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REGULAR ARTICLES

Prevalence and detection of antibiotic-resistant determinant in *Salmonella* isolated from food-producing animals

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Abstract *Salmonella* spp. infections are considered as the most common food-borne disease globally. The contamination of food products with *Salmonella* has given rise to severe health and economic challenges. This study assessed the prevalence of *Salmonella* in the faeces of cows and goats in the Eastern Cape province of South Africa, their antibiotic resistance patterns as well as antibiotic-resistant gene determinant. Antibiotic disc was used for antibiogram profiles while polymerase chain reaction was employed for the detection of antibiotic-resistant genes. A total of 150 *Salmonella* were isolated from the faecal samples. Eighty two (55 %) isolates were recovered from cow faeces while 68 (45 %) were isolated from goat faeces. All *Salmonella* isolates were sensitive to ciprofloxacin (100 %) while 95 % were sensitive to ofloxacin. Also, a high sensitivity of 93 and 89 % was observed against nalidixic acid and ofloxacin, respectively. *Salmonella* isolates demonstrated moderate sensitivity against cephalothin (70 %), chloramphenicol (75 %) and minocycline (68 %) while 49 % were resistant to tetracycline and erythromycin. The prevalence of the antibiotic-resistant genes in *Salmonella* isolates were detected as follows: integron conserved segment 28 % (42/150), bla_{TEM} gene 19.3 % (29/150), bla_{pse1} 7.3 % (11/150) and $bla_{\mathrm{amp}}C$ 4.7 % (7/150). The results obtained in the study imply that cow and goat faeces could be potential reservoirs of *Salmonella* and could possibly cause infections as a result of contamination of food products. There is a need for a surveillance system to track resistance patterns of *Salmonella* circulating in South Africa.

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Introduction

Salmonella is a well-known zoonotic pathogen causing diarrhoea, pyrexia and septicaemia in animals and humans. A wide range of clinical signs of *Salmonella* infections include acute septicaemia, abortion, arthritis and respiratory disease (Abouzeed et al. 2000; Akoachere et al. 2009). *Salmonella* could also be found present in animal feedstuffs, leading to subclinical gastrointestinal carriage or infectious disease in animals. A characteristic feature of this bacterium is its wide host range, which comprises most animal species including mammals, birds and cold-blooded animals (Wilfred et al. 2000; Curtello et al. 2013). Generally, animals may be infected without signs of clinical illness. As such, animals could be vital in respect to the spread of infection between flocks and herds and as sources of food contamination and human infection (Abouzeed et al. 2000). In most cases, salmonellosis is caused by contaminated food products, particularly those of animal origin such as poultry, eggs, beef and pork (Bouchrif et al. 2009).

Salmonella infections (salmonellosis) are considered as the most common food-borne disease and have been recognized globally in both developed and developing countries, resulting to high morbidity and economic costs (Antoine et al. 2008; Ammari et al. 2009). The contamination of food products with *Salmonella* has given rise to severe health and economic challenges, which have enthused various studies designed to investigate the transmission routes of these organisms in different farm animals and the environment (Winfield and Groisman 2003; Ammari et al. 2009).

Antibiotics are used in food-producing animals for prophylaxis, to treat diseases or to aid in the growth and development

of farm animals. However, animals could be a source of food-borne-resistant bacteria. Several studies carried out have documented the direct transfer of antibiotic-resistant bacteria from animals to humans (Zare et al. 2014). Antimicrobial-resistant *Salmonella* in food animals could acquire their resistance in animals, which might lead to human infections with food-borne-resistant bacteria through the food chain (Carattoli 2003; Stevens et al. 2006; Glenn et al. 2011). Also, they could perpetuate the spread of antibiotic-resistant genes to human by horizontal gene transfer through such mobile genetic elements as plasmids and integrons (Igbinosa et al. 2013). Class 1 integrons are one of the single biggest contributors to multidrug-resistant microbial infections, carrying resistance to many antibiotics in different pathogens globally (Stokes et al. 2006).

The digestive tract of humans and animals persists as the major reservoir of the contamination of food, and animals play a role in the distribution of salmonellosis (Nisbet and Ziprin 2001; Bouchrif et al. 2009). In meat production, the leading source of contamination of carcasses by *Salmonella* is the evisceration process; faecal bacteria may accidentally contaminate the meat and meat products (Stevens et al. 2006; Vandendriessche 2008; Bouchrif et al. 2009; Zare et al. 2014). Although non-typhoidal salmonellosis in humans is usually a self-limiting disease, when symptoms persist, or when immunocompromised persons are affected, it may have serious consequences requiring appropriate antimicrobial treatment. However, in animals, such symptoms may be deadly; thus, treatment with appropriate antimicrobial agents becomes vital. Therefore, the surveillance of antimicrobial resistance is necessary for successful treatment. The public health measures to lessen the burden of infection, thus, take into consideration the presence of the organism in animals (Woldemariam et al. 2005; Akoachere et al. 2009). This study was carried out to assess the antibiogram profiling and antibiotic-resistant gene determinant of *Salmonella* in the faeces of cows and goats in the Eastern Cape province of South Africa.

