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Original article

Occurrence and antimicrobial resistance of *Salmonella* isolates in farrow-to-finish pig farms in Bulgaria

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Abstract

Salmonella spp. is an important zoonotic and foodborne pathogen. It is spread worldwide and represents a public health risk. Pigs are a significant reservoir and are frequently subclinical carriers. The aim of this study was to detect the occurrence and antimicrobial resistance of *Salmonella* isolates being the five most important for public health *Salmonella* serovars in fattening pigs in Bulgaria.

The isolation of *Salmonella* spp. was carried out according to EN ISO 6579-1 for the detection of *Salmonella* bacteria in feces and *Salmonella* serotyping following the Kauffmann-White scheme. All confirmed *S. enterica* isolates were analysed by the disk-diffusion method for susceptibility to 14 antimicrobials. *Salmonella enterica* was detected in 13 out of 32 tested farms in Bulgaria. The overall percentage of *Salmonella* positive pooled fecal samples was 6.8% (43 of 630 samples). The highest occurrence was present in fattening pigs aged between 121-180 days (16.3%; 20/123), followed by dry sows (6.5%; 4/62) and gilts (4.8%; 12/248). About 75% of the isolated strains belonged to three serotypes: *Salmonella* Infantis (41.9%), *Salmonella* Give (16.3%) and *Salmonella* Typhimurium monophasic (16.3%). All the tested isolates were resistant to Tilmicosin (100%), 88.4% to Ampicillin, followed by 69.8% to Tiamulin, 25.6% to Amoxicillin and Chlortetracycline. Multidrug resistance was recorded in 62.8 % of the tested strains.

This study reports data regarding the circulation of the most important for public health five *Salmonella* serovars (*S.* Enteritidis, *S.* Typhimurium, *S.* Typhimurium monophasic, *S.* Infantis and *S.* Derby) in farrow-to-finish pig farms in Bulgaria and represent 74.4% of the total *Salmonella* spp. isolates. This phenomenon has critical effects for the health of consumers and therefore represents a key “one health” issue.

Keywords: antimicrobial resistance, farrow-to-finish pig farms, finishers, multidrug resistance, *Salmonella* spp.

Introduction

Salmonella is one of the main zoonotic agents with significant health and economic importance worldwide. It is responsible for gastrointestinal infections or more serious health problems in humans and is the second most common cause of disease in humans in the EU after campylobacteriosis (EFSA 2020). In 2022, 40 643 human cases with 81 deaths were reported in the EU. The overall trend for *Salmonella* infections in humans did not show any significant variations in the period 2018-2022. (EFSA 2023). However, it has been observed that the reported human cases of salmonellosis in the EU were lower in 2022 than in 2021, while the total number of foodborne outbreaks of salmonellosis was higher in 2022 than in 2021. In 2016-2020, the most frequently reported *Salmonella* serovars in humans were *S*. Enteritidis, *S*. Typhimurium, *S*. Typhimurium monophasic, *S*. Infantis and *S*. Derby (EFSA and ECDC 2021).

The infection is transmitted from animals to humans through the consumption of contaminated food of animal origin, including pork and pork products, which is in third place after turkeys and broilers (EFSA 2023). *S*. Typhimurium, including its monophasic variant, *S*. Derby and *S*. Rissen, are the top three *Salmonella* serovars in the European pork production chain (Roasto et al. 2023). The most common *Salmonella* serovars responsible for animal and human infections, with pork origin, were *S*. Typhimurium and *S*. Typhimurium monophasic (Sun et al. 2020). The disease in pigs is usually asymptomatic, but infected animals excrete the bacteria with feces, which is the main risk factor for meat contamination during slaughter (Bonardi 2017). Since the presence of *Salmonella* during fattening is a possible source of infection in slaughterhouses, the main preventive measures are usually focused on this stage of pig production (Alban et al. 2012, Argüello et al. 2013, Deane et al. 2022).

In 2008, the EU started the first process of monitoring zoonosis and zoonotic agents, in particular the control of *Salmonella*. A study was recently initiated by the EU to assess the prevalence, identify the main serotypes, and characterize the epidemiology and antimicrobial resistance of *Salmonella* infections in both fattening and breeding pigs in each Member State. The results of this survey indicate an average prevalence of *Salmonella* in fattening pigs of 10%, with a wide variation between Member States (from 0% in Finland to 29% in Spain) (EFSA 2008). Therefore, in 2020, the Commission Implementing Decision 2020/1729/EU laid down harmonised rules for the period 2021-2027 for the monitoring and reporting of *Salmonella* serovar occurrence and their antimicrobial resistance (minimal inhibitory concentration) in samples from slaughtered pigs. (Commission Implementing Decision (EU) 2020/1729).

Pig production has become an intensified industry and is frequently associated with the use of antimicrobials (Guardabassi et al. 2008). Therefore, antimicrobial resistance (AMR) is common among *Salmonella* isolated from food-producing animals. The transmission of these bacteria to humans by consumption of derived food products cannot be excluded (FAO 2021). Another important issue is resistance to three or more antimicrobial classes (multi-drug resistance) which could rapidly spread among susceptible populations.

