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Evaluation of the Antimicrobial Resistance of Different Serotypes of *Salmonella enterica* from Livestock Farms in Southern Italy

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Abstract: The antimicrobial susceptibility profiles of *Salmonella* spp. isolated from livestock production systems in Sicily were determined. The antibiotic sensitivity of isolated *Salmonella* spp. and broad-spectrum beta-lactamase strains were assessed by detecting β-lactamases blaCTX-M IV, TEM, and OXA SHV, and β-lactamases blaCMY II, CTX-M I, CTX-M II, and DHA. In total, 93.3% of *Salmonella* spp. strains showed multi-drug resistance (MDR). A total of seven serotypes (i.e., *Salmonella* Infantis, *S.* Typhimurium (monophasic), *S.* Derby, *S.* Hadar, *S. salamae*, *S. houtenae*, *S.* Cardoner) showed high resistance values (R) (100–47%) to sulfonamides, tetracyclines, diaminopyrimidines, penicillins, and quinolones. The gene for β-lactamase blaTEM was found in *S.* Typhimurium (monophasic) and *S.* Derby, isolated from swine meat and feces samples; *S.* Hadar isolated from an insect sample; *S. salamae* isolated from an abrasive sponge on swine skin; *S. houtenae* isolated from chicken skin samples; and *S.* Cardoner isolated from a chicken meat sample. The gene blaCTX-M I was found in *S.* Infantis isolated from a chicken meat sample. The results gathered in the current study suggest that the resistance to antibiotics is continuously increasing. This represents a worrying perspective since they should be usually used as the last option for therapy against bacterial infections.

Keywords: antimicrobial resistance; livestock; minimum inhibiting concentration; *Salmonella* spp.; zoonosis

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1. Introduction

Antibiotic resistance is the ability of some microorganisms to survive and multiply in the presence of various antimicrobials and represents a health problem that has afflicted the world population for years [1]. Various bacterial species are naturally resistant, while others acquire, through vertical and horizontal transfer, characteristics that make them resistant to the action of some antibiotics. Antimicrobial resistance is a serious global problem in the 21st century, strictly due to several factors linked with the growth and lifestyle of the population, the excretion of incompletely metabolized antibiotics by humans and animals, the disposal of unused drugs, and waste from pharmaceutical processes [2]. The livestock sector constitutes one of many focal points for the evolution and dissemination of antibiotic-resistant bacteria [3]. As a matter of fact, antibiotics are widely used in food-producing animals for therapeutic, prophylactic, and growth-promoting purposes [4–6]. In addition, the global sale of antimicrobials is continuously increasing: the sale of 93,309 tonnes of antimicrobials was estimated for 2017 and the global sale is expected to rise by 11.5% to 104,079 tonnes in 2023 [7]. Antimicrobial resistance represents a problem of worldwide interest, which includes animals and humans. In particular, a study found a resistance to two or more antimicrobials of 85% in food and animal strains and 77.4% in human strains [8]. Therefore, the tracking of this phenomenon is of fundamental importance to countering its spread, and the monitoring of antibiotic resistance in *Salmonella* is considered to be of high

priority by various global health organizations [9,10]. *Salmonella* species are associated with acute or chronic gastrointestinal diseases resulting from the consumption of food or water contaminated with fecal matter [11]. The *Salmonella* genus belongs to the *Enterobacteriaceae* family and includes two species, *S. bongori* and *S. enterica*. According to the Kauffman–White scheme, more than 2500 serotypes have been characterized [12,13]. *Salmonella enterica* represents one of the principal zoonotic agents that threatens public health and animal production worldwide [9]. Although poultry meat and eggs represent one of the principal sources of *S. enterica* infection in the food supply chain, other animal species could represent a means of spreading this zoonosis [14,15]. The contribution of the poultry industry to the dissemination of antibiotic-resistant *Salmonella* clones, and the associated dangers for human health, is well documented in certain countries [3,16–19]. Since antibiotic resistance patterns exhibit temporal and geographical variation, a continual evaluation of the situation is necessary in order to take opportune measures to limit the potential impact on public health systems and the food industry. This takes on considerable importance in an insular territory such as Sicily, where livestock breeding is mainly of extensive/breeding type and the transmission of zoonotic pathogens can occur between farm animals, wild animals, pets, and humans. Screening bacterial pathogens for the presence of antibiotic-resistance genes and detection by molecular methods enable researchers to determine whether a drug will be effective in a certain area and can be monitored. In view of the above considerations, the current study aimed to determine the antimicrobial susceptibility profiles and to detect resistance determinants of *Salmonella* spp. isolated from livestock production systems in Sicily, Southern Italy.

