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ANTIMICROBIAL RESISTANCE IN *SALMONELLA* SEROTYPES ISOLATED FROM SLAUGHTER ANIMALS IN KENYA

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ANTIMICROBIAL RESISTANCE IN *SALMONELLA* SEROTYPES ISOLATED FROM SLAUGHTER ANIMALS IN KENYA

G.M. KIKUVI, J.N. OMBUI, E.S. MITEMA and S. SCHWARZ

ABSTRACT

Objectives: **To isolate *Salmonella* from food animals and characterise the antimicrobial resistance of the isolates.**

Design: **A random sampling of slaughter animals was carried out.**

Setting: **Department of Public Health, Pharmacology and Toxicology, University of Nairobi, Kenya and Institute for Animal Breeding, Neustadt-Mariensee, Germany.**

Subjects: **Two hundred and eighty five samples, including faecal samples and carcass, cloacal and pharyngeal swab samples were analysed.**

Results: **Sixteen (5.6%) of 285 samples were positive for *Salmonella*. The prevalence of *Salmonella* on pig carcasses (19%) was higher than in faeces (8.6%). Three *Salmonella enterica* sub-species *enterica* serovars, namely Saintpaul (*S. Saintpaul*), Braenderup (*S. Braenderup*), and Heidelberg (*S. Heidelberg*), were identified, with *S. Saintpaul* being the predominant serovar. Antimicrobial resistance was found in 35.7% of all the isolates. The *S. Heidelberg* isolates were susceptible to all the antimicrobial agents tested. Multidrug resistance was found in 7.1% of the resistant *Salmonella* isolates. Plasmids were only detected in *S. Heidelberg*. Ampicillin resistance was based on expression of a bla_{TEM} gene, while chloramphenicol, streptomycin, and tetracycline resistances were encoded by the genes *catAl, strA,* and *tet(A),* respectively.**

Conclusion: **Pigs may serve as reservoirs of antimicrobial resistant *Salmonella* and slaughterhouse cross-contamination of pork may be a food safety risk. We recommended that slaughterhouse hygiene be improved to minimise contamination of pig carcasses.**

INTRODUCTION

Salmonellosis is considered as one of the most widespread foodborne zoonoses in industrialised as well as developing countries (1). In Kenya, non-typhoid *Salmonella* species (NTS) are common causes of bacteraemia among immunocompromised individuals, infants and newborns (2). Farm animals are the major reservoir for NTS in industrialised countries and large outbreaks of *Salmonella* infection have been associated with food-borne transmission including that from contaminated poultry and poultry products, meat and milk and other dairy products (3). In most of these countries, *Salmonella enterica* serovar Enteritidis (*S. Enteriditis*) is transmitted through consumption of foods containing raw or incompletely cooked eggs and improperly cooked products containing eggs (3). Strains of the multidrug resistant (MDR) *S.* Typhimurium phage type (DT) 104, which have

been responsible for epidemics particularly in Europe, the USA and Canada, represent reservoirs in cattle and is transmitted mainly through consumption of contaminated meat, milk and milk products (3).

MDR *Salmonella* strains have steadily increased, probably due to continuous antibiotic pressure in human and veterinary medicine (4). Resistance, particularly to the commonly available antibiotics, poses a major health concern, as alternative therapeutic choices are either unavailable or too expensive for most patients to afford (2). The genetic characterisation of antimicrobial resistance genes as well as their location and diversity is important in identifying factors involved in resistance (5).

To date, few studies have analysed the levels of resistance to antimicrobial agents in *Salmonella* serotype isolated from food animals in Kenya (2). Moreover, the mechanisms of resistance underlying the resistance phenotypes remain unknown. The present study was therefore carried out to estimate the prevalence of faecal carriage of *Salmonella* in healthy pigs, cattle and chicken at slaughter and of pig and cattle carcass contamination with *Salmonella.* Additionally, the antimicrobial susceptibility profiles and the genotypes of the resistant isolates were investigated.

