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Research Note

Multidrug-Resistant *Salmonella* Isolates from Swine in the Eastern Cape Province, South Africa

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ABSTRACT

The exposure of farm animals to antimicrobials for treatment, prophylaxis, or growth promotion can select for resistant bacteria that can be transmitted to humans, and *Salmonella* as an important zoonotic pathogen can act as a potential reservoir of antimicrobial resistance determinants. We assessed the antibiogram profiles of *Salmonella* species isolated from pig herds in two commercial farms in South Africa. Two hundred fifty-eight presumptive *Salmonella* isolates were recovered from the fecal samples of 500 adult pigs. Specific primers targeting *Salmonella* serogroups A, B, C1, C2, and D were used to determine the prevalence of different serogroups. Only serogroup A ($n = 48$) was detected, while others were not. Antimicrobial susceptibility of the confirmed *Salmonella* serogroup A isolates was performed by using the disk diffusion method against a panel of 18 antibiotics. All the 48 isolates were resistant to tetracycline and oxytetracycline, while 75% were resistant to ampicillin, sulphamethoxazole-trimethoprim, nalidixic acid, and streptomycin. All the isolates exhibited multidrug resistance, with the predominant phenotype being against 11 antibiotics, and multiple antibiotic resistance index ranged between 0.3 and 0.6. The incidence of genes encoding resistance against ampicillin (*ampC*), tetracycline (*tetA*), and streptomycin (*strA*) were 54, 61, and 44%, respectively. We conclude that healthy pigs are potential reservoirs of multidrug-resistant *Salmonella* that could be transmitted to humans through the food chain and, hence, a significant public health threat.

Key words: Antimicrobial resistance; *Salmonella*; Serogroup; South Africa; Swine

The use of antimicrobials for treatment, prevention of infections, as well as growth promotion in farm animals, is a major contributory factor to the development of antimicrobial resistance that can potentially lead to widespread transmission of antimicrobial-resistant bacteria through the food chain *(1)*. Antimicrobial resistance, as well as multidrug resistance patterns of *Salmonella* and other enteric pathogens, especially those of animal origin, have raised concerns all over the world *(4, 41)*. In South Africa, the highest level of antibiotics used in animal husbandry is in swine and poultry farms that are usually operated in intensive systems *(19)*. More so, organisms isolated from pigs have been reported to be more resistant than those recovered from other animal sources, due to a more intensive use of antimicrobials in pigs, hence posing a significant public health risk to consumers *(4)*.

Salmonella species are gram-negative flagellated and facultative intracellular pathogens causing a major global public health concern *(39, 41)*. *Salmonella* infection in pigs is often asymptomatic and sometimes causes less severe and transient diarrhea *(32)*. Infections caused by *Salmonella* are among the most common foodborne bacterial infections worldwide, with about 1.4 million cases and 600 deaths occurring annually in the United States *(28)*. Consumption of contaminated foods, including pork, predisposes humans to infection due to *Salmonella (1)*. Most infections in humans are self-limiting, and antimicrobial agents might not be necessary for treatment. However, severe forms of the disease, such as invasive infections, may occur, therefore requiring treatment with antibiotics *(13, 16)*.

The Kauffman-White serotyping scheme is used in most laboratories in characterizing *Salmonella* isolates in which a serotype is determined on the basis of somatic (O) and flagella (H) antigens present in the cell wall and flagella of the organism. The O factors determine the grouping, while the H factors define the serotype identity of a *Salmonella* strain *(20)*. Serotyping is the most commonly used method for phenotypic characterization and identification of *Salmonella* serogroups. Further, it is required to determine the relationship between the disease and source of infection. The traditional method has been shown to be time-consuming, expensive, and laborious and lacks standardized methods of determining antisera to be used for its detection. It is, therefore, imperative to integrate molecular methods to overcome these shortcomings *(30)*. Among the more than

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2,500 serotypes of *Salmonella* identified, over 95% of the strains responsible for infections in humans and animals belong to serogroups A, B, C1, C2, D, to E *(29)*. For example, *Salmonella* Paratyphi A, B, C, and *Salmonella* Typhi belong to the serogroups A, B, C1, and D, respectively. To our knowledge, studies showing the PCR identification of *Salmonella* serogroups obtained from swine fecal samples in the Eastern Cape Province of South Africa are scarce.

