

Antibiogram of *Salmonella* Species Obtained from Environmental Samples in Ilishan-Remo Ogun State, Nigeria

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Abstract: Multi-drug resistance in *Salmonella* species is becoming a great problem in healthcare institutions resulting in high mortality rate due to its infections. Hence, the purpose of this study was to evaluate the antibiogram of *Salmonella* species obtained from various environmental samples in Ogun state. Samples (150) comprising faecal droppings of cow, goat, bird and pig as well as pond wastewater were obtained and *Salmonella* species were isolated using Xylose Lysine Deoxycholate agar and identified using analytical profile index. The susceptibility of the species was evaluated and interpreted according to CLSI guidelines. Fifty (50) species of *Salmonella* species were obtained and all (100%) were resistant to ceftazidime, gentamicin, while 92%, 90%, 48%, 46%, and 44% were resistant to amoxicillin/clavulanate, cefuroxime, cefixime, ciprofloxacin, ofloxacin, and nitrofurantoin respectively. Multi-drug resistance was noted in *Salmonella* species to at least three antibiotic classes. The results revealed high prevalence and multi-drug resistant *Salmonella* species in our environment. Hence measures should be implemented urgently to curb further spread of multidrug resistant *Salmonella* species in our environment.

Keywords- Foodborne diseases, multi-drug resistance, *Salmonella* species, API, Antibiotics

I. INTRODUCTION

Salmonella species are Gram-negative, non-spore forming, motile bacterium belonging to the family of *Enterobacteriaceae* [1]. There are a plethora of *Salmonella* serovars including *S. Typhimurium*, *S. Enteritidis*, *S. Choleraesuis*, *S. Newport*, *S. Infantis* and *S. Panama* with the first two being the most common and have been implicated in human Salmonellosis [2]. The bacterium is known to be pathogenic when ingested and it can lead to different disease forms, for example, gastroenteritis, bacteremia and systemic infections in humans and animals. The reservoirs of this pathogen include poultry meat, eggs, dairy products, fruits, vegetables; pets including dogs, cats, reptiles, turtles and even humans [3]. Once the organism gains entry into the gut of the host following the consumption of contaminated food, the pathogen secretes a protein *sopE* which triggers immunological responses in the host that leads to the diarrhoeal symptoms. The severity and duration of the resulting gastroenteritis depends on the susceptibility of the host. The disease may be self-limiting and may last for 6-72 hours but can be life threatening in infants, elderly, pregnant women and immunocompromised persons [4].

Food contamination with antibiotic-resistant bacteria is an important public clinical problem of which antibiotic determinants can easily be transferred to clinical isolates of bacteria through many transfer mechanisms. The first genomic island of *Salmonella* with such potential was reported in *Salmonella enterica* serovar Typhimurium DT104 [5]. This genomic island has been reported to be transferable between *S. enterica* and *Escherichia coli* [6]. In developing countries, food hygiene is poor couple with lack of antimicrobial surveillance system. For this reason, the burden of *Salmonella* infections may be expensive primarily in children and patients with weak immunity. Hence, the purpose of this study was to evaluate the level of resistance of *Salmonella* species isolated from environmental samples.

II. SAMPLE COLLECTION AND IDENTIFICATION

Environmental samples (poultry droppings, cattle droppings, goat droppings, pig droppings and water from fish ponds) were obtained from various locations in Ilishan-Remo Ogun state. The samples were randomly collected from various markets and farms across the location. The collected samples were kept in sterile universal bottles and transported to the Microbiological laboratory of Babcock University in an ice pack. *Salmonella* species were isolated using method described elsewhere [7]. The identity of the species was confirmed with API 20E (BioMérieux, France) kit following the manufacturer's instructions.

III. SUSCEPTIBILITY TESTING

Following the recommendation by CLSI [8], the resistance level was determined and interpreted. Briefly, sterile swab stick was used to pick a pure culture suspension of the organism (equivalent of 0.5 McFarland standard) and streaked on already prepared Muller Hinton Agar (Oxoid, Hampshire, England). The antibiotic disk was placed on the plates evenly with a considerable distance maintained from the edge of the plates. The plates were incubated for about 18-24 hours at 37°C. Zone diameter was measured using ruler. The antibiotic disk (Abtek Biologicals Limited) contained cefixime (5µg), amoxicillin/clavulanate (30µg), ciprofloxacin (5µg), nitrofurantoin(300µg), gentamicin(10µg), cefuroxime(30µg), ceftazidime (30µg), and ofloxacin (5µg).