MATERIALS AND METHODS

Sources of samples: Fresh faecal and carcass swab samples were collected from cattle at the Dagoretti slaughterhouse complex and pigs at the Ndumbuini slaughterhouse in Nairobi during the period of June to December 2001. Cattle slaughtered at Dagoretti slaughterhouse complex originate from all parts of the country (6). Pigs are sent to the abattoir from farms in Kiambu and Nairobi districts which are among the main pig farming districts in Kenya. Animals were selected at random and about five grams of faeces aseptically removed from the large bowel after evisceration at the slaughtering line. The carcasses were sampled using sterile cotton wool swabs. Cloacal and pharyngeal swab samples were collected from indigenous free range chickens during slaughter at Burma and Kariokor markets in Nairobi. The samples were immediately placed into Stuart's transport medium (Oxoid, Basingstoke, United Kingdom), maintained on ice while being transported to the laboratory and processed on the same day.

Isolation and identification of Salmonella: Faecal and swab (carcass, cloacal and pharyngeal) samples were pre-enriched in peptone water (Oxoid, Basingstoke, England) and incubated for eighteen hours at 37°C. The pre-enrichment broth was transferred aseptically into tetrathionate broth (Oxoid) and incubated overnight at 37°C. This was followed by streaking aliquots from the enrichment broth onto desoxycholate citrate agar (DCA, Oxoid) plates, and incubated at 37°C for 18 hours. The DCA plates were examined for the presence of *Salmonella*-like colonies (colourless with black centres). Single colonies were tested for the appropriate reactions on Triple Sugar Iron agar (Oxoid) and urea agar (Oxoid) and their identification was confirmed biochemically using analytical profile index (API) 20E strips (bioMérieux, Marcy-I'Etoile, France).

The *Salmonella* isolates were serotyped and phage typed at the Robert Koch Institute, National Reference Centre for *Salmonella* and other Enteric Pathogens in Wernigerode, Germany. Serotyping was done based on 0- and H- group antigens according to the Kauffmann-White Scheme (7) using slide and microtitre agglutination. To phage type the isolates, routine test dilutions of each of the typing phages were applied to nutrient agar (Difco, Detroit, USA) plates with a lawn of the respective bacterial strain using a multipoint inoculator. These were incubated overnight at 37 °C until the phage lysis could be read. The lytic patterns were interpreted according to the Anderson phage typing scheme (8).

Minimal Inhibition Concentration (MIC) determination: MICs for ampicillin, tetracycline, streptomycin, kanamycin, gentamicin, sulphamethoxazole/trimethoprim (19:1), chloramphenicol, and nalidixic acid were determined using the standard broth dilution method with Mueller-Hinton (MH, Oxoid) medium according to the methods described by the Clinical and Laboratory Standard Institute (CLSI) (9). *E. coli* (ATCC 25922), was tested in parallel to control for growth of bacteria and potency of antibiotics. MICs were interpreted according to breakpoints given by CLSI (9) except for streptomycin. The breakpoints used for streptomycin were those recommended by the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (10).

Plasmid analysis and PCR assays: Plasmid DNA was prepared by alkaline lysis as previously described (11). PCR assays were used for the detection of the ampicillin resistance genes bla_{TEM} and bla_{PSE}, the tetracycline resistance genes *tet(A)*, *tet(B)*, *tet(C)*, and *tet(H)*, the chloramphenicol resistance genes *catA1*, *catA3*, and *cmlA*, and the streptomycin resistance genes *strA* and *aadA1*. PCR protocols and template preparation were as earlier described (11-13). The primers used are shown in Table 1. The plasmids and PCR products were detected by electrophoresis in 0.8% and 1.5% agarose gels, respectively. The plasmids of *E. coli V5 17* (molecular weight range 2.1 to 54 kb), the *Klebsiella pneumoniae* plasmid *R55* (150 kb), and the 90 kb plasmid of *Salmonella* Typhimurium LT2 (13) served as the molecular size standards for the plasmids while a 1 kb ladder (Gibco BRL, Eggestein, Germany) was used as a marker for estimation of the sizes of the PCR products. The migration distances of DNA bands were measured directly from photographs of the gels. Plasmid sizes were estimated by standard polynomial curves generated with the logarithm of the relative migration of DNA on the X axis and the logarithm of the molecular size of standard plasmids on the Y axis using the Microsoft Excel program (14).

