Prevalence and antimicrobial resistance profile in *Salmonella* spp. isolates from swine food chain

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Abstract

The aim of this survey was to examine the prevalence and the antimicrobial resistance (AMR) of *Salmonella* spp. isolated from swine food chain. A total of 435 samples were collected: 360 from slaughterhouse (150 carcasses, 30 cecal samples, 180 environmental samples) and 75 from Italian traditional pork dry sausages. Thirty-six *Salmonella* were isolated and identified by Polymerase Chain Reaction (PCR): 13,3% (4/30) in fecal samples, 5,5% (10/180) in environmental samples, 7,3% (11/150) in carcasses, and 14,6% (11/75) in Italian traditional dry sausages. *Salmonella* serotypes were: *S.* Typhimurium (44,4%), *S.* Typhimurium monophasic variant (8,3%), *S.* Typhi (2,8%), *S.* Enteritidis (22,2%), *S.* Rissen (16,6%) and *S.* Derby (5,5%). Phenotypic and genotypic characterization of AMR *Salmonella* spp. isolates was executed through automatic system (VITEK 2, bioMèrieux) and PCR assays. *Salmonella* spp. showed phonotypical and genotypical resistance to at least one or more classes of antibiotic. All *Salmonella* spp. were resistant to aminoglycoside (amikacin and tobramycin) and gentamicin, 86,1% strains were resistant to tetracycline, 55,5% strains were resistant to ampicillin and piperacillin, 25% strains to trimethoprim, 5,5% strains to chloramphenicol, 2,8% strains to amoxicillin/clavulanic acid, and nitrofurantoin. Among *Salmonella* isolates, the most detected AMR genes were *catA* for chloramphenicol (94,4%), nitrofuran *nfsA* (77.7%), *nfsB* (86,1%) and, for fluoroquinolone *par C* (100%) and *gyrA* (94,4%). This study reported epidemiological data regarding *Salmonella* spp. and AMR's circulation in the swine food chain. This phenomenon (AMR) has critical repercussions on the final consumer health; therefore, it represents a crucial One-Health issue.

Introduction

In 2019 Salmonellosis was the second reported zoonotic disease in the European Union (EU), affecting about 88,000 people (Molla *et al.,* 2003; Astorga *et al.,* 2007; Wang *et al.,* 2013; EFSA, 2021).

In swine, it is caused by *S.* Cholerasuis and, occasionally, may be responsible for human disease. However, ubiquitous *Salmonella* serovars are the unrestricted serovars that can cause symptomatic disease in a wide range of hosts, but more frequently cause self-limiting gastroenteritis. Typical symptoms in pigs are enteric and even fatal diseases; asymptomatic infected animals frequently carry these serovars in tonsils, gut, and gut-associated lymphoid tissue (Fedorka-Cray *et al*., 2000). These latent carriers could begin to shed *Salmonella* after leaving livestock, a process that might be triggered by stress factors such as transportation, holding pens at the slaughterhouse (Hurd *et al.,* 2002).

Pork is the most frequently consumed meat in Europe (especially in Northern countries), and parallelly to this trend, Mediterranean nations have increased its consumption (Valero *et al.*, 2014).

Italians are specialized in "*heavy pig*" livestock, which means that animals weight ranges between 150-160 kg with an age of 9 months (Di Ciccio *et al.,* 2016).

In Europe, contaminated pork and pork products are important sources for Salmonellosis in human cases (EFSA, 2021). The risk of infection is exacerbated by the high prevalence of *Salmonella* spp. in livestock, slaughterhouses, and asymptomatic animal acting as healthy carrier (Baptista *et al.*, 2010).

Since 2005, the EU has established strict microbiological criteria for pig carcasses (Regulation EU No. 2073/2005). Focusing on *Salmonella* carcasses' contamination, the European Legislator introduced new hygienic criteria (Regulation EU No. 217/2014) to reduce its prevalence.

Antibiotics' administration in animals has different aims: disease treating, metaphylaxis, and prophylaxis, especially in stress periods such as before slathering (Aarestrup, 2005). Antimicrobial misuses have increased AMR phenomenon's diffusion, and for this reason Regulation EC No. 1831/2003 was introduced to ban antibiotics usage for growth promotion.

In many countries the most used antibiotic in swine livestock were penicillin and tetracyclines. During pig production, sucking and post-weaning are periods in which there is a wide administration of oral medication (Lekagul *et al.*, 2019).

Antimicrobial resistance in pigs and pig products is an increasing global concern, with resistance to at least one antimicrobial observed in 92% of *Salmonella* isolates in the UK (Miller *et al.*, 2011). Treatment options of Salmonellosis in animal and humans has become more difficult due to the emerging of multidrug-resistant *Salmonella* spp. strains (Alcaine *et al.*, 2006; Hur *et al.,* 2012).

