Original article

Multiple antimicrobial-resistant *Salmonella* serotypes isolated from chicken carcass and giblets in Debre Zeit and Addis Ababa, Ethiopia

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Abstract

Background: Antimicrobial-resistant *Salmonella* and other zoonotic bacterial pathogens can be transferred from animals to humans through consumption of contaminated food and food products and thus present a public health risk. The increase in *Salmonella* resistance to the commonly used antimicrobials both in the public health and veterinary sectors is one of the major threats of health care worldwide.

Objectives: The present study was undertaken to determine the antimicrobial resistance pattern of eighty *Salmonella* strains isolated from chicken carcass and giblets (liver, gizzard and heart) obtained from processing plants at Debre Zeit and supermarkets in Addis Ababa, Ethiopia.

Methods: Eighty *Salmonella* strains were isolated from a total of 378 chicken carcass and giblet samples. Isolated *Salmonella* strains were serotyped and tested for resistance to 23 different antimicrobial agents with the agar dilution method at the Health Canada, Office International des Épizooties (OIÉ) Reference Laboratory for Salmonellosis in Guelph, Ontario, Canada.

Results: Fifty-one (63.7%) of the 80 *Salmonella* strains were resistant to one or more antimicrobials of which 42 (52.5%) displayed multiple-drug resistance. Among the strains, 51.2% were resistant to sulfisoxazole, 46.2% to spectinomycin, 45% to amoxicillin-clavulanic acid and ampicillin, 41.2% to tetracycline and 30% to chloramphenicol. Less than 27.5% of the strains showed resistance to florfenicol, streptomycin, cotrimoxazole and to trimethoprim. *S. typhimurium* var. Copenhagen (100%), *S. anatum* (62.5%), *S. typhimurium* (33.3%) and *S. braenderup* (34.3%) showed multiple antimicrobial resistance to up to eight antimicrobials. None of the strains were resistant to amikacin, apramycin, gentamicin, kanamycin, neomycin, tobramycin, quinolones, cephalosporins and nitrofurantoin.

Conclusion: Results of the present study indicated the potential importance of chickens as source of multiple antimicrobial-resistant *Salmonella* for human infections and suggest the need for detailed epidemiological and molecular studies in food animals, food products and humans in Ethiopia. [*Ethiop.J.Health Dev.* 2003;17(2):131-149]

Introduction

Resistance of *Salmonella* to commonly used antimicrobials is increasing both in the veterinary and public health sectors and has emerged as a global problem (1, 2, 3, 4, 5). The increasing proportion of single and multiple antimicrobial-resistant *Salmonella* strains isolated from human salmonellosis cases has been associated with the widespread use of antimicrobial agents in food animal production. A considerable number of antimicrobials commonly used in the treatment of salmonellosis and other bacterial infections of humans are also used in veterinary practices (3, 6). This may present a public health risk by the transfer of resistant *Salmonella* and other zoonotic bacterial pathogens or the resistant genes from food animals to humans through consumption of contaminated food and food products.

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The emergence and widespread of antimicrobial-resistant *Salmonella* strains in chickens and other food animals and humans may be associated with the use of medicated feeds in intensive animal husbandry systems, subtherapeutic doses and indiscriminate uses of antimicrobials both in animal and human treatments (2, 4, 7). Various antimicrobials in intensively managed food animals including chickens are often administered through the feed or drinking water either for therapy, prophylaxis or growth promotion. This enhances the risk of proliferation of resistant strains, which can have severe consequences for human health (4). In veterinary sciences, studies on the frequency and distribution of antimicrobial-resistant *Salmonella* and other foodborne bacterial pathogens are usually undertaken for the following reasons: (i) to monitor and improve management systems, (ii) to ensure the production of foods free of antimicrobials and drug-resistant pathogens, (iii) to decrease threats to the health of the consumer and (iv) to promote the international trade of food and food products (7, 8).

In developing countries like Ethiopia the situation of antimicrobial resistance is more complex and difficult. This is because Salmonellae and other major zoonotic bacterial pathogens are not routinely cultured and their resistance to commonly employed antimicrobials both in the public health and veterinary practices is rarely determined (9, 10). Previous studies undertaken in Ethiopia indicated the presence of a high level of antimicrobial resistance in *Salmonella* isolated from humans (11, 12, 13), food animals and food products (10, 14). The present study, which was part of a survey to estimate the prevalence and distribution of *Salmonella* in chicken meat and giblets (liver, gizzard and heart) in central Ethiopia was conducted from October 2001 to April 2002 (15). The purpose of the study was to determine the antimicrobial resistance pattern of eighty *Salmonella* strains belonging to eight different serotypes isolated from chicken samples obtained from processing plants at Debre Zeit and supermarkets in Addis Ababa, Ethiopia.

