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Multidrug Resistance and Distribution of *Salmonella* Serovars in Slaughtered Pigs

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Summary

The present study was undertaken to estimate the occurrence and distribution of multidrug resistance (MDR) among *Salmonella* serovars isolated from slaughtered pigs at Debre Zeit, Ethiopia. A total of 501 different samples were examined of which 42 (41.6%) of 101 mesenteric lymph nodes, 22 (21.8%) of 101 tongues, 17 (16.8%) of 101 caecal contents, 11 (11.1%) of 99 livers and two (2%) of 99 muscle (diaphragm and abdomen) samples were *Salmonella* positive. Of the 94 *Salmonella* isolates representing 15 different serovars, 69 (73.4%) were multidrug resistant (resistance to two or more antimicrobials). Among the *Salmonella* serovars a high level of MDR was observed in *S*. Hadar, *S*. Kentucky, *S*. Blockley and *S*. Enteritidis mainly to tetracycline (88.6%), streptomycin (82.9%), nitrofurantoin (74.3%), nalidixic acid and ciprofloxacin (42.9% each), sulfisoxazole (21.1%) and spectinomycin (20%). The pattern of MDR varied from two to eight antimicrobials among the resistant *Salmonella* serovars. The common profiles of resistance among the MDR serovars were the combined resistance to nitrofurantoin, streptomycin and tetracycline (R type NitStrTet, 51.4%), ciprofloxacin, nalidixic acid and nitrofurantoin (R type CipNalNit, 10%), ciprofloxacin, nalidixic acid, spectinomycin, streptomycin, sulfisoxazole and tetracycline (R type CipNalSptStrSulTet, 14.3%) and to ciprofloxacin, kanamycin, nalidixic acid, neomycin, nitrofurantoin, streptomycin and tetracycline (R type CipKanNalNeoNitStrTet, 10%). Results of the present study indicate the widespread occurrence and distribution of MDR *Salmonella* serovars in slaughtered pigs which could be a potential source of human MDR *Salmonella* infections.

Introduction

Non-typhoid *Salmonella* are considered as one of the most important zoonotic pathogens causing gastroenteritis both in developed and developing countries of the world (Gomez et al., 1997). Foods of animal origin such as pork, poultry and beef are regarded as the primary sources of salmonellosis in humans. Rapid emergence and dissemination of antimicrobial-resistant pathogens have become a public health concern worldwide and major foodborne pathogens such as *Salmonella* are frequently a focus of such discussions (Gebreyes et al., 2004; Larkin et al., 2004). In the European Union and in the United States antimicrobial resistance of *Salmonella* has been used in surveillance systems as an indicator of the status of resistance in other zoonotic pathogens (Marano et al., 2000; Wray and Gnanou, 2000; Johnson et al., 2005).

Despite the complexity and difficulty of evaluating the situation of antimicrobial resistance of zoonotic pathogens in sub-Saharan African countries like Ethiopia, few studies undertaken indicated a high level of antimicrobial resistance in *Salmonella* serovars isolated from food animals, food products and humans (Mache et al., 1997; Tibaijuka et al., 2002; Alemayehu et al., 2003; Molla et al., 2003, 2004). Although no published information is available on non-typhoid *Salmonella* in pigs in Ethiopia, extensive studies carried out elsewhere indicated that pigs have a significant role in foodborne infections due to *Salmonella* (Botteldoorn et al., 2003; Hald et al., 2003). They are also one of the major sources of MDR *Salmonella* serovars which are of veterinary and public health significance (Hald et al., 2003; Gebreyes et al., 2004). We report on the occurrence and distribution of MDR *Salmonella* serovars isolated from slaughtered pigs in Ethiopia.

Materials and Methods

Collection of samples

The present cross-sectional study was undertaken in Debre Zeit, 47 km southeast of Addis Ababa (Ethiopia) from November 2004 to February 2005. The study animals were apparently healthy slaughtered pigs at a slaughter plant that was supplied by individual farmers in and around Debre Zeit. A total of 501 samples consisting of caecal content, mesenteric lymph node and tongue ($n = 101$ each) and liver and muscle (diaphragm and abdominal) samples ($n = 99$ each) were collected from randomly selected 101 slaughter pigs during the study period.

