Evolution of antimicrobial resistance of *Salmonella enteritidis* (1972–2005)

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Dates:
Received: 23 Apr. 2014
Accepted: 09 July 2014
Published: 12 Nov. 2014

How to cite this article:
Khumalo, J., Saidi, B. & Mbanga, J., 2014, 'Evolution of antimicrobial resistance of *Salmonella enteritidis* (1972–2005)', *Onderstepoort Journal of Veterinary Research* 81(1), Art. #807, 6 pages. http://dx.doi.org/10.4102/ojvr.v81i1.807

With the extensive use of antibiotics in livestock production, surveillance revealed an increase in salmonella resistance to the commonly used antimicrobials in veterinary and public health. This serious threat to health care is further exacerbated by the limited epidemiological information about the common zoonotic agent, *Salmonella enteritidis*, required to determine antibiotic therapy. The aim was to characterise the antimicrobial resistance patterns of *S. enteritidis* isolates across different timelines (1972–2005) with accompanying genetic changes being investigated. Thirty-seven stored *S. enteritidis* isolates were collected from the Central Veterinary Laboratory, Harare, with antimicrobial susceptibility determined against eight antibiotics. Plasmids were isolated to analyse any genetic variation. An overall significant increase in resistance ($p < 0.05$) to nalidixic acid (0% – 10%), ampicillin (14.3% – 50%), tetracycline (14.3% – 30%) and erythromycin (71.4% – 100%) was observed across the timeline. However, the highest rates of susceptibility were maintained for gentamicin, sulphamethoxazole-trimethoprim, kanamycin and chloramphenicol. We report an increase in multidrug resistance (MDR) of 14.2% – 50% with an increase in resistotypes and plasmid profiles across the timeline. Eleven plasmid profiles were obtained in the 37 isolates studied with a minority of isolates (21.6%, 8/37) harbouring a 54 kb plasmid, commonly serovar-specific. A concerning increase in antimicrobial resistance to commonly administered drugs was observed across the timeline. The surge in MDR is of great concern and implies the need for consistent antimicrobial stewardship. No correlation was observed between the plasmid and antibiotic profiles.

Introduction

Salmonella enterica is causing particular concern because of its increasing prevalence and resistance to multiple antibiotics, with mostly animal-borne serovars being multidrug resistant (Alvarez-Fernández *et al.* 2012; Ngoi & Thong 2013; Sanpong *et al.* 2010; Singh *et al.* 2010). *Salmonella enteritidis* is one of the most prevalent serovars with an increased display of antimicrobial resistance globally (Hendriksen *et al.* 2011; Okeke *et al.* 2007; Parry 2003). It is currently the world's leading cause of non-typhoidal salmonellosis (Centers for Disease Control and Prevention [CDC] 2011; Ngoi & Thong 2013), predominantly associated with eggs, egg products, poultry, the farm environment and cross-contamination of other foods from eggs (Perry & Yousef 2012). It has adapted to be host specific and is still a major public health risk in developing nations (Betancor *et al.* 2010; Okeke *et al.* 2007).

The epidemiology of Salmonella and its antimicrobial resistome from Africa is underreported (Sow *et al.* 2007). Since 1993, annual reports from the Central Veterinary Laboratory (CVL), Harare, indicate that the isolation frequency of *S. enteritidis* is increasing in chickens and other animal species, with an concomitant rise in the number of cases of human gastroenteritis in which the bacterium was isolated from faecal cultures (CVL 2001; Makaya *et al.* 1998). An increased occurrence of *S. enteritidis* in both large-scale commercial (LSC) poultry and small-scale commercial (SSC) poultry is most likely a reflection of an increase in flock prevalence in Zimbabwe (Matope, Makaya & Pfukenyi 2012). However, the implementation of control programmes in many countries, including Zimbabwe in the 1970s and 1980s, significantly reduced infection in poultry (Matope *et al.* 2012). An emergence of *S. enteritidis* was noted post-1998 (Makaya *et al.* 1998). In South Africa, the incidence of *S. enteritidis* escalated after the first poultry-associated outbreak in 1991 (Mare 1997), reaching an incidence rate of 9.3% (223 incidents) between 1996 and 2006 (Kidanemariam, Engelbrecht & Picard 2010).