Methods

Salmonella isolates

A total of 378 samples (Table 1) consisting of liver (55), gizzard (56), heart (59), muscle (104) and skin (104) were randomly selected using a cross-sectional study design from two processing plants at Debre Zeit and 4 supermarkets supplied by these processing plants in Addis Ababa from October 2001 to April 2002. The samples were examined for the presence of *Salmonella* according to the techniques recommended by the International Organization for Standardization (ISO) 6579 (16). *Salmonella* isolates were further serotyped at Heath Canada, with slide agglutination tests as described by Ewing (17) and the flagellar antigens were detected by a technique utilizing microtitre plates (18). The isolates were also tested for resistance to 23 different antimicrobial agents at the Health Canada, Office International des Épizooties (OIÉ) Reference Laboratory for Salmonellosis in Guelph, Ontario, Canada.

Antimicrobial resistance tests

The antimicrobial resistance tests of *Salmonella* strains were carried out with the agar dilution method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS), (19). Briefly, the strains were grown to 0.5-1.0 McFarland density in Mueller Hinton (MH) broth (Difco, Detriot, USA) and replica plated using a Cathra Replicator (20) onto MH agar plates (Difco, Detriot, USA) containing antimicrobials at the concentration as follows: amikacin (amk) 16 and 64 µg/ml; amoxicillin-clavulanic acid (amox-clav) 20 µg/ml; ampicillin (amp) 32 µg/ml; apramycin (apr) 32 µg/ml; carbadox (car) 30 µg/ml; ceftiofur (ctf) 8 µg/ml; ceftriaxone (cef) 8 µg/ml; cephalotin (cep) 32 µg/ml; chloramphenicol (chl) 32 µg/ml; ciprofloxacin (cip) 0.125 µg/ml; florfenicol (fen) 16 µg/ml; gentamicin (gen) 16 µg/ml; kanamycin (kan) 64 µg/ml; nalidixic acid (nal) 32 µg/ml; neomycin (neo) 16 µg/ml; nitrofurantoin (nit) 64 µg/ml; spectinomycin (spc) 64 µg/ml; streptomycin (str) 64 µg/ml;

sulfisoxazole (sul) 512 µg/ml; tetracycline (tet) 16 µg/ml; tobramycin (tob) 8 µg/ml; cotrimoxazole (cot) 80 µg/ml and trimethoprim (tmp) 4 and 16 µg/ml. To determine resistance to florfenicol, Aquaflor® containing 50% florfenicol was dissolved in dimethylformamide and added to stock solution containing 20 mg/ml Aquaflor to MH agar to obtain plates with 16 and 32 µg/ml of florfenicol. The dilutions were chosen to detect incremental changes in resistance based on previous 2 years data. An isolate was defined as resistant if it was resistant to one or more of the antimicrobial agents tested whereas multiple resistance was defined as resistance to 2 or more antimicrobial agents. Standard and reference strains were used and interpretation of the strains as susceptible, intermediate or resistant was made following the recommendations of the NCCLS [19].

Results

A total of 80 *Salmonella* strains isolated from chicken carcass and giblets were tested for resistance and all strains were susceptible to the antimicrobial effect of quinolones (ciprofloxacin and nalidixic acid), nitrofurantoin, cephalosporins (ceftiofur, ceftriaxone and cephalotin) and aminoglycosides (amikacin, apramycin, gentamycin, kanamycin, neomycin and tobramycin). Fifty-one (63.7%) of the *Salmonella* strains were resistant to one or more antimicrobials (Table 1).