Isolation and identification of *Salmonella*

Salmonella were isolated and identified following conventional methods (ISO 6579, 1998; Quinn et al., 1999) as described previously (Molla et al., 2004; Woldemariam et al., 2005). All culture media used in this study were purchased from SIFIN (Berlin, Germany). Briefly, 25 g of each sample type was pre-enriched in buffered peptone water and incubated at 37°C for 16–20 h. Caecal contents and samples smaller than 25 g were pre-enriched in a ratio of 1 g of the sample to 9 ml of buffered

peptone water. Of the pre-enrichment broth, 1 and 0.1 ml were transferred to 10 ml of Rappaport-Vassiliadis and to another 10 ml of selenite cystine broth and incubated for 18–24 h at 42°C and 37°C respectively. About 100 μl from each enrichment broth was streaked on to xylose lysine desoxycholate agar and brilliant green-phenol red-lactose-sucrose agar plates and incubated at 37°C for 24–48 h. Presumptive *Salmonella* colonies were characterized using conventional biochemical tests (ISO 6579, 1998; Quinn et al., 1999). *Salmonella* isolates were sent to the Public Health Agency, Office International des Épizooties (OIÉ) Reference Laboratory for Salmonellosis, Guelph, ON, Canada for serotyping, phage typing and antimicrobial resistance testing.

Serotyping

For serotyping, the somatic (O) antigens of the *Salmonella* isolates were determined with a slide agglutination test as described by Ewing (1986) whereas the flagellar (H) antigens were identified by using a microtechnique that employs microtitre plates (Shipp and Rowe, 1980). The antigenic formulae of *Salmonella* serovars as listed by Le Minor and Popoff (1997) were used to name the serovars.

Phage typing

The standard phage typing technique described by Anderson and Williams (1956) was followed. *Salmonella* Enteritidis strains were phage typed with typing phages obtained from the International Centre for Enteric Phage Typing, Central Public Health Laboratory, Colindale, UK (Ward et al., 1987) via the National Laboratory for Enteric Pathogens, Health Canada, Winnipeg, MB, Canada.

Antimicrobial resistance testing

The antimicrobial resistance test of *Salmonella* isolates ($n = 94$) was conducted on a panel of 24 selected antimicrobial drugs by the agar dilution method as previously described (Poppe et al., 2002; Larkin et al., 2004). The susceptible and resistance breakpoint levels of the antimicrobials were based mainly on those specified by the National Committee for Clinical Laboratory Standards (NCCLS) M31-A2 (NCCLS, 2002) and M100-S15 (NCCLS, 2005). The antimicrobials, susceptible and resistance breakpoint levels are presented in Table 1. The ATCC reference strains *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *E. coli* ATCC 35218 (the latter strain for examining susceptibility to amoxicillin/clavulanic acid) were used for quality control purposes. In this study, *Salmonella* serovars with intermediate susceptibility classification were considered to be not resistant to that antimicrobial.

Table 1. Antimicrobials and concentrations used to test susceptibility of *Salmonella* isolates

Antimicrobials	Abbreviations	Susceptible and resistance breakpoint levels[a]	
		Susceptible at μg/ml	Resistant at μg/ml
Amikacin	Amk	16	NT[b]
Ampicillin	Amp	NT	32
Amoxicillin /clavulanic acid	Amc	NT	32/16
Apramycin	Apr	NT	32[c]
Carbadox	Car[d]	NT	32[e]
Cephalothin	Cef	NT	32
Ceftiofur	Ctf[d]	NT	8
Ceftriaxone	Cro	8[f]	NT
Cefoxitin	Fox	NT	32
Chloramphenicol	Chl	NT	32
Ciprofloxacin	Cip	0.125[g]	NT
Florfenicol	Fen	NT	16[h]
Gentamicin	Gen	NT	16
Kanamycin	Kan	NT	64
Nalidixic acid	Nal	NT	32
Neomycin	Neo[d]	NT	16[c]
Nitrofurantoin	Nit	NT	64[i]
Spectinomycin	Spt	NT	64[c]
Streptomycin	Str	NT	32[c]
Sulfisoxazole	Sul[d]	NT	512
Sulfamethoxazole /trimethoprim	Sxt	NT	76/4
Tetracycline	Tet	NT	16
Tobramycin	Tob	NT	8
Trimethoprim	Tmp	NT	16