2. Materials and Methods

2.1. Bacterial Strain Isolation and Identification

From January to December 2020, a total of 663 biological samples from livestock were received from the provinces of Palermo, Catania, and Ragusa at the Istituto Zooprofilattico Sperimentale of Sicily (Palermo, Italy) for routine diagnostic activity for *Salmonella* spp. culture research. A total of 43 Salmonella isolates were collected, and among these, 15 antibiotic-resistant strains were considered for this study. Salmonella strains originated from the following samples: meat, milk, skin, feces, and feathers. The isolation of *Salmonella* spp. was carried out by a conventional method [20]. The samples were pre-enriched with buffered peptone water and incubated at 38 °C for 24 h. The samples were then transferred to Modified Semisolid Rappaport Vassiliadis (MSRV) medium and incubated at 42 °C for 24–48 h. Finally, isolates were incubated on Xylose Lysine Deoxycholate agar (XLD) and Brilliant Green agar (BGA) at 37 °C for 24 h, and colonies were further identified biochemically and by means of a Vitek device (bioMerieux, Marcy l'Etoile, France). Serotyping was performed by means of agglutination with specific anti-sera for somatic O and flagellar H according to the Kauffmann–White scheme [21].

Strains were stored at −80 °C in Microbanks (Pro-Lab Diagnostics, Biolife Italiana Srl, Milan, Italy) until the analysis.

2.2. Antimicrobial Susceptibility Tests

The selected strains were sown in Hektoen enteric agar with the aim of obtaining pure colonies. Bacteria were collected from the gel surface and added to demineralized water to make a suspension equivalent to a 0.5 McFarland standard adjusted by using a nephelometer. A total of 10 µL of bacterial suspension was collected and mixed in tubes with 11 mL of Mueller Hinton Broth. The antimicrobial susceptibility profiles of *Salmonella* strains were determined by broth microdilution using the Sensititre EUVSEC kit (Thermo Fisher Scientific, Monza, Italy) according to the manufacturer's instructions. Fourteen antimicrobials were tested (test range): sulfamethoxazole-SMX (8–1024 µg/mL), trimethoprim-TMP (0.25–32 µg/mL), ciprofloxacin-CIP (0.015–8 µg/mL), tetracyclines-TET (2–64 µg/mL), meropenem-MERO (0.03–16 µg/mL), azithromycin- AZI (2–64 µg/mL), nalidixic acid-NAL (4–128 µg/mL), cefotaxime-FOT (0.25–4 µg/mL), chloramphenicol-CHL

(8–128 µg/mL), tigecycline-TGC (0.25–8 µg/mL), ceftazidime-TAZ (0.5–8 µg/mL), colistin-COL (1–16 µg/mL), ampicillin-AMP (1–64 µg/mL), and gentamicin-GEN (0.5–32 µg/mL). The minimum inhibitory concentration (MIC) for each antimicrobial was interpreted using the clinical breakpoints, established by the European Committee for Antimicrobial Susceptibility Tests (EUCAST) to categorize MIC results as susceptible or resistant.

2.3. Molecular Detection of Extended Spectrum β-Lactamase Genes

The DNA used for multiplex-PCR was extracted by the heat lysis method [22]. Molecular analyses were performed for the determination of extended-spectrum beta-lactamase (ESBL) strains by multiplex PCR [23]: two separate multiplexes were prepared, marked as Set 1, detecting β-lactamases blaCTX-M IV, TEM, and OXA SHV, and Set 2, detecting the β-lactamases blaCMY II, CTX-M I, CTX-M II, and DHA. Both PCR reactions were performed under identical conditions. Reactions were performed in a final volume of 25 µL containing 5 µL of template DNA, 1× reaction buffer, 0.2 mM of each deoxynucleoside triphosphate, 20 pM of each primer, and 3.5 units of Taq polymerase. Both assays used identical cycling conditions. Reactions were performed under the following conditions: denaturation at 94 °C for 5 min followed by 30 cycles of 94 °C for 1 min, 61 °C for 1 min, and 72 °C for 1 min, and a final extension of 72 °C for 5 min. The primers used for PCR reactions are listed in Table 1. To confirm the presence of antibiotic-resistance genes, one gene from each type was sequenced and used as a positive control [24]. The PCR products were analyzed by 2% agarose gel electrophoresis and the results were visualized on the trans-illuminator.

Table 1. Target gene, sequence, and amplicon size of forward and reverse primers used for the two separate multiplex PCR reactions (Set 1 detecting β-lactamases blaCTX-M IV, TEM, OXA, and SHV; Set 2 detecting the β-lactamases blaCMY II, CTX-M I, CTX-M II, and DHA).

	Target Gene	Primer Sequence (5′ → 3′)	Amplicon Size (bp)	Ref
Set 1	CTX-M IV F	GACAAAGAGAGTGCAACGGATG	501	[23]
	CTX-M IV R	TCAGTGCGATCCAGACGAAA		
	TEM F	AGTGCTGCCATAACCATGAGTG	431	
	TEM R	CTGACTCCCC GTCGTGTAGATA		
	OXA F	ATTATCTACAGCAGCGCCAGTG	296	
	OXA R	TGCATCCACGTCTTTGGTG		
	SHV F	ATTATCTACAGCAGCGCCAGTG	214	
	SHV R	CGCTGTTATCGCTCATGGTAA		
Set 2	CMY II F	AGCGATCCGGTCACGAAATA	695	[23]
	CMY II R	CCCGTTTTATG CACCCATGA		
	CTX M I F	TCCAGAATAAGGAATCCCATGG	621	
	CTX M I R	TGCTTTACCCAGCGTCAGAT		
	CTX M II F	ACCGCCGATAATTCGCAGAT	588	
	CTX M II R	GATATCGTTGGTGGTGCCATAA		
	DHA F	GTGGTGGACAGCACCATTAAA	314	
	DHA R	CCTGCGGTATAGGTAGCCAGAT		