As a consequence of the extensive use of antibiotics, especially in livestock production, surveillance networks have indicated that the incidence of human salmonellosis caused by antimicrobial resistant salmonella is rising in many countries (Breuil *et al.* 2000; Malorny,

Hauser & Dieckmann 2011). The use of medicated feeds in intensive animal husbandry systems and sub-therapeutic doses and indiscriminate use of antimicrobials that are often administered through the feed or drinking water for therapy, prophylaxis or growth promotion (Aarestrup 2005) have been implicated in the emergence of antibiotic resistance. This increase limits the empirical therapeutic options available for clinical cases that require antimicrobial treatment (Tamma, Cosgrove & Maragakis 2012). In this study, *S. enteritidis* animal isolates from different timelines were characterised and genotyped by antimicrobial susceptibility testing and plasmid profiling to map the bacterium's resistome profile and reveal its evolutionary pattern over time.

Methodology

Sample collection

A total of 37 stored *S. enteritidis* animal isolates were obtained from the CVL, Harare. The *S. enteritidis* isolates had been previously isolated and serotyped before preservation. They comprised seven freeze-dried isolates from 1972, 10 isolates from 1998, 10 from 2000 and 10 from 2005 which were cryopreserved in glycerol.

Antimicrobial susceptibility testing

Salmonella enteritidis isolates were subcultured overnight at 37 °C and microbial suspensions were made with the turbidity of the actively growing suspensions adjusted with sterile saline to obtain turbidity optically comparable to the 0.5% McFarland's standard for inoculation. The disc diffusion assay was used to determine antibiotic susceptibility of the isolates on Mueller Hinton agar (Oxoid, Basingstoke, Hampshire, UK). Each isolate was tested for antibiotic susceptibility using a panel of the following antibiotics: ampicillin (10 µg), gentamicin (10 µg), chloramphenicol (30 µg), erythromycin (15 µg), nalidixic acid (30 µg), kanamycin (30 µg), sulphamethoxazole-trimethoprim (25 µg) and tetracycline (25 µg). All antibiotic discs were from Oxoid (Basingstoke, Hampshire, UK). The plates were incubated at 37 °C for 24 h, and inhibition zones were measured. The results were interpreted according to Clinical and Laboratory Standards Institute guidelines (CLSI 2007). *Escherichia coli* (ATCC 25922) was used as a reference strain in all tests.

Plasmid isolation

Overnight cultures of *S. enteritidis* isolates were prepared in 5 mL nutrient broth (Sigma-Aldrich, St Louis, USA). Plasmid isolation was carried out using the GFX Microplasmid Preparation Isolation Kit (Amersham Biosciences, Uppsala, Sweden) with the isolated plasmids electrophoresed on 0.7% agarose gel and viewed using a Kodak Gel Logic 100 imaging system (Eastman Kodak, Rochester, New York, USA). V517 and 39R861 *E. coli* strain plasmids were used as plasmid markers.

Data analysis

The statistical analysis of the results obtained was performed using Microsoft Excel 2007 (Microsoft Corporation, Redmond, Washington, USA). Data were statistically analysed using one-way analysis of variance (ANOVA) to assess statistical variance between the obtained mean zones of inhibition across the different years of isolation relative to the panel of antibiotics. The results were considered to be statistically significant ($p < 0.05$) above a 95% confidence level.

Results

Antimicrobial susceptibility

The *S. enteritidis* isolates studied showed different antimicrobial susceptibility patterns across the different timelines (Table 1). Total susceptibility was observed for nalidixic acid in samples from 1972, kanamycin in samples from 1998, and both nalidixic acid and tetracycline in samples from 2000, with no significant trend across the timeline (Table 1). However, the isolates were significantly susceptible to sulphamethoxazole-trimethoprim, gentamicin, kanamycin and chloramphenicol across the timeline. There was a significant increase in ampicillin resistance (14.3% – 50%) from 1972 to 2005 (Table 1). Similar increases were observed for nalidixic acid (0% – 10%), tetracycline (14.3% – 30%) and erythromycin (71.4% – 100%) over the same time period.

TABLE 1: General percentages of antimicrobial resistance amongst *Salmonella enteritidis* isolates obtained from the Central Veterinary Laboratory (1972–2005).