From a microbiological perspective, our investigation wants to provide data regarding *Salmonella* spp. prevalence, AMR and antibiotic resistance genes circulation in swine food chain.

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Materials and methods

Sample collection

Samples were collected from six slaughterhouses located in North-Central Italy. These structures had different capabilities, as reported in Table 1.

Each slaughterhouse was visited three

times. Twenty-five carcasses and thirty environmental sites (15 before and 15 during slaughtering activities) were sampled on each sampling day. Finally, a total of 75 total of 150 carcasses, 180 environmental samples, and 30 cecal samples were collected. A total of 75 traditional pork dry sausages were collected.

Carcasses

A total of 150 carcasses (weight range: 140-160 kg) were randomly selected for sampling by using pre-hydrated sponges (International PBI S.P.A Milan, Italy) with Buffered Peptone Water (BPW; Oxoid, Milan, Italy) from different sites (4×100 cm^2): hind limb, abdomen lateral (belly), middorsal region (mid-back) and jowl. All samples were sent to the laboratory in cooled containers within the same day of analysis.

Cecal contents

A total of thirty pooled cecal samples (225 gr) were taken after evisceration. Every sample was collected aseptically from different pigs. All samples were individually packed and kept at a temperature of +4°C during storage and transportation to the laboratory.

Environmental samples

A total of 30 environmental samples (15 before slaughter activities and 15 during slaughter activities) were collected in each slaughterhouse. Evaluation included floor after bleeding, gut container, and run-off pit/drain well. The first sampling round was done before the starting of activities, and one at the end. A 100 cm^2 surface per site using a template, was sampled using sponges pre-soaked in 10 ml of Buffered Peptone Water (BPW; Oxoid, Milan, Italy) by collecting surface swabs in the floor after the bleeding stage, in runoff pit and gut container. All samples were stored at +4 C° and returned to the laboratory within the same day for the analysis.

Traditional dry sausages samples

A total of 75 traditional pork dry sausage samples were collected, they had an average weight of 150 gr and 3 week of curing time. The screened products were manufactured by a farmer, or a butcher or a small workshop. In the analyzed area all samples were stored at +4 C° and returned to the laboratory within the same day for the analysis.

Salmonella isolation, identification, and serotyping

Environmental and carcass samples were then pre-enriched in Buffered Peptone Water (BPW; Oxoid, Milan, Italy) and incubated at 37°C for 24h. Pools of fecal samples (25 gr) and dry sausages (25 gr) were transferred to 225 ml of sterile BPW solution and homogenized for 120 s in a stomacher machine and incubated at 37°C for 24h. All samples were analyzed following the ISO6579:2002 0,1 ml of the sample were transferred in 10 ml of Rappaport-Vassiliadis (RVS Oxoid, Milan, Italy) and incubated in 42°C for 24h. Xylose Lysine Doexycholate (XLD, Oxoid, Milan, Italy) was used as selective media than suspected colonies were transported in Tryptone Soya Agar (TSA, Oxoid, Milan, Italy) and they were performed by slide agglutination with *Salmonella* Rapid Latex Test (Oxoid, Milan, Italy).

All *Salmonella* spp. were differentiated biochemically and serologically by VITEK 2 system (bioMerieux, France), according to the manufacturer's instructions (bioMerieux, 2013).

The Identification Gram-Negative Bacteria (GNB) cards were used for the identification of bacterial strains, according to the manufacturer's instructions (bioMérieux, 2013).

Confirmation of serotyping was performed by qualitative Polymerase Chain Reaction (PCR) assay (Kikuvi *et al.*, 2010), while genomic DNA was analyzed by pulsed-field gel electrophoresis (PFGE), using Xbal (50U/sample), BlnI/AvrII (30U/sample) as restriction enzymes, according to the PULSENET protocol (PULSENET, 2010) (Di Ciccio, *et al.*2016).

Antimicrobial susceptibility testing

Card VITEK 2 AST GN-65 was performed for antibiograms susceptibility and Minimum Inhibitory Concentrations (MICs) detection according to the manufacturer's instructions (bioMérieux, 2013).

Fifteen antimicrobial agents were tested: ampicillin, amoxicillin and clavulanic acid, imipenem, cefpodoxime, ceftiofur, tobramycin, piperacillin, gentamicin, amikacin, enrofloxacin, marbofloxacin, chloramphenicol, tetracycline, nitrofurantoin, trimethoprim-sulfamethoxazole.

Detection of antibiotic resistance genes

According to the manufacturer's instructions, genomic DNA was extracted from the above-mentioned bacterial isolates by using High Pure PCR Template Preparation Kit (Roche, Indianapolis, Ind.).