3. Results

The 15 isolates were assigned to the species *Salmonella enterica* and to the subspecies *enterica* (13 isolates), (one isolate), (one isolate). Antigenic profiles were identified for *S. salamae* (O:1,23,22;-) and *S. houtenae* (O:43:z4,z23). Different serovars belonged to the 13 Salmonellae of subspecies enterica: Typhimurium (two isolates), Corvallis (three isolates), Derby (two isolates), Hadar, Enteritidis, Infantis (two isolates), Cardoner, Veneziana. Table 2 shows the Salmonella serotypes, the host, and the source from which they were collected.

Table 2. Antibiotic susceptibility results for *Salmonella* serotypes from different hosts and sources, obtained by means of the minimum inhibitory concentration (MIC) method. The following antibiotic drugs were tested: sulfamethoxazole (SMX), azithromycin (AZI), tigecycline (TGC), tetracyclines (TET), nalidixic acid (NAL), trimethoprim (TMP), ampicillin (AMP), ciprofloxacin (CIP), chloramphenicol (CHL), meropenem (MERO), cefotaxime (FOT), ceftazidime (TAZ), colistin (COL), and gentamicin (GEN). Resistances are represented in bold. EUCAST breakpoints are reported in red.

***Salmonella* serotypes**	**Host Species/Source**	**Antibiotic Drugs** *Breakpoint EUCAST*													
		SMX	**AZI**	**TGC**	**TET**	**NAL**	**TMP**	**AMP**	**CIP**	**CHL**	**MERO**	**FOT**	**TAZ**	**COL**	**GEN**
		S* ≤ *2 ***R* ≥ *4***	—	***S* ≤ *0.5*** ***R* ≥ *0.5***	—-	—	***S* ≤ *4*** ***R* ≥ *4***	***S* ≤ *8*** ***R* ≥ *8***	***S* ≤ *0.06*** ***R* ≥ *0.06***	***S* ≤ *8*** ***R* ≥ *8***	***S* ≤ *2*** ***R* ≥ *8***	***S* ≤ *1*** ***R* ≥ *2***	***S* ≤ *1*** ***R* ≥ *4***	***S* ≤ *2*** ***R* ≥ *2***	***S* ≤ *2*** ***R* ≥ *2***
S. Typhimurium	Cattle/feces	**≥1024**	**≥16**	**≥0.5**	**≥64**	**≥32**	**≥2**	**≥64**	**≥0.25**	**≥32**	**≥16**	≤0.5	0	≥1	0
S. Corvallis	Cattle/meat	**≥16**	**≥4**	**≥0.5**	0	0	0	≤1	≤0.015	0	≤0.12	0	0	≤1	≤0.5
S. Derby	Pig/feces	**≥1024**	**≥4**	**≥0.5**	**≥16**	**≥128**	**≥32**	**≥64**	**≥0.12**	**≥64**	≤1	**≥4**	**≥1**	0	0
S. Hadar	Insect	**≥512**	**≥4**	**≥0.5**	**≥32**	**≥128**	**≥16**	**≥64**	**≥4**	0	≤0.12	0	0	0	0
S. salamae	Pig/skin	**≥128**	**≥64**	**≥8**	**≥16**	**≥32**	**≥32**	**≥64**	**≥0.12**	**≥64**	**≥1**	**≥4**	**≥2**	≤1	≤2
S. houtenae	Chicken/skin	**≥1024**	**≥32**	**≥2**	**≥16**	**≥128**	**≥32**	**≥64**	**≥8**	**≥64**	≤0.03	0	0	**≥16**	≤0.5
S. Enteritidis	Chicken/feces	**≥32**	**≥64**	**≥0.5**	**≥64**	**≥8**	0	≤1	**≥2**	0	**≥2**	0	0	**≥2**	≤2
S. Corvallis	Chicken/feather	**≥8**	**≥4**	**≥0.5**	**≥2**	0	0	0	≤0.015	0	≤0.03	0	0	≤1	0
S. Infantis	Pig/feces	**≥16**	**≥8**	**≥2**	**≥2**	**≥128**	0	≤1	**≥0.5**	**≥16**	≤0.03	0	0	≤1	0
S. Corvallis	Goat/milk	**≥8**	**≥4**	**≥1**	0	0	0	≤2	≤0.015	0	0	0	0	**≥2**	0
S. Derby	Cattle/feces	**≥8**	**≥4**	**≥8**	**≥2**	**≥64**	≤0.25	≤1	≤0.015	**≥16**	≤0.03	0	**≥4**	≤1	0
S. Typhimurium	Pig/meat	**≥8**	**≥2**	**≥0.5**	0	0	**≥8**	≤1	≤0.03	0	0	0	0	0	0
S. Cardoner	Chicken/meat	**≥64**	**≥64**	**≥8**	**≥4**	0	**≥4**	**≥16**	0	0	**≥8**	0	0	**≥16**	**≥8**
S. Veneziana	Chicken/meat	**≥8**	**≥4**	≤0.25	0	0	≤0.25	≤1	≤0.015	0	0	0	0	0	0
S. Infantis	Chicken/meat	**≥1024**	**≥4**	**≥1**	**≥32**	**≥128**	**≥32**	**≥64**	**≥0.12**	0	0	**≥1**	**≥2**	0	0