Isolation year	Resistance	Antimicrobial profile %							
		AMP*	T*	K	SXT	GEN	C	NAL*	E*
2005	Sensitive	10	40	70	40	90	50	40	0
	Intermediate	40	30	30	30	10	20	50	0
	Resistant	50	30	0	30	0	30	10	100
2000	Sensitive	0	100	60	80	70	80	100	20
	Intermediate	20	0	40	10	10	20	0	10
	Resistant	80	0	0	10	20	0	0	70
1998	Sensitive	70	70	100	80	80	60	70	0
	Intermediate	10	20	0	0	20	40	30	10
	Resistant	20	10	0	20	0	0	0	90
1972	Sensitive	57.1	57.1	71.4	85.7	28.6	42.9	100	14.3
	Intermediate	28.6	28.6	28.6	0	71.4	57.1	0	14.3
	Resistant	14.3	14.3	0	14.3	0	0	0	71.4

AMP, ampicillin; T, tetracycline; K, kanamycin; SXT, sulphamethoxazole-trimethoprim; GEN, gentamicin; C, chloramphenicol; NAL, nalidixic acid; E, erythromycin.
*, $p < 0.05$ for comparison of mean zones of inhibition across the timeline for each antibiotic

Overall resistance was distinguished in the 35 isolates, which were then grouped into 14 resistotypes arbitrarily designated as A to N. Resistotype C was the most represented, in 37.8% of the isolates. Additionally, 21.6% (8/37) of the tested isolates were resistant to three or more antimicrobial drugs, that is, multidrug resistance (MDR) which increased from 14.2% (1/7) to 50% (5/10) across the timeline.

Plasmid profiles

Of the *S. enteritidis* isolates tested, 86.4% harboured plasmids similar in size and distribution. Each isolate had a different plasmid content, although with variation in the plasmid profile, and some profiles were shared across the different timelines (Table 2). A minority of isolates (27%; 10/37), harboured only one plasmid, and 78.4% (29/37) harboured a 54 kb plasmid that corresponds with the serovar-specific (*enteritidis*) 55 kb plasmid which has been found in several *S. enteritidis* isolates (Helmuth *et al.* 1985). Forty-eight percent of the isolates harboured two plasmids, and only four isolates harboured three plasmids. A total of 11 different plasmid profiles (PP) were identified amongst the 37 isolates studied (Table 2).

Discussion

Antimicrobial susceptibility profiles

In this study, a total of 37 stored *S. enteritidis* veterinary isolates were obtained from the CVL, Harare. The isolates showed a relatively high susceptibility to all the antimicrobials; this could be indicative of low resistance strains circulating in animals. This was similarly observed by Matope *et al.* (2012). Overall high susceptibility to gentamicin was observed across the timelines (Table 1). A similar susceptibility to gentamicin was reported in other parts of the world (Breuil *et al.* 2000; Cardoso *et al.* 2006; Ribeiro *et al.* 2007), even with *S. enteritidis* human isolates in Spain in 1998 (Cruchaga *et al.* 2001). Given the reports, gentamicin conclusively remains effective against *Salmonella* infections. Additionally, a maintained high susceptibility of all the isolates across the timelines to sulphamethoxazole-trimethoprim, kanamycin

TABLE 2: Antimicrobial resistance patterns and plasmid profiles of *Salmonella enteritidis* isolates obtained from the Central Veterinary Laboratory (1972–2005).

Isolation year	Sample identity	Resistotype	Drug resistance	Multidrug resistance %	Plasmid content (kb)	Plasmid profile
1972	S1	A	AMP, E, T	-	54, 4.5	PP1
	S1	B	E, SXT	-	54, 11.5, 5.6	PP2
	S3	C	E	-	54, 11.5, 5.6	PP3
	S4	C	E	-	54, 5.6	PP3
	S5	C	E	14.2	54	PP4
	S6	-	-	-	54	PP4
	S7	-	-	-	-	-
1998	P488	D	AMP, E	-	54, 4.5	PP1
	K350	D	AMP, E	-	54, 4.5	PP1
	P532	E	E, T, SXT	-	54, 4.5	PP1
	P539	F	SXT	-	54, 11.4, 4.5	PP5
	C422	C	E	14.2	-	-
	C4420	C	E	-	-	-
	C420	C	E	-	-	-
	P530	C	E	-	54, 4.5	PP1
	P533	C	E	-	54, 5.6, 4.5	PP6
	S11	C	E	-	54, 2.9	PP1
2000	23B	D	AMP, E	-	54, 2.9	PP7
	28H	D	AMP, E	-	54, 2.9	PP7
	16E	D	AMP, E	-	54	PP4
	61	D	AMP, E	14.2	54	PP4
	29A	G	AMP, E, GEN	-	2.9	PP8
	29B	H	AMP, GEN	-	59, 2.9	PP7
	23A	I	AMP, SXT	-	54, 2.9	PP7
	47	C	E	-	54	PP4
	53	C	E	-	54, 11.4	PP9
	16C	J	AMP	-	54	PP4
2005	23A	D	AMP, E	-	54, 2.9	PP7
	28	D	AMP, E	-	54, 4.5	PP1
	26A	K	AMP, SXT, C, E	-	-	-
	29A	C	E	50	54, 4.5	PP1
	26B	C	E	-	54	PP4
	23B	C	E	-	2.9	PP8
	30A	L	NAL, SXT, C, E	-	54, 34	PP10
	30B	M	T, SXT, C, E	-	54	PP4
	16D	A	AMP, T, E	-	54, 5.9	PP11
	16E	A	AMP, T, E	-	54, 5.9	PP11