[a]Susceptible and resistance breakpoint levels were those specified by the National Committee for Clinical Laboratory Standards (NCCLS) M31-A2 (NCCLS, 2002), M31-S1 (NCCLS, 2004) and M100-S15 (NCCLS, 2005).
[b]NT, not tested; the isolates were only tested at susceptibility and resistance breakpoint concentrations shown.
[c]There are no NCCLS interpretive standards for apramycin, neomycin, spectinomycin and streptomycin; the strains were considered to be resistant to these antimicrobials at 32, 16, 64 and 32 μg/ml, respectively.
[d]The abbreviations Car, Ctf, Neo and Sul were self-chosen.
[e]The strains were considered to be resistant to carbadox, a veterinary growth promoter for pigs, at 32 μg/ml (Dunlop et al., 1998).
[f]Strains that grew on Mueller Hinton agar with 8 μg/ml show reduced susceptibility to ceftriaxone (Allen and Poppe, 2002a).
[g]A 0.125 μg/ml of ciprofloxacin concentration determines reduced susceptibility to ciprofloxacin (Hakanen et al., 1999; Allen and Poppe, 2002b; Aarestrup et al., 2003).
[h]Strains were considered to be resistant to florfenicol at the level of 16 μg/ml (Poppe et al., 2002).
[i]Strains were considered to be resistant to nitrofurantoin at 64 μg/ml; human urinary tract isolates are considered to be resistant to nitrofurantoin at 128 μg/ml (NCCLS, 2005).

Results and discussion

Of the total 501 samples obtained from apparently healthy slaughtered pigs, 94 (18.8%) were *Salmonella* positive. The proportion of positive samples ranged from 2% in muscle to 41.6% in mesenteric lymph nodes (Table 2). Of the total caecal content and mesenteric lymph node samples ($n = 101$ each) examined, 22 (21.8%) and 42 (41.6%) respectively were *Salmonella* positive which could be a major source of contamination of carcass particularly during evisceration. The isolation of *Salmonella* in muscle, liver and tongue reflects the hygienic conditions during slaughter. Results of this study were comparable with previous studies undertaken elsewhere (Letellier et al., 1999; Swaneburg et al., 2001; Botteldoorn et al., 2003; Gebreyes et al., 2004). Lower prevalence of *Salmonella* in slaughtered pigs has also been reported (Käsbohrer et al., 2000; Hald et al., 2003). The occurrence and distribution of *Salmonella* in slaughtered pigs could vary depending upon the type, source and size of sample and analysis of more than one sample type per pig can usually

Table 2. Distribution of *Salmonella* serovars isolated from slaughtered pigs by source

Salmonella serovar	Type and number of samples positive for *Salmonella* by serovar						
	Mesenteric lymph nodes ($n = 101$)	Tongue ($n = 101$)	Caecal content ($n = 101$)	Liver ($n = 99$)	Muscle ($n = 99$)	Total (%) ($n = 501$)	MDR serovars (%) ($n = 69$)
S. Hadar	21	5	8	4	–	38 (40.4)	38 (100)
S. Kentucky	6	4	2	2	1	15 (15.6)	15 (100)
S. Anatum	3	3	1	1	–	8 (8.5)	–
S. Blockley	1	3	3	1	–	8 (8.5)	8 (100)
S. Leoben	1	4	1	–	–	6 (6.4)	–
S. Enteritidis	5	-	–	–	–	5 (5.3)	5 (100)
S. Havana	1	1	–	1	–	3 (3.2)	–
S. 1:9,12:-	1	–	–	1	–	2 (1.1)	2 (100)
S. Kiambu	2	–	-	–	–	2 (2.1)	1 (50)
S. Gaminara	–	1	1	–	–	2 (2.1)	–
S. Livingstone	1	–	–	–	–	1 (1.1)	–
S. Uganda	–	–	–	1	–	1 (1.1)	–
S. I:Rough-O:-	–	–	1	–	–	1 (1.1)	–
S. Newport	–	–	–	–	1	1 (1.1)	–
S. Eastbourne	–	1	–	–	–	1 (1.1)	–
Total	42 (41.6%)	22 (21.8%)	17 (16.8%)	11 (11.1%)	2 (2%)	94 (18.8%)	69 (73.4)

Table 3. Antimicrobial resistance of *Salmonella* serovars isolated from slaughtered pigs by antimicrobial type

