesebrouck, F.; Pasmans, F.; Chiers, K.; Maes, D.; Ducatelle, R.; Decostere, A. Efficacy of vaccines against bacterial diseases in swine: What can we expect? *Vet. Microbiol.* **2004**, *100*, 255–268. [CrossRef]
89. Yaeger, M.J.; Kinyon, J.M.; Songer, J.G. A prospective, case control study evaluating the association between *Clostridium difficile* toxins in the colon of neonatal swine and gross and microscopic lesions. *J. Vet. Diagn. Investig.* **2007**, *19*, 52–59. [CrossRef]
90. Cruz, E.C., Jr.; Salvarani, F.M.; Silva, R.O.S.; Silva, M.X.; Lobato, F.C.F.; Guedes, R.M.C. A surveillance of enteropathogens in piglets from birth to seven days of age in Brazil. *Pesqui. Vet. Bras.* **2013**, *33*, 963–969. [CrossRef]
91. Steele, J.; Feng, H.; Parry, N.; Tzipori, S. Piglet models of acute or chronic *Clostridium difficile* illness. *J. Infect. Dis.* **2010**, *201*, 428–434. [CrossRef]
92. Arruda, P.H.E.; Madson, D.M.; Ramirez, A.; Rowe, E.; Lizer, J.T.; Songer, J.G. Effect of age, dose and antibiotic therapy on the development of *Clostridium difficile* infection in neonatal piglets. *Anaerobe* **2013**, *22*, 104–110. [CrossRef] [PubMed]
93. McElroy, M.C.; Hill, M.; Moloney, G.; MacAogain, M.; McGettrick, S. Typhlocolitis associated with *Clostridium difficile* ribotypes 078 and 110 in neonatal piglets from a commercial Irish pig herd. *Ir. Vet. J.* **2016**, *69*, 10. [CrossRef] [PubMed]
94. Silva, R.O.S.; Salvarani, F.M.; Cruz, E.C.C., Jr.; Pires, P.S.; Santos, R.L.R.; Antunes de Assis, R.; Guedes, R.M.C.; Lobato, F.C.F. Detection of enterotoxin a and cytotoxin B, and isolation of *Clostridium difficile* in piglets in Minas Gerais, Brazil. *Cienc. Rural.* **2011**, *41*, 1430–1435. [CrossRef]

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06/09/2026 21:12:45 EEST - 216.73.216.250

95. Jonach, B.; Boye, M.; Stockmarr, A.; Jensen, T. Fluorescence in situ hybridization investigation of potentially pathogenic bacteria involved in neonatal porcine diarrhea. *BMC Vet. Res.* **2014**, *10*, 68. [CrossRef] [PubMed]
96. Alvarez-Perez, S.; Alba, P.; Blanco, J.L.; Garcia, M.E. Detection of toxigenic *Clostridium difficile* in pig feces by PCR. *Vet. Med.* **2009**, *54*, 360–366. [CrossRef]
97. Larsson, J.; Aspan, A.; Lindberg, R.; Grandon, R.; Baverud, V.; Fall, N.; Jacobson, M. Pathological and bacteriological characterization of neonatal porcine diarrhoea of uncertain aetiology. *J. Med. Microbiol.* **2015**, *64*, 916–926. [CrossRef]
98. Yaeger, M.; Funk, N.; Hoffman, L. A survey of agents associated with neonatal diarrhea in Iowa swine including *Clostridium difficile* and porcine reproductive and respiratory syndrome virus. *J. Vet. Diag.* **2002**, *14*, 281–287. [CrossRef]
99. Farzan, A.; Kircanki, J.; DeLay, J.; Soltes, G.; Songer, J.G.; Friendship, R.; Prescott, J.F. An investigation into the association between cpb2-encoding *C. perfringens* type A and diarrhea in neonatal piglets. *Can. J. Vet. Res.* **2013**, *77*, 45–53.