Materials and methods

Sample collection

The animal faecal samples were obtained from an animal farm in the Eastern Cape province of South Africa between February and August 2010. Faecal samples were collected at random using a sterile spatula into a sterile sample bottle. The samples were maintained in cold chain immediately after collection, and then transported to the laboratory for analysis. Samples collected were from beef cows and meat goats. The animal farm is a large commercial farm comprising of different farm animals including about 50 cows and 70 goats.

Microbiological analysis

Ten grams of animal faecal sample was inoculated into 10 ml of buffered peptone water as a pre-enrichment step and incubated at 37 °C for 18 h. Aliquots from pre-enrichment were inoculated into 10 ml of Rappaport-Vassiliadis Soya broth, selective enrichment media, and incubated at 42 °C for 18 h (Ammari et al. 2009). A loopful of the broth culture was streaked on plates of Hektoen selective media and *Salmonella-Shigella* agar. The plates were incubated at 37 °C for 24 h. Suspected colonies of *Salmonella* from each plate were purified (non-lactose fermenting with suitable colony morphology) and collected for presumptive identification. All strains were stored frozen at −80 °C in 20 % glycerol.

Biochemical identification

Pure presumptive colonies of *Salmonella* were subcultured on a nutrient agar plate and incubated at 37 °C for 18 h; afterwards, catalase and oxidase tests were carried out. Oxidase-negative, catalase-positive isolates were further suspended in normal physiological saline and turbidity adjusted to 0.5 McFarland standard. The bacteria suspension was inoculated onto API 20E strips (bioMérieux, Marcy-L'Étoile, France) following manufacturer's instructions. After 24 h incubation at 37 °C, strips were read according to manufacturer's manual and the results were secured using API 20E software (Shah and Korejo 2012).

Antibiogram profiling of isolates

Antimicrobial susceptibility testing was performed using disc diffusion method on Mueller-Hinton agar. Antibiotics were selected based on drugs commonly used in the treatment of diarhoea and those used in veterinary medicine. Antibiotics used in the study includes the following: penicillins (PEN; 10 μg), cefotaxime (CEF; 30 μg), nalidixic acid (NAL; 30 μg), cephalothin (CEP; 30 μg), ciprofloxacin (CIP; 5 μg), gentamicin (GEN; 10 μg), chloramphenicol (CHL; 30 μg), tetracycline (TET; 10 μg), erythromycin (ERY; 15 μg), minocycline (MIN; 30 μg), vancomycin (VAN; 30 μg), oxacillin (OXA; 1 μg), ofloxacin (OFL; 5 μg), ampicillin (AMP; 25 μg), trimethoprim-sulfamethoxazole (TXM; 25 μg), aztreonam (AZT; 30 μg) and streptomycin (STR; 10 μg). Discs were purchased from Mast Diagnostics (Mast Group, Merseyside, UK). Isolates were identified as susceptible, intermediate or resistant according to the guidelines of the clinical and laboratory standard institute (CLSI 2006).

Antibiotic-resistant gene determination

The genomic DNA of *Salmonella* isolates was extracted following the methods of Sambrook and Russell (2001) and

Igbinosa et al. (2013). The set of primers used for the detection of antibiotic-resistant genes is shown in Table 1. The PCR reaction was carried out in a total volume of 25 μl and the following conditions: integron conserved segment (initial denaturation at 94 °C for 12 min, 1 min of denaturation at 94 °C, 1 min of annealing at 55 °C and 5 min of extension at 72 °C for a total of 35 cycles; 5 s was added to the extension time at each cycle); bla_{TEM} gene (3 min at 93 °C, 40 cycles of 1 min at 93 °C, 1 min at 55 °C and 1 min at 72 °C and finally 7 min at 72 °C); $bla_{amp}C$ gene (94 °C for 5 min, 30 cycles of 25 s of denaturation at 94 °C, 40 s of annealing at 53 °C and 50 s of extension at 72 °C and a final cycle at 7 min at 72 °C); *Tet* C gene (3 min at 94 °C, followed by 30 cycles of 1 min at 94 °C, 1 min at 65 °C and 1 min at 72 °C followed by 10 min at 72 °C); bla_{pse1} (initial denaturation at 96 °C for 5 min, then 30 cycles of denaturation at 96 °C for 30 s, annealing at 60 °C and a single extension of 5 min at 72 °C). The amplified products were electrophoresed in 0.8 % agarose gels (Hispanagar, Spain) containing ethidium bromide 0.5 mg/l (Merck, South Africa) for 1 h at 100 V in 0.5×TAE buffer (40 mM Tris-HCl, 20 mM Na acetate, 1 mM EDTA, pH 8.5), visualized and photographed with an imaging system Alliance 4.7 XD-79 (UVITEC Cambridge) (Igbinosa et al. 2013).