Antimicrobial resistance genes were examinated using conventional PCR reaction (Table 2), which was performed in a final reaction volume of 25 µl: containing purified DNA 1 µl, DreamTaq Green PCR Master Mix (Thermo Fisher Scientific, US) 12,5 µl, forward primers 0.25 µl, reverse primers 0.25 µl, Nuclease-free water 11 µl. Twelve antimicrobial resistance genes were tested*: tetA, tetB, tetC* for tetracycline, *catA1* for chloramphenicol, *aadA2, aac(3)IV, aadB* for aminoglycoside, *bla TEM* and *bla PSE* for beta-lactamase, *nfsA* and *nfsB* for nitrofurantoin and *par C* and *gyrA* for fluoroquinolone, *dfrA1, dfrB, dfrA14* for trimethoprim-sulfametoxazole.

Results

In the present study, microbiological screenings permitted to identify different *Salmonella* serovars, as reported in Table 3.

The isolated *Salmonella* displayed a high spectrum of antibiotic resistance. All strains (100%) showed phenotypically and genotypically resistance to at least one or more examined classes of antibiotic.

All samples (100%) were resistant to amikacin, tobramycin, and gentamicin; 20 strains (55,5%) showed resistance to ampicillin and piperacillin; 31 strains (86%) were resistant to tetracycline, one strain was resistant to amoxicillin-clavulanic acid and nitrofurantoin, 2 strains (5,5%) were resistant of chloramphenicol and 9 strains were resistant of trimethoprim.

A lot of strains showed a multiple antimicrobial resistance: 27,8% (10/36) was resistant to 3 antimicrobial classes; 25% (9/36) to 4 antimicrobial classes, 2,8% (1/36) to 6 antimicrobial classes (Table 3).

All 36 *Salmonella* isolates, belonging to five different serovars recovered from the swine food chain, were investigated for antimicrobial resistance genes detection by uniplex PCR. The resistance genes most detected were *parC* (100%), *gyrA* (94,4%), c*atA* (94,4%), *nfsB* (86,1%), *nfsA* (77,7%), bla *TEM* (47,2%), *tetA* (47,2%) and *tetB* (41,6%). *tetC, aac(3)IV, aadB* and *dfraA1* showed a presence of 2.8%.

AadA2, *bla PSE* , *dfraB* and *dfraA14* genes resistance were not detected in *Salmonella* selected (Table 4).

Discussion

In swine food chain, *Salmonella* spp. prevalence has been extremely studied in all Europe (Bonardi, 2017); indeed, international agencies underlined high variability of *Salmonella* isolation data in different Member States (EFSA, 2021).

Our survey showed that 14,6% (11/75) of *Salmonella* was recovered from traditional dry sausages, 7,3% (11/150) from carcasses, 13,3% (4/30) from cecal pools, 5,5% (10/180) from environmental samples.

The different results observed could be attributed to different factors: slaughterhouses' capabilities, good hygienic standards, cross-contaminations between carcasses and equipment, presence of resident slaughterhouses microflora, and inappropriate food handling (Bonardi, 2017). For this reason, the European Legislator ((Regulation EU No 1474/2015) purposed a new approach to reduce prevalence, apply-

Table 1. Sampled slaughterhouses and their productive capabilities.

Slaughterhouse	Slaughtered animals / time
S 1	350 animals / 1 hour
S 2	450 animals / 1 hour
S 3	55 animals / 1 hour
S 4	115 animals / working daya
S 5	1000 animals / working day
S 6	350 animals / 1 hour

Table 2. Target antibiotic, PCR primers, forward and reverse sequence, annealing temperature of the primers, amplicon size and reference used to evaluate the presence of antibiotic resistance genes.