Antimicrobials[a]	Number of *Salmonella* serovars resistant to					
	S. Hadar ($n = 38$)	*S*. Kentucky ($n = 15$)	*S*. Blockley ($n = 8$)	*S*. Enteritidis ($n = 5$)	Others ($n = 28$)	Total resistant ($n = 70/94$)
Ampicillin	–	–	1	–	1	2 (2.9)
Gentamicin	–	1	1	–	–	2 (2.9)
Kanamycin	–	–	8	–	–	8 (11.4)
Neomycin	–	–	8	–		8 (11.4)
Spectinomycin	–	–	8	–	–	8 (11.4)
Streptomycin	37	12	8	–	1	58 (82.9)
Tetracycline	38	14	8	–	2	62 (88.6)
Ciprofloxacin	–	15	8	5	2	30 (42.9)
Nalidixic acid	–	15	8	5	2	30 (42.9)
Nitrofurantoin	37	–	8	5	2	52 (74.3)
Sulfisoxazole	–	14	–	–	1	15 (21.4)
Sulfamethoxazole /trimethoprim	–	–	–	–	1	1 (1.4)
Trimethoprim	–	–	–	–	1	1 (1.4)

[a]Resistance was not observed to amikacin, amoxicillin/clavulanic acid, apramycin, carbadox, cephalothin, ceftriaxone, ceftiofur, cefoxitin, chloramphenicol, florfenicol and tobramycin.

indicate the actual *Salmonella* status in slaughtered pigs (Swaneburg et al., 2001).

Of the total of 94 *Salmonella* isolates representing 15 serovars, *S*. Hadar was the most frequently occurring serovar followed by *S*. Kentucky, *S*. Blockley, *S*. Anatum, *S*. Leoben and *S*. Enteritidis (Table 2). *Salmonella* Kentucky was identified in all sample types examined (mesenteric lymph node, tongue, caecal content, liver and muscle). *Salmonella* Enteritidis, *S*. Kiambu and *S*. Livingstone were exclusively detected from mesenteric lymph nodes whereas *S*. Eastbourne, *S*. Newport and *S*. Uganda were isolated only from tongue, muscle and liver respectively indicating other sources of contamination such as the slaughterhouse environment. The occurrence and distribution of *Salmonella* serovars in food animals and food products could vary from country to country, region to region and from slaughterhouse to slaughterhouse (Letellier et al., 1999; Swaneburg et al., 2001). It is also well known that some *Salmonella* serovars maintain their dominant role over many years while others emerge, re-emerge or decrease over time. A rapid international trade in agriculture, aquaculture and manufactured products has facilitated the introduction of new *Salmonella* serovars into the importing countries (D'Aoust, 1994).

Among the *Salmonella* serovars tested for resistance ($n = 94$) to a panel of 24 antimicrobials, 69 (73.4%) were multidrug-resistant (MDR) type (Table 3). Of the *Salmonella* serovars, *S*. Hadar, *S*. Kentucky, *S*. Blockley and *S*. Enteritidis showed a high level (100%) of MDR (resistance to two to eight antimicrobial agents). More than 87% of the MDR *Salmonella* serovars originated from the lymph node (33/42), caecal content (15/17) and tongue (13/22) samples. Some works done previously indicated the widespread occurrence of antimicrobial-resistant *Salmonella* serovars in other food animals and food products as well as in humans in Ethiopia (Mache et al., 1997; Tibaijuka et al., 2002; Alemayehu et al., 2003; Molla et al., 2003, 2004) and other parts of the world (Hald et al., 2003; Gebreyes et al., 2004; Schroeter et al., 2004; Johnson et al., 2005). The high frequency of occurrence of MDR was in

agreement with the findings of other researchers on *Salmonella* serovars isolated from pigs in the United States (Farrington et al., 2001; Gebreyes et al., 2004) and Europe (Hald et al., 2003; Schroeter et al., 2004; Agustin et al., 2005).

The most common resistance phenotypes observed among *Salmonella* serovars in this study were to tetracycline (88.6%), streptomycin (82.9%), nitrofurantoin (74.3%), nalidixic acid (42.9%), ciprofloxacin (42. 9%), sulfisoxazole (21.4%), spectinomycin (20%), kanamycin and neomycin (each 11.4%) (Table 3). Resistance to the antimicrobial effects of ampicillin, kanamycin, sulphamethoxazole/trimethoprim and trimethoprim was low (< 3.0%). Resistance to streptomycin, a narrow-spectrum aminoglyoside, was high (82.9%) when compared with other aminoglycosides such as clavulanate-potentiated amoxicillin, ampicillin, amikacin and gentamycin. Antimicrobials within the same class usually possess slight structural differences to minimize cross-resistance (Farrington et al., 2001). Resistance to tetracycline, one of the commonly used antimicrobials in human and veterinary medicine practices, was expected and was consistent with other reports (Farrington et al., 2001; Gebreyes et al., 2004; Agustin et al., 2005).

