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Drug of Choice in the Treatment of Multiple Drug Resistant (MDR) Salmonellae Isolated from Wildlife in Nigeria

Oludairo O. O.[1], Kwaga J. K. P. [2], Dzikwi A. A., [2] Kabir J.[2]
[1]Department of Veterinary Public Health and Preventive Medicine, University of Ilorin, Ilorin, Nigeria
[2]Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Nigeria.

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ABSTRACT

Multiple drug resistant (MDR) strains of Salmonella are frequently encountered with increased rates in recent years. Many variants of the organism have developed MDR genes which they retain even when antimicrobial drugs are no more in use, limiting the choice of drugs for therapy of Salmonella infections resulting in morbidity and mortality in both man and animals and raising more public health questions. The objective of this study was to evaluate the susceptibility of Salmonella spp. to twelve antimicrobial agents using the disk diffusion method. Eight Salmonella spp. isolated from wildlife were tested. All the isolates exhibited MDR, showing resistance to at least four and up to nine antimicrobial agents. They were all highly resistant (100%) to ceftazidime, cephazoline, cefuroxidine and ampicillin but were susceptible to nalidixic acid and ciprofloxacin. Six resistant patterns were observed, with ampicillin-cefuroxime sodium-cephazolin-ceftazidime and streptomycin-ampicillin-cefuroxime sodium-cephazolin-ceftazidime resistant patterns exhibited by two isolates each. The substantial multiple resistance pointed to the fact that limitations could be faced in choosing drugs for the treatment of Salmonella infections and that mortality and economic losses could be experienced especially if sensitivity tests are not carried out before antimicrobial choice is made for treatments in both man and animals.

Keywords; multiple drug resistance, *Salmonella*, mortality, wildlife, man

Corresponding Author:
email:oludairo@hotmail.com

INTRODUCTION

The genus *Salmonella* is composed of motile bacteria which conform to the definitions of the family *Enterobacteriaceae* and tribe *Salmonellae* [1]. They have assumed increased significance due to their ubiquitous distribution, the growing number of serotypes, wide host range (including wildlife), complex pathogenesis and complicated epizootiology involving human, domesticated and wild animals and the environment [2-4]. The bacteria inhabit the intestinal tracts of vertebrate and invertebrate animals worldwide with recognized carrier states. The carrier states are the major sources of infection to human and animals. Excretion of the organism results in the contamination of water, food and the environment [5, 6].

Salmonella has assumed great zoonotic importance [1]. It causes Salmonellosis in both man and animals causing acute and chronic diarrhoea and deaths [7]. *Salmonella* Typhi causes typhoid fever (enteric fever) which is a global infection with a fatality rate of 10% (8). The disease is a cause for concern and a major public health problem in developing countries in Africa and Asia, due to poor sanitary conditions and inadequate portable water [8, 9]. It has been reported that worldwide, there are more than 1.3 billion cases of human salmonellosis annually, with three million deaths [10] while the World Health Organization (WHO) estimated an annual infection rate of 21.6 Million and approximate death rate of 600,000 [8].

Antibiotic resistance which is defined as resistance to two antimicrobial agents [11] has increased among *Salmonella* spp. in various areas of the world [12] and multiple drug resistance which is defined as resistance to four or more separate classes of antimicrobials [13] has become a significant trend with *Salmonella typhimurium* and several other non typhoidal serotypes [14-17].

Multidrug-resistant (MDR) strains of *Salmonella* are now encountered frequently and the rates of multidrug-resistance have increased considerably in recent years. Even worse, some variants of *Salmonella* have developed multidrug-resistance as an integral part of the genetic material of the organism. They are likely to retain their drug-resistant genes even when antimicrobial drugs are no longer used, a situation where other resistant strains would typically lose their resistance [18].

When fluoroquinolones were first licensed for human therapy, no immediate rise in *Salmonella* resistance was observed. In contrast, when fluoroquinolones were subsequently licensed for use in food animals, the rates of fluoroquinolone-resistant *Salmonella* in animals and food, and then subsequently in human infections, rapidly increased in several countries [18].

While resistance to the fluoroquinolones often emerges as a result of mutations in the bacterial genome, resistance to other antimicrobials often spread by transfer of DNA between bacterial strains [18]. In some cases multidrug-resistance is transferred through the plasmid. A Danish study found that although persons with susceptible *Salmonella* infections had a higher mortality than the general population, persons with resistant *Salmonella* infections had an even higher mortality. The death rate for persons with multidrug-resistant infections was estimated to be 10 times higher in the two years following specimen collection than for the general population [18].