Table 1: **Distribution of antimicrobial-resistant *Salmonella* isolates from chickens at Debre Zeit and Addis Ababa, Ethiopia**

Source	Number of samples Examined	Positive (%)	*Salmonella* serotypes (no.)	Number of strains resistant
Muscle	104	16 (15.4)	*S. braenderup* (8)	4
			S. anatum (2)	1
			S. hadar (2)	2
			S. typhimurium var. Copenhagen (1)	1
			S. infantis (1)	1
			S. kottbus (2)	-
Skin	104	8 (7.7)	*S anatum* (3)	3
			S. braenderup (2)	1
			S. typhimurium var. Copenhagen (1)	1
			S.bovismorbificans (1)	1
			S. infantis (1)	1
Liver	55	19 (34.5)	*S. braenderup* (8)	1
			S. typhimurium (2)	1
			S. typhimurium var. Copenhagen (9)	9
Gizzard	56	23 (41.1)	*S. braenderup* (11)	5
			S. kottbus (1)	-
			S. typhimurium (1)	1
			S. typhimurium var. Copenhagen (8)	8
Heart	59	14 (23.7)	*S. braenderup* (6)	4
			S. anatum (1)	1
			S. kottbus (2)	-
			S. typhimurium var. Copenhagen (5)	5
Total	378	80 (21.1)		51 (63.7%)

Forty-one (51.2%) of the *Salmonella* strains were resistant to sulfisoxazole, 37 (46.2%) to spectinomycin, 36 (45%) to amoxicillin-clavulanic acid and ampicillin, 33 (41.2%) to tetracycline, 24 (30%) to chloramphenicol, 22 (27.5%) to florfenicol and 18 (22.5%) to streptomycin. The remaining proportion of the isolates (less than 21 %) showed resistance to carbadox, cotrimoxazole and trimethoprim (Table 2).

Table 2: **Antimicrobial resistance of *Salmonella* isolates[1] from chickens at Debre Zeit and Addis Ababa, Ethiopia**

	Number of resistant serotypes (%)										
Serotype (Number tested)	AMP[2]	AMOX-CLAV	CAR	CHL	FEN	SPC	STR	SUL	TET	COT	TMP
S. braenderup *(35)*	7 (20)	7 (20)	8 (22.8)	0 (0.0)	0 (0.0)	8 (22.8)	12 (34.2)	12 (34.2)	1 (2.8)	12 (34.2)	12 (34.2)
S. typhimurium[3] (24)	24 (100)	24 (100)	0 (0.0)	24 (100)	22 (91.6)	24 (100)	1 (4.1)	24 (100)	24 (100)	0 (0.0)	0 (0.0)
S. *anatum* (8)	5 (62.5)	5 (62.5)	0 (0.0)	0 (0.0)	0 (0.0)	5 (62.5)	5 (62.5)	5 (62.5)	0 (0.0)	5 (62.5)	5 (62.5)
S. typhimurium (3)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (100)	0 (0.0)	0 (0.0)
S. *kottbus* (5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
S. bovismorbificans (1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)
S. infantis (2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)
S. hadar (2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)
Total	36 (45)	36 (45)	9 (11.2)	24 (30)	22 (27.5)	37 (46.2)	18 (22.5)	41 (51.2)	33 (41.2)	17 (21.2)	17 (21.2)

[1]All *Salmonella* strains tested were susceptible to the antimicrobial effects of amikacin, apramycin, ceftiofur, ceftriaxone, cephalotin, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, neomycin, nitrofurantoin and tobramycin.
[2]AMP = ampicillin; AMOX-CLAV = amoxicillin-clavulanic acid; CAR = carbadox; CHL = chloramphenicol; FEN = florfenicol; GEN = gentamicin; SPC = spectinomycin; STR = streptomycin; SUL = sulfisoxazole; TET = tetracycline; COT = cotrimoxazole; TMP = trimethoprim; [3]variety Copenhagen

All 24 isolates of *S. typhimurium* variety Copenhagen isolates were multiple resistant to up to seven antimicrobials (amox-clav/amp/chl/fen/spc/sul/tet), Table 3. Fifteen of the 35 (42.8%) *S. braenderup* isolates were resistant of which 12 were multiple resistant to up to eight antimicrobials (amox-clav/amp/car/spc/sul/str/cot/tmp) and 62.5% of *S. anatum* isolates showed multiple resistance to amox-clav, amp, spc, str, sul, cot and tmp. A total of 14 (17.5%) *Salmonella* strains (*S. braenderup* (7), *S .typhimurium* variety Copenhagen (6) and *S. infantis* (1) showed intermediate resistance to carbadox (13.7%), streptomycin (3.7%) and florfenicol (2.5%).

