Original Research Article

Multidrug Resistant *Salmonella* Species Isolated from Dam Water from Parts of Kaduna State, Nigeria

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A B S T R A C T

Dam water from parts of Kaduna was investigated for the presence of multidrug resistant *Salmonella* spp. A total of four hundred and fifty water samples) were sampled from (Galma, Shika and Kubanni Dams. The samples were collected over a period of Ten months (October 2016– July 2017). The isolates were enriched in Selenite broth and streaked on Salmonella Shigella agar (SSA). From these samples, 27 isolates were confirmed to be *Salmonella arizonae*, 14 were *Salmonella pullorum* while 2 were *Salmonella cholearesuis*. They were all selected for antibiotic susceptibility testing using the Kirby-Bauer method. The isolates were tested for resistance to ten antibiotics. The highest resistance observed was to Ampicillin, Nalidixic acid and Amoxiclav 43(100%) while all the isolates showed susceptibility to Imipenem and Ceftriaxone 43(100%). Multidrug resistance were observed in 24(55.8%) of the isolates which showed resistance to six of the antibiotics while the least was 3(7.0%) showing resistance to four antibiotics. The isolates that were resistant to the highest number of antibiotics were obtained from Shika dam and Galma water samples.. The isolation of antibiotic resistant bacteria in reservoir water is of public health concern due to the possibility of transferring multiple antibiotic resistances in the environment.

Introduction

Salmonella are facultative anaerobic, gram negative, small rods, motile (Bell and Kyriakides, 2002; Molbak, 2004). *Salmonella* species are classified and identified into serotypes according to the White-Kauffmann-Le Minor scheme; there are more than 2,500 *Salmonella* serotypes that have been described and reported (Wattiau *et al.,* 2011). The genus *Salmonella* consists of two species *S. enterica* and *S. bongori.* Most of the serotypes pathogenic to humans and animals belong to *S. enterica* (Pui *et al.,* 2011).

The *Salmonellae* are constantly found in environmental samples and in almost all types of aquatic environment, because they are excreted by humans, pets, farm animals, and wild life. Their presence in water, therefore, indicates faecal contamination. Municipal sewage effluents, pollution from agricultural

products, storm water runoff from contaminated environments and direct deposit of faecal materials from wild animals and birds are the main sources of these pathogens in natural waters (Bhatta *et al.,* 2007).

Salmonella causes four clinically distinguishable forms of infections in humans. These are gastroenteritis, enteric fever, bacteremia and other complications of non typhoidal salmonellosis as well as chronic carrier state in people of all ages. The transmission of Salmonella species takes place through the feacal-oral route, by means of contaminated food and water (Barry *et al.,* 2012).Several antibiotics are used in the treatment of Salmonella serovars in both human and veterinary medicine, the overuse of these antibiotics has lead to the emergence of antibiotic drug resistance (Zhang *et al.,* 2009).

The misuse of antimicrobial agents as chemotherapy in human and veterinary medicine or as growth promoter in food animals can potentially lead to widespread dissemination of antimicrobial resistance *Salmonella* and other pathogens via mobile genetic elements (Bouchrift *et al.,* 2009). Recent findings showed the consequences of antibiotic resistant on human health (Abatcha *et al.,* 2014). These consequences can be divided into two categories: first, the infections that may not have occurred and secondly, the high incidence of treatment failures and increase in the severity of disease (WHO, 2009). Moreover, resistance can spread from non-human sources to human by various routes such as animal, water and contaminated foods. However, contact with *Salmonella* carrier animal is the most important pathway in transmission of resistance to humans. Resistance to combinations of many classes of antimicrobial agents in *Salmonella* has led to the re-emergence of multidrug resistance *Salmonella*