IV. RESULTS AND DISCUSSION

Fifty (50) *Salmonella* species were obtained and were resistant to ceftazidime, gentamicin, while 92%, 90%, 48%, 46%, and 44% were resistant to amoxicillin/clavulanate, cefuroxime, cefixime, ciprofloxacin, ofloxacin, and nitrofurantoin respectively (TABLE 1). Multi-drug resistance was noted in *Salmonella* species to at least three antibiotic classes (TABLE 2).

Salmonella species are examples of common bacterial pathogens causing infections (food borne diseases). This organism causes Salmonellosis which is a leading cause of gastroenteritis in humans. It can be transmitted from the eating of animals used as food most especially when infection control practices and proper hygiene are not strictly adhere to. In this study, the overall resistance of *Salmonella* species to various antibiotics was very high and was comparable to the results obtained elsewhere [9]. The current study showed that all the *Salmonella* species obtained were resistance to aminoglycoside (gentamicin) and this was in contrast to other study previously [10]. The variation could be as a result differences geographical location as well as differences in stains encountered. *Salmonella* species express different phenotypes to different antibiotics in different studies [11-12].

Food contaminated with antimicrobial resistant microbes is of genuine danger to general wellbeing and the anti-microbial resistant determinants can undoubtedly be moved to therapeutically significant microorganisms through any route of genetic transfer methods. The first genomic island of *Salmonella* was noted in *Salmonella enterica* serovar Typhimurium DT104 [5]. This genomic island has been accounted for to be transferable between *S. enterica* and *Escherichia coli* [6]. In underdeveloped and developing nations where crude food cleanliness is habitually poor and antimicrobial surveillance system is at its formative stage, the effect of *Salmonella* contaminations might be expensive particularly in youngsters and immunocompromised patients. Food borne infection due to non-typhoidal *Salmonella* is behind both sporadic gastroenteritis and pandemic generally. In sub-Saharan Africa, disease because of *Salmonella enterica*, *Salmonella Typhi* and non-typhoidal *Salmonella* have been accounted for [13]. *Salmonella* causes intestinal-related diseases including fever and septicemia [14]. The disease is extreme particularly in old, youngsters and immunocompromised patients. The types of *Salmonella* pertinent to human contaminations are the *Salmonella enterica*.

V. CONCLUSION

The results revealed high prevalence and multi-drug resistant *Salmonella* species in our environment. Hence measures should be implemented urgently to curb further spread of multidrug resistant *Salmonella* species in our environment.

Table 1: The percentage of the antimicrobial susceptibility pattern of *Salmonella* species obtained from environmental samples

Antibiotics	Concentration (µg)	Resistant isolates		Sensitive isolates		Intermediate isolates	
		No	Per cent (%)	No	Per cent (%)	No	Per cent (%)
Ceftazidime (CAZ)	30	50	100	0	0	0	0
Cefuroxime (CRX)	30	46	92	0	0	4	8
Gentamicin (GEN)	10	50	100	0	0	0	0
Cefixime (CXM)	5	45	90	3	6	2	4
Ofloxacin (OFL)	5	23	46	19	38	8	16
amoxicillin/clavulanate	30	50	100	0	0	0	0
Nitrofurantoin (NIT)	300	22	44	19	38	9	18
Ciprofloxacin (CPR)	5	24	48	5	10	21	42

Key: No- number of species

Table 2: Multiple antibiotic resistance patterns of *Salmonella* species isolated from environmental samples

Number of Antibiotic(s)	Antibiotic Resistance Pattern	Frequency	Total Number
3	CAZ, GEN, AUG	1(2%)	2(4%)
	CAZ, CRX, GEN	1(2%)	
4	CAZ, CRX, GEN, AUG	1(2%)	1(2%)
	CAZ, CPX, GEN, AUG, CPR	1(2%)	
5	CAZ, CRX, GEN, CXM, AUG	14(28%)	15(30%)
	CAZ, CRX, GEN, CXM, AUG, CPR	1(2%)	
6	CAZ, CRX, GEN, CXM, AUG, CPR	4(8%)	13(26%)
	CAZ, CRX, GEN, CXM, AUG, NIT	6(12%)	
	CAZ, CRX, GEN, CXM, OFL, AUG	2(4%)	
	CAZ, GEN, CXM, OFL, AUG, CPR	1(2%)	
7	CAZ, CRX, GEN, CXM, OFL, AUG, CPR	4(8%)	6(12%)
	CAZ, CRX, GEN, CXM, OFL, AUG, NIT	2(4%)	
8	CAZ, CRX, GEN, CXM, OFL, AUG, NIT, CPR	13(26%)	13(26%)
TOTAL		50(100%)	50(100%)