The antibiotic sensitivity test showed that the *Salmonella* strains isolated from the analyzed biological samples were all resistant to a variable degree. All 15 resistant strains showed resistance to at least two classes of antibiotics and 14 (93.3%) strains were multiresistant, i.e., resistant to more than three classes of antibiotics, particularly the serotypes *S.* Typhimurium, *S.* Infantis, *S.* Hadar, *S.* Derby, *S.* Enteritidis, *S. houtenae*, *S. salamae*, and *S.* Cardoner. Moreover, seven serotypes showed high resistance values (100–47%) to the following molecules: sulfonamides, tetracyclines, diaminopyrimidines, penicillins, macrolides, and quinolones (Table 2).

The overall incidence of ESBLs-producing isolates was 46.66% (7/15) (Table 3). All isolates that tested positive for ESBLs were also resistant to more than four antibiotics (multi-drug resistance). Molecular analyses showed that the gene for β lactamase, blaTEM, was present in six serotypes of *Salmonella*, in particular *S.* Typhimurium (monophasic) and *S.* Derby, isolated from swine meat and feces samples; *S.* Hadar isolated from insects; *S. salamae* isolated from an abrasive sponge on swine skin; *S. houtenae* isolated from chicken skin; and *S.* Cardoner isolated from chicken meat. Meanwhile, blaCTX-M I was found in *S.* Infantis isolated from chicken meat.

Table 3. ESBLs genotype of Salmonella strains.

	ESLB Genes								
***Salmonella* serotypes**	**TEM**	**OXA**	**SHV**	**DHA**	**CTXM-1**	**CTXM-2**	**CTXM-4**	**CMY-2**	**EHXA**
S. Typhimurium	+	-	-	-	-	-	-	-	-
S. Corvallis	-	-	-	-	-	-	-	-	-
S. Derby	+	-	-	-	-	-	-	-	-
S. Hadar	+	-	-	-	-	-	-	-	-
S. salamae	+	-	-	-	-	-	-	-	-
S. houtenae	+	-	-	-	-	-	-	-	-
S. Enteritidis	-	-	-	-	-	-	-	-	-
S. Corvallis	-	-	-	-	-	-	-	-	-
S. Infantis	-	-	-	-	-	-	-	-	-
S. Corvallis	-	-	-	-	-	-	-	-	-
S. Derby	-	-	-	-	-	-	-	-	-
S. Typhimurium	-	-	-	-	-	-	-	-	-
S. Cardoner	+	-	-	-	-	-	-	-	-
S. Veneziana	-	-	-	-	-	-	-	-	-
S. Infantis	-	-	-	-	+	-	-	-	-

4. Discussion

Salmonella spp. are the main pathogens responsible for food zoonoses in both industrialized and developing countries [9,21,25,26]. About 95% of salmonellosis affecting humans is attributable to the food vehicle; the spread is favored by the wide variety of infection reservoirs and the complexity of the agri-food production chains, both of animal and vegetable origin [27,28]. Livestock has been implicated as a reservoir for antibiotic-resistant bacteria, and foods of animal origin can be vectors of transmission to humans [29]. The antibiotic resistance in *Salmonella* spp. isolated from animal sources and from their meat has been widely demonstrated, even beyond the serovariates on which the attention of microbiologists and clinicians is focused [29]. In Italy, *Salmonella* spp. strains showed higher resistance profiles than the European average, with sulfamethoxazole being ineffective in 44.9% of cases, followed by tetracycline (40.4%) and ampicillin (37.4%) [25]. Moreover, the latest EFSA report showed alarming values of resistance to the critically important antimicrobials, ciprofloxacin and cefotaxime and/or ceftazidime, of 18.9% and 23.5–31.6%, respectively [26].

The data gathered from the current survey showed that almost half of the serotypes of *Salmonella* spp. isolated from biological samples showed resistance towards at least three antibiotics and, among the isolated serotypes, *S.* Typhimurium, *S.* Infantis, *S.* Hadar, *S.* Derby, and *S.* Enteritidis showed the highest resistance. Noteworthy, 7 out of 10 *Salmonella*