Table 3: **Multiple antimicrobial resistance of *Salmonella* isolates by serotype**

	Number of strains				
Serotype	Tested	Resistant (%)	Multiple resistant (%)	Resistance pattern	No. of strains
S. braenderup	35	15 (42.8)	12 (34.3)	amp*/amox-clav/spc/str/sul/cot/tmp	5
				amp/amox-clav/car/spc/str/sul/cot/tmp	1
				car/spc/str/sul/cot/tmp	1
				car/str/sul/tet/cot/tmp	2
				car/spc/str/cot/tmp	2
				str/sul/cot/tmp	1
				Car	3
S. typhimurium var. Copenhagen	24	24 (100)	24 (100)	amp/amox-clav/chl/fen/spc/sul/tet	18
				amp/amox-clav/chl/fen/spc/str/sul	3
				amp/amox-clav/chl/fen/spc/sul	1
				amp/amox-clav/chl/spc/sul/str	2
S. typhimurium	3	2 (66.6)	1 (33.3)	tet/car	1
				tet	1
S. kottbus	5	0 (0.0)	0 (0.0)		
S. anatum	8	5 (62.5)	5 (62.5)	amp/amox-clav/spc/sul/str/cot/tmp	5
S. infantis	2	2 (100)	0 (0.0)	tet	2
S. hadar	2	2 (100)	0 (0.0)	tet	2
S. bovismorbificans	1	1 (100)	0 (0.0)	tet	1
Total	80	51 (63.7)	42 (52.5)		

* For key of abbreviations see Table 2.

Twelve different antimicrobial resistance patterns were displayed among the resistant *Salmonella* strains (Table 4). The common resistance pattern was to amox-clav, amp, chl, fen, spc, sul and tet seen in 18 (35.2%) of the resistant strains followed by resistance to amox-clav, amp, spc, str, sul, cot and tmp observed in 10 (19.6%) of them. Only 13 (23.5%) of the resistant isolates were resistant to five or fewer antimicrobials. The other strains were resistant to seven (38.7%), six (7.5%) or single (11.2%) antimicrobial agents tested. Twenty-nine (36.2%) of the strains were susceptible to all antimicrobial agents tested.

Table 4: **Multiple antimicrobial resistance patterns of *Salmonella* serotypes**

No. of antimicrobial resistance	Antimicrobial resistance pattern	Number of isolates (%)
Zero	-	29 (36.2)
One		9 (11.2)
	tet (6)*	
	car (3)	
Two	car/tet	1 (1.2)
Three	-	0 (0.0)
Four	str/sul/cot/tmp	1 (1.2)
Five	car/spc/str/cot/tmp	2 (2.5)
Six		6 (7.5)
	amp/amox-clav/chl/fen/spc/sul (1)	
	amp/amox-clav/chl/spc/sul/str (2)	
	car/spc/sul/str/cot/tmp (1)	
	car/str/sul/tet/cot/tmp (2)	
Seven		31 (38.7)
	amp/amox-clav/chl/fen/spc/sul/tet (18)	
	amp/amox-clav/spc/sul/str/cot/tmp (10)	
	amp/amox-clav/chl/fen/spc/str/sul (3)	
Eight	amp/amox-clav/car/spc/str/sul/cot/tmp	1 (1.2)

* Number of resistant strains

Discussion

The antimicrobial resistance pattern of the *Salmonella* strains isolated from chickens indicated that a large proportion of the strains were resistant to a variety of drugs tested. Antimicrobial resistant *Salmonella* isolates from animal and human sources have been reported in various countries of Africa including Ethiopia (9, 10, 11, 12, 13, 14, 21, 22), in Europe and America (5, 7, 23, 24, 25, 26).

