Incidence of multiple antibiotic resistance among *Salmonella* spp. isolated from poultry droppings and cow dung in Ado-Ekiti metropolis

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Abstract: Incidence of multiple antibiotic resistance has been observed among *Salmonella* species. The main objective of this study was to determine the multiple antibiotic resistance pattern in *Salmonella* isolated from poultry droppings and cow dung. Samples of poultry droppings and cow dung were randomly collected from poultry houses and abattoirs in Ado-Ekiti. Isolation was done using Salmonella-Shigella agar, after which the isolates were subjected to some biochemical tests. The isolates were also subjected to confirmatory test using *Salmonella* test kit. The confirmed *Salmonella* isolates were subjected to antibiotic susceptibility tests using antibiotic discs such as Pefloxacin (5µg), Ofloxacin (5µg), Ciprofloxacin (10µg), Norfloxacin (10µg), Amoxicillin (10µg), Nalidixic acid (30µg) and Nitrofurantoin (300µg) and their multiple antibiotics resistance (MAR) pattern were observed. Statistical analysis using student t-test was done to compare the mean resistance of *Salmonella* sp. isolated from different environment to the different antibiotics used. Eighty-seven isolates were recovered from the samples, forty six from cow dung and forty one from poultry droppings. When the isolates were subjected to confirmatory test using *Salmonella* test kit, sixty eight were found to be positive by showing agglutination within two minutes. The percentage resistance observed among *Salmonella* species isolated from cow dung and poultry droppings were amoxicillin (73.5%), nitrofurantoin (63.2%), nalidixic acid (60.3%), pefloxacin (52.9%), norfloxacin (36.8), ciprofloxacin (35.3%) and ofloxacin (20.9%). The highest resistance among the isolates was observed to amoxicillin and the least was to ofloxacin. The MAR patterns observed among the isolates include: PEF/OFX/AMX/CIP/NAL/NOR/NIT, PEF/AMX/NAL/NOR/NIT, PEF/AMX/NOR/NIT and CIP/NAL/NOR. Statistical analysis revealed that there was no significant difference in the resistance of *Salmonella* sp. from different sources to the different antibiotics used. It can be deduced from this study that those isolates showing multiple antibiotic resistance pose a threat to human population because if they find their way into the human population they can cause resistance to antibiotics in patients suffering from *Salmonella* infection.
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1. Introduction

Enteric fever also commonly known as typhoid fever, a systemic infection is one of the major diseases caused by *Salmonella typhi* or by the related but less virulent *Salmonella paratyphi* (Nataro *et al.*, 2000). *Salmonella* species are gram-negative pathogens which causes food-borne diseases that can result in public health concerns worldwide. These pathogens regularly infect humans after the consumption of contaminated food or as a result of direct contact with carrier animals (Pang *et al.*, 1995; Soto *et al*., 2003).

Animals such as cat, duck, cattle, fowls and persons chronically infected with typhoid fever serves as a reservoir for *Salmonella typhi.* Salmonellosis is caused by ingestion of contaminated poultry, beef, pork, eggs, milk, shellfish and canned meat. It can also be caused by drinking sewage contaminated water (CDC, 2000). A wide array of animal reservoir and commercial distribution of both animals and food products favour the spread of the disease. Poultry birds have frequently been incriminated as a means of *Salmonella* contamination and consequently act as major source of the pathogen in humans (Baeumler *et al.*, 2000). When infection spreads beyond the intestinal tract, appropriate antimicrobial therapy can be lifesaving (Shah and Korejo 2012). The use of antibiotics in humans unnecessarily and the use of antimicrobial agents in the feeds of animals for prophylaxis and as growth promoters which has been estimated to be million pounds per year (Angulo *et al.*, 2004) have resulted in resistance of *Salmonella* species to most antibiotics even in human population (Stevenson *et al., 2002*). The development of antimicrobial resistance among *Salmonella* species has become a serious problem, especially the emergence of multi-drug resistant (MDR) *Salmonella* strains (Lee *et al.*, 1994; Kristiansen *et al.*, 2003). *Salmonella* species isolated from clinical and environmental sources has shown

an increased resistance to antibiotics since it has developed a number of elaborate mechanisms for acquiring and disseminating plasmids, transposons, phages, and other genetic determinants (Harts and Kaariuki, 2003).

