PREVALENCE AND ANTIBIOTIC SENSITIVITY PROFILE OF Salmonella SPECIES IN EGGS FROM POULTRY FARMS IN UMUDIKE, ABIA STATE

E. O. Ekundayo* and J. C. Ezeoke

Department of Microbiology, Michael Okpara University of Agriculture, Umudike, P.M.B 7267, Umuahia, Abia State

ABSTRACT

The increasing reports of the role of poultry products and eggs in the transmission of food borne salmonellosis around the world are raising international public health concern. In this regard a survey was carried out to determine the prevalence and antibiotic profile of Salmonella species in eggs from poultry farms around Umudike, Abia State. A total of two hundred and forty (240) eggs were sampled from four poultry farms. Salmonella species were isolated from 58 of the 240 eggs. The prevalence rates ranged between 8 and 45% with an overall prevalence of 24% of salmonella contamination of the eggs. The antibiotic sensitivity testing was carried out using the disc agar diffusion technique. All the 58 isolates were sensitive to Nitrofurantoin, 42 (72.4%) were sensitive to Gentamicin, 22 (37.9%) to Ofloxacin, 17 (29.3%) to Nalidixic acid while only 4 (6.9%) were sensitive to both Amoxycillin and Tetracycline. All isolates were resistant to Cotrimoxazole. The level of contamination of eggs with Salmonella species in this study calls for urgent need to control the level of Salmonella contamination of poultry farms in the study area. The high level of resistance of the isolates to commonly used antibiotics is really alarming and has great public health significance.

Key words: Antibiotic sensitivity profile, Salmonellosis, Eggs, Salmonella spp

INTRODUCTION

Infection of humans with non-typhoid salmonellae from animal sources is an emerging public health problem of significant economic and health burdens in both developed and developing countries (Doughari, *et al.* 2007). An estimated 1.4 millions cases of Salmonellosis involving 16,000 hospitalizations and nearly 600 deaths occurred each year in the United States (Zhao *et al.*, 2003). Estimates of the total patient-related cost of salmonellosis in 1998 in the United States of America ranged from \$275 million to \$1.1 billion (Irwin *et al.*, 1998).

Poultry, poultry products and eggs are major sources of Salmonella-related foodborne illness (Boonmar *et al.*, 1998, Pignato *et al.*, 1995, Irwin *et al.*, 1998, Stevens *et al.*, 1989). International concern over rising isolations of Salmonellae, and in particular, Salmonella enteritidis among humans, and the association with poultry and poultry products has led to the call for establishment of measures to control Salmonella in the poultry industry (Rodrigue et al., 1990). Programmes for active surveys of prevalence of Salmonella in poultry and poultry products were active in some developed countries such as Canada, (Irwin et al., 1998), Italy (Nastasi et al., 1997, Germany (Schroeter et al., 1994), USA (USDA,1994), Britain (Humphrey *et al.*, 1988) and more advanced developing countries like Thailand (Sasipreeyajan et al., 1996, Boonmar et al., 2003). There is paucity of data on the prevalence and antimicrobial resistance profile of salmonellae from food animals in the developing countries especially in Sub-Saharan Africa (Okoli et al., 2002). This paper reports a survey

conducted to assess the prevalence and antimicrobial susceptibility profile of Salmonella species from eggs from four poultry farms in Umudike, Abia State.

Multidrug resistant Salmonella serotypes, resistant to commonly used antimicrobial agents have been reported (Zhao et al., 2003, Poppe et al., 1995). Indiscriminate use of antimicrobial agents in clinical and veterinary practices is fuelling the increasing problem of antimicrobial resistance among serotypes of salmonellae. common practice The of using antimicrobial agents in animal production as growth promoters has potential for creating drug resistant strains of bacteria which can be transferred to humans who handle the birds and through the food chain (Okoli et al., 2002). Resistant bacteria can also transfer their resistant genes to other bacteria.