Most *Salmonella* strains are sensitive to chloramphenicol, ampicillin, streptomycin, tetracycline, cotrimoxazole and some other antibiotics [17]. Chloramphenicol was considered to be the most effective drug in the treatment of typhoid fever [18]. Some strains however, are highly resistant to some of these antibiotics as a result of mutation or acquiring transmissible resistance plasmid. This therefore makes it necessary to test the antibiotic sensitivities of any *Salmonella* isolated [17, 19]. Multi-drug resistance is indeed becoming prominent with *Salmonella* [20] and is limiting the choice of drug for therapy of *Salmonella* infections in both man and animals leading to increased hospitalizations, health costs and mortality and is raising more public health questions [17, 21, 22].

MATERIALS AND METHODS

Eight *Salmonella* spp. isolated from randomly selected wild animals at the Jos Wildlife Park and National Zoological Garden, Jos as described by Oludairo *et al*. [23, 24] were subcultured on XLD and incubated for 24 hours at 37 ^{o}C. Pure cultures were inoculated into 5ml tryptone soya broth and incubated for 24 hours at 37 ^{o}C. One drop (0.1 ml) of the broth mixture was then added to 10 ml sterile normal saline to get turbidity optically comparable to 0.5 McFarland. The mixture was then inoculated into the Mueller Hinton agar with sterile swabs. Each isolate was tested for susceptibility to a panel of 12 antibiotics.

The antibiotics used and their disc contents were nalidixic acid (30 µg), streptomycin (10 µg), sulphamethoxazole/trimethoprim (25 µg), chloramphenicol (30 µg), ampicillin (10 µg), cefuroxime (30 µg), ciprofloxaxin (5 µg), tetracycline (30 µg), cephazolin (30 µg), gentamicin (10 µg), sulphamethoxazole (25 µg) and ceftazidime (10 µg).

The plates were then incubated for 18 hours at 37 ^{o}C before the zones of inhibition were measured to the nearest millimeter, using a transparent ruler. The interpretations of the zones of inhibition were done according to the recommendations of the Clinical Laboratory Standard Institute [25].

RESULTS

All the eight *Salmonella* spp. tested with a panel of 12 antimicrobial agents, showed high multiple antibiotic resistance. Two isolates (25%) showed resistance simultaneously to four antimicrobial agents, three isolates (37.5%) were resistant to five antimicrobial agents; two isolates (25%) were resistant to six antimicrobial agents while one isolate (12.5%) was resistant to nine antimicrobial agents (Table I). The isolate resistant to nine antimicrobial agents was obtained from the peafowl.

All the eight tested *Salmonella* isolates exhibited multiple drug resistance. They were resistant to at least four and up to nine antimicrobial agents. All the eight *Salmonella* isolates tested were resistant to ampicillin, cefuroxime, cephazolin and ceftazidime, four of the isolates were resistant to streptomycin, three were resistant to tetracycline and chloramphenicol, two were resistant to sulphamethoxazole / trimethoprim, one was resistant to sulphamethoxazole while none of the eight isolates was resistant to nalidixic acid, ciprofloxaxin and gentamicin (Table I & II).

The patterns of resistance of the eight tested *Salmonella* isolates showed that isolates from Be2C and 3Cm2C had the same pattern of resistance. They were resistant to streptomycin, ampicillin, cefuroxime sodium, cephazolin and ceftazidime. Isolates from 1L4C and Ahe1C also exhibited the same resistance pattern; they were resistant to

ampicillin, cefuroxime sodium, cephazolin and ceftazidime. These and other patterns of resistance of the other isolates are shown in Table III.

The list of antimicrobial agents used including their concentration and the classes they belong to are contained in Table IV.

DISCUSSION

All the eight *Salmonella* isolates tested were resistant to at least four and up to nine antibiotics thereby displaying high level multiple drug resistance. The antimicrobial agents to which many of the isolates were resistant are commonly used antimicrobial agents. Abuse of these antibiotics, underdosage, indiscriminate use when it is not indicated etc. could be possible causes of multidrug resistance in these organisms [8, 25-30].

There are reports of ways *Salmonella* resist antimicrobial agents [11]. These include the use of plasmids and integrons to spread antibiotic resistance and transfer resistance factors among members of the genus *Salmonella* and family *Enterobacteriaceae* [30]. Biofilms have also been reported to aid the resistance of *Salmonella* to antibiotics [29].

