Brief Original Article

Serotypes and antimicrobial resistance profiles of *Salmonella* isolates from pigs at slaughter in Kenya

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Abstract

Background: Salmonellosis is considered one of the most widespread food-borne zoonoses in industrialized as well as developing countries. The presence of *Salmonella* in food animals at slaughter and the consequent cross-contamination of edible carcass tissues present a significant food safety hazard.

Methodology: Samples were collected from randomly selected pigs at the Ndumbuini abattoir in Nairobi. Isolates were confirmed to be *Salmonella* by biochemical tests and characterised by serotyping, phage typing and plasmid analysis. Minimum inhibitory concentrations (MICs) of eight antimicrobials were determined and the resistant isolates were screened for resistance genes by PCR.

Results: Sixteen (13.8%) of 116 samples were positive for *Salmonella*. Three *Salmonella enterica* subsp. *enterica* serovars, namely Saintpaul, Braenderup, and Heidelberg were identified, *S.* Saintpaul being predominant. Antimicrobial resistance was found in 35.7% of the isolates. The *S.* Heidelberg isolates were susceptible to all the antimicrobials tested. Multidrug resistance was found in 7.1% of the *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 *catA1*, *strA*, and *tet*(A), respectively.

Conclusions: Pigs may serve as reservoirs of antimicrobial resistant *Salmonella* and slaughterhouse cross-contamination of pork may be a food safety risk.

Key words: serotypes; antimicrobial resistance; *Salmonella*; pigs; Kenya

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Introduction

Salmonellosis is one of the most widespread food-borne zoonoses in industrialized as well as developing countries [1]. In Kenya, non-typhoidal *Salmonella* species (NTS) are common causes of bacteraemia among immunocompromised individuals, infants and newborns [2]. Farm animals are the major reservoir for NTS in industrialized 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]. Strains of the multidrug resistant (MDR) *S.* Typhimurium phage type (DT) 104, which have been responsible for epidemics particularly in Europe, the United States of America and Canada, represent reservoirs in cattle and are 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 to be affordable for most patients [2]. The genetic characterization 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 analyzed the levels of resistance to antimicrobial agents in *Salmonella* serotypes isolated from pigs in Kenya [2]. Moreover, the mechanisms of resistance underlying the resistant phenotypes remain unknown. The present study was therefore conducted to estimate the prevalence of faecal carriage of *Salmonella* in healthy pigs at slaughter and of pig 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 pigs at the Ndumbuini slaughterhouse in Nairobi. Pigs are sent to the abattoir from farms in Kiambu and Nairobi districts, which are among the main pig farming districts in Kenya. Pigs were selected at random and approximately 5 g of faeces was aseptically removed from the large bowel after evisceration at the slaughtering line. The carcasses were sampled using sterile cotton wool swabs. A surface approximately 100 cm^2 around the hind limb (medial) and abdomen (lateral) was swabbed by wiping the cotton swabs on each sampling site five times in both vertical and horizontal directions. 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 samples were pre-enriched in buffered peptone water (Oxoid, Basingstoke, England) and incubated for 18 hours at 37^{o}C. A five millilitre aliquot of the pre-enrichment broth was transferred aseptically into tetrathionate broth (Oxoid) and incubated overnight at 37^{o}C. This step was followed by streaking aliquots from the enrichment broth onto deoxycholate citrate agar (DCA, Oxoid) plates, and incubating them at 37^{o}C for 18 hours. From DCA plates, *Salmonella*-like colonies were tested on triple sugar iron agar (Oxoid) and urea agar (Oxoid) and 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, according to the Kauffmann-White Scheme [6]. 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. Plates were incubated at 37^{o}C until the phage lysis result could be read and interpreted per the Anderson phage typing scheme [7].

Minimum inhibitory 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 Laboratory and Standards Institute (CLSI) [8]. *Escherichia coli* ATCC 25922 (*E. coli*) was tested in parallel as a control. MICs were determined according to CLSI [9] except for streptomycin, for which the method of the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme was followed [10].

Plasmid analysis and PCR assays

PCR assays were used for the detection of genes for ampicillin resistance (bla_{TEM} and bla_{PSE}), tetracycline resistance (*tet*(A), *tet*(B), *tet*(C), and *tet*(H)), chloramphenicol resistance (*catA1*, *catA3*, and *cmlA*), and streptomycin resistance (*strA* and *aadA1*) as described previously [11,12,13] using specific primers (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* V517 (2.1 to 54 kb), *Klebsiella pneumoniae* (R55, 150 kb), and *Salmonella* Typhimurium LT2 (90 kb) served as molecular size standards for the plasmid preps [13], with a 1 kb ladder (Gibco BRL, Eggestein, Germany) for the PCR products. The migration distances of DNA bands were measured directly from photographs of the gels to determine plasmid sizes as per standard methods [14].