Therefore, this study focuses on the resistance of *Salmonella* species isolated from poultry and abattoir sources to fluoroquinolone antibiotics and other types of antibiotics.

2. Materials and Methods:

Sample collection

Environmental samples such as cow dung and poultry droppings were collected from various abattoirs and poultry houses in Ado metropolis (South western, Nigeria). The samples were conveyed to the laboratory and isolation was performed immediately.

Isolation and identification of *Salmonella* isolates.

Isolation was carried out using selective media for *Salmonella*. The isolates obtained were purified by further subculturing and were observed for presumptive identification based on their morphological characteristics and various biochemical tests that included catalase, oxidase, hydrogen sulphide production, motility, indole, methyl red, and citrate utilization test. The colonies identified on the basis of biochemical tests were subjected to serological tests using polyvalent serum against O and H *Salmonella* antigens (Difco, Detroit, USA). The colonies that agglutinated during the period of one to two minutes were considered as positive for *Salmonella*, and were preserved on Nutrient agar at 4°C.

Antibiotic susceptibility test.

The antimicrobial susceptibility test was performed according to the CLSI method using Kirby-Bauer disk diffusion test on Muller-Hinton agar (Oxoid CM0337 Basingstoke, England). Each isolate was inoculated into nutrient broth separately and incubated for 24 hours at 37ºC. The broth were streaked using sterile cotton swabs on Mueller-Hinton Agar plates. The antimicrobial agents used were Pefloxacin (PEF) (5µg), Ofloxacin (OFX) (5µg), Ciprofloxacin (CIP) (10µg), Norfloxacin (NOR) (10µg), Amoxicillin (AMX) (10µg), Nalidixic acid (NAL) (30µg) and Nitrofurantoin (NIT) (300µg) all produced by Oxoid Ltd., UK. Zones of inhibition were evaluated following the recommendations by NCCLS, 2000.

Statistical analysis:

The mean of the resistance of *Salmonella* sp. isolated from poultry droppings and cow dung to the different antibiotics were compared using student t-test (paired samples test). The confidence limit was set at 95%. The null hypothesis was that there is no significant difference in the resistance of *Salmonella* sp. isolated from different sources to the different antibiotics used. All available data were analyzed using a computer program (SPSS version 15, Chicago, IL, USA).

3. Results

Eighty seven isolates were obtained from the samples collected i.e. forty six from cow dung and forty one from poultry droppings respectively. They were presumed to be *Salmonella* sp. based on their biochemical reactions. When the isolates were subjected to confirmatory test using *Salmonella* test kit, sixty eight were found to be positive by showing agglutination within two minutes (twenty eight from cow dung and forty from poultry droppings).

Table 1 shows the resistance of *Salmonella sp.* from abattoir and poultry sources to different antimicrobial agents. The figures in parenthesis indicate the percentage resistance of isolates from the different sources to different antibiotics. The percentage resistance among *Salmonella species* isolated from cow dung and poultry droppings are as listed: amoxicillin (73.5%), nitrofurantoin (63.2%), nalidixic acid (60.3%), pefloxacin (52.9%), norfloxacin (36.8%), ciprofloxacin (35.3%) and ofloxacin (20.9%). The highest resistance among the isolates was observed in amoxicillin and the least resistance among the isolates was observed when ofloxacin was used.

Based on the resistance of the isolates to the different antibiotics used, they were divided into three groups viz: Group I which consisted of those isolates that were susceptible (i.e. sensitive) to all the antibiotics used, group II which was made up of isolates that were resistant to only one antibiotics (single R-type) and the third group ie group III which consisted of isolates that showed multiple resistant to at least two antibiotics.