Antibiotic	Gene	Sequence (5'-3')	Annealing	Amplicon	Reference temp (C°)
Tetracycline	*tetA*	F- GTAATTCTGAGCACTGT R- CCTGGACAACATTGCTT	45	954	Kikuvi *et al.*,2010
Tetracycline	*tetB*	F- ACGTTACTCGATGCCAT R-AGCACTTGTCTCCTGTT	48	1170	Kikuvi *et al.*, 2010
Tetracycline	*tetC*	F-AACAATGCGCTCATCGT R-GGAGGCAGACAAGGTAT	50	1138	Kikuvi *et al.*, 2010
Chloramphenicol	*catA1*	F- GGCATTTCAGTCAGTTG R-CATTAAGCATTCTGCCG	50	551	Kikuvi *et al.*, 2010
Aminoglycosides	*aadA2*	F- CGGTGACCATCGAAATTTCG R-CTATAGCGCGGAGCGTCTCGC	54	250	Prasertsee *et al.*, 2016
Aminoglycosides	*aac(3)IV*	F- TGCTGGTCCACAGCTCCTTC R- CGGATGCAGGAAGATCAA	63	653	Kozak *et al.*,2009
Aminoglycosides	*aadB*	F- GAGGAGTTGGACTATGGATT R-CTTCATCGGCATAGTAAAAG	55	208	Kozak *et al.*,2009
Ampicillin	*blaTEM*	F-CCGTGTCGCCCTTATTCCC R-GCCTGACTCCCCGTCGTGT	51	780	Kikuvi *et al.*, 2010
Ampicillin	*blaPSE*	F-CGCTTCCCGTTAACAAGTAC R-CTGGTTCATTTCAGATAGCG	58	465	Kikuvi *et al.*, 2010
Nitrofurantoin	*nfsA*	F- CTGGCGCTTGCTCTGCTATC R-GCCCGCGTATCATACACTGG	60	964	Garcia *et al.*, 2017
Nitrofurantoin	*nfsB*	F-ATCACCGTCTCGCTACTCAAC R-CGCGCCATTGATCATTGAGG	58	921	Garcia *et al.*, 2017
Quinolone	*parC*	F- CTATGCGATGTCAGAGCTGG R- TAACAGCAGCTCGGCGTATT	62	270	El-Tayeb *et al.*, 2017
Quinolone	*gyrA*	F-AAATCTGCCCGTGTCGTTGGT R-GCCATACCTACGGCGATACC	55	343	El-Tayeb *et al.*, 2017
Trimethoprim	*dfrA1*	F-GTGAAACTATCACTAATGG R-TTAACCCTTTTGCCAGATTT	50	474	El-Tayeb *et al.*, 2017
Trimethoprim	*dfrB*	F-GATCACGTGCGCAAGAAATC R-AAGCGCAGCCACAGGATAAAT	60	141	El-Tayeb *et al.*, 2017
Trimethoprim	*dfrA14*	F-GAGCAGCTICTITTIAAAGC R-TTAGCCCTTTTIICCAATTTT	58	393	El-Tayeb *et al.*, 2017

Table 3. Prevalence of *Salmonella*.

Source	N. *Salmonella* isolates	Serovar. (%)
Environmental sample	10	4 *S.* Thiphimurium (4/10, 40%) 3 Monophasic variant *S.* Thiphimurium (3/10, 30%) 3 *S.* Rissen (3/10, 30%)
Traditional dry sausage	11	6 *S.* Enteritidis (6/11, 54,5%) 1 *S.* Thyphi (1/11, 9,1%) 4 *S.* Thiphimurium (4/11, 36,4%)
Carcass	11	2 *S.* Derby (2/11, 18,2%) 2 *S.* Rissen (2/11, 18,2%) 6 *S.* Thiphimurium (6/11, 54,5%) 1 *S.* Enteritidis (1/11, 9,1%)
Caecal sample	4	2 *S.* Thiphimurium (2/4, 50%) 1 *S.* Enteritidis (1/4, 25%) 1 *S.* Rissen (1/4, 25%)

ing hot waters to remove microbiological surface contamination from carcasses.

It was also found that *Salmonella* also persist in slaughterhouses environment. In our study, one *Salmonella* Typhimurium, detected from gut container, presented AMR to 6 antibiotic classes, and three monophasic *Salmonella* Typhimurium were isolated from runoff pit.

Therefore, the implementation of good manufacturing practice (GMP) is a crucial factor that allows to decrease environmental cross-contamination multi-drug resistance *Salmonella*.

Monophasic *Salmonella* Typhimurium is strongly associated with swine food chain, especially in Europe. This consideration permits to suggest a potential link between human infections with contaminated pork products consumption (de la Torre *et al*., 2003; Mossong *et al*., 2007; Hauser *et al.*, 2010; Lucarelli *et al*., 2010; Mourao *et al*., 2014).

Pork and dry ready-to-eat products are an important source of disease in southern Europe, where human Salmonellosis prevalence, derived from these products, is higher than in the rest of Europe (Bonardi, 2017). In fact, in our study, prevalence of *Salmonella* in traditional dry sausages is higher than in other groups of samples.

Our results, obtained from antimicrobial tests, showed that *Salmonella* is more resistant to ampicillin, piperacillin, tetracycline, gentamicin, amikacin, and tobramycin.

Table 4. Sources of sample, serovar., resistant antibiotic class, phenotypic resistance.