100. Gibert, X.; Puig, A.; Sabaté, D.; Vidal-Mas, J.; March, R. Effects of a new vaccine against *Clostridioides Difficile* and *Clostridium Perfigens* Type A on the incidence of diarrhoea and antibiotic treatments uder field conditions. In Proceedings of the European Symposium of Porcine Health Management (ESPHM 2021), Bern, Switzerland, 14–16 April 2021.
101. Dors, A.; Czyżewska-Dors, E.; Wasyl, D.; Pomorska-Mól, M. Prevalence and factors associated with the occurrence of bacterial enteropathogens in suckling piglets in farrow-to-finish herds. *Vet. Rec.* **2016**, *179*, 598. [CrossRef]
102. Estienne, M.J.; Hartsock, T.G.; Harper, A.F. Effects of antibiotics and probiotics on suckling pig and weaned pig performance. *Int. J. Appl. Res. Vet. Med.* **2005**, *4*, 303–308.
103. Szabó, I.; Wieler, L.H.; Tedin, K.; Scharek-Tedin, L.; Taras, D.; Hensel, A.; Appel, B.; Nöckler, K. Influence of a probiotic strain of enterococcus faecium on *salmonella enterica* serovar *Typhimurium* DT104 infection in a porcine animal infection model. *Appl. Environ. Microbiol.* **2009**, *96*, 219–233. [CrossRef] [PubMed]
104. Liao, S.F.; Nyachoti, M. Using probiotics to improve swine gut health and nutrient utilization. *Anim. Nutr.* **2017**, *3*, 331–343. [CrossRef] [PubMed]
105. Satora, M.; Magdziarz, M.; Rząsa, A.; Rypuła, K.; Płoneczka-Janeczko, K. Insight into the intestinal microbiome of farrowing sows following the administration of garlic (*Allium sativum*) extract and probiotic bacteria cultures under farming conditions. *BMC Vet. Res.* **2020**, *16*, 442. [CrossRef] [PubMed]
106. Betancur, C.; Martínez, Y.; Tellez-Isaias, G.; Castillo, R.; Ding, X. Effect of oral administration with *Lactobacillus plantarum* CAM6 strain on sows during gestation-lactation and the derived impact on their progeny performance. *Med. Inflamm.* **2021**, *2021*, 6615960. [CrossRef] [PubMed]
107. Satora, M.; Rząsa, A.; Rypuła, K.; Płoneczka-Janeczko, K. Field evaluation of the influence of garlic extract and probiotic cultures on sows and growing pigs. *Med. Weter.* **2021**, *77*, 21–29. [CrossRef]
108. Laskowska, E.; Jarosz, Ł.; Grądzki, Z. Effect of multi-microbial probiotic formulation bokashi on pro-and anti-inflammatory cytokines profile in the serum, colostrum and milk of sows, and in a culture of polymorphonuclear cells isolated from colostrum. *Probiotics Antimicrob. Proteins* **2019**, *11*, 220–232. [CrossRef]
109. Tsukahara, T.; Inatomi, T.; Otomaru, K.; Amatatsu, M.; Romero-Pérez, G.A.; Inoue, R. Probiotic supplementation improves reproductive performance of unvaccinated farmed sows infected with porcine epidemic diarrhea virus. *Anim. Sci. J.* **2018**, *89*, 1144–1151. [CrossRef]
110. Partanen, K.H.; Morz, Z. Organic acids for performance enhancement in pig diets. *Nutr. Res. Rev.* **1999**, *12*, 117–145. [CrossRef]
111. Thompson, J.L.; Lawrence, T.L.J. Dietary manipulation of gastric pH in the profilaxis of enteric disease in weaned pigs. Some field observations. *Vet. Rec.* **1981**, *109*, 120–122. [CrossRef]