Multiple drug resistance to *Salmonella* isolates reported in this study is in agreement with the reports of Kwaga *et al.* [25]; Hoff and Hoff [31]; Gopee et al. [31]; Patchanee et al. [32]; Donkor et al., [17]; Adetunji & Isola [8] and Perez-Montano et al. [33].

Multiple drug resistance makes treatment ineffective and therefore put human and animals infected with the organisms at risk of treatment failure and death [8, 17, 34].

The resistance and moderate resistance exhibited by *Salmonella* isolates to commonly used antibiotics like ampicillin, tetracycline, chloramphenicol, sulphamethoxazole/ trimethoprim in this study further affirm the submission that the phenomenon is attributed to the misuse and abuse of antimicrobial drugs due to poor enforcement of drug policies, insufficient control of drug prescription, easy access to antibiotics and ease of administration of some of the antimicrobial agents i.e. oral route [17, 21]. The isolates were highly susceptible to antimicrobial agents that are not commonly used like ciprofloxacin, nalidixic acid and gentimicin [31].

The isolates exhibited six multidrug resistance profiles, S-AMP-CXM-KZ-CAZ and AMP-CXM-KZ-CAZ profiles were observed in two different sets of *Salmonella* isolates, having exactly the same patterns of antibiotic resistance. This could be an indicator that there may be a link between the organisms in terms of antibiotics resistance, probably sharing genetic materials like plasmids which makes antibiotic resistance well coordinated and effective [35].

The public health importance of multiple drug resistance in *Salmonella* cannot be overemphasized. It is reported that in the last decade, many MDR strains of *Salmonella* are emerging, even those resistant to the drug of choice in invasive salmonellosis; such as cephalosporines [35]. This has been attributed to the spread of large resistance plasmid within the organisms with concern also for the possibility of the development of cross resistance to other drugs of choice to the organisms. It has been posited that extensive therapeutic use of the veterinary antimicrobial agents has been a major driving force in the dissemination of this resistance with the possibility of such resistant organisms infecting human beings (34). The emergence of more MDR strains of *Salmonella* may therefore make treatment of infections from the organism

difficult leading to higher morbidity and mortality in both man and animals [35].

Conclusion

Antibiotic sensitivity tests should be conducted on isolated organisms before antibiotics are used for treatment especially when dealing with diarrhoeic conditions to ensure drug effectiveness and the reduction of the development of antimicrobial resistance and multiple drug resistance.

Indiscriminate use of antibiotics should be discouraged in both humans and animals since drug resistance and multiple drug resistance strains of *Salmonella* could spread from wildlife to domestic animals and humans. Drug policies should be enforced and prescription drugs should be well controlled.

Periodic surveillance to monitor antibiotic resistance patterns should be done to guide in making decisions for chemotherapy in the treatment of salmonellosis and to detect emerging trends in antimicrobial resistance early in other to facilitate effective control measures.

Further studies could consider using more isolates for better understanding of multiple drug resistance strains and factors relating to resistance, multiple drug resistance, resistance pattern and similar resistance patterns in *Salmonella* isolates e.g. plasmids, integrons, biofilms etc.

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Table I : Inhibition zone measurement (mm) and interpretation for *Salmonella* isolates

Codes	NA	S	SXT	C	AMP	CXM	CIP	TE	KZ	CN	RL	CAZ
	26	0	0	0	0	0	39	0	0	21	0	0
Pf2A	(S)	(R)	(R)	(R)	(R)	(R)	(S)	(R)	(R)	(S)	(R)	(R)
Ahe1B	22	17	16	12	0	0	34	18	6	23	18	0
	(S)	(S)	(S)	(R)	(R)	(R)	(S)	(R)	(R)	(S)	(S)	(R)
2Sh1C	26	14	0	29	0	0	29	21	12	22	29	0
	(S)	(R)	(R)	(S)	(R)	(R)	(S)	(S)	(R)	(S)	(S)	(R)
	30	18	34	32	0	0	0	24	12	21	29	0
1L4C	(S)	(S)	(S)	(S)	(R)	(R)	(S)	(S)	(R)	(S)	(S)	(R)
	28	13	34	26	0	0	42	24	12	22	26	0
3Cm2C	(S)	(R)	(S)	(S)	(R)	(R)	(S)	(S)	(R)	(S)	(S)	(R)
	28	18	31	30	0	0	35	20	12	15	30	0
Ahe1C	(S)	(S)	(S)	(S)	(R)	(R)	(S)	(S)	(R)	(S)	(S)	(R)
	25	14	33	30	0	0	42	20	10	21	22	0
Be2C	(S)	(R)	(S)	(S)	(R)	(R)	(S)	(S)	(R)	(S)	(S)	(R)
	20	16	20	8	0	0	36	16	0	19	20	0
2SrhC	(S)	(S)	(S)	(R)	(R)	(R)	(S)	(S)	(R)	(S)	(S)	(R)