RESULTS

Isolation rate: A total of 285 samples comprising of 110 samples from cattle (carcass, n = 55 and faeces, n = 55), 116 from pigs (carcass, n = 58 and faeces, n = 58) and 59 from chickens (pharyngeal swab, n = 15 and cloacal swab, n = 44) were obtained and analysed. Sixteen (5.6%) of 285 samples were positive for *Salmonella*. The isolation rate for *Salmonella* was 13.8% for pigs. No *Salmonella* was isolated from cattle and chicken. Among the pigs, *Salmonella* was isolated from 11 (19.0%) of carcass and five (8.6%) of faecal

Table 1

Sequences of oligonucleotides used as primers and annealing temperatures for the detection of antimicrobial resistance determinants

Target gene / Primer	Oligonucleotide sequence (5′ → 3′)	Amplicon size (bp)	Annealing temp (°C)	Reference sequence (Genbank Accession number(s))
aadA1	f:- GTGGATGGCGGCCTGAAGCC	527	56	*Escherichia coli*
	r:- ATTGCCCAGTCGGCAGCG			(M10241, X02340)
catA1	f:- GGCATTTCAGTCAGTTG	551	50	*Tn9E. coli*
	r:- CATTAAGCATTCTGCCG			(V00622)
catA3	f:- ACCATGTGGTTTTAGCTTAACA	473	56	Uncultured eubacterium
	r:- GCAATAACAGTCTATCCCCTTC			(AJ271 879)
cmlA	f:- CCGCCACGGTGTTGTTGTTATC	698	40	*Pseudomonas aeruginosa*
	r:- CACCTTGCCTGCCCATCATTAG			(M64556)
bla_{TEM}	f:- CCGTGTCGCCCTTATTCCC	780	51	*Enterobacter cloacae*
	r:- GCCTGACTCCCCGTCGTGT			AY302260
bla_{PSE}	f:- CGCTTCCCGTTAACAAGTAC	465	58	*Salmonella Typhimurium*
	r:- CTGGTTCATTTCAGATAGCG			AF1 53200
strA	f:- GACTGGTTGCCTGTCAGAGG	646	64	Plasmid RSF 1010
	r:- CAGTTGTCTTCGGCGTTAGCA			M28829
tet(A)	f:- GTAATTCTGAGCACTGT	954	45	RPl from *E. coli*
	r:- CCTGGACAACATTGCTT			(X00006)
tet(B)	f:- ACGTTACTCGATGCCAT	1170	48	Tn*10* from *Shigella flexneri*
	r:- AGCACTTGTCTCCTGTT			(J01830)
tet(C)	f:- AACAATGCGCTCATCGT	1138	50	pSC101 from *Salmonella*
	r:- GGAGGCAGACAAGGTAT			Typhimurium (X01654)
tet(H)	f:- ATACTGCTGACACCGT	1076	50	pVM111 from *Pasteurella*
	r:- TCCCAATAAGCGACGCT			*multocida* (S52437)

f, forward primer; r, reverse primer

samples. *Salmonella* was isolated from faeces and carcass swabs of the same animal only in two cases. *Salmonella* was found on the carcass but not in the faeces in nine pigs as compared to only one in which *Salmonella* was isolated from faecal but not from the carcass swab sample of the same animal.

Distribution of Salmonella serotypes: Only 14 of the 16 *Salmonella* strains were able to grow from the lyophilised cultures and these strains were therefore sero- and phage typed. Three serovars were identified with *S. Saintpaul* being the predominant serovar (64.3%) followed by *S. Heidelberg* (21.4%) and *S. Braenderup* (14.3%). Only the *S. Heidelberg* isolates were phage typable and they belonged to phage type 02. The highest proportion of *S. Saintpaul* (77.8%) was identified from carcass swabs as compared to faecal samples (22.2%). Only in one pig was *S. Saintpaul* identified from both faecal and carcass swab samples. Both *S. Braenderup* isolates were from the same animal, of which one was obtained from faeces while the other came from carcass swab sample. All three *S. Heidelberg* isolates were obtained from carcass swabs from different animals. Not more than one serovar was isolated from the same animal.

Antimicrobial susceptibility: All three *S. Heidelberg* isolates were fully susceptible to all eight antimicrobials tested. In contrast, one *S. Braenderup* isolate obtained from a faecal sample was multiple resistant to ampicillin, tetracycline, and streptomycin while the other was fully susceptible to all antimicrobials tested. The three *S. Saintpaul* isolates were solely resistant to either ampicillin, chloramphenicol, or streptomycin. Resistance to ampicillin and chloramphenicol were only observed in the faecal isolates while resistances to streptomycin and tetracycline were observed in both faecal and carcass isolates. All isolates were fully susceptible to gentamicin, kanamycin, sulphamethoxazole/trimethoprim and nalidixic acid.