AMR in *Salmonella* of porcine origin is a crucial issue, especially for those serotypes that are responsible for most of the pork-related human cases of salmonellosis (Roasto et al. 2023). The alarming increase in antimicrobial resistance has forced European health authorities to develop new rules for the use of colistin in veterinary medicine and since 2015 its use as a preventive agent has been restricted (EMA 2016).

Data on the occurrence of *Salmonella* in pigs produced in Bulgaria are very limited. Therefore, the aim of this study was to identify the *Salmonella* spp. occurrence of the five most important serovars for human health: *S*. Enteritidis, *S*. Typhimurium, *S*. Typhimurium monophasic, *S*. Infantis and *S*. Derby in farrow-to-finish pig farms in Bulgaria. Also, a study was carried out to indicate the exposure of pigs at the age before slaughter to the *Salmonella* spp. respectively to the top five *Salmonella* serovars. In addition, we aimed to provide information on the phenotypic profiles of antimicrobial and multidrug resistance found in *Salmonella* serovars isolated from pigs in Bulgaria.

Materials and Methods

Sample collection

From October 2020 to June 2021, 630 pooled fecal samples were collected in the context of the One Health European Joint Programme project (773830) "Biosecurity practice for pig farming across Europe (BIOPIGEE)". All samples were collected from 32 industrial farms in 11 cities in Bulgaria (Table 1). The districts chosen are where farrow-to-finish farms are present. The farms were randomly selected from the National Register of pig establishments in Bulgaria in 2020; however, only some farmers were willing to participate in the study. From each district, 1 to 5 farms were visited, since in one district only one farrow-to-finish farm was present, and it was sampled, while in those districts with more farms of this type we chose to visit

Table 1. Fecal samples of visited farms by administrative district of Bulgaria.

Administrative District	Number of Sampled Farms	Total Pooled Samples per District
Razgrad	4	80
Pazardzhik	4	70*
Vratsa	1	20
Montana	2	40
Ruse	5	100
Lovech	3	40
Gabrovo	1	20
Stara Zagora	4	80
Yambol	4	80
Shumen	2	40
Varna	3	60
In total	32	630

* In Pazardzhik district, only 10 samples were collected (from dry sows and gilts) from a farm, as no finishers were present at the moment of sampling.

all farms (4-5) when possible. At the moment of the sampling in some districts, a few farms were empty and, subsequently, not enrolled in this study. All the farms visited had similar management. The population of finishers varied between the farms, but in general, farms with a capacity of >1000 finishers dominated (22 farms), followed by farms with a capacity between 200 and 1000 finishers (6 farms), and small farms with <200 finishers (4 farms).

Twenty pooled fecal samples per farm were obtained, with each consisting of 10 individual fecal samples. Each individual sample, used to prepare the pooled sample, contained 10 g of fresh feces, collected immediately after defecation with plastic gloves and a spoon, which were changed after each sample collection. In addition, it was recommended that the fecal samples were best collected from as many different pens as possible from the targeted groups of pigs (see below). This sample size provided sufficient sensitivity to detect at least one positive sample with 95% confidence even if the within-herd occurrence was as low as 2% and would estimate an expected within-farm occurrence of 10% with 15% precision (EFSA 2009, Powell et al. 2017, Sergeant ESG 2018).

The ratio of samples (finisher/gilt/dry sow) collected from each farm was 50-40-10% (10 pooled fecal samples from finishers, 8 pooled fecal samples from gilts, and 2 pooled fecal samples from dry sows). In the context of the study design, dry sows are pigs in the dry period (time interval from weaning to farrowing), gilts are female pigs of ~6 months of age before the first delivery of a litter of piglets, and finishers are pigs after weaning until they reach their market weight. In one of the tested farms in the Pazardzhik district, fatteners were not present at the sampling visit, and so we sampled only dry sows and gilts. The average slaughtering age of fattening pigs in Bulgaria is 4-6 months. The age of the fattening pigs sampled in our study was between 2.5 and 6 months. The sampled fattening pigs were split into two different age groups: finishers aged between 60 and 120 days (n=197 pooled fecal samples) and finishers aged between 121 and 180 days (n=123 pooled fecal samples). After collection, all samples (n=630) were immediately refrigerated and transported to the laboratory.

Salmonella isolation

The isolation of *Salmonella* spp. was performed according to the methodology of EN ISO 6579-1 (2017) for the detection of *Salmonella* bacteria in feces. Two suspected colonies were initially subjected to standard biochemical tests, triple sugar iron agar (TSI) and API 20E strips (bioMérieux, France). Thereafter, if both colonies were confirmed as *Salmonella* spp., only one was randomly selected for further analysis.

Serotyping of *Salmonella* isolates

Salmonella isolates were sub-cultured and serotyped using commercial O- and H- antisera by agglutination on a slide following the Kauffmann-White scheme (2007). Confirmed isolates were stored at -80°C for further analyses.