serotypes (i.e., *S.* Infantis, *S.* Typhimurium (monophasic), *S.* Derby, *S.* Hadar, *S. salamae*, *S. houtenae*, and *S.* Cardoner) were resistant to clinically important antibiotics such as sulfonamides, tetracyclines, diaminopyrimidines, penicillins, and quinolones, many of which are widely used in human and veterinary medicine. The decreased susceptibility to these conventional drugs suggests advising against their empirical use. Contrariwise, in agreement with previous studies [18,30,31], the third-generation cephalosporins herein tested (cefotaxime and ceftazidime) showed good effectiveness against *Salmonella* spp., with a sensitivity of 100% in most investigated serotypes. In addition, high resistance was found in a less common *Salmonella* serotype, namely the *S. enterica* subspecies *salamae*, from pig farms, which showed resistance to five antibiotic classes, including ciprofloxacin. Although the use of ciprofloxacin in animal husbandry is reduced, the continuous evidence of the antimicrobial resistance of *Salmonella* spp. towards this antibiotic are worrying. Worthy data gathered in the current study were the comparable antibiotic resistance profiles of some of the isolated *Salmonella* serotypes. This finding seems to confirm the ability of bacteria to communicate, and therefore, to share information such as the ability to develop mechanisms to avoid the antibacterial action of certain molecules. It should be pointed out that in livestock systems and, particularly, in intensive farming, the prophylactic use of certain antibiotics is common practice. The administration of small doses of antibiotics to healthy animals causes the microbiota in the guts of animals to become familiar with the drugs and gives them a chance to develop resistance to the given substances [5,32]. The bacteria then share the antibiotic resistance determinants via mobile genetic elements such as plasmids, transposons, integrons, and phages [33,34]. This renders healthy animals carriers of antibiotic-resistant bacteria [35]. Since antibiotic-resistant bacteria are found in the gut, fecal contamination is the main route for the transmission of these pathogens to humans. The environments in which the animals are reared and indirect contact with animals are possible sources of the transmission of resistant zoonotic pathogens to humans [36,37].

A previous epidemiological study from 1999 to 2002 carried out on Sicilian territory reported that *Salmonella* Enteritidis (39%) was the most prevalent serovar, followed by *S.* Typhimurium (16%), *S. newport* (6%), *S. salamae* (5%), and others. The highest rate of antibiotic resistance was observed in *S.* Typhimurium [38]. In the last decade, antimicrobial resistance to five antibiotic classes, including penicillins, tetracyclines, sulphonamides, ciprofloxacin, and third-generation cephalosporins, has increased from 20% to 80% in Abruzzo and in Molise (Italy) [39]. Among the numerous serotypes of *Salmonella*, *S.* Typhimurium has always represented the ubiquitous type most frequently isolated both in humans and in the animal sector, surpassed only in some periods by emerging serotypes. The interest in the *S.* Typhimurium serotype is linked not only to its widespread diffusion in nature and the high frequency of infection in humans but also to the appearance of poly-antibiotic-resistant characteristics, as already highlighted in the 1960s. The mechanisms encoded by antimicrobial resistance determinants include antimicrobial modification and inactivation, the alteration of the antimicrobial target site, efflux pumps, and membrane impermeability [40]. These protect the bacteria from being attacked by antibiotics [40].

In particular, *Salmonella* spp. uses the well-studied AcrAB-TolC efflux pump to extrude antibiotics such as tetracycline, chloramphenicol, and quinolones [41,42]. Bacteria resist antibiotic entry into the cell by reducing or modifying porin channels in the outer membrane, which are used by antibiotic molecules to enter the bacterial cell to reach their targets [43,44]. In addition to their high pathogenic potential, bacteria of the *Salmonella* genus are of particular interest for their contribution to the spread of antibiotic resistance, as they are able to accumulate and spread antibiotic-resistance genes [45]. Genes that encode antibiotic resistance are either located on the chromosome or plasmids within a bacterial cell and are mobilized by transposons and integrons during conjugation or phages through transduction [34,46]. The genetic plasticity of *Salmonella* bacteria allows them to accumulate and disseminate antibiotic-resistance genes that often are located in plasmids that also carry other virulence genes [47,48]. Thanks to this characteristic, the genus *Salmonella* spp. can easily transfer resistance genes horizontally to other bacteria [49]. The molecular

analysis of data for the presence of resistance genes highlighted the prevalent presence of blaTEM, whereas blaCTX-M I was found only in a *Salmonella* Infantis isolated from a chicken meat sample. Bacteria that are resistant to the beta-lactam class of antibiotics produce the beta-lactamase enzyme, which destroys the beta-lactam ring, thus deactivating the antibiotic [50,51]. The blaTEM gene is the most frequently reported worldwide, especially in Gram-negative bacteria. This is assumed to be due to its broad dissemination via migratory waterfowl and the large number of β-lactamase enzymes synthesized by bacteria [52,53]. These findings are a cause for concern considering the associated virulence that these strains, isolated from different sources, are proven to possess, and bearing in mind that *Salmonella* spp. is capable of considerable persistence under certain conditions [54].

5. Conclusions

Awareness of the consequences of the indiscriminate and unnecessary use of antimicrobials is slowly growing, but numerous studies still report highly virulent and resistant bacteria in areas of human activity. This study reported the results on the antibiotic resistance profiles of 10 different serotypes of *Salmonella* spp. in circulation in livestock-related samples in Sicily. Moreover, the *Salmonella* serotypes analyzed here exhibited the presence of blaTEM and blaCTX-M I. The results gathered in the current study confirm the scientific evidence available in the literature, according to which the resistance towards antibiotics is continuously increasing. This represents a worrying perspective since they should be usually used as a last option for therapy against bacterial infections. As a matter of fact, the current study highlights the need for the consolidation of surveillance activities for public health, with two necessary conditions: (I) the integration of veterinary data with those of clinical origin; and (II) the definition of analysis protocols that provide precise selection criteria of the serotypes to be subjected to molecular typing tests, since it is neither possible nor reasonable to extend the execution of very large and expensive analysis panels to all isolates. This information advocates the implementation of surveillance systems and the dissemination of guidelines on the correct use of antibiotics in both human and veterinary medicine, also in view of the ever more current holistic approach of the One Health concept.