Results

A total of 150 *Salmonella* were isolated from the faecal samples collected. The isolates were identified based on biochemical characteristics; however, further strain characterization was not carried out. Eighty two (54.7 %) isolates were recovered from cow faeces while 68 (45.3 %) were isolated from goat faeces. Antibiotic susceptibility carried out showed that these isolates were multidrug resistant (Table 2). Of the 150 isolates, 125 (83.3 %) were resistant to penicillin, 84 (56 %) were resistant to ampicillin while 74 (49.3 %) were resistant to erythromycin. In addition, none of the isolates were resistant to ciprofloxacin whereas absolute resistance was observed against oxacillin and vancomycin (Table 2). In general, isolates showed appreciable susceptibility (≥85 %) against ofloxacin and nalidixic acid.

Some of the *Salmonella* isolates were found to possess the antibiotic-resistant determinant screened. The prevalence of the antibiotic-resistant genes detected were as follows: integron conserved segment 42 (28 %), bla_{TEM} gene 29 (19.3 %), bla_{pse1} 11 (7.3 %), $bla_{\mathrm{amp}}C$ 7 (4.7 %) while *Tet* C was not detected in the *Salmonella* isolates. Figure 1 shows gel electrophoresis of PCR products of the integron gene of selected *Salmonella* isolates.

Discussion

Salmonella is a well-known zoonotic pathogen in animals and humans. Animals could possibly spread these organisms in the environment (Abouzeed et al. 2000; Akoachere et al. 2009). The occurrence of *Salmonella* in the faeces of cattle and goats have been documented (Zare et al. 2014), potentiating the fact that food-producing animals are carrier of faecal shedding of *Salmonella*. Gragg et al. (2013) reported the prevalence of *Salmonella enterica* subspecies *enterica* serotypes in cattle faeces among other sources in Mexico. Glenn et al. (2011) reported the presence of *Salmonella* from food-producing animals including cattle. Also, the occurrence of *Salmonella* at variable frequency from different samples obtained from goats (including faecal samples) in Ethiopia has been documented (Woldemariam et al. 2005). The recovery of *Salmonella* from cow faeces may not be surprising, as cattle have been regarded as an important reservoir for *Salmonella* (Gragg et al. 2013).

The recovery rate of *Salmonella* in the study was high. Some studies have reported the low prevalence of *Salmonella* in goat population (Mahmood et al. 2014). Similarly, a low prevalence rate was reported by D'Amico et al. (2008) who surveyed 133 milk samples, but *Salmonella* could not be recovered even from a single sample of goat, sheep and cow's milk. Also, Radostits et al. (2007) reported the prevalence of

Table 1 List of primer set used in the detection of antibiotic-resistant gene

Target genes	Primer sequence (5′–3′)	Size (bp)	References
bla_{TEM} gene	AGGAAGAGTATGATTCAACA CTCGTCGTTTGGTATGGC	535	Wang et al. (2006)
Tet C gene	GGT TGAAGG CTCTCAAGGGC GGTTGAAGGCTCTCAAGGGC	505	Agersø and Sandvang (2005)
Integron conserved segment	GGCATCCAAGCAGCAAG AAG CAGACTTGACCTGA	Variable	Fonseca et al. (2005)
$bla_{amp}C$	GGTATGGCTGTGGGTGTTA TCCGAAACGGTTAGTTGAG	882	Yang et al. (2008)
bla_{pse1}	ACC GTA TTG AGC CTG ATT TA ATT GAA GCC TGT GTT TGA GC	321	Bert et al. (2002)

Table 2 Prevalence of antibiotic-resistant *Salmonella* isolated from faecal samples

