Veterinaria Italiana, 2012, **48** (2), 125-132

Prevalence and antimicrobial resistance pattern of *Salmonella* in animal feed produced in Namibia

Renatus P. Shilangale[1], Elisabetta Di Giannatale[2], Percy M. Chimwamurombe[3] & Godwin P. Kaaya[4]

Summary

The occurrence of *Salmonella* is a global challenge in the public health and food production sectors. Our study investigated the prevalence, serovar and antimicrobial susceptibility of strains of *Salmonella* serovars isolated from animal feed (meat-and-bone and blood meal) samples from two commercial abattoirs in Namibia. A total of 650 samples ($n = 650$) were examined for the presence of *Salmonella*. Results showed that 10.9% ($n = 71$) were positive for *Salmonella*. Of the *Salmonella* serovars isolated, *S.* Chester was the most commonly isolated serovar (19.7%), followed by *S.* Schwarzengrund at 12.7%. From the *Salmonella* isolates, 19.7% ($n = 14$) were resistant to one or more of the antimicrobials (nalidixic acid, trimethoprim-sulfamethoxazole, sulfisoxazole, streptomycin and/or tetracycline), whereas 80.3% ($n = 57$) were susceptible to all 16 antimicrobials tested. Resistance to sulfisoxazole and the trimethroprim-suflamethoxazole combination were the most common. The resistant isolates belonged to ten different *Salmonella* serovars. The susceptibility of most of the *Salmonella* isolated to the antimicrobials tested indicates that antimicrobial resistance is not as common and extensive in Namibia as has been reported in many other countries. It also appears that there is a range of antimicrobials available that are effective in managing *Salmonella* infections in Namibia. However, there is some evidence that resistance is developing and this will need further monitoring to ensure it does not become a problem.

Keywords

Antimicrobial, Feed, Multidrug resistance, Namibia, Prevalence, Resistance, *Salmonella.*

Prevalenza e pattern di resistenza antimicrobica della *Salmonella* nel mangime per animali prodotto in Namibia

Riassunto

La Salmonella *è un problema globale per i settori della salute pubblica e della produzione alimentare. Questo studio ha indagato la prevalenza, il profilo sierotipico e la suscettibilità antimicrobica di ceppi di sierotipi di* Salmonella *isolati da campioni di mangime per animali (composti di farina di carne, ossa e sangue secco) prelevati da due mattatoi commerciali in Namibia. Su un totale di 650 campioni* (n=650) *controllati, il 10,9%* (n=71) *è risultato positivo alla* Salmonella. *Tra i sierotipi di* Salmonella *isolati, il più comune era il* S. *Chester (19,7%), seguito dal* S. *Schwarzengrund (12,7%). Tra gli isolati, il 19,7%* (n=14) *è risultato resistente a uno o più antimicrobici (acido nalidissico, trimetoprim-sulfametossazolo, sulfisossazolo, streptomicina e/o tetraciclina), mentre l'80,3%* (n=57) *è risultato suscettibile a tutti i 16 antimicrobici testati. La resistenza al sulfisossazolo e quella alla combinazione trimetoprim-sulfametossazolo sono*

(1) Central Veterinary Laboratory, Ministry of Agriculture Water and Forestry, Private Bag 13187, Windhoek, Namibia rpshilangale@yahoo.com
(2) Istituto 'G. Caporale', Via Campo Boario, 64100 Teramo, Italy
(3) Department of Biological Sciences, Faculty of Science, University of Namibia, Private Bag 13301, Windhoek, Namibia
(4) Department of Animal Science, University of Namibia, Private Bag 13301, Windhoek, Namibia

risultate le più frequenti. Gli isolati resistenti appartenevano a dieci sierotipi differenti di Salmonella. *La suscettibilità della maggior parte degli isolati di* Salmonella *agli antimicrobici testati indica che la resistenza antimicrobica non è tanto comune e diffusa in Namibia quanto in diversi altri paesi. I dati indicano inoltre che sono disponibili diversi antimicrobici efficaci nella gestione delle infezioni da* Salmonella *in Namibia. Tuttavia, alcune evidenze dimostrano lo sviluppo di resistenza, fenomeno che dovrà essere ulteriormente monitorato per evitare che diventi un problema.*

Parole chiave

Antimicrobico, Namibia, Mangime, Prevalenza, Resistenza, Resistenza multifarmaco, *Salmonella*.