(MDR) strains that may pose a risk to humans (White *et al.,* 2001; O'Brien, 2002). Many researches in the last two decades indicated the occurrence of MDR strains in different *Salmonella* serovars (Abatcha *et al.,* 2014, Chen *et al.,* 2004; Gebreyes and Thakur, 2005; Zhao *et al.,* 2008). The emergence and persistence of antibiotic resistance in *Salmonella* species continue to pose serious risks to human health (Joseph *et al.,* 2008). In Nigeria, morbidity associated with illnesses due to *Salmonella* continues to be on the increase and, in some cases, resulting in death. New concerns have been identified. Since the beginning of the 1990s, strains of *Salmonella* which are resistant to a range of antimicrobials, including first-choice agents for the treatment of humans, such as chloramphenicol and cotrimoxazole, and the third-generation cephalosporins, have emerged and are threatening to become a serious public health problem (Akinyemi *et al.,* 2006, Hakanen *et al.,* 2001).

Materials and Methods

Study area

The study was conducted in Kaduna State, Nigeria in three selected dams: The Galma dam, The Kubanni dam and The Shika dam.

Collection of samples

The water samples were collected in three different dams in Kaduna State, Nigeria which included the Galma dam, the Kubanni dam and the shika dam. A total of 450 water samples were taken from the three dams during a 10 month period. Using 2L sterile plastic containers, raw water samples were collected from the reservoirs. The water samples were collected in batches and each batch of the samples were taken to the laboratory at 4^0C in a cooler for analysis

Enrichment and isolation of *Salmonella* spp from water samples

Approximately 10ml of each water sample was enriched in 10ml Selenite cystine broth (Difco) and incubated for 18-24 hour at 37^0C. It was sub cultured onto *Salmonella-Shigella* agar (Oxoid) (SSA) and plates were incubated at 37^0C for 48 h for isolation of *salmonellae* (Cheesebrough, 2006). Representative colonies from *Salmonella-Shigellae* agar (Oxoid) (SSA)plates showing growth presumptive for *Salmonella* was examined based on cultural and morphological characteristics that is transparent colonies with black centre and Gram stained (Cheesebrough, 2006).

Biochemical characterization of isolates

The following biochemical tests were done to identify Salmonella Species following Standard methods. Sugar fermentation in Triple Sugar Iron (TSI) agar, Hydrogen sulphide formation Test, Citrate utilization Test, Oxidase Test, Indole Test, Motility Test, Urease Test, Methyl red Test, Voges-Proskauer Test, Lysine decarboxylase Test (Mcall,1955) It was further Confirmed using a commercial identification Microgen test kits

Determination of antibiotic susceptibility profile of the isolates

The antibiotic susceptibility patterns of the isolates was determined using disk diffusion method of Kirby-Bauer on Mueller-Hilton agar according to CLSI guidelines for antimicrobial susceptibility testing (CLSI, 2017).

Standardization of inoculum

Pure culture of identified *Salmonella* isolates from an 18-hour plate culture was selected. Sterile wire loop was used to select 3 colonies of each *Salmonella* serotype and emulsified in 5 ml of sterile normal saline which was compared to 0.5 McFarland standards. A sterile cotton swab was used to distribute the bacteria evenly over the entire surface of Mueller-Hinton agar. The inoculated plates were left at room temperature to absorb for 3-5 minutes. The antimicrobial disc concentration used were: Ampicillin, (AMP), (10µg); Ciprofloxacin, (CIP), (5µg); Sulphamethoxazole-trimethprim (Cotrimoxazole), (SXT), (25µg); Nalidixic acid, (NAL), (30µg); Imipenem (10µg), Chloramphenicol (C), (30µg); Ceftriaxone (CTX), (30µg), Cefoxitin (FOX), (30µg); Gentamycin (CN), (10µg) and Augmentin, (AUG), (30µg). The antibiotic discs (Oxoid) were placed on the surface of the seeded Muller-Hinton plate and incubated at 37^0C for 24 hours for growth. The diameter of the zone of clearance were measured to the nearest whole millimeter and interpreted on the basis of CLSI guidelines for antimicrobial susceptibility testing (CLSI, 2015). The multiple antibiotic resistance (MAR) index was calculated and interpreted according to Krumperman (1983).