AMP, ampicillin; T, tetracycline; GEN, gentamicin; C, chloramphenicol; NAL, nalidixic acid; E, erythromycin; SXT, sulphamethoxazole-trimethoprim; PP, plasmid profile.

and chloramphenicol supports their continued therapeutic use regardless of the overall increase in resistance.

A slight increase in resistance (0% – 10%) to nalidixic acid for the period 2000–2005 was observed (Table 1). This is similar to other reports of an increase in resistance to nalidixic acid from 0.8% to 8.5% (1995–2000) in France (Breuil *et al.* 2000) and from 0.4% to 1.3% (1994–1997) in England (Threlfall, Rowe & Ward 1999). This trend was reported to be ascribable to probable cross-resistance between quinolones and commonly used fluoroquinolones (Hwang *et al.* 2010; Oliveira, Brandelli & Tondo 2006). Studies have shown that the use of fluoroquinolones contributed to the emergence and dissemination of nalidixic acid cross-resistance in Salmonella amongst food animals (Ngoi & Thong 2013; Parry 2003; Stevenson *et al.* 2007; Velge, Cloeckaert & Barrow 2005). In Germany, a similar incidence of quinolone resistance for the period 1986–1998 in salmonella isolated from cattle, poultry and pigs increased in the years following the licensing of fluoroquinolones (Malorny, Schroeter & Helmuth 1999). However, there is reported quinolone resistance that is chromosomally mediated (point mutation of the gyrase A gene) (Parry 2003).

A significant increase in ampicillin resistance is rather worrying as the drug is commonly used in the treatment of human salmonellosis. This was similarly observed in Greece, where ampicillin resistance increased from 10% to 52% (1987–1991) (Tassios *et al.* 1997). In addition, some studies have reported high resistance patterns (in animals and humans) in France and Spain (Breuil *et al.* 2000; Cruchaga *et al.* 2001). The fact that 51.3% of the isolates exhibited resistance to two or more antibiotics is of public health concern because of the potential spread of resistant clones, in addition to the development of resistance to first-line therapy drugs. However, further investigation is required to confirm the seemingly high antimicrobial resistance. This percentage was higher than observed in *S. enteritidis* isolates exhibiting resistance in avian populations in Zimbabwe (25.7%) (Matope *et al.* 2012) and than observed for South African isolates tested (50%) (Mare, Dicks & Van der Walt 2001).

The period 1972–2000 saw reasonably controlled and intensified prevention strategies for drug use in the animal and human medical sectors. However, post-2000 saw the emergence of the fast-tracked land reform programme, which led to the economic meltdown and low socioeconomic status of the country. This affected the health system and policy on antibiotic distribution and usage (Asante 2012; Trane & Bate 2005). This period saw reduced availability of antibiotics to the population (thus increasing demand) and consequently an influx of counterfeit drugs, with antibiotics constituting an important part of the counterfeit medicines (Centre for Public Accountability [CPA] n.d.). With a collapse in legislation and implementation in the health sector, parallel street-market pharmacies were perpetuated with limited and weak deterrent surveillance authorities (CPA n.d.). This explains the significantly reduced susceptibility observed from 2000 to 2005.