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References

1. Cassini, A.; Högberget, L.D.; Plachouras, D.; Quattrocchi, A.; Hoxha, A.; Gunnar, S.S.; Colomb-Cotinat, M.; Kretzschmar, M.E.; Devleesschauwer, B.; Cecchini, M.; et al. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: A population-level modelling analysis. *Lancet Inf. Dis.* **2019**, *19*, 56–66. [CrossRef] [PubMed]
2. Kumar, A.; Pal, D. Antibiotic resistance and wastewater: Correlation, impact and critical human health challenges. *J. Environ. Chem. Eng.* **2017**, *6*, 52–58. [CrossRef]
3. Sánchez-Salazar, E.; Gudiño, M.E.; Sevillano, G.; Zurita, J.; Guerrero-López, R.; Jaramillo, K.; Calero-Cáceres, W. Antibiotic resistance of *Salmonella* strains from layer poultry farms in central Ecuador. *J. Appl. Microbiol.* **2020**, *128*, 1347–1354. [CrossRef] [PubMed]
4. Chattopadhyay, M.K. Use of antibiotics as feed additives: A burning question. *Front. Microbiol.* **2014**, *5*, 334. [CrossRef]
5. Eagar, H.; Swan, G.; Van Vuuren, M. A survey of antimicrobial usage in animals in South Africa with specific reference to food animals. *J. S. Afr. Vet. Assoc.* **2012**, *83*, 15–23. [CrossRef] [PubMed]
6. FAO/WHO. Codex Alimentarius. Commission Codex Texts on Foodborne Antimicrobial Resistance. 2015. Available online: http://www.fao.org/3/a-i4296t.pdf (accessed on 12 October 2021).
7. Tiseo, K.; Huber, L.; Gilbert, M.; Robinson, T.P.; Van Boeckel, T.P. Global Trends in Antimicrobial Use in Food Animals from 2017 to 2030. *Antibiotics* **2020**, *9*, 918. [CrossRef] [PubMed]
8. Alessiani, A.; Goffredo, E.; Mancini, M.; Occhiochiuso, G.; Faleo, S.; Didonna, A.; Fischetto, R.; Suglia, F.; De Vito, D.; Stallone, A.; et al. Evaluation of Antimicrobial Resistance in *Salmonella* Strains Isolated from Food, Animal and Human Samples between 2017 and 2021 in Southern Italy. *Microorganisms* **2022**, *10*, 812. [CrossRef]
9. European Food Safety Authority (EFSA). The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-Borne Outbreaks in 2016. *EFSA J.* **2017**, *15*, e05077.
10. WHO. Salmonella. 2017. Available online: http://www.who.int/foodsafety/areas_work/foodborne-diseases/salmonella/en/ (accessed on 12 October 2021).
11. Schatten, H.; Eisenstark, A. *Salmonella, Methods and Protocols*; Humana Press: New York, NY, USA, 2015.
12. Grimont, P.A.; Weill, F.X. *Antigenic Formulae of the Salmonella Servovars*, 9th ed.; Institut Pasteur: Paris, France, 2007; Volume 13.
13. Emond-Rheault, J.G.; Hamel, J.; Jeukens, J.; Freschi, L.; Kukavica-Ibrulj, I.; Boyle, B.; Tamber, S.; Malo, D.; Franz, E.; Burnett, E.; et al. The *Salmonella enterica* plasmidome as a reservoir of antibiotic resistance. *Microorganisms* **2020**, *8*, 1016. [CrossRef]
14. Foley, S.; Nayak, R.; Hanning, I.B.; Johnson, T.J.; Han, J.; Ricke, S.C. Population dynamics of *Salmonella enterica* serotypes in commercial egg and poultry production. *Appl. Environ. Microbiol.* **2011**, *77*, 4273–4279. [CrossRef]