NA; Nalidixic acid, S; Streptomycin; SXT; Sulphamethoxazole, C; Chloramphenicol, AMP; Ampicillin, CXM; Cefuroxime sodium, CIP ; Ciprofloxaxin, Te; Tetracycline, KZ; Cephazolin, CN; Gentamicin, RL; Sulphamethoxazole, CAZ; Ceftazidime.Pf2A; Peafowl, Ahe1B; African hawk eagle, Sh1C; Spotted hyena, 1L4C; Lion, 3Cm2C; Chimpanzee, Ahe1C; African hawk eagle, Be2C; Bateleur eagle, 2SrhC;Stripped hyena; R, Resistant; S, Sensitive

Table II: List of antimicrobial agents, number and percentages of *Salmonella* isolates resistant to the antimicrobial agents tested (n=8).

Antimicrobial Agents	Number / (%) of Isolates Resistant
Nalidixic Acid (NA)	0 (0)
Ciprofloxaxin (CIP)	0 (0)
Gentamicin (CN)	0 (0)
Sulphamethoxazole (RL)	1 (12.5)
Sulphamethoxazole / Trimethoprim (SXT)	2 (25)
Chloramphenicol (C)	3 (37.5)
Tetracycline (TE)	3 (37.5)
Streptomycin (S)	4 (50)
Ampicillin (AMP)	8 (100)
Cefuroxime Sodium (CXM)	8 (100)
Cephazolin (KZ)	8 (100)
Ceftazidime (CAZ)	8 (100)

Table III: Codes of *Salmonella* isolated from Wild animals and their Patterns of resistance to tested antimicrobial agents.

Wild Animal Code	*Salmonella* Isolates Resistance Pattern								
Pf2A	S	SXT	C	AMP	CXM	TE	KZ	RL	CAZ
Ahe1B	C	AMP	CXM	TE	KZ	CAZ			
2Sh1C	S	SXT	AMP	CXM	KZ	CAZ			
1L4C	AMP	CXM	KZ	CAZ					
3Cm2C	S	AMP	CXM	KZ	CAZ				
Ahe1C	AMP	CXM	KZ	CAZ					
Be2C	S	AMP	CXM	KZ	CAZ				
2SrhC	SXT	AMP	CXM	KZ	CAZ				

NA; Nalidixic acid, S; Streptomycin; SXT; Sulphamethoxazole, C; Chloramphenicol, AMP; Ampicillin, CXM; Cefuroxime sodium, CIP; Ciprofloxaxin, Te; Tetracycline, KZ; Cephazolin, CN; Gentamicin, RL; Sulphamethoxazole, CAZ; Ceftazidime.Pf2A; Peafowl, Ahe1B; African hawk eagle, Sh1C; Spotted hyena, 1L4C; Lion, 3Cm2C; Chimpanzee, Ahe1C; African hawk eagle, Be2C; Bateleur eagle, 2SrhC;Stripped hyena.

Table IV: List of antimicrobial agents, concentrations and their classes.

Nalidixic Acid (NA) 30 µg	Quinolone
Streptomycin (S) 10 µg	Aminoglycoside
Sulphamethoxazole/Trimetoprim (SXT) 25 µg	Folate pathway inhibitor
Chloramphenicol (C) 30 µg	Phenicol
Ampicillin (AMP) 10 µg	Penicillin
Cefuroxime Sodium (CXM) 30 µg	Cephem
Ciprofloxaxin (CIP) 5 µg	Fluoroquinolone
Tetracycline (TE) 30 µg	Tetracycline
Cephazolin (KZ) 30 µg	Cephem
Gentamicin (CN) 10 µg	Aminoglycoside
Sulphamethozazole (RL) 25 µg	Folate pathway inhibitor
Ceftazidime (CAZ) 10 µg	Cephem