Emergence of multidrug resistance

An overall increase in multidrug resistance (resistance to ≥ 3 antibiotics) (Zurfluh *et al.* 2013) from 14.2% in 1972 to 50% in 2005 was observed (Table 2). This was greater than the MDR prevalence (12.1%) previously reported by Matope *et al.* (2012). The widespread veterinary use of a number of antimicrobials for therapy, prophylaxis or growth promotion (Cardoso 2006; World Health Organization [WHO] 2007) are probable causes. *S. enteritidis* isolated from diarrheal patients in Zimbabwe showed low MDR (Simango & Mbewe 2000). Similar low MDR in human isolates was reported for the period 1996–2000 in England and Wales (Oliveira *et al.* 2006). Relative to other reported MDR incidences, namely 0.4% (Threlfall 2002), 21% (Yang *et al.* 2002) and 16.5% (Oliveira *et al.* 2006), the observed increase to 50% in this study is of great concern.

Plasmid profiles

A general inconsistency in distribution of plasmid content was observed in the tested isolates. Transferable genetic resistance factors have been implicated in inducing variable resistance in strains (Olsen *et al.* 1993) contributing to salmonella evolution (Guard *et al.* 2011). Besides the serovar-specific 54 kb plasmid, additional smaller plasmids of lower molecular weight were observed – these have been associated with antibiotic resistance (Brown *et al.* 1994).

No specific plasmid profile could be linked to any of the antimicrobial resistance profiles. This shows the unrelatedness of clonal lines even with similar plasmid profiles (Mare *et al.* 2001; Olsen *et al.* 1993). However, a marked increase in plasmid profiles was observed with increasing multidrug resistance. Unrestricted access to and use of antibiotics influences the acquisition of resistance. This emergence of MDR *S. enteritidis* strains and possible association with systemic illness is a cause for concern in public health. Further studies are required to determine the nature of these plasmids and to assay for specific resistance genes and the conditions which favour selection of antimicrobial resistance in *S. enteritidis* isolates in Zimbabwe. There is a need for continual antimicrobial resistance surveillance in Zimbabwe and other developing countries.

Limitations

Potential biases in our study are related to the relatively small sample size of unrelated *S. enteritidis* field isolates investigated. The lack of other molecular typing strategies (macrorestriction analysis, pulsed-field gel electrophoresis, polymerase chain reaction) limited the conclusion on the relatedness of the isolates and the evolutionary profile of their resistance genes.

Recommendations

Possible horizontal transfer of resistance genes could be speculated, thus more stringent methods for analysis are required to characterise evolutionary events, including an increased sample size of isolates for a definite molecular

epidemiological conclusion. Monitored surveillance of *Salmonella enteritidis*' antimicrobial resistance patterns is required to follow up on emerging resistance, for which the study provides a baseline.

Conclusion

The study reveals an evolution in the antimicrobial resistance profiles of *S. enteritidis*. An overall increase in antibiotic resistance was shown across the timeline for the *S. enteritidis* isolates studied, along with an additional increase in multidrug resistance. This calls for the implementation of antimicrobial stewardship, monitoring and control in the veterinary health sector.

Acknowledgements

Competing interests

The authors declare that they have no financial or personal relationship(s) which may have inappropriately influenced them in writing this article.

Authors' contributions

J.K. (National University of Science and Technology) was responsible for the project design and experimental work carried out in the project. J.M. (National University of Science and Technology) was the principle supervisor for the project, responsible for the management of the project and the guidance of J.K. in all aspects of the project. J.M. also assisted in the experimental design of the project. B.S. (Central Veterinary Laboratory) acted as co-supervisor and provided assistance with the isolates studied. All three authors contributed to the writing of this article.

References

Aarestrup, F.M., 2005, 'Veterinary drug usage and antimicrobial resistance in bacteria of animal origin', *Basic and Clinical Pharmacology and Toxicology* 96(4), 271–281. http://dx.doi.org/10.1111/j.1742-7843.2005.pto960401.x

Alvarez-Fernández, E., Alonso-Calleja, C., García-Fernández, C. & Capita, R., 2012, 'Prevalence and antimicrobial resistance of salmonella serotypes isolated from poultry in Spain: Comparison between 1993 and 2006', *International Journal of Food Microbiology* 153(3), 281–287. http://dx.doi.org/10.1016/j.ijfoodmicro.2011.11.011

Asante, A., 2012, 'National economic collapse and revival: The case of Zimbabwe', Bachelor's degree thesis, International Business, Arcada University of Applied Sciences, viewed 26 June 2014, from http://publications.theseus.fi/bitstream/handle/10024/65537/Ashley%20Asante.pdf?sequence=1