In addition, studies have been conducted in Sub-Saharan Africa to determine the prevalence and antimicrobial resistance of *Salmonella* in swine and other animals *(21)*; however, reports on antibiotic resistance in isolates from farm animals, especially from the feces of swine in Eastern Cape Province of South Africa are limited *(19)*. In this article, we report on the antibiogram characteristics of *Salmonella* serogroup A isolates recovered from the feces of healthy pigs as part of our larger study on the reservoirs of antibiotic resistance determinants in the environment.

MATERIALS AND METHODS

Description of sampling sites and sample collection. The sampling sites include two commercial farms within the Nkonkobe Municipality in Eastern Cape Province, South Africa, which is the second largest in the province, covering approximately 3,725 km^2 in size and a rural municipality largely involved in agriculture. Fecal samples were collected from 500 healthy adult pigs by using sterile swab sticks. The samples were transported immediately on ice to the Applied Environmental Microbiology Research Group laboratory at the University of Fort Hare, South Africa, for analyses. An inventory of the antibiotics commonly used in the farms was also taken.

Isolation of *Salmonella* species. *Salmonella* species were isolated following the method described by Karou et al. *(23),* with some modification. Briefly, samples were preenriched by inoculating into tryptic soy broth and incubated at 37°C for 18 to 24 h. This was followed by adding 1 ml of preenrichment to 9 ml of Muller-Kauffmann tetrathionate broth and incubating at 37°C for 48 h. Tubes showing growth were selectively plated onto xylose lysine desoxycholate agar (Merck, Modderfontein, Gauteng, South Africa) and incubated aerobically at 37°C for 22 to 24 h. Red colonies with black centers were selectively picked as presumptive *Salmonella* isolates and further purified on nutrient agar (Merck).

DNA extraction. The extraction of DNA from pure culture was done by using the boiling method, as described by Maugeri et al. *(31)*. Briefly, about 3 to 5 colonies of pure culture of the organism were picked by using a sterile wire loop and placed into sterile DNase- and RNase-free Eppendorf tubes (Biologix Research Co., Lenexa, KS) containing 200 μl of sterile nuclease-free water (Thermo Fisher Scientific, Lafayette, CO). The suspension was vortexed, and the cells were lysed by boiling in a heating block (Thermo Fisher Scientific, Leicestershire, UK) at 100°C for 15 min. The cell debris was removed by centrifugation using a centrifuge (Lasec-Sigma Laborzentrifugen, Osterode, Germany) at 13,500 rpm for 10 min. The supernatant containing the genomic DNA template was carefully transferred into another Eppendorf tube and stored at −20°C for further assays.

Identification of *Salmonella* serogroups by PCR. The presumptive isolates were screened for the different *Salmonella* serogroups, including A, B, C1, C2, and D by using a PCR technique. Primer sets name, primer sequences, and product sizes are listed in Table 1. Each reaction mixture consisted of 12.5 μl of 2× Dream *Taq* Master mix (Thermo Fisher Scientific, Gauteng, South Africa), 10 pmol each of the forward and reverse primers, 5 μl DNA template, and nuclease-free water (Thermo Fisher Scientific) to make up the final volume to 25 μl. PCR amplification for all the serogroups was carried out in MyCycler Thermal Cycler PCR system (Bio-Rad, Hercules, CA), with an initial denaturation of 94°C for 3 min, 35 cycles of 94°C for 50 s, 60°C for 50 s, and 72°C for 50 s, and then with a final extension at 72°C for 10 min *(30)*. Five-microliter aliquots of the amplicons were resolved in 1.5% agarose gel (Separations, Johannesburg, South Africa) at 100 V for 60 min. The gel, stained with ethidium bromide, was visualized under the UV transilluminator (Alliance 4.7, UVItec, Cambridge, UK). A 100-bp DNA ladder (Promega, San Luis Obispo, CA) was used on each gel as a molecular weight marker.