15. Cosby, D.E.; Cox, N.A.; Harrison, M.A.; Wilson, J.L.; Buhr, R.J.; Fedorka-Cray, P.J. *Salmonella* and antimicrobial resistance in broilers: A review: Table 1. *J. Appl. Poult. Res.* **2015**, *24*, 408–426. [CrossRef]
16. Antunes, P.; Mour~ao, J.; Campos, J.; Peixe, L. Salmonellosis: The role of poultry meat. *Clin. Microbiol. Infect.* **2016**, *22*, 110–121. [CrossRef] [PubMed]
17. Liljebjelke, K.A.; Hofacre, C.L.; White, D.G.; Ayers, S.; Lee, M.D.; Maurer, J.J. Diversity of antimicrobial resistance phenotypes in *Salmonella* isolated from commercial poultry farms. *Front. Vet. Sci.* **2017**, *4*, 96. [CrossRef] [PubMed]
18. Khadka, P.; Thapaliya, J.; Thapa, S. Susceptibility pattern of *Salmonella enterica* against commonly prescribed antibiotics, to febrile-pediatric cases, in low-income countries. *BMC Pediatr.* **2021**, *21*, 38. [CrossRef]
19. Mthembu, T.P.; Zishiri, O.T.; El Zowalaty, M.E. Molecular detection of multidrug-resistant *Salmonella* isolated from livestock production systems in South Africa. *Infect. Drug Resist.* **2019**, *12*, 3537–3548. [CrossRef]
20. Jensen, A.N.; Sørensen, G.; Baggesen, D.L.; Bødker, R.; Hoorfar, J. Addition of Novobiocin in pre-enrichment step can improve Salmonella culture protocol of modified semisolid Rappaport-Vassiliadis. *J. Microbiol. Methods* **2003**, *55*, 249–255. [CrossRef]
21. Popoff, M.Y.; Le Minor, L. *Antigenic Formulas of the Salmonella Serovars*; WHO Collaborating Centre for Reference and Research on Salmonella: Paris, France, 1997.
22. Sugumar, M.; Kumar, K.M.; Manoharan, A.; Anbarasu, A.; Ramaiah, S. Detection of OXA-1 β-lactamase gene of Klebsiella pneumoniae from blood stream infections (BSI) by conventional PCR and in-silico analysis to understand the mechanism of OXA mediated resistance. *PLoS ONE* **2014**, *9*, e91800. [CrossRef]
23. Kim, J.; Jeon, S.; Rhie, H.; Lee, B.; Park, M.; Lee, H.; Lee, J.; Kim, S. Rapid Detection of Extended Spectrum beta-Lactamase (ESBL) for Enterobacteriaceae by use of a Multiplex PCR-based Method. *Infect Chemother.* **2009**, *41*, 181–184. [CrossRef]
24. Gargano, V.; Gambino, D.; Migliore, S.; Vitale, M.; Sciortino, S.; Costa, A.; Vicari, D. Can Human Handling Increase the Presence of Multidrug Resistance (MDR) in *Salmonella* spp. Isolated from Food Sources? *Microorganisms* **2021**, *9*, 2018. [CrossRef]
25. European Food Safety Authority (EFSA); European Centre for Disease Prevention and Control (ECDC). The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2017. *EFSA J.* **2018**, *16*, e05500.
26. European Food Safety Authority (EFSA); European Centre for Disease Prevention and Control. The European Union One Health 2019 Zoonoses Report. *EFSA J.* **2021**, *19*, e06406.
27. Murakami, K.; Horikawa, K.; Ito, T.; Otsuki, K. Environmental survey of *Salmonella* and comparison of genotypic character with human isolates in Western Japan. *Epidemiol. Infect.* **2001**, *126*, 159–171. [CrossRef] [PubMed]