MATERIALS AND METHODS

Sources and Collection of Eggs

A total of 240 eggs were collected from four poultry farms located around Michael Okpara University of Agriculture, Umudike. Sixty (60) eggs were collected, ten eggs on six different days from each of the farms. The eggs were collected into clean plastic bags. The eggs were processed for microbiological analysis at Microbiology Laboratory, Department of Microbiology, Michael Okpara University of Agriculture, Umudike.

Preparation of Culture Media

The culture media used were Salmonella – Shigella Agar (SSA) (Fluka Biochemika, Fluka Chemie GmbH, India), Muller Hinton Agar (Fluka Biochemika, Fluka Chemie GmbH, Spain) and Selenite F broth Base (Biotec Laboratories Ltd, Martleham Heath Ipswich, UK). All the culture media were prepared according to the manufacturers' instructions.

Isolation of Organisms

The eggs were processed in batches of 10. The shell of each egg was washed with 70% alcohol and then rinsed in sterile distilled water in a beaker. The egg was transferred to a dry sterile beaker and aseptically broken with sterile forceps. The shell was removed and the content was homogenized using a sterile glass rod. 1ml of the homogenate was introduced into 9ml of Selenite F broth in a sterile culture bottle. Serial dilutions to 10⁻³ were made from this by pipetting 1ml into 9ml of Selenite F broth. The dilutions were incubated overnight. 1ml of the broth culture was then plated out on Salmonella-Shigella Agar. The SSA plates were read after an overnight incubation at 37°C. The number of colonies on each plate was counted.

Characterization of Isolates

Colonies that showed characteristics of *Salmonella* species on SSA were presumptively identified as *Salmonella* species on the basis of Gram Stain reaction and motility test. Gram Staining and Motility test were done according to standard procedures (Cheesborough, 2000).

Antibiotic Sensitivity Testing

Disc diffusion susceptibility test by Bauer method was carried out as previously described by Ekundayo and Omodamiro (2008). Abtek Sensitivity discs (Abtek Biologicals Ltd, UK) were used.

RESULTS

A total of 240 eggs were collected from four poultry farms in Umudike, Abia State. *Salmonella* species were isolated from 58 eggs. This represents a prevalence of 24 .17%. The prevalence of *Salmonella* isolates in the eggs from the farms ranged from 8% to 45%. Table 1 presents the prevalence of Salmonella isolates in the eggs from the four farms.

All the fifty-eight isolates were sensitive to Nitrofurantoin and forty-two (72.4%) to Gentamycin. Twenty-two and seventeen of the isolates were sensitive to Ofloxacin and Nalidixic acid respectively. The isolates manifested high level of resistance to Amoxycillin and Tetracycline with 93.1% of the isolates resistant to both antimicrobial agents. All the isolates were resistant to cotrimoxazole.

Many of the isolates manifested high level of multidrug resistance, sixteen (27.6%) of the isolates were resistant to seven of the antimicrobial agents tested. Thirty-four (58.6%) were resistant to six of the antimicrobial agents, 44(75.9%) to 5 and 93.1% to 3 antimicrobial agents. The antibiotic sensitivity profile of the 58 Isolates is presented in Table 2. Fig. 3 presents the comparative sensitivity of the isolates to the antimicrobial agents tested.

Farm	No. of eggs tested	No. of eggs with growth	% of eggs showing growth
Α	60	14	23
В	60	27	45
C	60	5	8
D	60	12	20

Table 1: Prevalence of Salmonella isolates in eggs from poultry farms in Umudike

Antimicrobial Agents	Disc Potency (µg)	No. (%) of Isolates sensitive	No. (%) of isolates with intermediate sensitivity	No.(%) of isolates Resistant
Amoxycillin	25	4 (6.9)	0 (0)	54 (93.1)
Cotrimoxazole	25	0 (0)	0 (0)	58 (100)
Nitrofurantoin	300	58 (100)	0 (0)	0 (0)
Gentamycin	10	42 (72.4)	0 (0)	16 (27.5)
Ofloxacin	30	22 (37.9)	2 (3.4)	34 (58.6)
Augmentin	30	12 (20.6)	2(3.4)	44 (75.9)
Nalidixic acid	30	17 (29.3)	7 (12.0)	34 (58.6)
Tetracycline	30	4 (6.9)	0 (0)	54 (93.1)