Overall, the high frequency of resistant *Salmonella* serovars to the various antimicrobials could be probably an indication of indiscriminate and continuous uses of subtherapeutic doses of commonly available antimicrobials both in the veterinary and public health sectors in Ethiopia. It has been reported that the misuse or overuse of antimircrobials for treatment, prophylaxis and growth promotion in food animals has contributed to the emergence and spread of resistance to foodborne pathogens including *Salmonella* (Agustin et al., 2005; Zhao et al., 2005). This is particularly more striking in developing countries where there is a widespread misuse of antimicrobials due to the lack of access to appropriate treatment and under use due to inadequate dosing, poor drug quality and incomplete treatment courses (WHO, 2001).

In our study all *Salmonella* isolates tested were susceptible to the antimicrobial effects of amikacin, amoxicillin/clavulanic acid, apramycin, carbadox, cephalosporins, chloramphenicol, florfenicol and tobramycin. The absence of resistance particularly to some first-, second- and third-generation cephalosporins in this study could be perhaps due to their limited usage in veterinary and public health sectors in Ethiopia. Other researchers, however, have reported a considerable level of resistance to cephalothin, a first-generation cephalosporin, among *Salmonella* isolates from pigs (Gebreyes et al., 2004; Agustin et al., 2005). It has also been indicated that resistance particularly to third-generation cephalosporins (e.g. ceftriaxone), an important drug for the treatment of invasive *Salmonella* infections in humans, especially for children, is of great concern (Zhao et al., 2005). The high level of resistance to quinolones (42.9%) observed in present study was in accordance with the reports of others (Schroeter et al., 2004; Agustin et al., 2005). In this study we considered isolates growing on Mueller Hinton agar with 0.125 μg/ml ciprofloxacin as having a reduced susceptibility to the antimicrobial (Allen and Poppe, 2002b). This was in agreement with the occurrence of treatment failures when *Salmonella* strains showed reduced susceptibility to ciprofloxacin at 0.125 μg/ml (Hakanen et al., 1999; Aarestrup et al., 2003). The results of antimicrobial susceptibility testing of the *Salmonella* strains (*S*. Kentucky, $n = 15$; *S*. Blockley, $n = 8$ and *S*. Enteritidis, $n = 5$) showed that there was a complete agreement between *Salmonella* strains showing reduced susceptibility to ciprofloxacin at 0.125 μg/ml and to nalidixic acid at 32 μg/ml. Resistance to quinolones such as nalidixic acid is a good indicator for reduced susceptibility to fluoroquinolones like ciprofloxacin (Malorny et al., 2003; Schroeter et al., 2004). Allen and Poppe (2002b) indicated that the mutations affecting reduced susceptibility to ciprofloxacin at 0.125 μg/ml and resistance to nalidixic acid at 32 μg/ml are commonly found on the gyrA gene at codons 82, 83 or 87. Resistance to fluoroquinolones such as ciprofloxacin undermines the value of these drugs for the treatment of human clinical salmonellosis in adults (Zhao et al., 2005). As we do not have reliable information on the use of fluoroquinolones both in the veterinary and public health sectors in study area, it is difficult to establish an association between the observed resistance and use of the antimicrobials. Previous studies on *Salmonella* strains isolated from Ethiopian chickens and slaughter camels indicated that all strains tested were susceptible to the antimicrobial effects of ciprofloxacin and nalidixic acid (Molla et al., 2003, 2004). A study undertaken in Germany indicated that the main reservoir for resistant *Salmonella* strains to quinolones was poultry and poultry meat (Malorny et al., 2003). Poppe et al. (2001) reported that the increase of fluoroquinolone resistance has been associated with the use of enrofloxacin in veterinary practices.