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed on all confirmed *Salmonella* serogroup A isolates by using a disk diffusion assay, as recommended by the Clinical and Laboratory Standards Institute guideline *(8)*. Isolates grown overnight in nutrient agar (Merck) at 37°C were suspended in 0.85% sterile normal saline, and the cell density adjusted to a 0.5 McFarland turbidity standard. The entire surface of the Mueller-Hinton agar was evenly spread with the standardized bacterial test suspension, after which appropriate antibiotic discs (Mast Diagnostics, Merseyside, UK) were placed on the dried surface, by using an antibiotic disc dispenser (Mast Diagnostics). After incubation at 37°C for 24 h, the results were interpreted according to the Clinical and Laboratory Standards Institute standard *(8)*. The panel of antibiotics tested included the following: tetracycline (30 μg), oxytetracycline (30 μg), ampicillin G (10 μg), sulphamethoxazole-trimethoprim (25 μg), streptomycin (10 μg), gentamycin (10 μg), amikacin (30 μg), ceftazidime (30 μg), cephalothin (30 μg), cefotaxime (30 μg), chloramphenicol (10 μg), norfloxacin (10 μg), ciprofloxacin (5 μg), nalidixic acid (30 μg), cefuroxime (30 μg), imipenem (10 μg), and polymycin B (300 μg). *Escherichia coli* 25922 was used as a quality control. The multiple antibiotic resistance (MAR) patterns were generated for all the serogroup A isolates following the protocol described by Ateba et al. *(5)*.

Multiple antibiotic resistance indexing. The multiple antibiotic resistance index (MARI) of the isolates was calculated and interpreted according to Krumperman *(27),* by using the following formula: MARI = a/b, where a represents the number of antibiotics to which a particular isolate was resistant and b the total number of antibiotics tested. A MAR index of ≥ 0.2 indicates high-risk environment where antibiotics are often used *(36)*.

Detection of antibiotic resistance genes. The *ampC, tetA,* and *strA* genes that code for resistance to ampicillin, tetracyclines, and streptomycin, respectively, were assessed following the method described by Iweriebor et al. *(22),* using the primer sequences described elsewhere *(12, 18, 35)*. The same PCR conditions were used as follows: 5-min initial denaturation at 94°C, followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1.5-min final incubation at 72°C for 5 min.

RESULTS

Isolation and molecular serogrouping. A total of 258 presumptive isolates were recovered from 500 pig fecal samples. They were screened for the five serogroups, and only serogroup A was detected as 48 of the isolates (18.6%) from farm A only. None of the other serogroups were detected.

TABLE 1. *Antimicrobial susceptibility pattern of the* Salmonella *serogroup A isolates isolated from feces of pigs in Nkonkobe Municipality, Eastern Cape Province, South Africa*

Antimicrobial agent	Abbreviation	Potency (μg)	*n* (%)[a]: R	I	S
Tetracycline	T	30	48 (100)	0 (0)	0 (0)
Oxytetracycline	OT	30	36 (75)	12 (25)	0 (0)
Ampicillin	AMP	10	36 (75)	12 (25)	0 (0)
Cephalothin	KF	30	4 (8)	4 (8)	40 (83)
Cefuroxime	CXM	30	8 (17)	8 (17)	32 (67)
Ceftazidime	CTX	30	12 (25)	24 (50)	12 (25)
Cefotaxime	CAZ	30	20 (42)	16 (33)	12 (25)
Erythromycin	E	15	48 (100)	0 (0)	0 (0)
Sulphamethoxazole-trimethoprim	TS	25	36 (75)	0 (0)	12 (25)
Chloramphenicol	C	10	12 (25)	32 (67)	4 (8)
Nalidixic acid	NA	30	36 (75)	12 (25)	0 (0)
Ciprofloxacin	CIP	5	0 (0)	16 (33)	32 (67)
Norfloxacin	NOR	10	4 (8)	0 (0)	44 (92)
Gentamycin	GM	10	4 (8)	4 (8)	40 (83)
Amikacin	AK	30	8 (17)	20 (42)	20 (42)
Streptomycin	S	10	36 (75)	0 (0)	12 (25)
Imipenem	IMI	10	0 (0)	0 (0)	48 (100)
Polymycin B	PB	300	44 (92)	4 (8)	0 (0)

[a] R, resistant; I, intermediate; S, susceptible.

Antimicrobial susceptibility testing of the *Salmonella* group A isolates. The distribution of antimicrobial resistance in the *Salmonella* group A isolates obtained in this study is summarized in Table 1. All the isolates were sensitive to imipenem, whereas 91.7, 83.3, and 66.7% were sensitive to norfloxacin, gentamycin, and ciprofloxacin, respectively. All the isolates were resistant to erythromycin, tetracycline, and oxytetracycline. A large proportion was also resistant to ampicillin (75%), sulphamethoxazole-trimethoprim (75%), nalidixic acid (75%), and streptomycin (75%). Furthermore, 42% of the isolates were resistant to a third-generation cephalosporin, cefotaxime, while 25% of the isolates showed resistance to chloramphenicol.