Source	Serovar.	Resistance phenotype	Resistance pattern	Resistance genotype
Traditional dry sausage	*S.* Typhimurium	AMI, AMP,GEN,PIP, TOB	2	*gyrA, parC*
Traditional dry sausage	*S.* Typhimurium	AMI, AMP,GEN,PIP, TET, TOB,TRI	4	*catA1,drfA, gyrA, parC, te A, tetB*
Traditional dry sausage	*S.* Typhimurium	AMI, GEN, TET, TOB	2	*catA1, gyrA, nfsB, parC, tetA, tetB*
Traditional dry sausage	*S.* Typhimurium	AMI, GEN, TET, TOB	2	*catA1, gyrA, nfsA, parC, tetB*
Caecal sample	*S.* Typhimurium	AMI, AMP, GEN, PIP, TET, TOB	3	*catA1, gyrA, nfsA, nfsB, parC, tetB*
Carcass	*S.* Typhimurium	AMI, GEN, TET, TOB	2	*blaTEM, catA1, gyrA, nfsA, nfsB, parC, tetA*
Carcass	*S.* Typhimurium	AMI, GEN, TET, TOB	2	*catA1, gyrA, nfsA, nfsB, parC, tetA*
Carcass	*S.* Typhimurium	AMI, GEN, TET, TOB	2	*catA1, gyrA, nfsA, nfsB, parC, tetA*
Caecal sample	*S.* Typhimurium	AMI, AMP, GEN, PIP, TET, TOB	3	*blaTEM, catA1, gyrA, nfsA, nfsB, parC, tetB*
Environmental sample	*S.* Typhimurium	AMI, AMP, GEN, PIP, TET, TOB	3	*blaTEM, catA1, gyrA, nfsA, nfsB, parC, tetB*
Environmental sample	*S.* Typhimurium	AMI, GEN, TET, TOB	2	*catA1, gyrA, nfsA, nfsB, parC, tetC*
Environmental sample	*S.* Typhimurium	AMI, AMP, GEN, PIP, TET, TOB	3	blaTEM, *catA1*, *gyrA*, *nfsA*, nfsB, *parC*, *tetB*
Carcass	*S.* Typhimurium	AMI, AMP, GEN, PIP, TET, TOB	3	blaTEM, *catA1*, *gyrA*, *nfsA*, *nfsB*, *parC*, *tetB*
Carcass	*S.* Typhimurium	AMI, AMP, GEN, PIP, TET, TOB	3	blaTEM, *catA1*, *gyrA*, *nfsA*, *nfsB*, *parC*, *tetB*
Carcass	*S.* Typhimurium	AMI, AMP, GEN, PIP, TET, TOB	3	blaTEM, *catA1*, *gyrA*, *nfsA*, *nfsB*, *parC*, *tetB*
Environmental sample	*S.* Typhimurium	AMI, AMP, AMX, CEF, CLO, GEN, NIT, PIP, TET, TOB	6	*catA1*, *gyrA*, *parC*, *tetB*
Environmental sample	Monophasic Variant *S.* Typhimurium	AMI, AMP, GEN, PIP, TET, TOB		*aadB, blaTEM, catA1, nfsA, nfsB, parC, tetB*
Environmental sample	Monophasic Variant *S.* Typhimurium	AMI, AMP, GEN, PIP, TET, TOB		*aadB, blaTEM, catA1, nfsA, nfsB, parC tetB*
Environmental sample	Monophasic Variant *S.* Typhimurium	AMI, AMP, GEN, PIP, TET, TOB		*blaTEM, gyrA, nfsA, nfsB, parC, tetB*
Traditional dry sausage	*S.* Enteritidis	AMI, AMP, GEN, PIP, TET, TOB, TRI	4	*blaTEM, catA1, gyrA, nfsA, nfsB, parC, tetA*
Traditional dry sausage	*S.* Enteritidis	AMI, AMP, GEN, PIP, TET, TOB, TRI	4	*blaTEM, catA1, gyrA, nfsA, nfsB, parC, tetA*
Traditional dry sausage	*S.* Enteritidis	AMI, AMP, GEN, PIP, TET, TOB .TRI	4	*blaTEM catA1, gyrA, nfsA, nfsB, parC, tetA*
Traditional dry sausage	*S.* Enteritidis	AMI, AMP, GEN, PIP, TET, TOB, TRI	4	*blaTEM, catA1, gyrA, nfsB, parC, tetA*
Traditional dry sausage	*S.* Enteritidis	AMI, AMP, GEN, PIP, TET, TOB, TRI	4	*blaTEM , catA1, gyrA, nfsA, nfsB, parC, tetA*
Traditional dry sausage	*S.* Enteritidis	AMI, AMP, GEN, PIP, TET, TOB, TRI	4	*blaTEM, catA1, gyrA, nfsB, parC, tetA*
Carcass	*S.* Enteritidis	AMI, GEN, TOB	1	*catA1, gyrA, nfsA, nfsB, parC*
Caecal sample	*S.* Enteritidis	AMI, GEN, TOB	1	*catA1, gyrA, nfsA, nfsB, parC*
Traditional dry sausage	*S.* Typhi	AMI, AMP, GEN, PIP, TET, TOB, TRI	4	*catA1, blaTEM, gyrA, par C, tetA, tetB*
Caecal sample	*S.* Rissen	AMI, GEN, TET, TOB	2	*catA1, gyrA, nfsA, nfsB, parC*
Environmental sample	*S.* Rissen	AMI, GEN, TET, TOB	2	*catA1, gyrA, nfsA, nfsB, parC, tetA*
Environmental sample	*S.* Rissen	AMI, GEN, TET, TOB	2	*aac(3)IV, catA1, gyrA, nfsB, parC, tetA*
Environmental sample	*S.* Rissen	AMI, GEN, TET, TOB	2	*catA1, gyrA, nfsA, nfsB, parC, tetA*
Carcass	*S.* Rissen	AMI, CLO, GEN, TET, TOB, TRI	4	*catA1, gyrA, nfsA, nfsB, parC, tetA*
Carcass	*S.* Rissen	AMI, GEN, TET, TOB	2	*catA1, gyrA, nfsA, nfsB, parC, tetA*
Carcass	*S.* Derby	AMI, GEN, TOB	1	*catA1, gyrA, nfsA, nfsB, parC*
Carcass	*S.* Derby	AMI, GEN, TOB	1	*catA1, gyrA, nfsA, nfsB, parC*