Table 2:	Antimicrobial	Sensitivity	profile o	of Salmonella	isolates	in eggs
from p	oultry farms in	Umudike				



Fig. 1: Percentages of Isolates Sensitive to different Antimicrobial Agents

DISCUSSION

An overall prevalence of 24.17% of Salmonella species were isolated from the eggs collected from four farms in Umudike, Abia State. This shows that there is significant level a of contamination of eggs in the four farms from which the eggs were obtained. In one of the farms, up to 45% of the eggs were contaminated with Salmonella species. This study only assessed the internal contamination of the eggs. The prevalence of contamination would probably be higher if one takes into consideration the external contamination. The public health impact of contamination of eggs with Salmonella species cannot be directly determined from this study. However, reports from advanced countries where public health systems are better organized suggest that transmission of Salmonella related food borne illness from eggs is a real public health hazard and that it is associated with huge economic losses and considerable health burdens (Mead et al., 1999, Rodrigue et al., 1990, Stevens et al., 1989, St. Louis et al., 1988). If poultry and egg related Salmonella infections remain public health serious problems in developed countries where better sanitation measures and infection controls are in place, it is most likely that the problems are more serious in developing countries. However, there are no data to show the extent of the problem. Most cases of Salmonellosis are self-limiting and in developing countries such diseases are not usually reported, so it is difficult to assess the real impact on the populace. The poultry industry is at infancy in Nigeria, if the expectations of sustainable poultry industry are to be realized, control measures need to be established to control Salmonella infections.

The level of antimicrobial resistance of the isolates in this study is really alarming. Many of the isolates are resistant to multiple antimicrobial agents tested. All the isolates were resistant to Cotrimoxazole and 93.1% to Amoxicillin

and Tetracycline. These drugs are the most commonly used antimicrobial agents for treatment of Salmonella infections. In a retrospective study covering data for Salmonella isolates over a period of four years, Doughari et al. (2007) reported a resistance of 88.8% to Cotrimoxazole and various levels of resistance to other drugs in Yola, Northern Nigeria. As in that study, we also recorded high level of resistance to Amoxicillin, 93.1% in this study. Indiscriminate use of antimicrobial agents in medical and veterinary practices is fuelling the antimicrobial resistance The ultimate source of problems. contamination of poultry and animal houses is the environment. Amaechi and Ezeronye (2006) reported high level of contamination of piggery environment with Salmonella species in the study area.

CONCLUSION:

This study shows that a considerable level of contamination of eggs occurs in the study area. This study therefore provides the basis for further study on this subject and also the need to assess the public health impact of Salmonella-related food borne illness in the poultry industry in Nigeria. There is need to adopt a more efficient hygienic and infection control measures in the poultry industry to control Salmonella infections of poultry and prevent the possible public health hazards.

REFERENCES

- Amaechi, N. and Ezeronye, O.U.(2006) Piggery Environment as a source of *Salmonella* contamination for swine *Journal of Animal and Veterinary Advances* 5 (2) : 102-107.
- Boonmar, S., Bangtrakulnonth, A., Pornrunangwong, S., Terajima, J., Watanabee, H., Kaneko, K. and Ogawa, M. (1998) Epidemiological Analysis of *Salmonella enteritidis* Isolates from Humans and Broiler Chickens in Thailand by phage Typing and Pulsed-Field Gel