112. Tanaka, T.; Imai, Y.; Kumagae, N.; Sato, S. The effect of feeding lactic acid to *Salmonella typhimurium* experimentally infected swine. *J. Vet. Med. Sci.* **2010**, *72*, 827–831. [CrossRef]
113. Ferronato, G.; Prandini, A. Dietary Supplementation of Inorganic, Organic, and Fatty Acids in Pig: A Review. *Animals* **2020**, *10*, 1740. [CrossRef] [PubMed]
114. Sampath, V.; Park, J.H.; Pineda, L.; Han, Y.; Kim, I.H. Impact of synergistic blend of organic acids on the performance of late gestating sows and their offspring. *Ital. J. Anim. Sci.* **2022**, *21*, 1334–1342. [CrossRef]
115. Devi, S.M.; Lee, K.Y.; Kim, I.H. Analysis of the effect of dietary protected organic acid blend on lactating sows and their piglets. *Rev. Bras. Zootech.* **2016**, *45*, 39–47. [CrossRef]
116. Turner, J.L.; Dritz, S.; Minton, J.E. Alternatives to Conventional Antimicrobials in Swine Diets1. *PAS* **2001**, *17*, 4. [CrossRef]
117. Greeff, A.; Schokker, D.; Roubos-van den Hil, P.; Ramaekers, P.; Vastenhouw, S.A.; Harders, F.; Bossers, A.; Smits, M.A.; Rebel, J.M.J. The effect of maternal antibiotic use in sows on intestinal development in offspring. *J. Anim. Sci.* **2020**, *98*, skaa181. [CrossRef]
118. Lessa, F.C.; Gould, C.V.; McDonald, L.C. Current status of *Clostridium difficile* infection epidemiology. *Clin. Infect. Dis.* **2012**, *55*, S65–S70. [CrossRef]
119. Kociolek, L.K.; Gerding, D.N. Breakthroughs in the treatment and prevention of *Clostridium difficile* infection. *Nat. Rev. Gastroenterol. Hepatol.* **2016**, *13*, 150–160. [CrossRef]
120. Nale, J.Y.; Redgwell, T.A.; Millard, A.; Clokie, M.R.J. Efficacy of an Optimised Bacteriophage Cocktail to Clear *Clostridium difficile* in a Batch Fermentation Model. *Antibiotics* **2018**, *7*, 13. [CrossRef]
121. Fry, P.R.; Thakur, S.; Abley, M.; Gebreyes, W.A. Antimicrobial resistance, toxinotype, and genotypic profiling of *Clostridium difficile* isolates of swine origin. *J. Clin. Microbiol.* **2012**, *50*, 2366–2372. [CrossRef]

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122. Alvarez-Perez, S.; Blanco, J.L.; Bouza, E.; Alba, P.; Gibert, X.; Maldonado, J.; Garcia, M.E. Prevalence of *Clostridium difficile* in diarrhoeic and non-diarrhoeic piglets. *Vet. Microbiol.* **2009**, *137*, 302–305. [CrossRef]
123. Avbersek, J.; Janezic, S.; Pate, M.; Rupnik, M.; Zidaric, V.; Logar, K.; Vengust, M.; Zemljic, M.; Pirs, T.; Ocepek, M. Diversity of *Clostridium difficile* in pigs and other animals in Slovenia. *Anaerobe* **2009**, *15*, 252–255. [CrossRef] [PubMed]
124. Susick, E.K.; Putnam, M.; Bermudez, D.M.; Thakur, S. Longitudinal study comparing the dynamics of *Clostridium difficile* in conventional and antimicrobial free pigs at farm and slaughter. *Vet. Microbiol.* **2012**, *157*, 172–178. [CrossRef] [PubMed]
125. Schneeberg, A.; Neubauer, H.; Schmoock, G.; Baier, S.; Harlizius, J.; Nienhoff, H.; Brase, K.; Zimmermann, S.; Seyboldt, C. *Clostridium difficile* genotypes in piglet populations in Germany. *J. Clin. Microbiol.* **2013**, *51*, 3796–3803. [CrossRef] [PubMed]

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