Antimicrobial agent susceptibility testing

All confirmed *S. enterica* isolates were analyzed using the Kirby Bauer technique, according to the

Table 2. Occurrence of *Salmonella* spp. in pooled fecal samples according to different age categories in pigs from Bulgaria.

Production stage		Total number of tested pooled fecal samples	Number of positive pooled fecal samples	% of positive pooled fecal samples
Dry sows		62	4	6.5
Gilts		248	12	4.8
Fattening pigs	between 60 – 120 days (2-4 month)	197	7	3.6
	between 121 – 180 days (>4 - 6 month)	123	20	16.3
Total fattening pigs		320	27	11.6
In total		630	43	6.8

recommendations of the Clinical and Laboratory Standards Institute (Humphries et al. 2018, M100Ed32 2020). Reference strains of *Escherichia coli* ATCC 25922 were used for quality control. Susceptibility testing was done using 14 antibiotics (total of ten classes) commonly used in swine production for the treatment of infections caused by *Enterobacteriaceae*. The bacterial suspension was standardized to 0.5 McFarland units using a nephelometer. Analyzed antibiotics included Ampicillin (AMP, 10 μg.disk^{-1}), Amoxicillin (AMX, 10 μg.disk^{-1}), Cefuroxime (CXM, 30 μg.disk^{-1}), Ceftazidime (CAZ, 10 μg.disk^{-1}), Gentamicin (GEN, 10 μg.disk^{-1}), Amikacin (AK, 30 μg.disk^{-1}), Ciprofloxacin (CIP, 5 μg.disk^{-1}), Nalidixic acid (NX, 30 μg.disk^{-1}), Chlortetracycline (C, 30 μg.disk^{-1}), Tetracycline (TE, 30 μg.disk^{-1}), Florfenicol (FFC, 30 μg.disk^{-1}), Tilmicosin (TIL, 15 μg.disk^{-1}), Tiamulin (TIA, 30 μg.disk^{-1}), and Colistin sulphate (CT, 25 μg.disk^{-1}), using commercial disks (Oxoid, Great Britain). All *Salmonella* isolates with resistance to more than three classes of antimicrobials were identified as multidrug-resistant isolates (MDR).

Statistical analysis

Statistical analysis was performed using Excel 2007 (Microsoft, Redmond, WA, USA). Chi-square tests were performed to detect differences between categories of variables and the *Salmonella* results. When the result was $p<0.05$ this was determined to be statistically significant.

Results

Isolation of *S. enterica*

Salmonella enterica was detected in at least one pooled fecal sample in 30.2% of the tested farms in Bulgaria (13/32). The mean occurrence in pooled fecal samples was 6.8% (43/630). The highest occurrence was in fattening pigs aged between 121-180 days (16.3%; 20/123), followed by dry sows (6.5%; 4/62) and gilts (4.8%; 12/248). The occurrence was lowest in fattening pigs between 60-120 days of age (3.6%; 7/197) (Table 2).

There was no significant difference between the results of dry sows and gilts, but fatteners had a significantly higher change of probability of positive results (Pchi-sq<0.001) than sows and gilts. The occurrence of *Salmonella* spp. in pooled fecal samples from total fattening pigs (11.6%) was higher than in gilts (4.8%). *Salmonella* spp. was significantly (Pchi-sq = 0.04) more likely to be detected in pooled fecal samples from fattening pigs aged between 121 and 180 days, 16.3%. (20/123), than in fattening pigs aged between 60 and 120 days (3.6%).

Salmonella spp. was detected in farrow-to-finish farms with more than 1000 fattening pigs (40.9%, 9/22) and in 40.0% (4/10) of farms with 1000 fattening pigs or less (Pchi-sq > 0.05). In addition, *Salmonella* spp. detection levels varied significantly between administrative districts. In the Pazardzhik, Vratsa, Montana and Gabrovo districts all tested farms were negative for *Salmonella* spp. (100%). The results were similar in farrow-to-finish farms in the Stara Zagora and Yambol districts with *Salmonella* spp. occurrence in two of the four farms in each district. The majority of the farms were *Salmonella*-positive in Razgrad, Varna and Stara Zagora, 75% (3/4), 67% (2/3) and 75% (3/4) respectively. In the Ruse district, only two out of five tested farms were positive for *Salmonella* spp. (Fig.1). The number of farms in each district was too small to make useful statistical comparisons.

Salmonella serotyping

Nine different serovars were identified. *Salmonella* Infantis (41.9%), *Salmonella* Give (16.3%), and *Salmonella* Typhimurium monophasic (16.3%) were the most frequent serovars in pig samples. *Salmonella* Infantis was present in 9.1% of the positive pig farms, followed by *Salmonella* Typhimurium monophasic (6.1%) and *Salmonella* Derby (6.1%) (Table 3).

Two circulating serovars, *Salmonella* Give and *Salmonella* Rissen were detected together in one farm in the Varna district.