AMP- ampicillin, AMX-amoxicillin and clavulanic acid, CEF-Ceftiofur, TOB-Tobramycin, PIP-Piperacillin, GEN-Gentamicin, AMI-Amikacin, CLO-Chloramphenicol, TETRA-Tetracycline, NIT-Nitrofurantoin, TRI-Trimethoprim-Sulfamethoxazole

Ampicillin and tetracycline are commonly used in swine livestock as first-choice antibiotics to cure disease. Ampicillin and tetracycline are commonly used in swine livestock as first-choice antibiotics to cure disease worldwide (Prasertesee *et al.,* 2016, Kozak *et al*., 2009). In devolving country such as China, antibiotics have been used as growth promoter (Yang *et al.,* 2019), in Europe instead they had been banned since 2006 (Regulation EC No 1831/2003). For this reason, it is crucial detect the prevalence of AMR, especially in a global trade prospective. Indeed, microbiological and genetic evaluation are powerful tools to investigate Salmonella spp. prevalence, AMR and antibiotic resistance genes circulation in swine food chain.

The highest priority critically important antimicrobials are still used in pig production, for treatment and prevention of infection. This evidence requires global efforts for a prudent use of antibiotics to reduce the emergence of AMR in agricultural, veterinarian, and foodborne sectors (Lekagul *et al.*, 2019).

The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals, and food in 2018/2019 shows resistance to ampicillin, sulfonamides, and tetracyclines >20% (in Italy 68-72 % of the isolates were resistant), while the resistance to third generation cephalosporins was <10% (in Italy this resistance was <2% in human isolates and <5% in animal isolates).

In our study, AMR against third generation cephalosporins and fluoroquinolones, classified as "Critically important antimicrobials" (CIA), were not discovered. All examined strains were susceptible to cefpodoxime, marbofloxacin, and enrofloxacin, and only 2.8% resulted resistant to ceftifur.

On the other hand, the presence of gene par C has been detected in all sample and, gyrA has been detected in 94.4% of samples.

The fluoroquinolone ciprofloxacin and the third-generation cephalosporin ceftriaxone are now the recommended drugs to treat human invasive Salmonella infections or patients at risk of developing an invasive infection (Shane *et al.,* 2017).

For the strategic importance of fluoroquinolones, it could be useful investigate the presence of mutation and sequencing DNA.

In our study, antimicrobial resistance phenotype more present in the swine food chain are ampicillin, streptomycin, tetracycline, and chloramphenicol, in agreement with previous research papers (Calayag *et al.* 2017, Lekagul *et al.*, 2019).

Two strains are phenotypic resistant to chloramphenicol, and 94,4% of strains showed *catA* gene typical antimicrobial resistance gene of this antibiotics. In European Union, it is not authorized for use in food-producing animals (EFSA, 2014). Deekshit demonstrated in his study that the ubiquitarian strain of non-typhoidal *Salmonella* can have a silent gene of antimicrobial resistance and there isn't a correlation between phenotypic and genotypic resistance (Deekshit *et al.* 2012).

Previous studies demonstrated that the main important resistance factor to chloramphenicol is in an auto-transmissible plasmid (IncHI). This type of plasmid carries other resistance genes responsible for streptomycin, sulfonamide, and tetracycline (Crump et al. 2015).

All strains are phenotypically resistant to at least three antibiotic classes. It is an important concern for human health.