Most of the isolates were resistant to multiple antibiotics showing different multiple antibiotic resistance (MAR) patterns. Some of the MAR patterns observed include: PEF/OFX/AMX/CIP/NAL/NOR/NIT, AMX/CIP/NAL/NOR/NIT and AMX/NAL/NIT among others. Tables 2 and 3 shows the MAR patterns of the isolates based on their sources of isolation. Table 4 shows the result for the statistical analysis comparing the mean of the resistance of *Salmonella* sp. isolated from poultry droppings and cow dung to the different antibiotics.

Table 1: Comparison of resistance of *Salmonella* species isolated from poultry droppings and cow dung to different antibiotics.

Antibiotics	Resistance of isolates from Poultry droppings % (n=40)	Resistance of isolates from Cow dung % (n=28)	Total number of microorganisms resistant to the antibiotics	Resistant microorganisms (%)
Amoxicillin (AMX)	32 (80%)	18 (64.3%)	50	73.5
Nalidixic acid (NAL)	27 (65.9%)	14 (50%)	41	60.3
Nitrofurantoin (NIT)	28 (70%)	15 (53.6%)	43	63.2
Ciprofloxacin (CIP)	12 (30%)	12 (42.8%)	24	35.3
Ofloxacin (OFX)	6 (15%)	8 (28.6%)	14	20.9
Norfloxacin (NOR)	13 (32.5%)	12 (42.8%)	25	36.8
Pefloxacin (PEF)	19 (47.5%)	17 (60.7%)	36	52.9

Table 2: Multiple antibiotic resistance (MAR) pattern of *Salmonella* species from isolated cow dung.

Source of isolation	Number of microorganisms isolated (n)	Number of isolates sensitive to all antibiotics used	Single R-type	Multiple antibiotic resistance pattern	Frequency
Cow dung	28	4	5	PEF,OFX,AMX,CIP,NAL,NOR,NIT	4
				PEF,OFX, CIP,NAL,NOR,NIT	2
				PEF, AMX,CIP,NAL,NIT	2
				PEF, AMX,CIP,NAL,NOR	1
				PEF,AMX,NAL,NOR,NIT	1
				PEF,AMX,NAL,NIT	1
				AMX,NAL,NOR,NIT	1
				AMX,NAL,NIT	1
				AMX,NAL,NOR	1
				PEF,NAL,NIT	1
				AMX,NOR,NIT	1
				AMX,NIT	2
				NAL,NIT	1

Table 3: Multiple antibiotic resistance (MAR) pattern of *Salmonella* species from poultry droppings.

Source of isolation	Number of microorganisms isolated (n)	Number of isolates sensitive to all antibiotics used	Single R-type	Multiple antibiotic resistance pattern	Frequency
Poultry droppings	40	2	7	PEF,OFX,AMX,CIP,NAL,NOR,NIT	5
				PEF,AMX,CIP,NAL,NOR,NIT	3
				PEF,OFX, CIP,NAL,NOR,NIT	1
				PEF, AMX,NAL,NOR,NIT	1
				PEF,OFX,AMX,NAL,NIT	1
				PEF,AMX,CIP,NAL,NIT	1
				PEF,AMX,NOR,NIT	1
				PEF,AMX,NAL,NIT	2
				AMX,NAL,NIT	6
				PEF,AMX,NAL	1
				CIP,NAL,NOR	1
				AMX,NIT	5
				PEF,NAL	1
				AMX,NAL	2

Table 4: Statistical comparison of mean resistance of *Salmonella* sp. from poultry droppings and cow dung to different antibiotics.