Betancor, L., Pereira, M., Martinez, A., Giossa, G., Fookes, M. & Flores, K., 2010, 'Prevalence of *Salmonella enterica* in poultry and eggs in Uruguay during an epidemic due to *Salmonella enterica* serovar Enteritidis', *Journal of Clinical Microbiology* 48(7), 2413–2423. http://dx.doi.org/10.1128/JCM.02137-09

Breuil, J., Brisabois, A., Casin, I., Armand-Lefèvre, L., Frémy, S. & Collatz, E., 2000, 'Antibiotic resistance in salmonellae isolated from humans and animals in France: Comparative data from 1994 and 1997', *Journal of Antimicrobial Chemotherapy* 46(6), 965–971. http://dx.doi.org/10.1093/jac/46.6.965

Brown, D.J., Baggesen, D.L., Hansen, H.B., Hansen, H.C. & Bisgaard, M., 1994, 'The characterization of Danish isolates of *Salmonella enterica* serovar Enteritidis by phage typing and plasmid profiling: 1980–1990', *Acta Pathologica, Microbiologica, et Immunologica Scandinavica* 102(3), 208–214. http://dx.doi.org/10.1111/j.1699-0463.1994.tb04866.x

Cardoso, M.O., Ribeiro, A.R., Santos, L.R., Pilotto, F., Moraes, H.L.S., Salle, C.T.P. *et al.*, 2006, 'Antibiotic resistance in *Salmonella enteritidis* isolated from broiler carcasses', *Brazilian Journal of Microbiology* 37, 368–371. http://dx.doi.org/10.1590/S1517-83822006000300030

Centers for Disease Control and Prevention (CDC), 2011, *FoodNet Surveillance Report for 2009*, US Department of Health and Human Services, Atlanta.

Clinical and Laboratory Standards Institute (CLSI), 2007, *Performance standards for antimicrobial susceptibility testing*, 17th information supplement, M100-S17, vol 27(1), Clinical and Laboratory Standards Institute, Wayne.

Centre for Public Accountability (CPA), n.d., *Zimbabwe battles counterfeit drugs*, Centre for Public Accountability, Harare, viewed 26 June, 2014 from http://www.cpazimbabwe.org.zw/index.php?option=com_content&view=article&id=9:zimbabwe-battles-counterfeit-drugs&catid=31:news&Itemid=9

Cruchaga, S., Echeita, A., Aludea, A., Garcia-Pena, J., Frias, N. & Usera, M.A., 2001, 'Antimicrobial resistance in salmonellae from humans, food and animals in Spain in 1998', *Journal of Antimicrobial Chemotherapy* 47(3), 315–321. http://dx.doi.org/10.1093/jac/47.3.315

Central Veterinary Laboratory (CVL), 2001, 'Monthly report, February 2001', Central Veterinary Laboratory, Harare.

Guard, J., Cesar, A., Fedorka-Cray, M.P. & Gast, R.K., 2011, 'Single nucleotide polymorphisms that differentiate two subpopulations of *Salmonella enteritidis* within phage type', *BMC Research Notes* 4(1), 369. http://dx.doi.org/10.1186/1756-0500-4-369

Helmuth, R., Stephan, R., Bunge, C., Hoog, B., Steinbeck, A. & Bulling E., 1985, 'Epidemiology of virulence-associated plasmids and outer membrane protein patterns with seven common salmonella serotypes', *Infection and Immunology* 48(1), 175–182.

Hendriksen, R.S., Vieira, A.R., Karlsmose, S., Lo Fo Wong, D.M., Jensen, A.B., Wegener, H.C. *et al.*, 2011, 'Global monitoring of salmonella serovar distribution, World Health Organization Foodborne Infections Network Country Data Bank: Results of quality assured laboratories from 2001 to 2007', *Foodborne Pathogens and Disease* 8(8), 887–900. http://dx.doi.org/10.1089/fpd.2010.0787

Hwang, I.S., Song, J.Y., Kim, W.Y., Jeong, H.W., Kim, M.S. & Cheong, H.J., 2010, 'Prevalence and mechanisms of low level quinolone resistance among non-typhoidal salmonella isolates from human and poultry/livestock in Korea: Usefulness of nalidixic acid resistance test', *Infection and Chemotherapy* 42(4), 230–236. http://dx.doi.org/10.3947/ic.2010.42.4.230