Plasmid analysis and antimicrobial resistance genes: Eleven (78.6%) of the 14 *Salmonella* strains investigated were plasmid-free (Table 2). Plasmids were only detected in the three *S. Heidelberg* isolates. Two of the isolates harboured two large plasmids of approximately 40 and 80 kb in size while the third isolate carried these two plasmids in addition to a third plasmid of approximately 54 kb. The PCR results were consistent with the antimicrobial susceptibility phenotypes. The bla_{TEM} gene was detected in each of the ampicillin-resistant *Salmonella* isolates while the *strA* gene was detected in each of the streptomycin-resistant isolates. The *catAl* and *tet(A)* genes were detected in the chloramphenicol and tetracycline resistant isolates, respectively.

Table 2

Serotype, phage type and antimicrobial resistance profiles of Salmonella isolates from pig faecal and carcass samples

Pig No.	Source	Serotype	Phage type	Resistance pattern	Resistance gene(s) detected	Approximate plasmid size (kb)
1	Carcass	S. Heidelberg	HB PT 02	Susceptible	ND	40, 80
2	Carcass	S. Heidelberg	HB PT 02	Susceptible	ND	40, 80
3	Carcass	S. Heidelberg	HB PT 02	Susceptible	ND	40, 54, 80
4	Carcass	S. Braenderup	NT	$Amp^RSm^RTet^R$	bla_{TEM}, *strA, tet(A)*	None
	Faeces	S. Braenderup	NT	Susceptible	ND	None
5	Carcass	S. Saintpaul	NT	Susceptible	ND	None
6	Carcass	S. Saintpaul	NT	Susceptible	ND	None
7	Faeces	S. Saintpaul	NT	Amp	bla_{TEM}	None
8	Carcass	S. Saintpaul	NT	Susceptible	ND	None
9	Carcass	S. Saintpaul	NT	Tet^I	*tet(A)*	None
	Faeces	S. Saintpaul	NT	Cm^R	*catA1*	None
10	Carcass	S. Saintpaul	NT	Sm^R	*strA*	None
11	Carcass	S. Saintpaul	NT	Susceptible	ND	None
12	Carcass	S. Saintpaul	NT	Susceptible	ND	None

Amp = ampicillin; Cm = chloramphenicol; Sm = streptomycin; Tet = tetracycline; R = resistant; I = intermediately resistant; ND = Not Done; NT = Not Typable

DISCUSSION

The present study detected *Salmonella* spp. in 13.8% of the pigs, but not in the cattle and chicken samples analysed. In agreement with our findings, Bywater *et al (15)* did not find *Salmonella* in caecal content samples from chickens in Sweden and in faeces from cattle in Germany, France and Italy. In Kenya, Kariuki *et al* (2) reported the failure to isolate *Salmonella* from goats' faeces and camel carcass swabs and a low frequency (1.5%) of *Salmonella* from beef carcass swab and abattoir effluent samples. The fact that *Salmonella* infection in pig herds is subclinical while clinical disease is more common in the other animal species and that most often pigs are clinically inapparent carriers of *Salmonella* (16) may be the reason why *Salmonella* was found in apparently healthy pigs and not in the other animal species.

The prevalence of *Salmonella* on pig carcasses (19%) was higher than in faeces (8.6%). This observation is in line with a previous study in Belgium (16) that reported finding higher frequency of *Salmonella* on pig carcasses than in their faeces. A higher prevalence of *Salmonella* on carcasses than in faeces suggests the presence of severe cross-contamination during the slaughtering process and points towards poor hygienic conditions during subsequent dressing operations (17). In nine pigs, *Salmonella* was found on the carcass but not in the faeces. The fact that 78.6% of the pigs in this study had culture-positive carcass swab samples and culture negative faeces suggest slaughterhouse cross-contamination may be a real food safety risk. Cross-contamination of a pig carcass could result from adjacent positive carcasses on the dressing line (18), contaminated abattoir equipments, floors or from *Salmonella*-shedding slaughterhouse personnel (1). The initial source of pork carcass contamination is a carrier pig (16), therefore monitoring of *Salmonella* infection in pig herds is essential in order to reduce *Salmonella* slaughter pigs. In 18.2% of the positive carcasses, the same *Salmonella* serovar was isolated from the carcass and faeces from the same pig. This observation clearly indicates that such pathogens can and do transfer from faeces onto carcass meat, posing direct threats to consumer health.