28. Rumyantsev, S. Toward molecular level of the "*Salmonella*-Victim" Ecology, Genetics, and Evolution. *Sci. World J.* **2004**, *4*, 193–199. [CrossRef] [PubMed]
29. Van den Bogaard, A.E.; Stobberingh, E.E. Epidemiology of resistance to antibiotics: Links between animals and humans. *Intern. J. Antimicr. Agents* **2000**, *4*, 327–335. [CrossRef] [PubMed]
30. Crump, J.A.; Sjölund-Karlsson, M.; Gordon, M.A.; Parry, C.M. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive *Salmonella* infections. *Clin. Microbiol. Rev.* **2015**, *28*, 901–937. [CrossRef]
31. Kariuki, S.; Gordon, M.A.; Feasey, N.; Parry, C.M. Antimicrobial resistance and management of invasive *Salmonella* disease. *Vaccine.* **2015**, *33*, C21–C29. [CrossRef] [PubMed]
32. van Vuuren, M. Bacterial resistance against antibiotics: Global and local trends. *Stockfarm* **2017**, *7*, 51.
33. Gillings, M.R. Integrons: Past, present, and future. Microbiol. *Mol. Biol. Rev.* **2014**, *78*, 257–277. [CrossRef] [PubMed]
34. Hall, R.M. Integrons and gene cassettes: Hotspots of diversity in bacterial genomes. *Ann. N. Y. Acad. Sci.* **2012**, *1267*, 71–78. [CrossRef]
35. Marshall, B.M.; Levy, S.B. Food animals and antimicrobials: Impacts on human health. *Clin. Microbiol. Rev.* **2011**, *24*, 718–733. [CrossRef]
36. Tauxe, R.V. Emerging foodborne pathogens. *Int. J. Food Microbiol.* **2002**, *78*, 31–41. [CrossRef] [PubMed]
37. Dhama, K.; Rajagunalan, S.; Chakraborty, S.; Verma, A.K.; Kumar, A.; Tiwari, R.; Kapoor, S. Food-borne pathogens of animal origin-diagnosis, prevention, control and their zoonotic significance: A review. *Pak. J. Biol. Sci.* **2013**, *15*, 1076–1085. [CrossRef] [PubMed]
38. Bellissima, P.; Amato, R.; Aurnia, G.; Cannizzo, R.; Bonfante, S. Epidemiology of salmonellosis in Caltagirone area (Sicily). *Infez. Med.* **2004**, *12*, 60–64. [PubMed]
39. Di Marcantonio, L.; Romantini, R.; Marotta, F.; Chiaverini, A.; Zilli, K.; Abass, A.; Di Giannatale, E.; Garofolo, G.; Janowicz, A. The Current Landscape of Antibiotic Resistance of *Salmonella* Infantis in Italy: The Expansion of Extended-Spectrum Beta-Lactamase Producers on a Local Scale. *Front. Microbiol.* **2022**, *13*, 812481. [CrossRef] [PubMed]
40. Frye, J.G.; Jackson, C.R. Genetic mechanisms of antimicrobial resistance identified in *Salmonella enterica*, *Escherichia coli*, and *Enteroccocus* spp. isolated from US food animals. *Front. Microbiol.* **2013**, *4*, 135. [CrossRef]
41. Parry, C.M.; Threlfall, E. Antimicrobial resistance in typhoidal and nontyphoidal *Salmonellae*. *Curr. Opin. Infect. Dis.* **2008**, *21*, 531–538. [CrossRef]
42. Sun, J.; Deng, Z.; Yan, A. Bacterial multidrug efflux pumps: Mechanisms, physiology and pharmacological exploitations. *Biochem. Biophys. Res. Commun.* **2014**, *453*, 254–267. [CrossRef]
43. Delcour, A.H. Outer membrane permeability and antibiotic resistance. *Biochim. Biophys. Acta* **2009**, *1794*, 808–816. [CrossRef]
44. van der Heijden, J.; Reynolds, L.A.; Deng, W.; Mills, A.; Scholz, R.; Imami, K.; Foster, L.J.; Duong, F.; Finlay, B.B. *Salmonella* rapidly regulates membrane permeability to survive oxidative stress. *MBio* **2016**, *7*, e01216–e01238. [CrossRef]
45. McDermott, P.F.; Zhao, S.; Tate, H. Antimicrobial Resistance in Nontyphoidal *Salmonella*. *Microbiol. Spectr.* **2018**, *6*, ARBA-0014-2017. [CrossRef]
46. Guerra, B.; Soto, S.; Helmuth, R.; Mendoza, M.C. Characterization of a self-transferable plasmid from *Salmonella enterica* serotype Typhimurium clinical isolates carrying two integron-borne gene cassettes together with virulence and drug resistance genes. *Antimicrob. Agents Chemother.* **2002**, *46*, 2977–2981. [CrossRef] [PubMed]
47. Lian, X.; Wang, X.; Liu, X.; Xia, J.; Fang, L.; Sun, J.; Liao, X.; Liu, Y. OqxAB-Positive IncHI2 Plasmid PHXY0908 Increase *Salmonella enterica* serotype *typhimurium* strains tolerance to ciprofloxacin. *Front. Cell. Infect. Microbiol.* **2019**, *9*, 242. [CrossRef] [PubMed]
48. Soares, F.B.; Camargo, C.H.; Cunha, M.P.V.; de Almeida, E.A.; de Jesus, A.M.B.; de Carvalho, E.; de Paiva, J.B.; Fernandes, S.A.; Tiba-Casas, M.R. Subtyping of plasmid-mediated quinolone resistance among *Salmonella* serotypes by whole genome sequencing. *Diagn. Microbiol. Infect. Dis.* **2019**, *94*, 403–406. [CrossRef] [PubMed]
49. Munita, J.M.; Arias, C.A. Mechanisms of Antibiotic Resistance. *Microbiol. Spectr.* **2016**, *4*, 4.2.15. [CrossRef] [PubMed]
50. Aminov, R.I. The role of antibiotics and antibiotic resistance in nature. *Environ. Microbiol.* **2009**, *11*, 2970–2988. [CrossRef] [PubMed]
51. Bush, K.; Bradford, P.A. β-Lactams and β-lactamase inhibitors: An overview. *Cold Spring Harb. Perspect. Med.* **2016**, *6*, a025247. [CrossRef]
52. Jang, J.; Suh, Y.S.; Di, D.Y.; Unno, T.; Sadowsky, M.J.; Hur, H.G. Pathogenic *Escherichia coli* strains producing extended-spectrum β-lactamases in the Yeongsan River Basin of South Korea. *Environ. Sci. Technol.* **2012**, *47*, 1128–1136. [CrossRef]
53. Ouedraogo, S.A.; Sanou, M.; Kissou, A.; Sanou, S.; Solaré, H.; Kaboré, F.; Poda, A.; Aberkane, S.; Bouzinbi, N.; Sano, I.; et al. High prevalence of extended-spectrum β-lactamase producing enterobacteriaceae among clinical isolates in Burkina Faso. *BMC Infect. Dis.* **2016**, *16*, 326. [CrossRef]
54. Wales, A.; Davies, R.H. Environmental aspects of *Salmonella*. In *Salmonella in Domestic Animals*; Barrow, P.A., Methner, U., Eds.; Centre for Agriculture and Bioscience International (CABI): Wallingford, UK, 2013.