Kidanemariam, A., Engelbrecht, M. & Picard, J., 2010, 'Retrospective study on the incidence of Salmonella isolations in animals in South Africa, 1996 to 2006', *Journal of the South African Veterinary Association* 81, 37–44. http://dx.doi.org/10.4102/jsava.v81i1.94

Makaya, P.V., Matope, G., Schlundt, J., Aabo, S. & Baggesen, D.L., 1998, '*Salmonella enteritidis* infection in poultry in Zimbabwe: An emerging zoonosis in Zimbabwe', *Zimbabwe Veterinary Journal* 29(1), 32–139.

Malorny, B., Hauser, E. & Dieckmann, R., 2011, *Salmonella: From genome to function – New approaches in subspecies-level Salmonella classification*, Academic Press, Caister.

Malorny, B., Schroeter, A. & Helmuth, R., 1999, 'Incidence of quinolone over the period of 1986 to 1998 in veterinary salmonella isolated from Germany', *Antimicrobial Agents and Chemotherapy* 43(9), 2278–2282.

Mare, L., 1997, 'An epidemiological study of *Salmonella enterica* serovar Enteritidis in South Africa', M.Sc. thesis, Department of AgriSciences, University of Stellenbosch.

Mare, L., Dicks, L.M.T. & Van der Walt, M.L., 2001, 'Characterization of South African isolates of *Salmonella enteritidis* by phage typing, numerical analysis of RAPD-PCR banding patterns and plasmid profiles', *International Journal of Food Microbiology* 64(3), 237–245. http://dx.doi.org/10.1016/S0168-1605(00)00448-7

Matope, G., Makaya, P. & Pfukenyi, D., 2012, 'Distribution of salmonella serovars and antimicrobial susceptibility of *Salmonella enteritidis* from poultry in Zimbabwe', *Avian Pathology* 41(2), 221–226. http://dx.doi.org/10.1080/03079457.2012.667558

Ngoi, S.T. & Thong, K.L., 2013, 'Molecular characterization showed limited genetic diversity among *Salmonella enteritidis* isolated from humans and animals in Malaysia', *Diagnostic Microbiology and Infectious Disease* 77(4), 304–311. http://dx.doi.org/10.1016/j.diagmicrobio.2013.09.004

Okeke, I.N., Aboderin, O.A., Byarugaba, D.K., Ojo, K.K. & Opintan, J.A., 2007, 'Growing problem of multidrug-resistant enteric pathogens in Africa', *Emerging Infectious Diseases* 13(11), 1640–1646. http://dx.doi.org/10.3201/eid1311.070674

Oliveira, F.A., Brandelli, A. & Tondo, E.C., 2006, 'Antimicrobial resistance in *Salmonella enteritidis* from foods involved in human salmonellosis outbreaks in southern Brazil', *New Microbiologica* 29, 49–54.

Olsen, J.E., Brown, D.J., Skov, M.N. & Christensen, J.P., 1993, 'Bacterial typing methods suitable for epidemiological analysis: Application in investigations of salmonellosis among livestock', *Veterinary Quarterly* 15, 125–135. http://dx.doi.org/10.1080/01652176.1993.9694390

Parry, C.M., 2003, 'Antimicrobial drug resistance in *Salmonella enterica*', *Current Opinion in Infectious Diseases* 16(5), 467–472. http://dx.doi.org/10.1097/00001432-200310000-00014

Perry, J. & Yousef, A., 2012, '*Salmonella enteritidis* in shell eggs: Evolving concerns and innovative control measures', *Advanced Applied Microbiology* 81, 243–267. http://dx.doi.org/10.1016/B978-0-12-394382-8.00007-1

Ribeiro, A.R., Kellermann, A., Santos, L.R., Bessa, M.C. & Nascimento, V.P., 2007, '*Salmonella* spp. in raw broiler parts: Occurrence, antimicrobial resistance profile and phage typing of the *Salmonella enteritidis* isolates', *Brazilian Journal of Microbiology* 38(2), 296–299. http://dx.doi.org/10.1590/S1517-83822007000200021

Sanpong, P., Theeragool, G., Wajjwalku, W. & Amavisit, P., 2010, 'Characterization of multiple antimicrobial resistant salmonella isolated from pig farms in Thailand', *Kasetsart Journal* 44, 643–651.