The resistance genes most commonly detected were *parC* (100%), *gyrA* (94,4%), *catA* (94,4%), *nfsB* (86,1%), *nfsA* (77,7%), *bla TEM* (47,2%), *tetA* (47,2%) and *tetB* (41,6%). *TetC, aac(3)IV* and *aadB, dfraA1* show a presence of 2.8%.

Conclusions

In accordance with EU Regulation No 2160/2003, all Member States elaborated national control plans for *Salmonella* serovars in poultry and pig's food chains. The aim of these plans is to guarantee human health.

The findings in this survey may suggest that there is a strict correlation between the prevalence of *Salmonella* spp. and antimicrobial resistance for human and animal health. Pig products could be an important carrier of AMR and a potential risk for public health.

The data relating to the frequency of isolation and presence of multiple resistances in the isolates of dry products demonstrate that problem must be carefully evaluated, especially in those situations where the domestic slaughter of pigs and the preparation of traditional products are used.

The monitoring of antimicrobial resistance and the rapid identification of trends that could further reduce the effectiveness of therapeutic antibiotics require a comprehensive and integrated One-Health approach.

References

Aarestrup FM, 2005. Veterinary drug usage and antimicrobial resistance in bacteria of animal origin. Bas Clin Phar Tox 96:271-81.

Alcaine SD, Warnick LD, Wiedmann M, 2006. Antimicrobial Resistance in nontyphoidal Salmonella. J Food Prot 70:780-90.

Astorga RJM, Salaberria AE, Garcìa AM, Jimenez SV, Martinez AC, Garcìa AA, Casas A, 2007. Surveillance and antimicrobial resistence of Salmonella strains isolated from slaughtered pigs in Spain. J Food Prot 70:1502-6.

Baptista FM, Dahl J, Nielsen LR, 2010. Factors influencing Salmonella carcass prevalence in Danish pig abattoirs. Prev Vet Med 95:231-8.

Bonardi S, 2017. Salmonella in the pork production chain and its impact on human health in the European Union. Epid Infec 145:1513-26.

Calayag AMB, Paclibare PAP, Santos PDM, Bautista CAC, Rivera WL, 2017. Molecular characterization and antimicrobial resistance of *Salmonella enterica* from swine slaughtered in two different types of Philippine abattoir. Food Microbiol 65:51-56.

Crump AJ, Sjoulond-Karlsson M, Gordon MA, Parrye CM, 2015. Epidemiology clincial presentation, laboratory diagnosis, antimicrobial resistance and antimicrobial management of invasive *Salmonella* infection. Clin Microbiol Rev Oct 28:901-37.

de la Torre E, Zapata D, Tello M, Mejia W, Frias N, Garcia Pena FJ, Mateu EM, Torre E, 2003. Several Salmonella enterica subsp. enterica serotype 4,5,12:i:- phage types isolated from swine samples originate from serotype typhimurium DT U302. J Clin Microbiol 41:2395–400.

Deekshit VK, Kumar BK, Rai P, Srikumar S, Karunasagar I, 2012. Detection of class 1 integrons in Salmonella Weltevreden and silent antibiotic resistance genes in some seafood-associated nontyphoidal isolates of Salmonella in south-west coast of India. J App Microbiol 112:1113-22.

Di Ciccio P, Ossiprandi MC, Zanardi E, Ghidini S, Belluzzi G, Vergara , Ianieri A, 2016. Microbiological contamination in three lare-scale pig slaughterhouses in Northern Italy. Ital J Food Saf 5:6151.

EFSA, 2014. The European Union Summary Report on Trends and

Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2012. EFSA Journal 12.

EFSA, 2021. The European Union One Health 2019 Zoonoses Report. EFSA Journal 19:6406.

EFSA, 2021. The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2018/2019. EFSA Journal 19:6490

El-Tayeb M, Ibrahim ASS, Al-Salamah, AA, Almaary K, and Elbadawi YB, 2017. Prevalence, serotyping and antimicrobials resistance mechanism of Salmonella enterica isolated from clinical and environmental samples in Saudi Arabia. Braz J Microbiol 48:499–508.

European commission, 2003. Regulation (EC) No 1831/2003 of the European Parliament and of the council on additives for use in animal nutrition. In Official Journal, L268, pp. 29-39.

European commission, 2005. Regulation (EC) N.2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. In: Official Journal, L338, pp. 1-26.

European commission, 2014. Regulation (EU) n. 217/2014 of 7 March 2014 amending Regulation (EC9 n. 2073/2005 as regards Salmonella in pig carcasses. In Official Journal, L69, pp. 93-94.

European commission, 2015. Regulation (EU) n. 1474/2015 of 27 August 2015 concerning the use of recycled hot water to remove microbiological surface contamination from carcasses. In Official Journal, L225/7, pp. 83-85.