Electrophoresis. J. Clin. Microbio. 36(4): 971-974

- Cheesborough, M. (2000) District Laboratory Practice in Tropical Countries Part 2 pp
- Chiu, C., Su, L. and Chu (2004) Salmonella enterica serotype Choleraesuis: Epidemiology, Pathogenesis, Clinical Disease, and Treatment. Clin. Microbio. Reviews 17 (2): 311-322
- Doughari, J. H., Elmahmood, A.M. and Nggada, H. P. (2007)Retrospective study on the antibiotic resistant pattern of Salmonella typhi from some clinical samples. African Journal Microbiology of Research. Available online http://www.academicjournals.org/a jmr
- Ekundayo, E.O. and Omodamiro, O.D.
 (2008) Evaluation of the Quality of locally Manufactured Antimicrobial Susceptibility Testing discs used in South Eastern Nigeria Afr. J. Clin Exp. Microbiology 9 (3) 122-128
- Holt, P. S. (1993) Effect of induced molting on the susceptibility of White Leghorn hens to a *Salmonella enteritidis* infection. *Avian Dis* 37: 412-417
- Irwin, R. J., Poppe, C., Messier, S., Finley, G.G. and Oggel, J. A. (1998)National Survey to Estimate the Prevalence of *Salmonella* species Among Canadian Registered Commercial Turkey Flocks *Can J. Vet Res.* 58 : 263-267
- Mead, P. S., Slutsker, L. Dietz, V., McCaig, F., Bresee, J. S., Shapiro, C., Griffin, P. M. and Tauxe, R. V. (1999) Food –related illness and death in the United States. *Emerging Infect. Dis.* 5: 607-625
- Nastasi, A., Mammina , C., Fantasia, M. and Pontello, M (1997)

Epidemiological analysis of strains of *Salmonella enterica* serotype enteritidis from foodborne outbreak occurring in Italy, 1980-1994 j Med. Microbiol. 46: 377-382

- Okoli, I. C., Nwogu, C. I. Okoli, G. C., Okeudo, N. J. and Ibekwe, V. (2002) Management of Antimicrobial Resistance in Avian Bacterial Pathogens in Nigeria. *Environmental Health and Human Development* 3: 35-42
- Pignato, S., Marino, A., Emmanuele, M. C., Iannotta, V., Caracappa, S. and Giammanco, G. (1995) Evaluation of New Culture Media for Rapid Detection and Isolation of Salmonellae in Foods *Applied and Environmental Microbiology* 61 (5): 1996-1999
- Poppe, C., Kolar, J.J., Demczuk, W.H.B. and Harris , J. E. (1995). Drug Resistance and Biochemical Characterizations of Salmonell from Turkeys. *Can. J. Vet. Res.* 59: 241-248
- Rodrigue, D. C., Tauxe, R.V., and Rowe, B. (1990) International increase in Salmonella enteritidis phage type 4: a new pandemic? *Epidemiology and Infection* 105: 21-27
- Sasipreeyajan, J., Jeingklinchan, J., Koowatananukul, C. and Saitanu,
 K. (1996) Prevalence of Salmonella in broiler, layer and breeder flocks in Thailand. Trop. Anim. Health Prod 28: 174-180
- Schroeter, A., Ward, L. R., Rowe. B., Protz, D., Hartung, M. And Helmutth, R.(1994) Salmonella enteritidis phage types in Germany. Eur. J. Epidemiology 10: 645-648
- St. Louis, M. E., Morse, D. L., Potter, M.E., Melfi, T. M., Guzewich, J.J. Tauxe, R.V., Blake, P. A. and the *Salmonella enteritidis* Working Group (1988) The emergence of grade A eggs as a major source of

Salmonella enteritidis infections JAMA 259: 2103-2107

- Stevens, A., Joseph. C., Bruce, J., Fento,
 D. O'Mahony, M., Cunigham, D.,
 O'Connor, B. and Rowe, B. (1998)
 A large outbreak of Salmonella enteritidis phage type 4 associated with eggs from overseas. Epidemiology and Infection 103: 425-433
- Us Department of Agriculture, Animal and Plant Health Inspection Service 1994. Salmonella enteritidis (SE) control programme. SE pilot Project Summary October, 1994. US Government Printing Office. Washington, D. C.
- Zhao, S., Qaiyumi. S., Friedman, S., Singh, R., Foley, S.L., White, D.G., McDermott, P.F., Donkar, T., Bolin, C., Munro, S., Baron, E.J. and Walker, R.D. (2003) Characterization of *Salmonella enterica* Serotype Newport Isolated from Humans and Food Animals *Journal of Clin. Microbio.* 41 (12) : 5366-5371.