Introduction

The occurrence of *Salmonella* is now a global challenge in the public health and food production sectors, as the awareness of consumers in regard to food safety is on the increase. These foodborne pathogens are primarily transmitted to humans through the consumption of contaminated food of animal origin. Contamination of food with *Salmonella* is associated with a foodborne disease in humans known as salmonellosis. Animals may become infected from other *Salmonella*-infected animals, directly or through a contaminated environment, including contaminated feed (7).

The global transformation and intensification of agriculture during the last century has led to increasing reliance on manufactured feed products to feed food-producing animals (5). Animal feed is usually made up from bone, meat trimmings, blood and other slaughter by-products. However, there is evidence of the possible transmission of *Salmonella* from animal feed to animals consuming the feed and then to food products derived from the animals (7). These pathogens may be transmitted to humans through the food chain and cause illness (5, 17).

Although the current global impact of *Salmonella* on public health is not very clear, available data estimates indicate that there are 93.8 million cases of *Salmonella* infection and 155 000 deaths each year (16). In the United States, cases of *Salmonella* infection are estimated to stand at 1.4 million with an estimated cost of US$2.7 billion annually (25). In 2008, salmonellosis accounted for about 132 000 cases, thereby becoming the second most reported zoonotic disease in humans, after campylobacteriosis, in the European Union (8). The estimated cost of this burden on the European Union is believed to be between €0.2 billion and €3 billion (9).

In addition, the control of infectious diseases is seriously threatened by the steady increase in the number of micro-organisms that are resistant to antimicrobial agents (21). The emergence and spread of antimicrobial resistance are complex problems driven by numerous interconnected factors, many of which are linked to the misuse of antimicrobials (26). The consequences of antimicrobial resistance can be severe, especially when infections caused by the resistant micro-organism fail to respond to the prescribed treatment. Previous studies suggest that antimicrobial resistant bacteria or antimicrobial resistance genes can be transmitted via feed (7).

This study was undertaken to investigate the prevalence, serovar and antimicrobial resistance pattern of *Salmonella* in animal feed produced from by-products at two commercial abattoirs in Namibia.

Materials and methods

Sample collection

A total of 650 bovine and ovine meat-and-bone meal and blood meal samples were collected from two local abattoirs from January 2008 to December 2009. As a routine test, samples were analysed for the presence of *Salmonella* at the Central Veterinary Laboratory in Windhoek. Of these, 564 samples were meat-and-bone meal and 86 samples were blood meal. Samples were collected by the State veterinary officials at each respective abattoir using sterile dilution bags and were transported to the laboratory for analysis. The samples were kept at between 2°C to 8°C during transportation, using a cooler box with ice bricks. When received at the laboratory,

samples were stored in accordance with ISO 7218:2007 and kept refrigerated at between 1°C and 5°C prior to analysis. The isolation of *Salmonella* was performed within 24 h from the time of receipt of the samples.

Isolation and identification

Detection of *Salmonella* in feed samples was performed according to the standard culture method ISO-6579:2002. A 25 g sample was pre-enriched into 225 ml of buffered peptone water (Merck, Darmstadt) and incubated at 37°C for 24 h. Subsequently, 0.1 ml of the pre-enrichment culture was added to 10 ml of Rappaport-Vassiliadis broth (Merck, Darmstadt) and 10 ml to 100 ml of selenite cystine broth (Merck, Darmstadt) and incubated for 24 h at 41.5 and 37°C, respectively. Selenite cystine broth was used instead of Mueller-Kauffmann tetrathionate novobiocin (MKTTn) broth. The culture was then streaked onto two selective agars: xylose lysine desoxycholate (XLD) (Merck, Wadeville) and brilliant green agar (BGA) (Scharlau Chemie SA, Barcelona) and incubated at 37°C for 24 h. The presumptive *Salmonella* colonies were then confirmed serologically and biochemically. For serological confirmation, omnivalent antisera (Siemens, Marburg) were used. For biochemical confirmation, the following tests were performed: triple sugar iron reactions, urea production, the Voges-Proskauer reaction, indole reaction, lysine decarboxylase reaction and the detection of β-galactosidase.