Results

Isolation rate

A total of 116 samples from pigs (carcass, n = 58 and faeces, n = 58) were obtained and analysed. Sixteen (13.8%) of 116 samples were positive for *Salmonella*. *Salmonella* was isolated from 11 (19.0%) of the carcass swabs and five (8.6%) of the faecal 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 of nine pigs as compared to only one in which *Salmonella* was isolated from the faecal but not the carcass swab of the same animal.

Distribution of Salmonella *serotypes*

Only 14 of the 16 *Salmonella* strains were able to grow from the lyophilized cultures and these strains were therefore sero- and phage typed. Three serovars were identified, with *S.* Saintpaul being predominant (9 strains) followed by *S.* Heidelberg (3 strains) and *S.* Braenderup (2 strains) (Table 2). Only the *S.* Heidelberg isolates were phage typable and they belonged to phage type 02. The highest proportion of *S.* Saintpaul (7/9, 77.8%) was

identified from carcass swabs as compared to faecal samples (2/9, 22.2%). On only one occasion *S.* Saintpaul was identified from the faecal and carcass

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 (^{o}C)	Reference sequence (Genbank Accession number(s))
aadA1	f:- GTGGATGGCGGCCTGAAGCC	527	56	*Escherichia coli*
	r:- ATTGCCCAGTCGGCAGCG			(M10241, X02340)
catA1	f:- GGCATTTCAGTCAGTTG	551	50	Tn*9 E. coli*
	r:- CATTAAGCATTCTGCCG			(V00622)
catA3	f:- ACCATGTGGTTTTAGCTTAACA	473	56	Uncultured eubacterium
	r:- GCAATAACAGTCTATCCCCTTC			(AJ271879)
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			AF153200
strA	f:- GACTGGTTGCCTGTCAGAGG	646	64	Plasmid RSF 1010
	r:- CAGTTGTCTTCGGCGTTAGCA			M28829
tet(A)	f:- GTAATTCTGAGCACTGT	954	45	RP1 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

Table 2. Serotype, source and antimicrobial resistance profiles of *Salmonella* isolates from pig faecal and carcass samples.

Pig Number	*Salmonella* serovar	Source	Resistance pattern	Resistance gene(s) detected	Approximate plasmid size (kb)
1	*S.* Heidelberg	carcass	susceptible	ND	40, 80
2	*S.* Heidelberg	carcass	susceptible	ND	40, 80
3	*S.* Heidelberg	carcass	susceptible	ND	40, 54, 80
4	*S.* Braenderup	faeces	$Amp^{R}Sm^{R}Tet^{R}$	bla_{TEM}, *strA*, *tet*(A)	none
	S. Braenderup	carcass	susceptible	ND	none
5	*S.* Saintpaul	carcass	susceptible	ND	none
6	*S.* Saintpaul	carcass	susceptible	ND	none
7	*S.* Saintpaul	faeces	Amp^{R}	bla_{TEM}	none
8	*S.* Saintpaul	carcass	susceptible	ND	none
9	*S.* Saintpaul	carcass	Tet^{I}	*tet*(A)	none
	S. Saintpaul	faeces	Cm^{R}	*catA1*	none
10	*S.* Saintpaul	carcass	Sm^{R}	*strA*	none
11	*S.* Saintpaul	carcass	susceptible	ND	none
12	*S.* Saintpaul	carcass	susceptible	ND	none

Amp, ampicillin; Cm, chloramphenicol; Sm, streptomycin; Tet, tetracycline; ^{R}, resistant;
^{I}, intermediately resistant; ND, not done. N.B. Two faeces samples could not be grown from lyophilized culture and are therefore not shown in this table.

swab samples obtained from the same animal. Both *S.* Braenderup isolates were from the same animal. All three *S.* Heidelberg isolates were obtained from carcass swabs. No more than one serovar was isolated from the same animal.

Antimicrobial susceptibility

All three *S.* Heidelberg and five of the nine *S.* Saintpaul isolates were susceptible to all eight antimicrobials tested. In contrast, one *S.* Braenderup isolate, from a faecal sample, was resistant to ampicillin, tetracycline, and streptomycin while the other was susceptible to all antimicrobials tested. Three out of the four *S.* Saintpaul isolates showing resistance were fully resistant to one of chloramphenicol, streptomycin or ampicillin, whereas the fourth strain was intermediately resistant to tetracycline. Resistance to ampicillin and chloramphenicol was only observed in the faecal isolates while resistance to streptomycin or tetracycline was observed in both faecal and carcass isolates. All isolates were susceptible to gentamicin, kanamycin, sulphamethoxazole/trimethoprim and nalidixic acid.