MAR phenotypes and MARIs. All the isolates were resistant to more than five antibiotics tested. The MAR pattern of each isolate is as shown in Table 2. The highest frequency of MAR phenotype was against 11 antibiotics and was demonstrated by 16.7% of the isolates ($n = 8$), whereas 4.2% ($n = 2$) of the isolates showed the lowest frequency against six antibiotics. The MAR indices ranged between 0.3 to 0.6.

Prevalence of resistance genes. Approximately 54, 61, and 44% and of the resistant *Salmonella* serogroup A isolates were positive for *ampC, tetA,* and *strA* resistance genes, respectively.

DISCUSSION

In this study, molecular serogrouping of *Salmonella* isolates obtained from pig fecal materials showed that only the serogroup A was detected in about 19% of the presumptive isolates, while other serogroups tested were not detected. The remaining presumptive isolates might either belong to other serogroups not tested or are not *Salmonella*. This is in contrast with other studies in which other serogroups were identified, although they were mainly from clinical sources, and other animal sources, such as poultry *(6, 30),* as studies that showed molecular serogrouping of *Salmonella* isolates from swine samples are scarcely available. The primers used in detection of serogroups in this study were used succesfully elsewhere *(30)*; hence, the variation in our results could be attributed to the sampling source, geographical differences, or both. In addition, the *Salmonella* serogroup A isolates are suspected to belong to *Salmonella* Paratyphi, *Salmonella* Typhimurium, or both, the most common serovars belonging to this serogroup. For instance, in South Africa, an 11-year (1996 to 2006) retrospective study on the occurrence of *Salmonella* in pigs showed that the majority was due to *Salmonella* Typhimurium *(24)*.

From the prevalence of *Salmonella* obtained in this study, it is evident that healthy pigs can be reservoirs of *Salmonella*. A similar prevalence of 17.2% was reported by Molla et al. *(34)* from certain swine production units in the United States, while a relatively similar finding was observed by Kishima et al. *(26)* who reported a 15.1% (26 of 172) prevalence from the feces of pigs in Japan. On the other hand, a lower prevalence was reported by Kikuvi et al. *(25)* from a slaughterhouse in Kenya, where the prevalence of *Salmonella* in fecal samples of pigs was 8.6%. A higher prevalence of 19% observed from carcass samples suggested that environmental contamination during slaughtering can increase the prevalence of *Salmonella* in pork. Another study done elsewhere *(11),* reported a prevalence of 31.5% that was higher than our finding in this current study.

Results of antimicrobial susceptibility showed high sensitivity of *Salmonella* isolates to gentamycin, which is

TABLE 2. *Multiple antimicrobial resistance pattern and MARI of* Salmonella *spp. isolated from feces of pigs in Nkonkobe Municipality, Eastern Cape Province, South Africa*