Confirmed strains were then identified biochemically using miniaturised commercial systems (Vitek-BioMérieux, Marcy L'Étoile). Serological identification with commercially available antisera (State Serum Institute, Copenhagen) for detection of somatic and flagellar antigens was performed and the isolates were identified and named in accordance with the Kauffman-White scheme (22).

Antimicrobial susceptibility test

Antimicrobial susceptibility testing was performed on confirmed *Salmonella* strains using the disk diffusion method on Mueller-Hinton agar (Merck, Darmstadt) plates according to the National Committee for Clinical Laboratory Standards Guidelines (19). The zone of inhibition (sensitive, intermediate and resistant) was interpreted according to Popoff (22) and Kirby *et al.* (15). The resistance of *Salmonella* isolates was examined against 16 antimicrobial substances, as follows:

- nalidixic acid (30 µg)
- ampicillin (10 µg)
- amoxicillin-clavulanic acid (20 µg/10 µg)
- cefazolin (30 µg)
- gentamicin (10 µg)
- kanamycin (30 µg)
- enrofloxacin (5 µg)
- trimethoprim-sulfamethoxazole (1.25 µg /23.75 µg)
- tetracycline (30 µg)
- cefotaxime (30 µg)
- sulfisoxazole (250 µg-300 µg)
- colistin (10 µg)
- streptomycin (10 µg)
- chloramphenicol (30 µg)
- cephalothin (30 µg)
- ciprofloxacin (5 µg).

The diameters of zones of inhibition were recorded to the nearest millimetre and classified as susceptible, intermediate and resistant.

Results

Prevalence rate

From a total of 650 samples of blood meal and meat-and-bone meal examined for the presence of *Salmonella*, 10.9% ($n = 71$) were found to be positive for *Salmonella*. The *Salmonella* prevalence in blood meal and in meat-and-bone meal samples are reported in Table I. The prevalence rate of *Salmonella* in meat-and-bone meal samples ($n = 564$) was 10.3%, whereas in blood meal samples ($n = 86$), the prevalence rate was 15.1%. A total of 29 *Salmonella* serovars were identified from one or both sample types, with *S.* Chester being the most frequently isolated, followed by *S.* Schwarzengrund and *S.* Chartres (Table I). Four strains were found to be positive for *Salmonella* group C1 but could not be identified because they did not express the phase 2 'H' antigens.

Table I
Distribution of *Salmonella* serovars isolated in animal feeds, by source, total number of isolates and prevalence

Salmonella serovar	Number of isolates per source		Number of isolates	Prevalence (%) (n = 71)
	Blood meal	Meat-and-bone meal		
S. Eppendorf	0	2	2	2.8
S. Schwarzengrund	5	4	9	12.7
S. Reading	0	2	2	2.8
S. Chartres	1	3	4	5.6
S. Braenderup	0	3	3	4.2
S. Chester	1	13	14	19.7
S. Anatum	2	0	2	2.8
S. Sandiego	0	1	1	1.4
S. Onderstepoort	0	2	2	2.8
S. Beaudesert	0	1	1	1.4
S. Fischerkietz	0	2	2	2.8
S. Ball	0	1	1	1.4
S. Sarajane	0	1	1	1.4
S. Brezany	0	2	2	2.8
S. Stanley	0	1	1	1.4
S. Southbank	0	1	1	1.4
S. Djugu	0	1	1	1.4
S. Petahtikve	0	2	2	2.8
S. Vaertan	0	1	1	1.4
S. Mbandaka	0	1	1	1.4
S. Djermaia	0	1	1	1.4
S. Infantis	0	3	3	4.2
S. Parkroyal	0	1	1	1.4
S. Kaapstad	1	0	1	1.4
S. Typhimurium	2	1	3	4.2
S. Bahrenfeld	1	0	1	1.4
S. Svedvi	0	2	2	2.8
S. Aflao	0	1	1	1.4
S. Saintpaul	0	1	1	1.4
Salmonella Group C1*	0	4	4	5.6
Total	13 (18.3%)	58 (81.7%)	71 (100.0%)	100.0