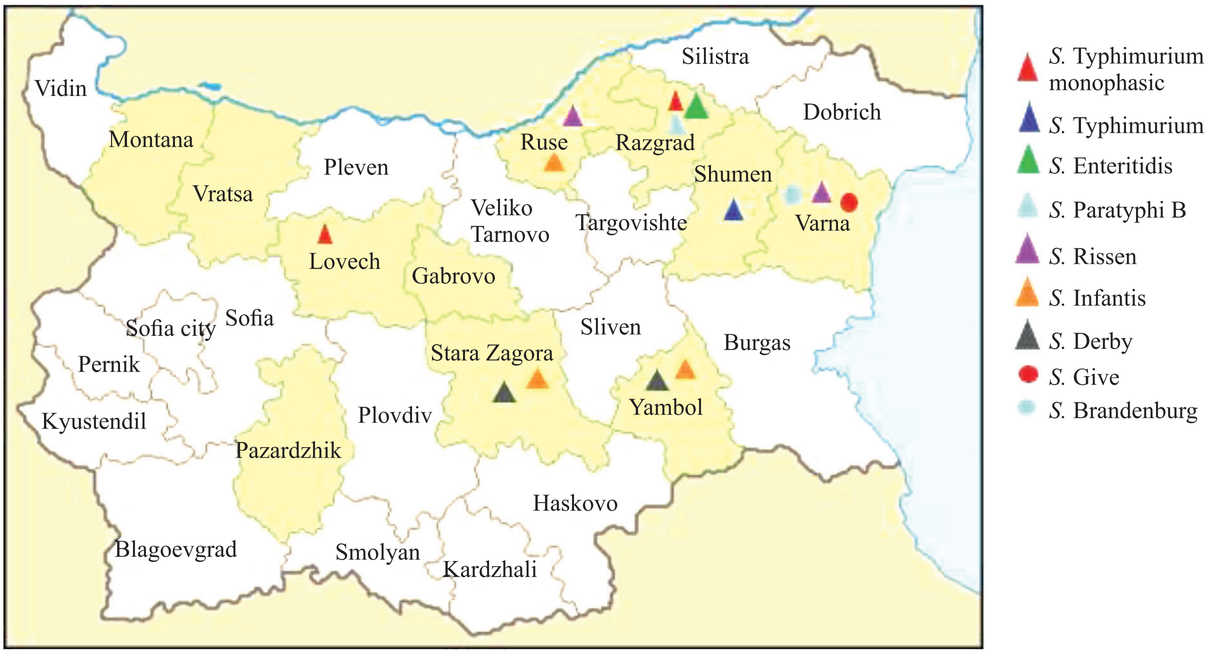

Fig. 1. Occurrence of *Salmonella* serovars in different administrative districts.

Table 3. Serovar occurrence of *Salmonella* spp. in positive farrow-to-finish farms in Bulgaria.

Salmonella serotype	Number of positive farms	% positive farms of the total tested farms (n=32)
Salmonella Typhimurium monophasic	2	6.1
Salmonella Typhimurium	1	3.0
Salmonella Enteritidis	1	3.0
Salmonella Paratyphi B	1	3.0
Salmonella Rissen	2	6.1
Salmonella Infantis	3	9.1
Salmonella Derby	2	6.1
Salmonella Give	1	3.0
Salmonella Brandenburg	1	3.0

Antimicrobial resistance of *S. enterica* strains

All tested isolates showed antimicrobial resistance to at least one of the antibiotics and were present on 100% of the positive pig farms. Antimicrobial susceptibility tests showed that 100% (43) of the strains were susceptible to Amikacin and Nalidixic acid, while all the tested isolates were resistant to Tilmicosin (100%), 88.4% to Ampicillin, followed by 69.8% to Tiamulin, 25.6% to Amoxicillin and Chlortetracycline. Less pronounced resistance was observed to Florfenicol (7.3%), Cefuroxime, Ceftazidime and Ciprofloxacin (4.9%), Gentamicin and Colistin (2.4%).

Many strains showed multidrug resistance (62.8%). The antimicrobial resistance profiles of 43 isolates are presented in Table 4.

Overall, the profiles of the two *S.* Typhimurium isolates showed multidrug resistance and differed from those of the monophasic variant. The predominant pattern in the monophasic variant isolates was resistance to C TIL TE AMX and AMP. *S.* Infantis, which was the most frequently isolated serovar in our study, had a predominant resistance profile to TIL (94.4%) and an antimicrobial profile: TIL AMP (55.5%).

Discussion

People can frequently become infected with *Salmonella* from contaminated fattening pig carcasses. For this reason, many studies have been associated with the detection of *Salmonella* in fattening pigs (Bolton et al. 2013, Harrison et al. 2023). All research has shown that it is necessary to test all pig categories and their facilities in order to fully control and reduce *Salmonella* circulation (Sisak et al. 2011, De Busser et al. 2013,

Table 4. Antimicrobial resistance profiles of *Salmonella* isolates (n=43).