Isolate no.	Resistance pattern[a]	No. of antibiotics	MARI
FP1	T-CAZ-S-E-OT-AMP-NA-AK-PB	9	0.5
FP2	T-S-E-OT-T/S-NA-PB	7	0.4
FP3	T-S-E-OT-AMP-T/S-NA-PB	7	0.4
FP4	T-S-E-OT-AMP-NA-AK-CTX-NOR-PB	10	0.6
FP5	T-CAZ-S-E-OT-AMP-T/S-NA-CTX-CXM-PB	11	0.6
FP6	T-CAZ-S-E-OT-AMP-T/S-NA-C-CTX-CXM	11	0.6
FP7	T-S-E-OT-KF-AMP-T/S-NA-PB	9	0.5
FP8	T-E-OT-AMP-C-CTX-PB	7	0.4
FP9	T-S-E-OT-T/S-PB	6	0.3
FP10	T-CAZ-S-E-OT-AMP-T/S-NA-AK-PB	10	0.6
FP11	T-CAZ-S-GM-E-OT-AMP-T/S-NA-CTX-PB	11	0.6
FP12	T-S-C-E-OT-T/S-NA-PB	8	0.4
FP13	T-S-E-OT-AMP-NA-AK-CTX-NOR-PB	11	0.6
FP14	T-CAZ-S-E-OT-AMP-NA-AK-PB	9	0.5
FP15	T-S-E-OT-AMP-NA-AK-CTX-NOR-PB	10	0.6
FP16	T-S-E-OT-KF-AMP-T/S-NA-PB	9	0.5
FP17	T-S-E-OT-KF-AMP-T/S-NA-PB	7	0.4
FP18	T-S-E-OT-T/S-NA-PB	7	0.4
FP19	T-E-OT-AMP-C-CTX-PB	7	0.4
FP20	T-S-E-OT-KF-AMP-T/S-NA-PB	9	0.5
FP21	T-CAZ-S-E-OT-AMP-T/S-NA-AK-PB	10	0.6
FP22	T-CAZ-S-E-OT-AMP-NA-AK-PB	9	0.5
FP23	T-S-E-OT-T/S-PB	6	0.3
FP24	T-CAZ-S-E-OT-AMP-T/S-NA-AK-PB	9	0.5
FP25	T-CAZ-S-GM-E-OT-AMP-T/S-NA-CTX-PB	11	0.6
FP26	T-S-E-OT-T/S-NA-PB	7	0.4
FP27	T-CAZ-S-E-OT-AMP-NA-AK-PB	7	0.4
FP28	T-S-E-OT-AMP-T/S-NA-PB	7	0.4
FP29	T-S-E-OT-T/S-PB	6	0.3
FP30	T-S-E-OT-AMP-NA-AK-CTX-NOR-PB	10	0.6
FP31	T-CAZ-S-GM-E-OT-AMP-T/S-NA-CTX-PB	11	0.6
FP32	T-CAZ-S-E-OT-AMP-T/S-NA-C-CTX-CXM	11	0.6
FP33	T-S-E-OT-AMP-T/S-NA-PB	7	0.4
FP34	T-CAZ-S-E-OT-AMP-T/S-NA-AK-PB	10	0.6
FP35	T-S-C-E-OT-T/S-NA-PB	8	0.4
FP36	T-S-E-OT-AMP-NA-AK-CTX-NOR-PB	10	0.6
FP37	T-CAZ-S-E-OT-AMP-T/S-NA-AK-PB	10	0.6
FP37	T-CAZ-S-E-OT-AMP-T/S-NA-CTX-CXM-PB	11	0.6
FP38	T-S-E-OT-T/S-PB	6	0.3
FP39	T-CAZ-S-E-OT-AMP-T/S-NA-C-CTX-CXM	11	0.6
FP40	T-CAZ-S-GM-E-OT-AMP-T/S-NA-CTX-PB	11	0.6
FP41	T-S-C-E-OT-T/S-NA-PB	8	0.4
FP42	T-S-E-OT-AMP-T/S-NA-PB	7	0.4
FP43	T-E-OT-AMP-C-CTX-PB	7	0.4
FP44	T-CAZ-S-E-OT-AMP-T/S-NA-C-CTX-CXM	11	0.6
FP45	T-S-C-E-OT-T/S-NA-PB	8	0.4
FP46	T-S-E-OT-T/S-NA-PB	7	0.4
FP47	T-CAZ-S-E-OT-AMP-T/S-NA-CTX-CXM-PB	11	0.6
FP48	T-CAZ-S-E-OT-AMP-T/S-NA-CTX-CXM-PB	11	0.6

[a] See Table 1 for definitions of abbreviations.

consistent with the findings of some authors *(13, 38)*. This could be attributed to its infrequent use in the farms visited. Furthermore, low resistance to ciprofloxacin, which is the drug of choice in the treatment of salmonellosis in humans, was observed is in accordance with reports of some studies in both developed and developing countries *(9, 13)*. The relatively low resistance of isolates to chloramphenicol could probably be owing to its rare use in farms following the ban of its use in veterinary medicine in South Africa *(17)*.

High frequency of resistance was observed against tetracyclines (100%), followed by ampicillin (75%), sulphamethoxazole-trimethoprim (75%), and streptomycin (75%). Similar results have been reported in other studies from Spain *(3, 13)* and other parts of the world, including New Zealand *(14)*. Resistance to these antimicrobials is not

surprising as they are frequently used in the treatment of infections, as well as growth promoters in animals, most likely because of their affordability and availability in South Africa *(19, 25)*. Generally, all the isolates were observed to be resistant to more than five antibiotics. The most predominant resistance phenotype was observed against 11 antibiotics, implying that these isolates are highy multidrug resistant *(33)*. Furthermore, four resistant isolates from this study demonstrated the penta resistance pattern AMP-C-S-T/S-T, typical of the *Salmonella* Typhimurium DT104, which has the gene-encoded resistance pattern ACSSuT (ampicillin, chloramphenicol, streptomycin, sulphonamides, and tetracycline) *(15, 40)*. This serovar has been isolated from pigs and pork products in many countries *(26)* and has become a potential threat for animal husbandry and human medicine *(10)*.