More than half of the *Salmonella* strains (52.5%) tested exhibited multiple resistance to up to eight antimicrobials. Salmonellae are among those most known to carry plasmids, which encode for drug resistance (R plasmids). This implies that widespread use of antimicrobials in animals or humans may cause an increase in the frequency of occurrence of bacteria resistant to other antimicrobials as the R plasmid may encode resistance to additional antimicrobials (7, 25). Drug resistance occurred at high frequency to sulfisoxazole, amoxacillin-clavulanic acid, ampicillin, spectinomycin, tetracycline, chloramphenicol, florfenicol, streptomycin, cotrimoxazole, carbadox, and trimethoprim. This could be partly associated with the widespread use of a number of these antimicrobials in the mass production of chickens for therapy, prophylaxis or growth promotion. The findings of the present study support results of previous studies on antimicrobial resistance of *Salmonella* isolated in Ethiopia from different food animals, food products and humans (10, 11, 12, 13, 14). Alemayehu et al (14) isolated *Salmonella* strains from apparently healthy slaughtered cattle in Debre-Zeit (Ethiopia) and they were resistant to ampicillin, cotrimoxazole, sulfamethoxazole, tetracycline, streptomycin and cephalothin. *Salmonella* isolates from minced beef samples in Addis Ababa were resistant to commonly used antimicrobials including ampicillin, furazolidone, nitrofurantoin, streptomycin and cotrimoxazole (10). According to Gedebou and Tasew (11) a high level of single and multiple resistant *Salmonella* strains (79%) isolated from blood, stool and other specimens in Addis Ababa were resistant to sulphadiazine (63%) and streptomycin (51.5%). Mache et al (13) reported that among 45 *Salmonella* strains isolated from diarrhoeal samples collected from adult patients in Addis Ababa, 71.1% were resistant to tetracycline, 68.9% to ampicillin, 66.7% to cephalothin, 57.8% to cotrimoxazole, 53.9% to kanamycin and 46.7% to chloramphenicol. The high level of antimicrobial resistance of *Salmonella* isolates in Sub-Saharan Africa (9) is probably due to indiscriminate and widespread uses of the commonly available antimicrobials both in the veterinary and public health practices since, in these countries, people have easy access to various antimicrobials and can purchase them without prescription.

In other parts of the world several studies conducted on *Salmonella* isolated from chickens have shown a high level of resistance to different antimicrobial agents (7, 23, 26). *Salmonella* strains resistant to amoxacillin-clavulanilic acid were also resistant to ampicillin. This might be associated partly with the fact that both antimicrobials are derivatives of 6-aminopenicillanic acid (7, 25). All *Salmonella* strains resistant to chloramphenicol also showed resistance to florfenicol. This may be associated with the fact that both drugs are pharmacologically related. As expected *Salmonella* strains resistant to cotrimoxazole, a potentiated sulphonamide (diaminopyrimidines in combination with sulphonamides) displayed resistance to sulfisoxazole and there exists cross-resistance between sulphanoamides. *Salmonella* isolates were susceptible to apramycin but not to spectinomycin. This might be attributed to the significant structural difference between them and the little cross-resistance among aminocyclitols (7).

In our study, *S. typhimurium* var Copenhagen strains showed resistance to amoxacillin-clavulanic acid, ampicillin, chloramphenicol, florfenicol, spectinomycin, sulfisoxazole, tetracycline, streptomycin, cotrimoxazole and carbadox. All these strains were multiple resistant. Gebre-Yohannes et al (12) reported that *S. typhimurium* strains isolated from hospitalised patients in Addis Ababa were multiple-drug resistant to ampicillin, chloramphenicol, cephalothin, kanamycin, neomycin, streptomycin, tetracycline and trimethoprim. Poppe et al (25) reported *S. typhimurium* DT 104 to be one of the top human and animal *Salmonella* isolates with high multiple resistance pattern (to ampicillin, chloramphenicol, florfenicol, sulfisoxazole, tetracycline, spectinomycin, streptomycin, kanamycin and neomycin), and has become a serious public health problem.

Salmonella braenderup isolates showed multiple-drug resistance to up to eight different antimicrobials. A study undertaken in Canada also indicated that *S. braenderup* strains were multiple resistant to gentamicin, sulfisoxazole and tetracycline (7). In our study, *S. hadar* strains were resistant to tetracycline and *S. infantis* to carbadox and tetracycline. Poppe et al (7) reported that *S. hadar* strains were resistant to ampicillin, carbenicillin, cotrimoxazole, gentamicin, kanamycin, neomycin, nitrofurantoin, sulfisoxazole and tetracycline and *S. infantis* to gentamicin, sulfisoxazole and tetracycline. In the present study all *Salmonella* isolates were susceptible to quinolones, nitrofuran, and cephalosporins. This may be explained by the limited availability and high cost of the above groups of antimicrobials that would reduce their frequent utilization both in the veterinary and public health practices in Ethiopia. Results of the present study indicated the potential importance of chickens as a source of single and multiple antimicrobial-resistant *Salmonella* strains to commonly used antimicrobials including chloramphenicol, ampicillin, amoxacillin, tetracycline and cotrimoxazole for the treatment of *Salmonella* infections and other bacterial pathogens in humans. Further detailed epidemiological and molecular studies are essential on the frequency, sources of acquisition of resistant genes and distribution of antimicrobial-resistant *Salmonella* among food animals, food products and humans in Ethiopia.

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