Simango, C. & Mbewe, C., 2000, '*Salmonella enteritidis* diarrhoea in Zimbabwe', *Tropical Medicine and International Health* 5(7), 503–506. http://dx.doi.org/10.1046/j.1365-3156.2000.00587.x

Singh, B.R., Agarwal, M., Chandra, M., Verma, M., Sharma, G. & Verma, J.C., 2010, 'Plasmid profile and drug resistance pattern of zoonotic salmonella isolates from Indian buffaloes', *Journal of Infection in Developing Countries* 4, 477–483. http://dx.doi.org/10.3855/jidc.713

Sow, A.G., Wane, A.A., Diallo, M.H., Boye, C.S. & Aïdara-Kane, A., 2007, 'Genotypic characterization of antibiotic-resistant *Salmonella enteritidis* isolates in Dakar, Senegal', *Journal of Infection in Developing Countries* 1(3), 284–288.

Stevenson, J., Gay, K., Barrett, T., Medalla, F., Chiller, T. & Angulo, F., 2007, 'Increase in nalidixic acid resistance among non-typhi *Salmonella enterica* isolates in the United States from 1996 to 2003', *Antimicrobial Agents and Chemotherapy* 51(1), 195–197. http://dx.doi.org/10.1128/AAC.00222-06

Tamma, P.D., Cosgrove, S.E. & Maragakis, L.L., 2012, 'Combination therapy for treatment of infections with gram-negative bacteria', *Clinical Microbiology Reviews* 25(3), 450–470. http://dx.doi.org/10.1128/CMR.05041-11

Tassios, P.T., Markogiannakis, A., Vatopoulos, A.C., Katsanikou, E., Velonakis, E.N., Koureakremastinou, J. *et al.*, 1997, 'Molecular epidemiology of antibiotic resistance of *Salmonella enteritidis* during a 7-year period in Greece', *Journal of Clinical Microbiology* 35(6), 1316–1321.

Threlfall, E.J., 2002, 'Antimicrobial drug resistance in salmonella: Problems and perspectives in food and water-borne infections', *FEMS Microbiology Reviews* 26, 141–148. http://dx.doi.org/10.1111/j.1574-6976.2002.tb00606.x

Threlfall, E.J., Rowe, B. & Ward, L.R., 1999, 'Increase in multiple resistance in nontyphoidal salmonellas from humans in England and Wales: A comparison of data for 1994 and 1996', *Microbial Drug Resistance* 3(3), 263–266. http://dx.doi.org/10.1089/mdr.1997.3.263

Trane, R. & Bate, R., 2005, 'Despotism and disease: A report into the health situation of Zimbabwe and its probable impact on the region's health', Africa Fighting Malaria and American Enterprise Institute, Washington DC, viewed 26 June 2014, from http://www.aei.org/files/2005/03/11/20050311_AFM_Zim_disease_paper_hires.pdf

Velge, P., Cloeckaert, A. & Barrow, P., 2005, 'Emergence of salmonella epidemics: The problems related to *Salmonella enterica* serotype Enteritidis and multiple antibiotic resistance in other serotypes', *Veterinary Research* 36, 267–288. http://dx.doi.org/10.1051/vetres:2005005

World Health Organization (WHO), 2007, 'Critically important antimicrobials for human medicine: Categorization for the development of risk management strategies to contain antimicrobial resistance due to non-human antimicrobial use: Report of the 2nd WHO Expert Meeting', 29–31 May, Copenhagen, Denmark.

Yang, S.J., Park, K.Y., No, K.M., Besser, T.E., Yoo, H.S., Kim, S.H. *et al.*, 2002, 'Antimicrobial resistance in *Salmonella enterica* serovars Enteritidis and Typhimurium isolated from animals in Korea: Comparison of phenotypic and genotypic resistance characterization', *Veterinary Microbiology* 86, 295–301. http://dx.doi.org/10.1016/S0378-1135(02)00009-3

Zurfluh, K., Hachler, H., Nüesch Inderbinen, M. & Stephan, R., 2013, 'Characteristics of extended spectrum beta-lactamase (ESBL) and carbapenemase-producing Enterobactericeae isolated from rivers and lakes in Switzerland', *Applied and Environmental Microbiology* 79(9), 3021–3026. http://dx.doi.org/10.1128/AEM.00054-13