AMR Profile	*S.* Infantis	*S.* Give	*S.*Typhimurium	Monophasic S.Typhimurium	Other serovars	Total
TIL TIA GEN CIP AMP CAZ CXM CT					1	1
C FFC TIL TIA TE AMX AMP					2	2
C FFC TIA TE AMX AMP			1			1
C TIL TIA TE AMX AMP				4		4
C TIL TE AMX AMP				2		2
TIL TIA AMP CAZ CXM	1					1
TIL TIA TE AMX AMP			1			1
C TIL TIA AMP					1	1
TIL TIA AMP	5	4			4	13
TIL TIA		2			2	4
TIL AMP	10			1		11
TIA AMP	1					1
TIL	1					1
Total number of isolates	18	6	2	7	10	43

AMP – ampicillin, AMX – amoxicillin, CXM – cefuroxim, CAZ – ceftazidime, GEN – gentamicin, AK – amikacin, CIP – ciprofloxacin, NX – nalidixic acid, C – chlortetracycline, TE – tetracycline, FFC – florfenicol, TIL – tilmicosin, TIA – tiamulin, CT – colistin sulfate

Roldan-Henao et al. 2023). As pigs are well known to be easily infected by the fecal-oral route with *Salmonella* (Schwartz 1999) and contaminated environments, such as feed and bedding, direct contact with each other is considered to be the main source of infection. Since, in Bulgarian farrow-to-finish farms, sows, gilts, and fattening pigs are co-housed on the same farm, it should be recognized that sows and gilts may play an important role in *Salmonella* transmission.

The results of this study are part of the international BIOPIGEE project, the aim of which was to identify on-farm biosecurity measures, related to limiting the possibility of *Salmonella* introduction and transmission on pig farms (Smith et al. 2023), but not the overall occurrence of *Salmonella* and its antimicrobial resistance in different participating countries.

Salmonella spp. was commonly found on farrow--to-finish farms in Bulgaria (13/32; 30.2%). There were no *Salmonella* spp found on the sampled farms located in the Western part of Bulgaria. The Northeastern part had a higher proportion of samples that were positive for *Salmonella* spp. This may be explained by the fact that this part of Bulgaria is characterized by a large number of pig farms with >1000 finishers.

The highest percentage of *Salmonella*-positive pooled fecal samples was in fattening pigs between 4 and 6 months of age, shortly before slaughter (16.3%). The population of younger fattening pigs (2-4 months of age) also showed a positivity rate of about 11.6%. This result indicates the potential risk of *Salmonella* entering the food chain, presenting a hazard to consumers. Our results are similar to the European Food Safety Authority results for Greece, Luxembourg, Portugal, and Spain in 2008 (EFSA 2009), which reported prevalence between 25% and 30%. Lower prevalence was found in other countries, e.g. Austria, Estonia, Finland, Norway, Poland, and Slovakia, where the prevalence was around 5%. These differences may be related to the strict biosecurity standards and better farming practices in these countries.

Studies on the prevalence of *Salmonella* on pig farms and in slaughterhouses have been carried out in many countries in the European Union. It is known from the published data that *Salmonella* isolation from pigs younger than 2 weeks is much less frequent than in fattening pigs (Costinar et al. 2021). A study conducted by Asai et al. (2000) showed the highest percentage of *Salmonella* in fattening pigs (17.3%) and lower in sows (4.2%), which is fully comparable with our data. A recent study on the *Salmonella* prevalence in the fattening pig population in Estonia also reported similar data - 27.7% (Kuus et al. 2021). In Ireland, a study of pigs at a slaughterhouse also found a high prevalence of *Salmonella* (55.5%) in pig carcasses (Deane et al. 2022). Similar data have been reported from the UK. A total prevalence of *Salmonella* was found in 32.2% of the cecum contents in a study of 286 slaughterhouses (Martelli et al. 2021). Research on the prevalence of *Salmonella* in pigs has also been carried out in Eastern Europe. A study conducted on seven farms in Serbia reported 3.3% *Salmonella* prevalence (3/90) in fattening pigs between 130 and 180 days of age. In contrast, they found that *Salmonella* spread increased from weaning to 75 days, then slowed down and, by 180 days of age, when the fatteners were sent to the slaughterhouse, all fecal samples were free of *Salmonella* (Stojanac et al. 2014). Probably, the different results are due to the increased resistance of pigs and the use of antibiotics for preventive purposes, which is common on farms in Serbia. A study of 8 fattening farms carried out in western Romania demonstrated *Salmonella* spp. in 8 out of 10 fecal samples from pigs before slaughter (around 130 days of age) (Costinar et al. 2021).