The pattern of MDR varied from two to eight antimicrobials (Table 4). Forty-eight (51.1%) of the multidrug-resistant serovars were resistant to five or fewer antimicrobials whereas 21 (22.3%) of them were multiple resistant from six to eight antimicrobial agents suggesting that resistance to multiple antimicrobial agents is widespread among *Salmonella* serovars in slaughtered pigs. The common profiles of resistance among the MDR serovars were the combined resistance to NitStrTet (51.4%), CipNalNit (10%), CipNalSptStrSulTet (14.3%) and CipKanNalNeoNitStrTet (10%) (Table 4). Of the 38 multidrug-resistant *S*. Hadar strains, 36 (94.7%) displayed NitStrTet resistance pattern. On the contrary 66.6% of the multidrug-resistant *S*. Kentucky strains were resistant to CipNalSptStrSulTet. All *S*. Enteritidis phage types tested (PT 8, 13a, 911) were resistant to CipNalNit. Some of these phage types have been reported previously in other food animals in Ethiopia (Molla et al., 2003). Seven of the eight *S*. Blockley strains were resistant to CipKanNalNeoNitStrTet. Even though all *S*. Hadar strains (38/38) were multidrug resistant, the number of antimicrobials to which they were resistant was small (two to three antimicrobials) in contrast to other serovars which showed resistance to up to seven antimicrobials (Table 4). This was consistent with previous reports of Johnson et al. (2005) on *S*. Hadar antimicrobial resistance patterns.

The MDR *S*. Typhimurium strains including definitive type (DT)104 which had been isolated in other food animals previously in Ethiopia (Alemayehu et al., 2003; Molla et al., 2004) was not detected in the present study. *Salmonella* Typhimurium DT104 and other phage types could be present in Ethiopia pigs in such low numbers that our sample size was not large enough to detect it. Multiple (two to three) *Salmonella* serovars (isolates recovered from two or more different samples from same individual animal) with different antimicrobial resistance patterns were also identified from individual pigs (data not shown). A similar observation was made from slaughtered pigs elsewhere (Farrington et al.,

Table 4. Multidrug resistance pattern of *Salmonella* serovars from slaughtered pigs

Salmonella serovar	Number of *Salmonella* serovars		Resistance pattern (number)
	Tested	MDR (%)	
S. Hadar	38	38 (100)	NitStrTet[a](36) StrTet (1) NitTet (1)
S. Kentucky	15	15 (100)	CipNalSptStrSulTet (10) CipGenNalSptStrSulTet (2) CipNalSptSulTet (2) CipNal (1)
S. Anatum	8	–	–
S. Blockley	8	8 (100)	CipKanNalNeoNitStrTet (7) AmpCipKanNalNeoNitStrTet (1)
S. Leoben	6	–	–
S. Enteritidis	5	5 (100)	CipNatNit (5)
S. Havana	3	–	–
S. Kiambu	2	1 (50)	AmpStrSulSxtTetTmp (1)
S. I:9,12:-:-	2	2 (100)	CipNalNit (2)
S. Gaminara	2	–	–
S. Livingstone	1	–	–
S. Uganda	1	–	–
S. I:Rough-O:-:-	1	–	–
S. Newport	1	–	–
S. Eastbourne	1	–	–
Total	94/501 (18.7%)	69 (73.4%)	

[a]For abbreviations of antimicrobials refer to Table 1.

2001). In general in most sub-Saharan African countries like Ethiopia where salmonellae and other zoonotic bacterial pathogens are not routinely isolated and identified and their resistance to commonly used antimicrobials are rarely assessed suggest that the high level of antimicrobial resistance among *Salmonella* strains and other zoonotic pathogens will remain a major health problem. The magnitude of the problem should be reduced at various levels through the prudent use of antimicrobials both in the veterinary and public health sectors.

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References

Aarestrup, F. M., C. Wiuff, K. Mølbak, and E. J. Threlfall, 2003: Is it time to change fluoroquinolone breakpoints for *Salmonella* spp? Antimicrob. Agents Chemother. **47,** 827–829.

Agustin, A. I., J. J. Carraminana, C. Rota, and A. Herrera, 2005: Antimicrobial resistance of *Salmonella* spp. from pigs at slaughter in Spain in 1993 and 2001. Lett. Appl. Microbiol. **41,** 39–44.

Alemayehu, D., B. Molla, and A. Muckle, 2003: Prevalence and antimicrobial resistance pattern of *Salmonella* isolates from apparently healthy slaughtered cattle in Ethiopia. Trop. Anim. Health Prod. **35,** 309–319.

Allen, K. J., and C. Poppe, 2002a: Occurrence and characterization of resistance to extended-spectrum cephalosporins mediated by β-lactamase CMY-2 in *Salmonella* isolated from food-producing animals in Canada. Can. J. Vet. Res. **66,** 137–144.

Allen, K. J., and C. Poppe, 2002b: Phenotypic and genotypic characterization of food animal isolates of *Salmonella* with reduced sensitivity to ciprofloxacin. Microb. Drug Resist. **8,** 375–383.