Antibiotics	Cow faeces *n*=82 (%)	Goat faeces *n*=68 (%)	Total *n*=150 (%)	*p* value
PEN	65 (79.2)	60 (88.2)	125 (83.3)	0.021
CEF	23 (28)	37 (54.4)	60 (40)	0.011
NAL	6 (7.3)	4 (5.9)	10 (6.7)	0.051
CEP	20 (24.4)	25 (36.8)	45 (30)	0.010
CIP	0 (0)	0 (0)	0 (0)	ns
GEN	1 (1.2)	16 (23.5)	17 (11.3)	0.025
CHL	17 (20.7)	20 (29.4)	37 (24.7)	0.020
TET	51 (62.2)	22 (32.3)	73 (48.7)	0.001
ERY	35 (42.7)	39 (57.4)	74 (49.3)	0.002
MIN	38 (46.3)	10 (14.7)	48 (32)	0.001
VAN	82 (100)	68 (100)	150 (100)	ns
OXA	82 (100)	68 (100)	150 (100)	ns
OFL	7 (8.5)	0 (0)	7 (4.6)	0.041
AMP	67 (81.7)	17 (25)	84 (56)	0.015
TXM	51 (62.2)	48 (70.5)	99 (66)	0.021
STR	33 (40.2)	20 (29.4)	53 (35.3)	0.013

PEN penicillins, *CEF* cefotaxime, *NAL* nalidixic acid, *CEP* cephalothin, *CIP* ciprofloxacin, *GEN* gentamicin, *CHL* chloramphenicol, *TET* tetracycline, *ERY* erythromycin, *STR* streptomycin, *MIN* minocycline, *VAN* vancomycin, *OXA* oxacillin, *OFL* ofloxacin, *AMP* ampicillin, *TXM* trimethoprim-sulfamethoxazole, *ns* not significant

Salmonella in other species but none was detected in goats. However, the source of high prevalence of *Salmonella* observed in these animals in the study could be via ingestion of contaminated feed (Kidd et al. 2002) or grazing plants that may have been contaminated with untreated effluents or sludge through fertilization (Akoachere et al. 2009). Another speculation could be the hygiene status of the farm and farm animals, as this could be a predisposing factor of high prevalence of *Salmonella* in the faeces of farm animals.

Salmonella isolates obtained in the study were highly multidrug resistant. The isolates from cow and goat faeces were found to demonstrate variable resistance patterns. Isolates from cow faeces were highly sensitive to gentamicin while isolates from goat faeces were moderately sensitive. Another observation was that isolates from goat were less sensitive to some antibiotics including cephalothin, cefotaxime and chloramphenicol among others (Table 2). It can therefore be deduced that isolates from cow faeces were more susceptible to cephalosporin compared to isolates from goat faeces. On the other hand, isolates from goat faeces were more sensitive to the tetracycline (tetracycline and minocycline). In general, most of the isolates were highly sensitive to quinolones (ciprofloxacin, nalidixic acid and ofloxacin), indicating that quinolones are mainly the active antibiotics against the *Salmonella* isolates. *Salmonella* sensitivity to the quinolones has been reported from Cameroun (Akoachere et al. 2009), Iran (Dallal et al. 2010), India (Bhatia et al. 2007) and Austria (Mayrhofer et al. 2004), suggesting a wide distribution of *Salmonella* isolates sensitive to the quinolones. β-Lactamases confer resistance to the penicillin group of antibiotics, first to fourth generation cephalosporins and monobactams (Wu et al. 2011). This may be a reason for the resistance observed against isolates to penicillin and oxacillin and the moderate sensitivity observed with *Salmonella* isolates against cephalothin and cefotaxime.

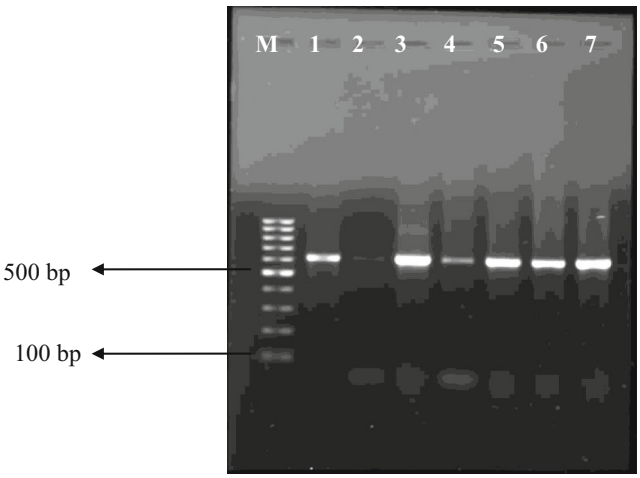

Fig. 1 Agarose gel electrophoresis of PCR products of encoded integrase gene from positive *Salmonella* isolates; *lane M* indicates the DNA ladder 100 bp, and *lanes 1–7* the *Salmonella* isolates. Expected amplicon size is variable