* non-typeable isolates of *Salmonella* group C1

Antimicrobial resistance

In our study, all *Salmonella* isolates that tested positive for antimicrobial resistance showed resistance to one or more of the 16 antimicrobials employed, namely, to nalidixic acid, trimethoprim-sulfamethoxazole, sulfisoxazole, streptomycin and/or tetracycline (Table II). In total, 14 of 40 isolates belonging to 10 different *Salmonella* serovars showed antimicrobial resistance to one or more of the antimicrobials tested. Eleven (15.5 %) of 71 isolates that belonged to eight of 29 different serovars showed resistance to sulfisoxazole and the trimethoprim-sulfamethoxazole combination. Only two (2.8%) of the isolates showed resistance to nalidixic acid.

Table II
Salmonella antimicrobial resistance pattern and the prevalence of resistant strains

Salmonella serovar	Antimicrobial resistance pattern	No. of resistant strains	Prevalence of resistant strain (%)
S. Eppendorf	AN[a]	2 (2)	100.0
S. Schwarzengrund	SXT[a], SULF[a]	2 (9)	22.0
S. Reading	SXT[a], SULF[a]	2 (2)	100.0
S. Chartres	SXT[a], SULF[a], S[b]	2 (4)	50.0
S. Chester	SULF[a]	1 (14)	7.1
S. Sandiego	SXT[a], SULF[a]	1 (1)	100.0
S. Onderstepoort	SXT[a], SULF[a]	1 (2)	50.0
S. Beaudesert	SXT[a], SULF[a]	1 (1)	100.0
S. Fischerkietz	SXT[a], SULF[a], TE[b]	1 (2)	50.0
S. Typhimurium	SXT[a], SULF[a]	1 (3)	33.3
Total		14 (40)	35.0

a) resistant
b) intermediate

AN nalidixic acid
SXT trimethoprim-sulfamethoxazole
SULF sulfisoxazole
CTX cefotaxime
S streptomycin
TE tetracycline

Discussion

Animal feed (or at least the precursors of animal feed) can be a common source of *Salmonella* infection in humans (5). This may happen when the food supply becomes contaminated with *Salmonella* and the pathogen subsequently comes into contact with humans. In Namibia, animal feed made from animal protein such as blood meal, meat-and-bone meal and carcass meal are produced locally, but they are not allowed to be fed to animals produced for food. These types of feeds are processed for pet food and for export to other countries. However, pets may also be a source of *Salmonella* infection for humans (4). Several other countries in the world are reported to have banned the use of feed derived from animal products for ruminants, including the United States (6) to prevent the potential occurrence of transmissible spongiform encephalopathy (TSE) in humans. Thus, microbial monitoring of animal feeds remains important in food animal production.

Our study on the prevalence of *Salmonella* in animal feed is the first of its kind in Namibia. This study showed that the prevalence of *Salmonella* in feed was lower than in previously reported findings from other studies (20, 23). A study conducted by Sartorelli *et al.* (23) on the nutritional and microbial evaluation of meat-and-bone meal found *Salmonella* contamination to be as high as 90% in the samples tested. In an Australian study, the prevalence rate of *Salmonella* in meat-and-bone meal samples was as high as 69.5% (2). However, a study by Gopo and Banda (11) found no *Salmonella* contamination in all bone-and-meat meal samples processed in Zimbabwe for export purposes. The differences between these findings could be due to the species of animals slaughtered, the relative prevalence of *Salmonella* serovars in different animal species, processing methods and possible recontamination during handling, storage and transportation after processing. Recontamination of animal by-products after processing is usually believed to be the principal factor accounting for the presence of *Salmonella* in the final product (6, 10).