Anderson, E. S., and R. E. O. Williams, 1956: Bacteriophage typing of enteric pathogens and Staphylococci and its use in epidemiology. J. Clin. Pathol. **9,** 94–114.

Botteldoorn, N., M. Heyndrickx, N. Rijpens, K. Grijspeerdt, and L. Herman, 2003: *Salmonella* on pig carcasses: positive pig and cross contamination in the slaughter house. J. Appl. Microbiol. **95,** 891–903.

D'Aoust, J. -Y. 1994: *Salmonella* and the international food trade. Int. J. Food Microbiol. **24,** 11–31.

Dunlop, R. H., S. A. McEwen, A. H. Meek, W. D. Black, R. C. Clarke, and R. M. Frienship, 1998: Individual and group antimicrobial usage rates on 34 farrow-to-finish swine farms in Ontario, Canada. Prev. Vet. Med. **34,** 247–264.

Ewing, W. H. 1986: Serologic identification of *Salmonella*. In: Ewing, W. H. (ed.), Edwards and Ewing's Identification of *Enterobacteriaceae*, 4th edn. Elsevier Science Publishing Co., Inc., New York.

Farrington, L. A., R. B. Harvey, S. A. Buckley, R. E. Droleskey, D. J. Nisbet, and P. D. Inskip, 2001: Prevalence of antimicrobial resistance in salmonellae isolated from market-age swine. J. Food Prot. **64,** 1496–1502.

Gebreyes, W. A., P. R. Davies, P. -K. Turkson, W. E. M. Morgan, J. A. Funk, C. Altier, and S. Thakur, 2004: Characterization of antimicrobial-resistant phenotypes and genotypes among *Salmonella enterica* recovered from pigs on farm, from transport trucks and from pigs after slaughter. J. Food Prot. **67,** 698–705.

Gomez, T. M., Y. Motarjemi, S. Miyagawa, F. K. Käferstein, and K. Stöhr, 1997: Foodborne salmonellosis. World Health Stat. Q. **50,** 81–89.

Hakanen, A., P. Kotainen, J. Jalava, A. Siitonen, and P. Huovinen, 1999: Detection of decreased fluoroquinolone susceptibility in salmonellas and validation of nalidixic acid screening test. J. Clin. Microbiol. **37,** 3572–3577.

Hald, T., A. Wingstrand, M. Swanenburg, A. von Altrock, and B. Thornberg, 2003: The occurrence and epidemiology of *Salmonella* in European pig slaughterhouse. Epidemiol. Infect. **131,** 1187–1203.

ISO 6579, 1998. Microbiology of Food and Animal Feeding Stuff: Horizontal Method for the Detection of *Salmonella*. ISO, Geneva.

Johnson, J. M., R. Andrijana, and L. M. McMullen, 2005: Antimicrobial resistance of selected *Salmonella* isolates from food animals and foods in Alberta. Can. Vet. J. **46,** 141–146.

Käsbohrer, A., D. Protz, R. Helmuth, K. Nockler, T. Blaha, F. Conraths, and L. Geue, 2000: *Salmonella* in slaughter pigs of German origin: an epidemiological study. Eur. J. Epidemiol. **16,** 141–146.

Larkin, C., C. Poppe, B. McNa, B. McEwen, A. Mahdi, and J. Odumeru, 2004: Antibiotic resistance of *Salmonella* isolated from hog, beef and chicken carcass samples from provincially inspected abattoirs in Ontario. J. Food Prot. **67,** 448–455.

Le Minor, L., and M. Y. Popoff, 1997: Antigenic Formulas of the *Salmonella* Serovars, WHO Collaborating Centre for Reference and Research on *Salmonella*. Pasteur Institute Paris, France.

Letellier, A., S. Messier, J. Pare, J. Menard, and S. Quessy, 1999: Distribution of *Salmonella* in pig herds in Quebec. Vet. Microbiol. **67,** 299–306.

Mache, A., Y. Mengistu, and S. Cowley, 1997: *Salmonella* serogroups identified from adult diarrhoeal outpatients in Addis Ababa, Ethiopia: antibiotic resistance and plasmid profile analysis. East Afr. Med. J. **74,** 183–187.

Malorny, B., A. Schroeter, B. Guerra, and R. Hemuth, 2003: Incidence of quinolone resistance in strains of *Salmonella* isolated from poultry, cattle and pigs in Germany between 1998 and 2001. Vet. Rec. **153,** 643–648.