Fedorka-Cray PJ, Gray JT, Wray C. Salmonella infections in pigs. In: Wray C, Wray A, eds. Salmonella in Domestic Animals. Wallingford: CAB International, 2000, pp. 191–207.

Garcia V, Montero I, Bances M, Rodicio R, and Rodicio R, 2016. Indigence and genetic bases of nitrofurantoin resistance in clinical isolates of two successful multidrug-resistant clone if Salmonella enterica Serovar Typhimurium: pandemic "DT 104" and pUO-StVR 2. Microb Drug Resist 2:405–12.

Hauser E, Tietze E, Helmuth R, 2010. Pork contaminated with Salmonella enterica serovar 4,[5],12:i:-, an emerging health risk for humans. Appl Environ Microbiol 76:4601–10.

Hur J, Jawale C, Lee JH, et al, 2012. Antimicrobial resistance of Salmonella isolated from food animals: A review. Food Res Inter 45:819-30.

Hurd HS, Mc Kean JD, Griffith RW, Wesley IV, Rostagno MH, 2002. Salmonella enterica infections in market swine before and after transport and holding. Appl Envirom Microbiol 68:2376–81.

Kikuvi GM, Ombui JN, Mitema ES, 2010. Serotypes and antimicrobial residence profiles of Salmonella isolates from pigs at slaughter in Kenya. J Infect Dev Ctries 4:243-8.

Kozak GK, Boerlin P, Janecko N, Reid-Smith RJ, Jardine C, 2009.Antimicrobial Resistance in Escherichia coli Isolates from Swine and Wild Small Mammals in the Proximity of Swine Farms and in Natural Environments in Ontario, Can Appl Environ Microbiol, 559-566.

Lekagul A, Tangcharoensathien V, Yeung S, 2019. Patterns of antibiotic use in global pig production: a systemic review. Vet and Anim Scien 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 Ty- phimurium and its monophasic variant circulating in Italy, Denmark, and the United Kingdom. J Clin Microbiol 48:2103–9.

Miller AJ, Twomey DF, Davies RH, Teale CJ, Williamson SM, Reichel R, Featherstone CA, Cook AJC, Snow LC, Armstrong JD, 2011. Salmonella serovars and antimicrobial resistance patterns on a sample of high seroprevalence pig farms in England and Wales (2003-2008). Zoon Publ Health 58:549–59.

Molla B, Alemayehu D, Salah W, 2003. Spruces and distribution of Salmonella serotypes isolated from food animals, slaughterhouse personnel and retail meat products in Etiopia 1997-2002. Ethiop J Health Dev 17:63-70.

Mossong J, Marques P, Ragimbeau C, Huberty-Krau P, Losch S, Meyer G, Moris G, Strottner C, Rabsch W, Schneider F, 2006. Outbreaks of monophasic Salmonella enterica serovar 4,[5],12: i:- in Luxembourg. Euro Surveill 2007;12:E11–2.

Mourao J, Novais C, Machado J, et al, 2015. Metal tolerance in emerging clinically relevant multidrug-resistant Salmonella enterica serotype 4,[5],12:i:- clones circulating in Europe. Int J Antimicrob Agents 45:610–6.

Prasertsee T, Nattakarn K, Panuwat Y, Pannita S, Chokesajjawatee N, Patchanee P, 2016. Repertitive sequence-based PCR fingerprinting and the relationship of antimicrobial-resistence characteristics and corresponding genes among Salmonella strains from pig production. Asian Pac J Trop Dis 6:390-5.

Shane AL, Mody RK, Crump JA, 2017. Infectious Diseases Society of America clinical practice guidelines for the diagnosis and management of infectious diarrhea. Clin Infect Dis 65:e45–80.

Valero A, Hernandez M, De Cesare A, Manfreda G, Garcia-Gimero RM, Gonzalez-Garcia P, Rodriguez-Lazaro D, 2014. Probabilistic approach for determining Salmonella spp. and L. monocytogenes concentration in pork meat from presence/absence microbiological data. Int J Food Microbiol 184:60-3.

Yang X, Wu Q, Zhang J, Huang J, Chen L, Wu S, Zeng H, Wang J, Chen M, Wu H, Gu Q, Wei X, 2019. Prevalence, Bacterial Load, and Antimicrobial Resistance of Salmonella Serovars Isolated From Retail Meat and Meat Products in China. Front Microbiol 10 DOI=10.3389/fmicb.2019.02121

Wang H, Ye K, Wei X, Cao J, Xu X, Zhou G, 2013. Occurrence, antimicrobial resistance and biofilm formation of Salmonella isolated from resistance and biofilm formation of Salmonella isolates from a chicken slaughter plant in China. Food Control 33:378-84.