The prevalence of the isolated *Salmonella* strains in feed samples in Namibia showed *Salmonella* serovar Chester to be isolated more often than other serovars. The six most

frequently isolated serovars were *S.* Chester, followed by *S.* Schwarzengrund and *S.* Chartres and then by *S.* Infantis, *S.* Typhimurium and *S.* Braenderup, each having the same number of isolates. *Salmonella* Enteritidis was not isolated at all. These findings were different from other studies where *S.* Enteritidis was found to be the most commonly isolated *Salmonella* serovar. Bouchrif *et al.* (3) reported *S.* Enteritidis and *S.* Anatum to be the most common serovars isolated in Africa, whereas *S.* Infantis, followed by *S.* Enteritidis and *S.* Typhimurium, constituted the largest proportion of isolates in Europe. However, few studies, if any, have been performed on this subject in this part of Africa to enable comparison of our results with previous findings. Findings reported in Kenya, Malawi and Mozambique (12, 14, 18) showed that *S.* Enteritidis and *S.* Typhimurium were the most common clinical isolates from humans. However, our findings show that *S.* Enteritidis may not be the most common *Salmonella* serovar in this part of the southern African region.

Although our study did not focus on biomedical aspects, the present findings indicate that *Salmonella* in animal feed may be a significant cause of bacterial diseases in Namibia. The association between contaminated animal feed and *Salmonella* infections in humans through the food chain has been reported previously by different authors (5, 7, 17). While *S.* Chester is not commonly isolated from or linked to outbreaks of salmonellosis in humans in Africa, a recent report of the United States Centres for Disease Control and Prevention showed that 44 people were infected with this serovar in 18 states in the United States from April to August 2010 (4). These findings show that *S.* Chester could be an important foodborne pathogen of public health concern worldwide. Such conclusions cannot be reached in this particular study unless further clinical studies are conducted on the pathogens isolated from humans in Namibia. The isolation of *S.* Onderstepoort, which is usually referred as the 'mutton type' of *Salmonella*, was not surprising because the meat-and-bone meal was produced from bovine and ovine meat by-products.

Concerning the antimicrobial resistance pattern of *Salmonella* strains, the susceptibility reported in this study was not comparable to findings reported previously. A study in the United States has reported *Salmonella* isolates to have a susceptibility rate of 40% against the 13 antimicrobials used (1). A different study conducted in Alberta found that of 3 553 antimicrobial susceptibility tests conducted with 17 antimicrobials on each of 209 *Salmonella* strains isolated from food animals and foods, 11.8% of *Salmonella* isolates were positive for resistance (13). Comparing our findings to the studies in the United States and Canada, we noted that the prevalence of *Salmonella* in animal feed in Namibia does not appear to be similar to that observed in many other countries where fewer effective antimicrobials are available for the treatment of *Salmonella* infections.

The findings of the present study on antimicrobial resistance were different from those reported elsewhere. In other studies, most of the *Salmonella* strains were shown to be resistant to tetracycline (24). This study showed that only 11 of the 14 resistant strains were resistant to sulfisoxazole and the trimethoprim-sulfamethoxazole combination, whereas two strains were additionally resistant to streptomycin and one to tetracycline. These results were unexpected, since tetracycline has long and extensively been used in Namibia as it has in many other parts of the world (24). Our findings suggest that there is a need to control the introduction of tetracycline-resistant pathogens into Namibia and ensure the prudent use of tetracycline, in order to avoid the development of resistance to this drug.

Resistance to nalidixic acid was present in two isolates of one serovar and to sulfisoxazole and trimethoprim-sulfamethoxazole in isolates of eight serovars. Although these frequencies were low, resistance to these antimicrobial substances may still be a public health concern if these foodborne pathogens enter the food chain. The animal feed used in this study was produced from meat by-products which suggest the possibility of such pathogens being present in the food chain.