The MAR index observed in this study, which ranged between 0.3 and 0.6, suggests a high level of antibiotic use in the swine farm either for treatment or as growth promoters in the feed, which is common in pig farms *(2, 36)*.

The *ampC, tetA,* and *strA* genes that code for resistance to ampicillin, tetracyclines, and streptomycin, respectively, and the commonly used drugs in the farms studied were assessed for their possible involvement in observed phenotypic resistance. From our findings, the *ampC* gene was detected in 61.1% of isolates resistant to β-lactams, lower than the finding of Chander et al. *(7)* who reported 11 (92%) of 12 resistant *Salmonella* harboring the *ampC* gene. In addition**,** this study showed that *tetA* and *strA* genes were detected in 54 and 44% of resistant isolates, respectively, in contrast with the findings of Pezzella et al. *(37)* in Italy who reported the prevalence of *tetA* and *strA* genes to be 84 and 68%, respectively.

Results obtained in this study reveal that swine from this province could harbor multidrug-resistant *Salmonella* group A isolates, which could be attributed to the intensive use of antibiotics in pig farms. This poses a significant public health concern and calls for strategies to reduce the incidence of *Salmonella* carriage in pigs, and more stringent policies to reduce the excessive use of clinically important antibiotics in farms animals should be implemented in South Africa.