Salmonella isolates obtained in the study were found to express the beta lactamase genes examined. The onset of resistance of non-typhoidal *Salmonella* to extended-spectrum cephalosporin antibiotics is noteworthy in public health (Kruger et al. 2004). Studies have shown the occurrence of a wide range of beta lactamase genes in *Salmonella* (Baraniak et al. 2002; Casin et al. 2003; Kruger et al. 2004; Sjolund-Karlsson et al. 2013; Lee et al. 2014); however, these were basically clinical isolates. On the other hand, a broad distribution of bla_{TEM} gene in *Salmonella* isolated from

animal samples has been documented, for instance, bla_{TEM} gene was detected in *Salmonella* isolated from cattle faeces in Egypt (Dahshan et al. 2010) and in food-producing animal in the USA (Glenn et al. 2011), bla_{TEM} gene was found in *Salmonella* recovered from cow and goat faeces in a study. Although this is the first report of the occurrence of bla_{TEM} gene in *Salmonella* recovered from animal faeces in the Eastern Cape province of South Africa, nonetheless, the prevalence of bla_{TEM} gene in *Salmonella* from clinical samples in South Africa including Eastern Cape province has been documented (Kruger et al. 2004). A relatively higher number of *Salmonella* isolates in the study were found to harbour bla_{TEM} gene compared to clinical isolates reported (Kruger et al. 2004), but lower than *Salmonella* isolates from human and food sources in Bulgaria (Archambault et al. 2006). $bla_{amp}C$ gene was detected in low prevalence in *Salmonella* isolates in the study, and the occurrence of $bla_{amp}C$ in *Salmonella* isolates from human and animal sources has been documented in the USA (Winokur et al. 2000), Romania (Miriagou et al. 2002), Taiwan (Yan et al. 2003) and in clinical isolates in South Africa (Kruger et al. 2004) and South Korea (Lee et al. 2014).

Salmonella isolates in the study were found to harbour bla_{pse1} gene, this result is consistent with the findings of Dahshan et al. (2010), as *S. enterica* serovars Stanley isolated from cattle faeces was found to possess bla_{pse1} gene. Also, *Salmonella* isolates from food animal sources were found to possess bla_{pse1} gene (Glenn et al. 2011). It is interesting to find out that all isolates that possessed bla_{pse1} also had either bla_{TEM}, $bla_{amp}C$ or both genes simultaneously. A similar observation of the direct association of bla_{pse1} and bla_{TEM} genes in *Salmonella* isolates has been documented (Dahshan et al. 2010). The reason for this strong relationship is not clear, but it reveals that *Salmonella* could possess beta lactamase genes of several types, thereby conferring resistance to β-lactamase antibiotics. A wide distribution of *pse1* gene in South African aquatic ecosystem has been speculated (Igbinosa and Okoh 2012). Hence, source water may be a possible source of these antibiotic-resistant determinants in the animals. Therefore, good hygiene status and safe drinking water are agitated in animal farms.

Tet C gene was not detected despite tetracycline resistance observed in the *Salmonella* isolates. This was quite intriguing despite tetracycline resistance exhibited by some isolates; however, since *Tet* C is not the only tetracycline-resistant gene that confers resistance to tetracyclines, there is a possibility that other tetracycline-resistant determinant may be present in the isolates. This fact is potentiated as other tetracycline-resistant determinant has been detected in *Salmonella* isolates elsewhere. For instance, Glenn et al. (2011) reported the prevalence of tetracycline-resistant genes in *Salmonella* isolates from food animals in Georgia. The capability of a microorganism to develop multiple drug resistances partly results in their ability to acquire new antibiotic-resistant genes. Mobile genetic elements called integrons determine a site-specific recombination system that is responsible for the acquisition of many antibiotic-resistant determinants (Hall and Collis 1995; Igbinosa et al. 2013). Integron conserved segment of class 1 integron was found in some *Salmonella* isolates. The presence of class 1 integron in *Salmonella* isolates from animal sources has been documented (Dahshan et al. 2010; Glenn et al. 2011). The detection of integron in some *Salmonella* isolates indicates that these animals are potential reservoir of antibiotic-resistant genes and are capable of transferring antibiotic-resistant determinant to other competent bacteria.

The results obtained in the study imply that cow and goat faeces could be potential reservoirs of *Salmonella* and could possibly cause infections as a result of contamination of food products. There is a need for surveillance system to track resistance patterns of *Salmonella* circulating in South Africa.

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Conflict of interest The author declares that there is no conflict of interest.

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