Plasmid analysis and antimicrobial resistance genotyping

All the isolates were plasmid free except for the three *S.* Heidelberg isolates. All three isolates harboured two large plasmids (~40 and 80 kb in size) while one also possessed a third plasmid (~54 kb). The PCR results were consistent with the antimicrobial susceptibility phenotypes. The $bla_{\mathbf{TEM}}$ 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 *catA1* and *tet*(A) genes were detected in the chloramphenicol and tetracycline resistant isolates, respectively.

Discussion

The present study detected a moderately high prevalence (12/58 pigs; 20.7%) of *Salmonella* spp. in pigs. In fact, *Salmonella* infection persists in pig herds in the subclinical stage and pigs are often clinically asymptomatic carriers of *Salmonella* [15], which might be attributable to the isolation of *Salmonella* from apparently healthy pigs. The prevalence of *Salmonella* on pig carcasses (19%) was higher than in faeces (8.6%). A higher prevalence of apparently different *Salmonella* strains on carcasses than in faeces suggests the presence of environmental contamination during the slaughtering process. About 79% of the pigs in this study had culture-positive carcass swab samples and culture-negative faeces (data not shown). The major contamination sources of pig carcasses have been shown to be either pig-(faeces, pharynx and stomach) or environment-related (contact surfaces and handling by *Salmonella*-carrying slaughterhouse personnel) [1]. Serological monitoring of pig herds is essential for the identification of *Salmonella* in the pork production chain [16]. In nine pigs, *Salmonella* was found on the carcass but not in the faeces. This may have been a result of cross-contamination from adjacent positive carcasses on the dressing line [17] that may or may not have been part of the sample carcasses. Alternatively, contaminated abattoir equipment or floors, or *Salmonella*-shedding slaughterhouse personnel [2] may have been the source. In 18.2% (2/11) of the positive carcasses, the same *Salmonella* serovar was isolated from the carcass and faeces of the same pig. Poor evisceration technique was probably the reason for this contamination. Paying attention to the technique can reduce faecal contamination of the carcasses although transmission of *Salmonella* from the environment could also occur, via the slaughterhouse personnel or the equipment [18].

S. Saintpaul was the most frequently isolated serovar followed by *S.* Heidelberg and *S.* Braenderup. This observation is contrary to that of a previous study in Kenya [2] which found *S.* Agona to be the main serotype in pigs. As demonstrated by Botteldoorn *et al.*, this disparity might be due to differences in the period of sampling, the various slaughterhouses, or the origin and number of infected pigs [16]. The observation that *S.* Heidelberg accounted for approximately one fifth of the total isolates appears to be the first report of this strain in food animals in Kenya.

The majority (9/14, 64.3%) of the *Salmonella* isolates in this study were susceptible to all eight antimicrobials tested with all the isolates susceptible to gentamicin, kanamycin, sulphamethoxazole/trimethoprim and nalidixic acid. Similar observations were made in the analysis of NTS serovars from chicken, pigs, and beef cattle in Kenya [2]. 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]. Although, in Kenya, neither chloramphenicol nor its fluorinated analog,

florfenicol is approved for use in food animals, chloramphenicol resistance was still detected in one faecal isolate. This observation might be due to the acquisition of resistance genes from other sources, such as bacteria in water contaminated with human sewage or due to illegal use of chloramphenicol. Chloramphenicol resistance genes have often been associated with either multiresistance conjugative transposons or plasmid-borne multiresistance integrons which are mobile genetic elements detected in a variety of enterobacterial species [19].

Plasmids were detected only in the *S.* Heidelberg isolates. Each of the two ampicillin-resistant isolates in this study contained the bla_{TEM} gene. The bla_{TEM} gene is usually part of transposon Tn*3* and has been found previously among *Salmonella* isolates [10]. The *strA* gene was detected in each of the two streptomycin-resistant isolates. The gene *strA*, which may be part of transposon Tn*5393*, has been found frequently among streptomycin-resistant isolates, such as *Salmonella* Typhimurium [6,11]. Chloramphenicol resistance was based on expression of the *catA1* gene. The Tn*9*-associated *catA1* 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 Tn*1721*, and the gene has been found to be widespread among Gram negative bacteria including salmonellae [5,20].

The present study revealed the occurrence of antimicrobial resistant *Salmonella* in pigs at slaughter and on pork carcasses in Kenya. This observation indicates the potential importance of pigs as a source of single and multiple antimicrobial-resistant *Salmonella* isolates to commonly used antimicrobials including ampicillin, chloramphenicol, streptomycin and tetracycline. This calls for measures to control the occurrence of *Salmonella* in pigs and pork carcasses. Resistance was encoded by genes that are widespread in other *Enterobacteriaceae* and that are known to be commonly located on transposons, mobile genetic elements that play an important role in the transmission and dissemination of antimicrobial resistance determinants.

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