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REFERENCES

1. Abatcha, M. G., Z. Zakaria, G. Kaur, and K. L. Thong. 2014. Review article: A trends of *Salmonella* and antibiotic resistance. *Adv. Life Sci. Technol.* 17:9–21.
2. Adzitey, F., G. Rusul, and N. Huda. 2012. Prevalence and antibiotic resistance of *Salmonella* serovars in ducks, duck rearing and processing environments in Penang, Malaysia. *Food Res. Int.* 45:947–952.
3. Agustín, A. I., J. J. Carramiñana, 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.
4. Astorga, R. J. M., A. E. Salaberria, A. M. García, S. V. Jimenez, A. C. Martinez, A. A. García, and A. A. Casas. 2007. Surveillance and antimicrobial resistance of *Salmonella* strains isolated from slaughtered pigs in Spain. *J. Food Prot.* 70:1502–1506.
5. Ateba, C. N., and C. C. Bezuidenhout. 2008. Characterisation of *Escherichia coli* O157 strains from humans, cattle and pigs in the North-West Province, South Africa. *Int. J. Food Microbiol.* 128:181–188.
6. Cardona-Castro, N., M. Sánchez-Jiménez, L. Lavalett, N. Múñoz, and J. Moreno. 2009. Development and evaluation of a multiplex polymerase chain reaction assay to identify *Salmonella* serogroups and serotypes. *Diagn. Microbiol. Infect. Dis.* 65:327–330.
7. Chander, Y., S. Oliveira, and S. M. Goyal. 2011. Characterisation of ceftiofur resistance in swine bacterial pathogens. *Vet. J.* 187:139–141.
8. Clinical and Laboratory Standards Institute. 2012. Performance standards for antimicrobial susceptibility testing: twenty-second informational supplement. M100-S22. Clinical and Laboratory Standards Institute, Wayne, PA.
9. De Jong, A., A. Smet, C. Ludwig, B. Stephan, E. De Graef, M. Vanrobaeys, and F. Haesebrouck. 2014. Antimicrobial susceptibility of *Salmonella* isolates from healthy pigs and chickens (2008–2011). *Vet. Microbiol.* 171:298–306.
10. Esaki, H., A. Morioka, K. Ishihara, A. Kojima, S. Shiroki, Y. Tamura, and T. Takahashi. 2004. Antimicrobial susceptibility of *Salmonella* isolated from cattle, swine and poultry (2001–2002): report from the Japanese Veterinary antimicrobial resistance monitoring program. *J. Antimicrob. Chemother.* 53:266–270.
11. Farzan, A., R. M. Friendship, A. Cook, and F. Pollari. 2010. Occurrence of *Salmonella, Campylobacter, Yersinia enterocolitica, Escherichia coli* O157 and *Listeria monocytogenes* in swine. *Zoonoses Public Health* 57:388–396.
12. Forward, K. R., B. M. Willey, D. E. Low, A. Mcgeer, M. A. Kapala, M. M. Kapala, and L. L. Burrows. 2001. Molecular mechanisms of cefoxitin resistance in *Escherichia coli* from the Toronto area hospitals. *Diagn. Microbiol. Infect. Dis.* 41:57–63.
13. García-Feliz, C., J. A. Collazos, A. Carvajal, S. Herrera, M. A. Echeita, and P. Rubio. 2008. Antimicrobial resistance of *Salmonella enterica* isolates from apparently healthy and clinically ill finishing pigs in Spain. *Zoonoses Public Health* 55:195–205.
14. Gebreyes, W. A., P. R. Davies, W. E. M. Morrow, J. A. Funk, and C. Altier. 2000. Antimicrobial resistance of *Salmonella* isolates from swine. *J. Clin. Microbiol.* 38:4633–4636.
15. Gebreyes, W. A., and S. Thakur. 2005. Multidrug-resistant *Salmonella enterica* serovar Muenchen from pigs and humans and potential interserovar transfer of antimicrobial resistance. *Antimicrob. Agents Chemother.* 49:503–511.
16. Glenn, L. M., R. L. Lindsey, J. F. Frank, R. J. Meinersmann, M. D. Englen, P. J. Fedorka-Cray, and J. G. Frye. 2011. Analysis of antimicrobial resistance genes detected in multidrug-resistant *Salmonella enterica* serovar Typhimurium isolated from food animals. *Microb. Drug Resist.* 17:407–418.
17. Global Antibiotic Resistance Partnership. 2011. Situation analysis: antibiotic use and resistance in South Africa. Part 2. *S. Afr. Med. J.* 101:549–596. Available at: http://www.cddep.org/publications/situation_analysis_antibiotic_use_and_resistance_south_africa. Accessed 21 April 2014.
18. Guillaume, G., D. Verbrugge, M. Chasseur-Libotte, W. Moens, and J. Collard. 2000. PCR typing of tetracycline resistance determinants (Tet A-E) in *Salmonella enterica* serotype Hadar and in the microbial community of activated sludges from hospital and urban wastewater treatment facilities in Belgium. *FEMS Microbiol. Ecol.* 32:77–85.
19. Henton, M. M., H. A. Eagar, G. E. Swan, and M. van Vuuren. 2011. Part VI. Antibiotic management and resistance in livestock production. *In* Situation analysis: antibiotic use and resistance in South Africa. Part 2. *S. Afr. Med. J.* 101:583–586.
20. Herrera-León, S., R. Ramiro, M. Arroyo, R. Díez, M. A. Usera, and M. A. Echeita. 2007. Blind comparison of traditional serotyping with three multiplex PCRs for the identification of *Salmonella* serotypes. *Res. Microbiol.* 158:122–127.
21. Ikwap, K., J. Erume, D. O. Owiny, G. W. Nasinyama, L. Melin, B. Bengtsson, N. Lundeheim, C. Fellstrom, and M. Jacobson. 2014. *Salmonella* species in piglets and weaners from Uganda: prevalence,