Conclusions

In conclusion, most of the *Salmonella* strains isolated in Namibia were susceptible to the antimicrobials tested, suggesting that widespread antimicrobial resistance is not a significant problem in Namibia, as it is in other parts of the world. This may be due to the fact that in Namibia both veterinary and humans drugs can only be obtained on prescription, thereby reducing the potential misuse of antimicrobials unless such use is deliberate. However, the evidence of antimicrobial resistance in some strains emphasises that resistance is developing and that further monitoring will be required to ensure that it does not become a problem in Namibia. Unlike other parts of the world, *S.* Chester appears to be the most prevalent serovar in animal feed ingredients in Namibia. However, only one of the 14 strains of this serovar was drug-resistant and only to sulfisoxazole. These findings suggest that in case of infections from this serovar, a range of antibiotics that are available in Namibia may be used to control the disease. Yet there is little information, if any, on either the prevalence of *S.* Chester in feed, or the antimicrobial resistance pattern of this serovar in other countries which could be compared with our findings. Although the surveillance of foodborne pathogens in Namibia and other developing countries is minimal, antimicrobial resistance is still a major public health concern in Africa. Nevertheless, the findings of the present study may help improve the prudent use of antimicrobials in Namibia and may help direct the proper selection of antimicrobials for the treatment of infections in both humans and animals in Namibia.

Acknowledgments

The authors thank Ms R. Heita and Ms E. Namupala of the Central Veterinary Laboratory in Windhoek, Namibia for their assistance in isolating *Salmonella*. The authors would also like to thank the *Istituto G. Caporale* in Teramo, Italy, for their support in the serotyping and testing of antimicrobial resistance of the *Salmonella* isolates.

References

1. Arthur T.M., Brichta-Harhay D.M., Bosilevac J.M, Guerin M.N., Kalchayanand N., Wells J.E., Shackelford S.D., Wheeler T.L. & Koohmaraie M. 2008. Prevalence and characterization of *Salmonella* in bovine lymph nodes potentially destined for use in ground beef. *J Food Prot*, **71** (8), 1685-1688.
2. Bensink J.C. 1979. *Salmonella* contamination of meat and bone meal. *Aust Vet J*, **5**, 13-15.
3. Bouchrif B., Paglietti B., Murgia M., Piana A., Cohen N., Ennaji M.M., Rubino S. & Timinouni M. 2009. Prevalence and antibiotic-resistance of *Salmonella* isolated from food in Morocco. *J Infect Dev Ctries*, **3** (1), 35-40 (www.jidc.org/index.php/journal/article/view/19749447 accessed on 2 May 2012).
4. Centres for Disease Control and Prevention (CDC) 2010. Investigation update: Multistate outbreak of human *Salmonella* Chester infections. (www.cdc.gov/salmonella/chester/ accessed on 2 May 2012).
5. Crump J.A., Griffin P.M. & Angulo F.J., 2002. Bacterial contamination of animal feed and its relationship to human foodborne illness. *Clin Infect Dis*, **35**, 859-865.
6. Denton J.H., Coon C.N., Pettigrew J.E. & Parsons C.M. 2005. Historical and scientific perspectives of same species feeding of animal by-products. *J Appl Poult Res*, **14**, 352-361.
7. European Food Safety Authority (EFSA) 2008. Microbiological risk assessment in feedingstuffs for food-producing animals. Scientific opinion of the Panel on Biological Hazards. *EFSA J*, **720**, 1-84 (www.efsa.europa.eu/en/efsajournal/doc/720.pdf accessed on 23 May 2012).
8. European Food Safety Authority (EFSA) 2010. Scientific opinion on a quantitative estimate of the public health impact of setting a new target for the reduction of *Salmonella* in laying hens. EFSA Panel on Biological Hazards (BIOHAZ). *EFSA J* **8** (4), 1546 (86 pp) (www.efsa.europa.eu/fr/efsajournal/doc/1546.pdf accessed on 23 May 2012).