Data analysis

The results were presented in the form of tables 1–4 while the association between the resistant rates of *Salmonella* isolates and also the antibiotic activities were also analysed using SPSS IBM (Version 20 for window) statistical package at $p<0.05$ level of significance

Results and Discussion

Distribution of organisms isolated from the water samples from the three dams

A total of 450 water samples were collected from three selected dams in parts of Kaduna State. *S. arizonae* was the most predominant accounting for 6.0%, followed by *S. pullorum* (3.1%) and *S. choleraesuis* (0.4%). The Shika

Dam (7.3%) water samples were the most contaminated while Kubanni Dam (2.7%) the least contaminated. There were significant differences among water samples which were positive for *Salmonella* spp (p≤0.05);

The antimicrobial susceptibility pattern of the 43 *Salmonella* spp isolated from water samples using ten antibiotics. All the 43 isolates (100%) were resistant to ampicillin, nalidixic acid and augmentin. They were also resistant to cefoxitin and cotrimoxazole with 95.3%. Intermediate resistance occurred for chloramphenicol (60.5%).The antibiotics effective against all the *Salmonella* isolates tested were ceftriaxone and imipenem (100%), Gentamicin (93%) and ciprofloxacin(74.4%).

The antibiotic resistance profiles of *S. arizonae, S. choleraesuis* and *S. pullorum* from water samples to different antibiotics. High resistance to antibiotics were seen in most of the antibiotics used except for ceftriaxone and imipenem (100%), Gentamicin (93%) and ciprofloxacin(74.4%).

The antibacterial resistance patterns and the MAR index of selected bacterial species isolated from water samples to different antibiotics. Highest levels of multidrug resistant were observed in 24(55.8%) of the isolates which showed resistance to six of the antibiotics; 9(20.9%) of the isolates were resistant to five antibiotics; 7(16.3%) of the isolates were resistant to seven antibiotics; followed by 3(7.0%) that were resistant to four antibiotics. The highest resistance was seen with the 24(55.8%) isolates showing resistance to six antibiotics.

The study revealed the presence of *Salmonella* in dam samples analyzed with an overall isolation rate of 9.5%. This finding, in itself, is not surprising since higher isolation rates of *Salmonella* is reported to be present in water samples in Nigeria (Akinyemi *et al.,* 2006, 2011; Oguntoke *et al.,* 2009; Oluyege *et al.,* 2009 and Jasini *et al.,* 2016).

Table.1 Distribution of *Salmonella* spp from water samples from selected dams in Kaduna State

Location (N=150)	*S. arizonae* No. isolated (%)	*S. pullorum* No. isolated (%)	*S. choleraesuis* No. isolated (%)	p-value
Shika dam (150)	11(7.3)	9(6.0)	0(0.0)	0.015
Kubanni dam (150)	4(2.7)	4(2.7)	1(0.7)	
Galma dam (150)	12(8.0)	1(0.7)	1(0.7)	
Total Frequency N=450	**27(6.0)**	**14(3.1)**	**2(0.4)**	

Table.2 Antimicrobial susceptibility of *Salmonella* spp isolated from selected dams in Kaduna State

S/N	Antibiotic	Disc potency (µg)	Susceptibility (n=43)		
			Sensitive No (%)	**Intermediate No (%)**	**Resistant No (%)**
1	AMP	10	0(0.0)	0(0.0)	43(100)
2	NAL	30	0(0.0)	0(0.0)	43(100)
3	AMC	30	0(0.0)	0(0.0)	43(100)
4	FOX	30	1(2.3)	1(2.3)	41(95.3)
5	SXT	25	2(4.7)	0(0.0)	41(95.3)
6	C	30	8(18.6)	9(20.9)	26(60.5)
7	GEN	10	40(93.0)	0(0.0)	3(7.0)
8	IPM	10	43(100)	0(0.0)	0(0.0)
9	CIP	5	32(74.4)	1(2.3)	10(23.3)
10	CEF	30	43(100)	0(0.0)	0(100)