antimicrobial resistance and herd-level risk factors. *Prev. Vet. Med.* 115:39–47.
22. Iweriebor, B. C., L. C. Obi, and A. I. Okoh. 2015. Virulence and antimicrobial resistance factors of *Enterococcus* spp. isolated from fecal samples from piggery farms in Eastern Cape, South Africa. *BMC Microbiol.* 15:136.
23. Karou, G. T., A. C. Bonny, G. Honoré, A. Dadie, and S. L. Ahonzo-Niamke. 2013. Prevalence of *Salmonella* resistance of serovars in gizzards and retail chicken. *Int. J. Med. Appl. Sci.* 2:223–233.
24. Kidanemariam, A., M. Engelbrecht, and J. Picard. 2010. Retrospective study on the incidence of *Salmonella* isolations in animals in South Africa, 1996 to 2006. *J. S. Afr. Vet. Assoc.* 81:37–44.
25. Kikuvi, G. M., J. N. Ombui, and E. S. Mitema. 2010. Serotypes and antimicrobial resistance profiles of *Salmonella* isolates from pigs at slaughter in Kenya. *J. Infect. Dev. Ctries.* 4:243–248.
26. Kishima, M., I. Uchida, T. Namimatsu, T. Osumi, S. Takahashi, K. Tanaka, H. Aoki, K. Matsuura, and K. Yamamoto. 2008. Nationwide surveillance of *Salmonella* in the faeces of pigs in Japan. *Zoonoses Public Health* 55:139–144.
27. Krumperman, P. H. 1983. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. *Appl. Environ. Microbiol.* 46:165–170.
28. Lan, R., P. R. Reeves, and S. Octavia. 2009. Population structure, origins and evolution of major *Salmonella enterica* clones. *Infect. Genet. Evol.* 9:996–1005.
29. Lim, B. K., and K. L. Thong. 2009. Application of PCR-based serogrouping of selected *Salmonella* serotypes in Malaysia. *J. Infect. Dev. Ctries.* 3:420–428.
30. Liu, B., L. Zhang, X. Zhu, C. Shi, J. Chen, W. Liu, X. He, and X. Shi. 2011. PCR identification of *Salmonella* serogroups based on specific targets obtained by comparative genomics. *Int. J. Food Microbiol.* 144:511–518.
31. Maugeri, T. L., M. Carbone, M. T. Fera, G. P. Irrera, and C. Gugliandolo. 2004. Distribution of potentially pathogenic bacteria as free living and plankton associated in a marine coastal zone. *J. Appl. Microbiol.* 97:354–361.
32. Miller, A. J., D. F. Twomey, R. H. Davies, C. J. Teale, S. M. Williamson, R. Reichel, C. A. Featherstone, A. J. C. Cook, L. C. Snow, and J. D. Armstrong. 2011. *Salmonella* serovars and antimicrobial resistance patterns on a sample of high seroprevalence pig farms in England and Wales (2003–2008). *Zoonoses Public Health* 58:549–559.
33. Mohanta, T., and S. Goel. 2014. Prevalence of antibiotic-resistant bacteria in three different aquatic environments over three seasons. *Environ. Monit. Assess.* 186:5089–5100.
34. Molla, B., A. Sterman, J. Mathews, V. Artuso-Ponte, M. Abley, W. Farmer, P. Rajala-Schultz, W. E. M. Morrow, and W. A. Gebreyes. 2010. *Salmonella enterica* in commercial swine feed and subsequent isolation of phenotypically and genotypically related strains from fecal samples. *Appl. Environ. Microbiol.* 76:7188–7193.
35. Nori, E. M. E., and K. L. Thong. 2010. Differentiation of *Salmonella enterica* based on PCR detection of selected somatic and flagellar antigens. *Afr. J. Microbiol. Res.* 4:871–876.
36. Osundiya, O. O., R. O. Oladele, and O. O. Oduyebo. 2013. Multiple antibiotic resistance (MAR) indices of *Pseudomonas* and *Klebsiella* species isolates in Lagos University Teaching Hospital. *Afr. J. Clin. Exp. Microbiol.* 14:164–168.
37. Pezzella, C., A. Ricci, E. Digiannatale, I. Luzzi, and A. Carattoli. 2004. Tetracycline and streptomycin resistance genes, transposons, and plasmids in *Salmonella enterica* isolates from animals in Italy. *Antimicrob. Agents Chemother.* 48:903–908.
38. Sisak, F., H. Havlickova, H. Hradecka, I. Rychlik, I. Kolackova, and R. Karpiskova. 2006. Antibiotic resistance of *Salmonella* spp. isolates from pigs in the Czech Republic. *Vet. Med.* 2006:303–310.
39. Suez, J., S. Porwollik, A. Dagan, A. Marzel, Y. I. Schorr, P. T. Desai, V. Agmon, M. McClelland, G. Rahav, and O. Gal-Mor. 2013. Virulence gene profiling and pathogenicity characterization of non-typhoidal *Salmonella* accounted for invasive disease in humans. *PLoS One* 8:e58449.
40. Threlfall, E. J. 2002. Antimicrobial drug resistance in *Salmonella*: problems and perspectives in food- and water-borne infections. *FEMS Microbiol. Rev.* 26:141–148.
41. Wang, H., K. Ye, X. Wei, J. Cao, X. Xu, and G. Zhou. 2013. Occurrence, antimicrobial resistance and biofilm formation of *Salmonella* isolates from a chicken slaughter plant in China. *Food Control* 33:378–384.