9. European Food Safety Authority (EFSA) 2010. Trends and sources of zoonoses and zoonotic agents and food-borne outbreaks in the European Union in 2008. Community summary report. *EFSA J*, **8** (1), 1496 (410 pp) (www.efsa.europa.eu/en/efsajournal/doc/1496.pdf accessed on 23 May 2012).
10. Franco D.A. 2006. The rendering industry's role in feed and food safety. *In* Essential rendering: all about the animal by-products industry (D.L. Meeker, ed). Kirby Lithographic Company, Inc., Arlington, Virginia, 53 pp (assets.nationalrenderers.org/essential_rendering_book.pdf accessed on 5 March 2012).
11. Gopo J.M. & Banda G.N. 1997. Occurrence of *Salmonella* on meat and meat products in an ostrich abattoir as determined with a DNA probe. *S Afr J Anim Sci,* **27** (1), 1-6.
12. Gordon M.A., Graham S.M., Walsh A.L., Wilson, L, Phiri A., Molyneux E., Zijlstra E.E., Heyderman R.S., Hart C.A. & Molyneux M.E. 2008. Epidemics of invasive *Salmonella enterica* serovar Enteritidis and *S. enterica* serovar Typhimurium infection associated with multidrug resistance among adults and children in Malawi. *Clin Infect Dis*, **46**, 963-969.
13. Johnson J.M., Rajic A. & McMullen L.M. 2005. Antimicrobial resistance of selected *Salmonella* isolates from food animals and food in Alberta. *Can Vet J*, **46**, 141-146.
14. Kariuki S., Revathi G., Gakuya F., Yamo V., Muyodi J. & Hart A. 2002. Lack of clonal relationship between non-Typhi *Salmonella* strain types from humans and those from animals living in close contact. *FEMS Immunol Med Microbiol*, **33**, 165-171.
15. Kirby J., Bauer A.W., Sherris C. & Turck M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol*, **45**, 493-496.
16. Majowicz S.E., Musto J., Scallan E., Angulo F.J., Kirk M., O'Brien S.J., Jones T.F., Fazil A. & Hoekstra R.M. 2010. The global burden of nontyphoidal *Salmonella* gastroenteritis. *Clin Infect Dis*, **50** (6), 882-889.
17. Molla B., Alemayehu D. & Salah W. 2003. Sources and distribution of *Salmonella* serotypes isolated from food animals, slaughterhouse personnel and retail meat products in Ethiopia: 1997-2002. *Ethiop J Health Dev*, **17** (1), 63-70.
18. Morpeth S.C., Ramadhani H.O. & Crump J.A. 2009. Invasive non-Typhi *Salmonella* disease in Africa. *Clin Infect Dis*, **49**, 606-611.
19. National Committee for Clinical Laboratory Standards (NCLLS) 2002. Performance standards for antimicrobial susceptibility testing; approved standards – M100-S12. NCCLS, Wayne, Pennsylvania, 22 pp.
20. Newell K.W., McClarin R., Murdock C.R., MacDonald W.N. & Hutchinson H.L. 1959. Salmonellosis in Northern Ireland, with special reference to pigs and *Salmonella* contaminated pig meal. *J Hyg (Lon)*, **57**, 92-105.
21. Okeke I.N., Laxminarayan R., Bhutta Z.A, Duse A.G., Jenkins P., O'Brien T.F., Pablos-Mendez A. & Klugman K.P. 2005. Antimicrobial resistance in developing countries. Part I: recent trends and current status. Review. *Lancet Infect Dis*, **5**, 481-493.
22. Popoff M.Y. 2001. Antigenic formulas of the *Salmonella* serovars, 8th Ed. WHO Collaborating center for reference and research on *Salmonella*. Institut Pasteur, Paris, 150 pp.
23. Sartorelli S.A., Bertechini A.G., Fassani E.J., Kato R.K. & Fialho E.T. 2003. Nutritional and microbiological evaluation of meat-and-bone meal produced in the State of Minas Gerais. *Braz J Poult Sci*, **5** (1), 51-60.
24. Speer B.S., Shoemaker N.B. & Salyers A.A. 1992. Bacterial resistance to tetracycline: mechanisms, transfer, and clinical significance. *Clin Microbiol Rev*, **5** (4), 387-399.
25. United States Department of Agriculture (USDA) 2011. Foodborne illness cost calculator. USDA, Washington, DC (www.ers.usda.gov/Data/FoodborneIllness/ accessed on 19 May 2012).
26. World Health Organization (WHO) 2002. Antimicrobial resistance. WHO, Geneva, Factsheet No. 194 (www.who.int/mediacentre/factsheets/fs194/en/ accessed on 23 May 2012).