KEY: AMP= ampicillin, IMP=Imipenem., SXT= cotrimoxazole, NAL =nalidixic acid, AMC= amoxicillin &clavulanic acid, CN= gentamycin, FOX=cefoxitin, C=chloramphenicol, CIP=ciprofloxacin and CEF=Ceftriaxone

Table.3 Antibiotic resistance profile of *S.arizonae, S.choleraesuis* and *S.pullorum* isolated from water sampled from dams in Kaduna State

	Percentage of resistant isolates		
	S.arizonae (n =27)	*S.pullorum* (n=14)	*S.cholearesuis* (n=2)
Antibiotics			
AMP	100	100	100
AMC	100	100	100
SXT	92.6	100	100
NAL	100	100	100
FOX	92.6	100	100
GEN	7.4	7.1	100
IPM	0.0	0.0	0.0
CIP	29.6	14.3	0.0
C	55.5	71.4	50.0
CEF	0.0	0.0	0.0

N = Total Number(%) of *Salmonella* isolates resistant to antibiotics

n =Number of isolates that are resistant.

KEY:AMP= ampicillin, IMP=Imipenem., SXT= cotrimoxazole, NAL =nalidixic acid, AMC= amoxicillin &clavulanic acid, CN= gentamycin, FOX=cefoxitin, C=chloramphenicol, CEF=ceftriaxone, CIP=ciprofloxacin

Table.4 Antibacterial resistance patterns of *Salmonella* species isolated from water samples to 10 different antibiotics

Number of Antibiotics	Number of resistant isolates (%)	Resistant pattern	MARI
4	3(7.0)	AMP-NAL-AMC-FOX AMP-SXT-NAL-AMC	0.4
5	9(20.9)	AMP-SXT-NAL-AMC-FOX AMP-NAL-AMC-FOX-C	0.5
6	24(55.8)	AMP-SXT-NAL-AMC-FOX-C AMP-SXT-NAL-AMC-CIP-FOX AMP-SXT-NAL-AMC-CN-FOX	0.6
7	7(16.3)	AMP-SXT-NAL-AMC-CN-FOX-C AMP-SXT-NAL-AMC-CIP-FOX-C	0.7

KEY:AMP= ampicillin, SXT= cotrimoxazole, NAL =nalidixic acid, AMC= amoxicillin &clavulanic acid, CN= Gentamicin, FOX=cefotaxime, C=chloramphenicol, CIP=ciprofloxacin

Moreover, previous studies reported that *Salmonella* may survive and remain virulent for long periods in the environment,, they may undergo a viable but nonculturable stage as a survival strategy when they encounter environmental stresses and cause infection (Martinez *et al.,* 2005) The 9.5% isolation rate of *Salmonella* obtained is however, lower than the 16% isolation rate by Adzitey *et al.,* (2016) from Dam water used for drinking in Tamale, Ghana and 13.3% by Oluyege *et al.,* (2009) in Nigeria.

The distribution of *Salmonella* species showed that a total of Forty three *Salmonella* isolates were obtained from the water samples collected from these selected dams and the most common specie isolated in this study were *S. arizonae*, *S. pullorum* and *S. choleraesuis*. *S. arizonae* and *S. pullorum*, were isolated from all the three dam sites

while *S. choleraesuis* were isolated from only Kubanni and Galma dam. These result showed that their presence in water, therefore, indicates faecal contamination, Sewage effluents, agricultural run-off and direct deposit of faecal materials from wild animals, reptiles and birds which are the major sources of the contamination in aquatic environments. The results agreed with the findings of (Arii *et al.,* 2002; Chen *et al.,* 2004, Parry *et al.,* 2002, Martinez *et al.,* 2005) who had faecal contamination in there samples.