According to the European Union One Health Zoonoses Report, *S*. Enteritidis (67.3%), *S*. Typhimurium (13.1%), *S*. Typhimurium monophasic (4.3%), *S*. Infantis (2.3%) and *S*. Derby (0.89%) were the main serotypes associated with disease in humans (EFSA and ECDC 2023). Pork meat is most commonly associated with *S*. Typhimurium and its monophasic variant, which means that pigs and pork are a major source of this foodborne pathogen for humans. They were among the most isolated serovars from pigs in the EU (Soliani et al. 2023). Although *S*. Derby is not a dominant cause of outbreaks in humans, it has a high prevalence in pigs and pork products (EFSA and ECDC 2021). *S*. Derby was the fourth most commonly detected serovar in our reseach. Kuus et al. (2021) reported that *S*. Derby was the most often isolated serotype from all stages of pork production in Estonia. *Salmonella* Typhimurium monophasic and *Salmonella* Infantis predominated among the positive samples in our study. These data are consistent with studies from many countries (Stojanac et al. 2014, Martelli et al. 2021, Deane et al. 2022). In contrast to the widespread data that *S*. Derby is more common in the EU (EFSA 2020), in our study it was found only in 7% of tested samples.

Antimicrobial resistance to *Salmonella* spp. in the swine food chain is under continuous monitoring and has been thoroughly studied across Europe (Bonardi 2017). Moreover, EFSA and ECDC (2023) have highlighted the significant variation in *Salmonella* isolation data amongst member states.

Our study found high levels of resistance to ampicillin and tetracycline that were comparable to previous studies (Thi et al. 2020, Deane et al. 2022, Karabasanavar et al. 2022, Lauteri et al. 2022). Ampicillin and tetracycline are commonly used in swine production as first-choice antibiotics for the treatment of bacterial diseases in piglets (Prasertsee et al. 2016, Lekagul et al. 2019), which accounts for the high resistance to these agents. The reported data are of concern, because ampicillin and tetracycline belong to category D (precautionary) and should be used as first-line treatments, but only if medically necessary (EMA 2016).

Antimicrobial resistance to nalidixic acid and ciprofloxacin for all serotypes in our study was comparable to data in Ireland (Deane et al. 2022). At this time, ciprofloxacin is the one of the recommended drugs for the treatment of invasive *Salmonella* infections in humans or in patients at risk of developing an invasive infection (Shane et al. 2017) and has a high significance in the prevention of the diseases. Roasto et al. (2023) reported extremely frequent resistance of *Salmonella* to sulfamethoxazole and streptomycin in the European pork production chain. Most of the *Salmonella* isolates were sensitive to colistin and ciprofloxacin, which was in agreement with the present study.

In the present study, 62.8% were resistant to more than three antimicrobial classes. Of these, 6 (22.2%) were *S.* Typhimurium monophasic, 2 (7.4%) were *S.* Typhimurium, and 19 (70.4%) were a combination of other serotypes. These results clearly indicate that *S.* Typhimurium monophasic and *S.* Typhimurium have a high level of resistance, particularly to older antibiotics that have been used over a long period of time in swine production. Similar to our data, Retamal et al. (2022) reported high levels of multidrug resistance in *Salmonella* isolates from swine samples. In contrast, however, these strains showed resistance to more than 10 antimicrobials. *Salmonella* Typhimurium monophasic has been associated with pig food production, especially in Europe. This indicates a potential link between human infections and consumption of contaminated pork products (Lucarelli et al. 2010, Mourao et al. 2015). The different results obtained on the prevalence of *Salmonella* spp. and its antimicrobial resistance can be explained by differences in pig production technologies, vaccination programmes, the type of antimicrobials, and the therapy duration or metaphylaxis of bacterial gastrointestinal and respiratory co-infections in industrial pig production.

In conclusion, domestic pigs are an important zoonotic reservoir for *Salmonella* infection. This survey provides useful insight into the *Salmonella* prevalence and circulating serotypes in the pork production chain. The high prevalence of *Salmonella* spp. in fattening pigs and the high levels of MDR in fecal pig samples confirm the current risk for human health. Therefore, the present study indicates the epidemiological importance of monophasic *S*. Typhimurium, *S*. Typhimurium and *S*. Derby in Bulgarian pig farms and could be a good basis in epidemiological surveys for human cases.

To prevent the possible spread of *Salmonella* through the production of pig meat, measures to limit the spread should be considered. The consumption of safe food products remains one of the most important responsibilities of any public policy worldwide. Assessment of the risk factors associated with the introduction and spread of *Salmonella* on pig farms remains a fundamental requirement to prevent pathogen transmission.

Our recommendation is to develop a national surveillance system to monitor the prevalence of *Salmonella* spp. and levels of antimicrobial resistance. This will allow the identification of the sources of *Salmonella* spp. at different stages of pig production and provide evidence to improve the regulation of antimicrobial use in livestock production.

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References

Alban L, Baptista FM, Møgelmose V, Sørensen LL, Christensenet H, Aabo S, Dahl J (**2012**) *Salmonella* surveillance and control for finisher pigs and pork in Denmark – A case study. Food Res Int 45: 656-665.

Argüello H, Álvarez-Ordoñez A, Carvajal A, Rubio P, Prieto M (**2013**) Role of slaughtering in *Salmonella* spreading and control in pork production. J Food Prot 76: 899-911.

Asai T, Otagiri Y, Osumi T, Namimatsu T, Hirai H, Sato S (**2000**) Isolation of *Salmonella* from diarrheic feces of pigs. J Vet Med Sci 64: 159-160.