Marano, N. N., S. Rossiter, K. Stamey, K. Joyce, T. J. Barrett, L. K. Tollefson, and F. J. Angulo, 2000: The National Antimicrobial Resistance Testing Monitoring System (NARMS) for enteric bacteria, 1996–1999: surveillance for action. J. Am. Vet. Med. Assoc. **217,** 1829–1830.

Molla, B., A. Mesfin, and D. Alemayehu, 2003: Multiple antimicrobial resistance pattern of *Salmonella* serotypes isolated from chicken carcass and giblets in Debre Zeit and Addis Ababa, Ethiopia. Ethiop. J. Health Dev. **17,** 131–149.

Molla, B., S. Woubit, D. Alemayehu, and A. Mohamed, 2004: Antimicrobial resistance pattern of *Salmonella* serotypes isolated from apparently healthy slaughtered camels (*Camelus dromedarius*) in eastern Ethiopia. Berl. Münch. Tierärztl. Wochenschr. **117,** 39–45.

National Committee for Clinical Laboratory Standards (NCCLS), 2002: Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard, 2nd edn. NCCLS document M31-A2. NCCLS, Wayne, PA.

National Committee for Clinical Laboratory Standards (NCCLS), 2004: Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Informational Supplement (May 2004), NCCLS document M31-S1. NCCLS, Wayne, PA.

National Committee for Clinical Laboratory Standards (NCCLS), 2005: Performance Standards for Antimicrobial Susceptibility Testing; Fifteenth Informational Supplement. NCCLS document M100-S15, NCCLS, Wayne, PA.

Poppe, C., M. Ayroud, G. Ollis, M. Chirino-Trejo, N. Smart, S. Quessy, and P. Michell, 2001: Trends in antimicrobial resistance of *Salmonella* isolated from animals, foods of animal origin and the environment of animal production in Canada, 1994–1997. Microb. Drug Resist. **7,** 197–212.

Poppe, C., K. Ziebell, L. Martin, and K. Allen, 2002: Diversity in antimicrobial resistance and other characteristics among *Salmonella* Typhimurium DT104 isolates. Microb. Drug Resist. **8,** 107–122.

Quinn, P. J., M. E. Carter, B. Markey, and G. R. Carter, 1999: Veterinary Clinical Microbiology. Wolf publishing, London.

Schroeter, A., B. Hoog, and R. Helmuth, 2004: Resistance of *Salmonella* isolates in Germany. J. Vet. Med. B **51,** 389–392.

Shipp, C. R., and B. Rowe, 1980: A mechanized microtechnique for *Salmonella* serotyping. J. Clin. Pathol. **33,** 595–597.

Swaneburg, M., H. A. Urlings, J. M. Snijders, D. A. Keuzenkamp, and F. van Knapen, 2001: *Salmonella* in slaughter pigs: prevalence serotypes and critical control points during slaughter in two slaughterhouses. Int. J. Food Microbiol. **70,** 243–254.

Tibaijuka, B., B. Molla, G. Hildebrandt, J. Kleer, and W. Salah, 2002: Antimicrobial resistance to salmonellae isolated from retail raw chicken meat and giblets in Ethiopia. Bull. Anim. Health Prod. Afr. **50,** 86–95.

Ward, L. R., J. D. H. De Sa, and B. Rowe, 1987: A phage-typing scheme for *Salmonella* Enteritidis. Epidemiol. Infect. **99,** 291–294.

WHO, 2001: WHO Global Strategy for Containment of Resistance. Department of Communicable Disease Surveillance and Response. WHO/CDS/CSR/DRS/2001.2. World Heath Organization, Geneva.

Woldemariam, E., B. Molla, D. Alemayehu, and A. Muckle, 2005: Prevalence and distribution of *Salmonella* in apparently healthy slaughtered sheep and goats in Debre Zeit, Ethiopia. Small Rumin. Res. **58,** 19–24.

Wray, C., and J. C. Gnanou, 2000: Antibiotic resistance monitoring in bacteria of animal origin: analysis of national monitoring programs. Int. J. Antimicrob. Agents **14,** 291–294.

Zhao, S., P. J. Fedor-Cray, S. Friedman, P. F. McDermont, R. D. Walker, S. Qaiyumi, S. L. Foley, S. K. Hubert, S. Ayers, L. English, D. A. Dargatz, B. Salamone, and D. G. White, 2005: Characterization of *Salmonella* Typhimurium of animal origin obtained from the national antimicrobial resistance monitoring system. Foodborne Pathog. Dis. **2,** 169–181.