Key; CAZ= ceftazidime, CRX= cefuroxime, GEN = gentamicin, CXM = cefixime, OFL = ofloxacin, AUG (amoxicillin/clavulanate), NIT = nitrofurantoin, CPR = ciprofloxacin.

REFERENCES

- Wiley, J. M., Sherwood, L. M., & Woolverton, C. J. (2008). *Prescott, Harley and Klein's Microbiology*. 7th Edition. New York: McGraw Hill ISBN 978-0-071-10231 pp 309-311; 332-346; 816-833.
- Rahman, S. H., & Othman, H. H (2017). *Salmonella* infection: The common cause of human food poisoning. *Progress in Bioscience and Bioengineering*. 1(1), 5-10.
- Brooks, G. F., Carroll, K. C., Butel, J. S., Morse, S. A. & Mietzner, T. A. (2013). *Jawetz, Melnick and Adelberg's Medical Microbiology*. 26th Edition. New York: McGraw Publishers ISBN 978-0-07-181578-9 pp 150-163.
- Antunes, P., Mourao, J., Campos J., & Peixe, L. (2016). Salmonellosis: The role of poultry meat. *Clin. Microbiol. Infect*, 22, 110-121.
- Hao Van, T., Moutafis, G., Istivan, T., Thuoc, T.L. and Coloe, P.J. (2007). Detection of *Salmonella* Spp. in retail raw food samples from Vietnam and characterization of their antibiotic resistance. *Appl. Environ. Microbiol.* 73(21), 6885-6890.
- Doublet, D., Boyd, D., Mulvey, M.R. and Cloeckert, A. (2005). The *Salmonella* genomic island 1 is an integrative mobilizable element, 55(6):1911-1924.
- Ahmed AKM, Islam MT, Haider MG, Hossain MM (2008) Sero prevalence and pathology of naturally infected Salmonellosis in

- poultry with isolation and identification of causal agents. *J Bangladesh Agric Univ* 6:327–334.
- [8]. CLSI (Clinical and Laboratory Standards Institute). (2016). Performance Standards for Antimicrobial Susceptibility Testing: CLSI M100-S26.
- [9]. Detha, A. I. R. and Datta, F. U. (2016). Antimicrobial activity of traditional wines (sopi and moke) against *Salmonella* sp. and *Escherichia coli*. *J. Adv. Vet. Animal Research*, 3, 282-285.
- [10]. Khan, M. F., Rahman, M. B., Khan, M. S., Nazir, K. H. and Rahman, M. . (2005). Antibigram and plasmid profile analysis of isolated poultry *Salmonella* of Bangladesh. *Pakistan J. Biol. Sci.* 1614-1619.
- [11]. Selvaraj, R., Das, R., Ganguly, S., Ganguli, M., Dhanalakshmi, S. and Mukhopadhyay, S. K. (2010). Characterization and antibiogram of *Salmonella spp.* from poultry specimens. *J. Microbiol Antimicrob*, 2(9), 123-126.
- [12]. Divek, V. T., Nair, Kumar Venkitanarayanan, and Anup Kollanoor Jonny. (2018). Antibiotic- resistant *Salmonella* in the food supply and the potential role of antibiotic alternatives for control. *Nat. Lib. Med*, 7(10), 167.
- [13]. Msemo, O.A., Mbwana, J., Mahende, C., Malabeja, A., Gesase, S., Crump, J.A., Dekker, D. and Lusingu, J.P.A. (2019). Epidemiology and Antimicrobial Susceptibility of *Salmonella enterica* Bloodstream Isolates Among Febrile Children in a Rural District in Northeastern Tanzania: A Cross-sectional Study. *Clinical Infectious Diseases*. 68 (Suppl 2).
- [14]. Bjelland, A.M., Sandvik, L.M., Skarstein, M.M., Svendal, L. and Debenham, J.J. (2020). Prevalence of *Salmonella* serovars isolated from reptiles in Norwegian zoos. *Acta Vet. Scand* 62, 3.