Bolton DJ, Ivory C, McDowell D (**2013**). A study of *Salmonella* in pigs from birth to carcass: serotypes, genotypes, antibiotic resistance and virulence profiles. Int J Food Microb 160: 298-303.

Bonardi S (**2017**) *Salmonella* in the pork production chain and its impact on human health in the European Union. Epidemiol Infect 145: 1513-1526.

CLSI M100 (**2022)** Performance Standards for Antimicrobial Susceptibility Testing, 32nd ed., Clinical & Laboratory Standards Institute, Malvern, PA, USA, pp 416.

Commission Implementing Decision (EU) 2020/1729 (**2020**) on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria and repealing Implementing Decision 2013/652/EU. Official Journal EU 387: 8-21.

Costinar L, Heman V, Iancu I, Pascu C (**2021**) Phenotypic characterization and antimicrobial resistance of *Salmonella* strains isolated from pigs from fattening farms. Rev Rom Med Vet 31: 31-34.

Deane A, Murphy D, Leonard FC, Byrne W, Clegg T, Madigan G, Griffin M, Egan J, Prendergast DM (**2022**) Prevalence of *Salmonella* spp. in slaughter pigs and carcasses in Irish abattoirs and their antimicrobial resistance. Ir Vet J 75: 4.

De Busser EV, De Zutter L, Dewulf J, Houf K, Maes D (**2013**) *Salmonella* control in live pigs and at slaughter. Vet J 196: 20-27.

EFSA (European Food Safety Authority) (**2008**) Report of the task force on zoonoses data collection on the analysis of the baseline survey on the prevalence of *Salmonella* in slaughter pigs. Part A. EFSA J 135: 1-111.

EFSA (European Food Safety Authority) (**2009**) Analysis of the baseline survey on the prevalence of *Salmonella* in holdings with breeding pigs in the EU, 2008 – Part A: *Salmonella* prevalence estimates. EFSA J 7: 1377.

EFSA (European Food Safety Authority) (**2020**) The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017/2018. EFSA J 18: 6007.

EFSA (European Food Safety Authority) & ECDC (European Centre for Disease Prevention and Control) (**2020**) The European Union One Health 2021 Zoonoses Report. EFSA J 20: 7666.

EFSA (European Food Safety Authority) & ECDC (European Centre for Disease Prevention and Control) (**2021**) European Union One Health 2020 Zoonoses Report. EFSA J 19: 6971.

EFSA (European Food Safety Authority) & ECDC (European Centre for Disease Prevention and Control) (**2023**) The European Union One Health 2022 Zoonoses Report. EFSA Journal, 21: e8442.

EN ISO 6579-1:2017 (**2017**) Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of Salmonella – Part 1: Detection of Salmonella spp. International Organization for Standardization: Geneva, Switzerland.

European Medicines Agency (**2016**) EMA/CVMP/CHMP/231573/2016 – Updated Advice on the Use of Colistin Products in Animals within the European Union: Development of Resistance and Possible Impact on Human and Animal Health, pp 1-56

FAO (Food and Agriculture Organization) (**2021**) The FAO action plan on antimicrobial resistance 2021-2025 Rome, pp 1-46.

Grimont PAD & Weill F-X (**2007**) Antigenic formulae of the *Salmonella* serovars. 9th ed., WHO Collaborating Centre for Reference and Research on Salmonella, Institut Pasteur, France, pp 166.

Guardabassi L, Jensen LB, Kruse H (**2008**) Guide to antimicrobials used in animals. 1st ed 2008, Blackwell Publishing Ltd, Oxford, UK 407, pp 102-125.

Harrison OL, Li KW, Gebhardt JT, Paulk CB, Woodworth JC, Rensing S, Jones CK,Trinetta V (**2023**) Investigation of the environmental presence of *Salmonella* spp. in finishing pigs at commercial swine farms in Kansas (USA). Lett Appl Microbiol 76: ovad065.

Humphries RM, Ambler J, Mitchell SL, Castanheira M, Dingle T, Hindler J, Koeth L, Sei K (**2018**) CLSI methods development and standardization working group best practices for evaluation of antimicrobial susceptibility tests. J Clin Microbiol 56:e01934-17.

Karabasanavar N, Sivaraman GK, Satheesha SP, Nair AS, Vijayan A, Rajan V, Girish PS (**2022**) Non-diarrhoeic pigs as source of highly virulent and multidrug-resistant non--typhoidal *Salmonella*. Braz J Microbiol 53: 1039-1049.

Kuus K, Kramarenko T, Sõgel J, Mäesaar M, Fredriksson--Ahomaa M, Roasto M (**2021**) Prevalence and serotype diversity of *Salmonella* enterica in the Estonian meat production chain in 2016-2020. Pathogens 10: 1622.

Lauteri C, Festino AR, Conter M, Vergara A (**2022**) Prevalence and antimicrobial resistance profile in *Salmonella* spp. isolates from swine food chain. Ital J Food Saf 11: 9980.