The importance of susceptibility testing cannot be over emphasized. It provides guidance to selective use of drug for therapy and information on the spectrum of an antimicrobial agent. Therefore, it is of great importance to monitor antibiotic resistance among pathogenic bacteria isolates from water samples to detect emerging resistant

pathogens and antibiotic resistance trends (Heuer *et al.,* 2011). From the results obtained in this study, The antimicrobial susceptibility pattern of the 43 *Salmonella* spp isolated from the water samples using ten antibiotics revealed that Ceftriaxone and Imipenem was the most susceptible with 43(100%) followed by Gentamycin and Ciprofloxacin with 93% and 74.4% respectively.

43 isolates were 100% resistant to Ampicillin, Nalidixic acid, and Amoxiclav followed by Cotrimoxazole and Cefoxitin with 95.3% while Chloramphenicol had 60.5% in order of decreasing resistance. The findings compares with Effa *et al.,* (2011), Tesfaw *et al.,* (2013), Poonia *et al.,* (2014), Jasini *et al.,* (2016) who stated that third-generation cephalosporin such as ceftriaxone and fluoroquinolones are the drugs of choice for first line of treatment. The origin of resistance in water samples can be traced to the faecal constituent of the raw water from the dams. The high rate of multidrug resistance by *Salmonella* isolates correlates with the works of Adley *et al,* (2011) and Oluyege *et al.,* (2009), which reported that *Salmonella* exhibit multidrug resistant patterns to more than four antibiotics. This result could be due to the overuse of antibiotics in animals as growth promoter. The mass treatment and long-term administration of antimicrobial growth promoters to animals may lead to the emergence of multidrug-resistant strains of enteric bacteria, including *Salmonella* spp. highest resistance seen in Ampicillin, Nalidixic acid and Amoxiclav could also be due to the usage of these drugs both in human medicine. This has resulted in the increase in the development of increasing number of resistance strains to so many antibiotics through the horizontal gene transfer by mobile genetic elements. The high resistance could also be due to the use of fake or expired drugs, abuse and misuse of the drugs among the general populace. In the developing world

it is a serious problem particularly when the drugs are purchased in local drug stores without appropriate prescription thereby taken at low dosages and at times results in incomplete dosages only to alleviate symptoms. Most of the time the guidelines regarding selection of the drug prescription are not communicated to individuals that purchase this drugs thereby leading to self limiting infections especially when they are not bacterial infections but viral infections prescribed when not needed. Many countries don't have effective surveillance systems thereby leading to high morbidity and mortality rates, longer period of infectivity, increased cost and prolong hospital stay (Abdullahi *et al.,* 2013; Collingnon, 2003). The situation in the developing countries like Nigeria is however different, where antimicrobial agents are readily available to people in local drug stores without prescription (Kwaga and Adesiyun, 1984). The isolates being susceptible to Ceftriaxone, Imipenem, and Gentamycin could be due to cost effective and parenteral route for administration (Crump *et al.,* 2008). Antibiotic susceptibility patterns vary regionally and geographically and have been reported to change rapidly over time (Martinez *et al.,* 2005). The multiple antibiotics resistant patterns of *Salmonella* spp from water samples in this study is greater than 0.2 which implies that the strains of such *Salmonella* spp originated from an environment where several antibiotics are in use (Ehinmidu, 2003; Poonia *et al.,* 2014).

In conclusion, the study revealed the presence of *Salmonella* spp in dam samples analyzed with an overall isolation rate of 9.5%. All the *Salmonella* spp isolates were resistant to at least one of the antibiotics used in the study, indicating multiple resistance due to possible transfer of resistance genes. All the 43 isolates (100%) were resistant to ampicillin, nalidixic acid and Augmentin. The antibiotics

effective against all the *Salmonella* isolates tested were ceftriaxone and imipenem (100%), Gentamicin (93%) and ciprofloxacin (74.4%).

Recommendation

Based on the findings it is therefore recommended that attention should be focussed by both the public and government on putting in place antibiotic resistance surveillance programs in the country and infections due to multidrug resistant salmonella can be eliminated not by massive use of antibiotics but by improvement in the conditions of animal husbandry and reduction in the opportunities for the initiation and spread of the diseases.

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