S. Saintpaul was the most frequently isolated serovar followed by *S. Heidelberg* and *S. Braenderup*. This observation was contrary to a previous study in Kenya (2), which found *S. Agona* to be the main serotype in pigs. This difference may have probably been due to the differences in the period of sampling, slaughter-houses, the origin and number of infected pigs that were delivered to the slaughterhouse during the sampling period as demonstrated by Botteldoorn *et al* (16). *S. Heidelberg* accounted for approximately one fifth of the serotypes identified. To our knowledge this is the first observation of *S. Heidelberg* in food animals in Kenya.

The majority (64.3%) of the *Salmonella* isolates in this study were fully susceptible to all the eight antimicrobials tested with all isolates being susceptible to gentamicin, kanamycin, sulphamethoxazole/trimethoprim or nalidixic acid. Similar observations were made in analyses of non-typhoidal *Salmonella* serovars from food animals in Kenya (2). Only four *S. Saintpaul* and one *S. Braenderup* isolates showed resistance or exhibited intermediate resistance to at least one of the antimicrobial agents analysed. The *S. Braenderup* isolate exhibited multidrug resistance to ampicillin, tetracycline and streptomycin. Three out of the four *S. Saintpaul* isolates showing resistance were only resistant to chloramphenicol, streptomycin or ampicillin whereas the fourth strain was intermediately resistant to tetracycline. Due to the relatively low cost and ready availability of ampicillin, tetracycline and streptomycin, these antimicrobial agents are widely used by farmers for therapeutic and prophylactic applications (2). In Kenya, as in the European Union or the USA, chloramphenicol is not approved for use in food animals and its fluorinated analog, florfenicol has not been in use. Chloramphenicol resistance may be acquired via horizontal transmission of genes from other sources, such as water contaminated with human sewage or due to illegal use of chloramphenicol (19).

Resistance was encoded by genes that are widespread in other *Enterobacteiaceae* and known to be commonly located on transposons, which are mobile DNA elements that play an important role in transmission and dissemination of antimicrobial resistance determinants.

Each of the two ampicillin-resistant isolates in this study contained the bla_{TEM} gene. The bla_{TEM} gene is usually part of transposon Tn3 and has been found previously among *Salmonella* isolates (20). The *strA* gene was detected in each of the two streptomycin-resistant isolates. The gene *strA*, which may be part

of transposon Tn5393, has been found frequently among streptomycin-resistant isolates, such as *Salmonella* Typhimurium (6,11). Chloramphenicol resistance was based on the expression of *catAl* gene. The Tn9-associated *catAl* gene has been detected previously in *Salmonella* isolates, but is also widespread among other Gram-negative bacteria (11). Tetracycline resistance was mediated by the *tet(A)* gene. The *tet(A)* gene is located frequently on transposons such as Tnl *721*, and the gene has been found widespread among gram-negative bacteria including *salmonellae* (5).

The present study revealed occurrence of resistant *Salmonella* in slaughter pigs in Kenya and underscores the importance of slaughter pigs as sources of mono and multidrug resistance to commonly available antimicrobials. The presence of *Salmonella* in pigs at slaughter and the consequent cross contamination of edible carcass tissue presents a significant public health hazard. This calls for adequate control measures to decrease *Salmonella* infection during production, improve slaughterhouse hygiene and decontaminate carcasses after slaughter.

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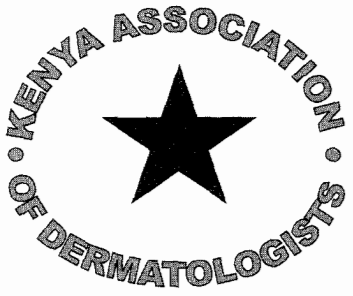
ANNOUNCEMENT



KENYA ASSOCIATION OF DERMATOLOGISTS

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