Lekagul A, Tangcharoensathien V, Yeung S (**2019**) Patterns of antibiotic use in global pig production: A systematic review. Vet Anim Sci 7: 100058.

Lucarelli C, Dionisi AM, Torpdahl M, Villa L, Graziani C, Hopkins K, Threlfall J, Caprioli A, Luzzi I (**2010**). Evidence for a second genomic island conferring multidrug resistance in a clonal group of strains of *Salmonella* enterica serovar Typhimurium and its monophasic variant circulating in Italy, Denmark, and the United Kingdom. J Clin Microbiol 48: 2103-2109.

Martelli F, Oastler C, Barker A, Jackson G, Smith RP, Davies R (**2021**). Abattoir based tudy of *Salmonella* prevalence in pigs at slaughter in Great Britain. Epidemiol Infect 149: e218.

Mourao J, Novais C, Machado J, Peixe L, Antunes P (**2015**) Metal tolerance in emerging clinically relevant multidrug-resistant *Salmonella* enterica serotype 4,[5],12:i:- clones circulating in Europe. Int J Antimicrob Agents 45: 610-616.

Powell BJ, Mandell DS, Hadley TR, Rubin RM, Evans AC, Hurford MO, Beidas RS (**2017**) Are general and strategic measures of organizational context and leadership associated with knowledge and attitudes toward evidence-based practices in public behavioral health settings? A cross-sectional observational study. Implement Sci 12: 64.

Prasertsee T, Khantaprab N, Yamsakul P, Santiyanont P, Chokesajjawatee N, Patchanee P (**2016**) Repertitive sequence-based PCR fingerprinting and the relationship of antimicrobialresistence characteristics and corresponding genes among *Salmonella* strains from pig production. Asian Pac J Trop Dis 6: 390-535.

Retamal P, Gaspar J, Benavides MB, Saenz L, Galarce N, Aravena T, Cornejo J, Lapierre L (**2022**) Virulence and antimicrobial resistance factors in *Salmonella* enterica serotypes isolated from pigs and chickens in central Chile. Front Vet Sci 9: 971246.

Roasto M, Bonardi S, Maesaar M, Alban L, Gomes-Neves E, Vieira-Pinto M, Vågsholm I, Elias T, Lindegaard L, Blagojevic B (**2023**) *Salmonella* enterica prevalence, serotype diversity, antimicrobial resistance and control in the European pork production chain. Trends Food Sci & Technol 131: 210-219.

Roldan-Henao M, Dalsgaard A, Cardona-Castro N, Restrepo-Rivera L, Veloza-Angul LC, Alban L (**2023**) Pilot study of the productivity and *Salmonella* seroprevalence in pigs administered organic acids. Front Vet Sci 10: 1123137.

Schwartz KJ (**1999**) Salmonellosis. In: Taylor DJ (ed) Diseases of swine. 8th ed., Ames: Iowa State University Press, pp 535-551.

Sergeant ESG (**2018**). Epitools Epidemiological Calculators, Ausvet. Australia, https://epitools.ausvet.com.au/

Shane AL, Mody RK, Crump JA, Tarr PI, Steiner TS, Kotloff K, Langley JM, Wanke C, Warren CL, Cheng AC, Cantey J, Pickering LK, Pickering LK (**2017**) 2017 Infectious Diseases Society of America clinical practice guidelines for the diagnosis and management of infectious diarrhea. Clin Infect Dis 65: e45-e80.

Sisak F, Havlickova H, Matiasovic J, Karpíšková R (**2011**) Serological and Bacteriological Evaluation of *Salmonella* status in swine herds. Czech J of Food Science 29: S102-S108.

Smith RP, May HE, Burow E, Meester M, Tobias TJ, Sassu E-L, Pavoni E, Di Bartolo I, Prigge C, Wasyl D, Zmudzki J, Viltrop A, Nurmoja I, Zoche-Golob V, Alborali GL, Romantini R, Dors A, Krumova-Valcheva G, Koláčková I, Aprea G, Daskalov H (**2023**). Assessing pig farm biosecurity measures for the control of *Salmonella* on European farms. Epidemiol Infect, 151: e130.

Soliani L, Rugna G, Prosperi A, Chiapponi C, Luppi A (**2023**). *Salmonella* Infection in Pigs: Disease, Prevalence, and a Link between Swine and Human Health. Pathogens 12: 1267.

Sun H, Wan Y, Du P, Bai L (**2020**). The epidemiology of monophasic *Salmonella* Typhimurium. Foodborne Pathog Dis 17: 87-97.

Stojanac N, Stevancevic O, Potkonjac A, Savić B, Stančićet I, Vračar V (**2014**) Importance of breeding pigs in the spread of *Salmonella* on farms. Acta Sci Vet 42: 1180.

Thi HN, Pham TTT, Turchi B, Fratini F, Ebani VV, Cerri D, Bertelloni F (**2020**) Characterization of *Salmonella* spp. Isolates from Swine: Virulence and Antimicrobial Resistance. Anim 10: 2418.