Source of variation	N	Mean	Standard deviation	df	t.cal	t. table	Remark
Poultry droppings	6	19.6	9.7	6	2.18	2.40	Not significant
Cow dung	6	13.7	3.4				

4. Discussion

Salmonella species are organisms of enormous importance due to their pathogenicity and have been associated with various infections in human such as septicaemia, typhoid or enteric fever, enterocolitis etc (Ogunleye *et al.,* 2005; Martin *et al.,* 2004; Witte, 1998). In this study, *Salmonella* species were isolated from environmental sources such as cow dung from abattoir and chicken droppings from poultry houses, these sources constitute part of the major sources through which *Salmonella* is introduced into human population either as food-borne or water-borne pathogen (CDC, 2000). Antimicrobial susceptibility test revealed that *Salmonella sp.* isolated in this study were more resistant to antibiotics such as amoxicillin (10 µg), nalidixic acid (30µg) and nitrofurantoin (300µg) than they were to fluoroquinolone antibiotics such as ciprofloxacin (5µg), pefloxacin (5µg), ofloxacin (5µg) and norfloxacin (10µg). A major reason for this could be because of the indiscriminate use of none fluoroquinolone antibiotics in feeds of animals for prophylaxis and as growth promoters (Angulo *et al., 2004*). Use of antimicrobials in any environment creates selection pressures that favour the survival of antibiotic-resistant pathogens. The routine practice of giving antimicrobials to domestic livestock for growth promotion and prophylaxis is an important factor in the emergence of antibiotic-resistant bacteria in the food chain (Shah and Korejo, 2012; Su *et al.*, 2004).

It could be observed from table 2 that of twenty eight isolates obtained from cow dung which were confirmed to be *Salmonella sp.* only four (14%) belonged to group I i.e. were sensitive to all the antibiotics used, five (18%) belonged to group II (single R-type) i.e. were resistant to only one antibiotic out of the seven antibiotics used while the remaining nineteen isolates belonged to group III i.e. they were resistant to multiple antibiotics. Based on the MAR pattern of the isolates, it was observed that 14% of the isolates were resistant to all the antibiotics used, 7% of the isolates were resistant to six of the antibiotics, 14% of the isolates were resistant to at least five of the antibiotics used, 7% of the isolates were resistant to at least four of the antibiotics used, 14% of the isolates were resistant to at least three of the antibiotics used and 11% of the isolates were resistant to at least two of the antibiotics used.

It could be observed from table 3 that of forty isolates obtained from poultry droppings which were confirmed to be *Salmonella sp.* only two (5%) belonged to group I i.e. were sensitive to all the antibiotics used, seven (17.5%) belonged to group II (single R-type) i.e. were resistant to only one antibiotic out of the seven antibiotics used while the remaining thirty-one isolates belonged to group III i.e. they were were resistant to multiple antibiotics. Based on the MAR pattern of the isolates, it was observed that 12.5% of the isolates were resistant to all the antibiotics used, 10% of the isolates were resistant to six of the antibiotics, 7.5% of the isolates were resistant to at least five of the antibiotics used, 7.5% of the isolates were resistant to at least four of the antibiotics used, 20% of the isolates were resistant to at least three of the antibiotics used and 20% of the isolates were resistant to at least two of the antibiotics used.

From table 4, it can be observed that the t-table is greater than the t-calculated, hence the null hypothesis is accepted and this implies that there is no significant difference resistance of *Salmonella* sp. isolated from different sources to the different antibiotics used. This therefore means that resistance of *Salmonella* sp. to antibiotics is not dependent on the source of isolation.

Incidence of multiple antibiotic resistance observed in this study is in agreement with previous studies carried out by Su *et al* (2004) and Coovadia *et al* (1992), who reported the emergence of multi drug resistant *Salmonella sp.* which were resistant to ampicillin, chloramphenicol, cotrimoxazole, cephalosporin and fluoroquinolones which were the drugs of choice in the treatment of most infections caused by *Salmonella* species.

In conclusion, in agreement with Witte (Martin *et al.*, 2004), resistance of microorganisms to antibiotics jeopardizes the effectiveness of the treatment of bacterial diseases; hence in developing countries like Nigeria, in order to reduce infections and diseases caused by *Salmonella* species, practicing good hygiene must be encouraged while discouraging the indiscriminate use of antibiotics in both humans and animals.

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