



Multidrug Resistance Patterns and Multiple Antibiotic Resistance Index of *Salmonella* species Isolated from Waste Dumps in Zaria Metropolis, Nigeria

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ABSTRACT: Several items containing residual antimicrobial agents are disposed of in dumpsites, placing a pressure on the microbial flora and a potential for development of resistance in these microorganisms. Multidrug resistance patterns and multiple antibiotic resistance index of *Salmonella* spp. isolated from waste dumps in Zaria Metropolis were thus assayed in this study using one hundred and twelve (112) soil samples collected from four waste dumpsites located in Sabon-Gari, Samaru, Tudun-Wada and Zaria City. *Salmonella* spp. were isolated by culture methods on selective media and characterized using a series of biochemical tests. The isolates were confirmed using microgen identification kits. Results were statistically analysed using percentages. The antibiotic resistance patterns were determined, using the disc-diffusion method. Ten antibiotics belonging to eight different classes, namely B-lactams, aminoglycosides, tetracycline, cephalosporins, fluoroquinolone, Nitrofurantoin, sulphonamides, and phenicols were tested. The result of the study revealed that 57.2% of the isolates exhibited multidrug resistance (MDR) taken as resistance to four or more antibiotics tested. On the other hand, all the isolates showed 100% susceptibility to Chloramphenicol (30µg) and Gentamicin (30µg) while 76.2% had Multiple Antibiotic Resistance (MAR) Index of 0.2 and above. The isolates showing resistance to the highest number of antibiotics were obtained from refuse dumpsites in Zaria City while an isolate from Sabon-Gari was found to be resistant to six antibiotics. These results could be indicative of possible disposal of these drug residues in the waste dump locations making them hot spots for development of resistance.

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Antibiotics are present in different environments as a result of their use in human and veterinary medicine. Inappropriate and irrational uses of antimicrobial medicines provide favourable conditions for resistant microorganisms to emerge, spread and persist (WHO, 2011). Antimicrobial resistance (AMR) is considered one of the most important global threats to human health in the 21st century (Liu *et al.*, 2016). Increased outbreaks of enteric illnesses have invariably led to increased use of antibiotics for managing infections. AMR threatens the effective prevention and treatment of an ever increasing range of infections caused by bacteria, parasites, viruses and fungi; a post antibiotic era, in which common infections and minor injuries can kill and is a real possibility in the 21st century. *Salmonella* is the most common causative agent of a wide range of human diseases such as enteric fever, gastroenteritis, bacteraemia and other complications of non typhoidal salmonellosis. The *Salmonellae* are constantly found in environmental samples because they are excreted by humans, pets, farm animals and wild life (Nazzal *et al.*, 2012). The presence of *Salmonella* spp. and other bacteria genera such as *Pseudomonas*, *Staphylococcus*, *Vibrio*, *Neisseria*,

Escherichia coli, *Shigella*, *Bacillus* and *Klebsiella* in refuse dumps is indicative of possibly serious public health risk to waste handlers, scavengers and people living in the vicinity (Awisan *et al.*, 2011). Increase antibacterial drug resistance in *Salmonella* species has been a serious problem for public health worldwide and are of concern (WHO, 2014). The concentrations currently found in different environments and concentrations representing action limits used in environmental risk assessment may be high enough to exert a significant selective pressure on clinically relevant bacteria (Liu *et al.*, 2016). Multidrug resistance (MDR) is a common problem that hinders chemotherapy. To overcome this problem, it is important to identify the multidrug resistance pattern of an isolate. An attempt was made in this study to isolate *Salmonella* spp. from refuse dumps, and to determine their MDR pattern by subjecting the isolates to antibiotic susceptibility study.

MATERIALS AND METHODS

Study area: The survey was conducted in parts of Zaria Metropolis, Nigeria. The locations were Sabon-Gari, Samaru, Tudun-Wada and Zaria City. Zaria

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Metropolis is located on the high plains of Northern Nigeria, on latitudes 11°07'N to 11°51'N and longitudes 7°43'E to 7°45'E (Uba *et al.*, 2013). These are semi-urban with a high population density with the attendant generation of wastes. These wastes are seen disposed within the community owing to the poor waste disposal systems posing a risk of transmission of various disease agents.

Sample Collection: A total of 112 soil samples were collected from 4 dumpsites locations in Samaru (28), Sabon-Gari (28), Tudun-Wada (28) and Zaria City (28). At each sampling site, surface debris was removed and soil was dug to a depth of 15cm using a hand trowel (Isirima *et al.*, 2005). Soil was then scooped into a clean low density polythene bag and transported in cool boxes to the Bacteriology Laboratory in the Department of Microbiology, Ahmadu Bello University, Zaria for analysis. Samples were stored at 4°C if not analysed immediately.

Identification and Characterization of *Salmonella* spp: Briefly, 25g of each soil sample was suspended in 225ml sterile distilled water, and mixed using a sterilized glass rod. Then 1ml of the resultant solution was enriched in 9ml of sterile Selenite cysteine broth (Difco) in sterile duplicate McCartney bottles and incubated for 18-24hrs at 37°C for 24hours. Aseptically, inoculations were made from the overnight cultures on prepared sterile plates of *Salmonella Shigella* Agar (SSA) (Oxoid). All inoculated plates were incubated aerobically at 37°C for 48 hours for isolation of *Salmonellae* (Cheesebrough, 2006). Discreet colonies showing transparent colonies with dark centres were picked as presumptive *Salmonella* spp. The presumptive isolates were subjected to routine IMViC tests (Indole, Methyl red, Voges Proskaur, citrate utilization tests), Sugar fermentation in Triple Sugar Iron (TSI) agar, Hydrogen sulphide formation test among other tests. Isolates giving atypical responses for any of the above named tests were examined further using Microgen™ Gram negative Identification A system. The data obtained by the Microgen GN-ID A microwell strip was designed to generate a 4 digit octal code which was used to interpret the result from the Micogen Identification System Software (Nyandjou *et al.*, 2017).

Antibiotic Susceptibility Testing: Susceptibility of *Salmonella* spp. to ten antibiotics was determined using the disc-diffusion method as recommended by Clinical Laboratory Institute Standards (CLSI, 2014). The bacterial isolates were grown for 18 hours on nutrient agar at 37°C. They were suspended in 2 ml normal saline and turbidity adjusted to match

McFarland Optical Standard 0.5 (equivalent to 1.5×10^8 cfu/ml). Bacterial suspensions (0.1ml) were dispensed on the surface of sterile Mueller-Hinton agar (Oxoid, UK) plate and spread evenly using a sterile spreader. This was allowed to dry for 5 min and antibiotic discs were placed on the surface of the agar plates and incubated aerobically at 37°C for 18 hours. The susceptibility patterns of the isolates to the different antibiotics were noted as Sensitive (S), Intermediate (I) or resistant (R) following the standard of the CLSI (CLSI, 2014). The following antibiotics (single discs, Oxoid Ltd., Basingstoke, Hampshire, England) were tested: Ampicillin (10µg), Amoxicillin-clavulanic acid (30µg), Chloramphenicol (30µg), Ceftriaxone (30µg), Ciprofloxacin (10µg), Gentamicin (30µg), Ofloxacin (10µg), Tetracycline (30µg), Nitrofurantoin (30µg) and Sulphamethoxazole/Trimethoprim (25µg) (CLSI, 2014). Isolates that were observed to be resistant to at least four different antibiotics were classified as being multidrug resistant (Ezekiel *et al.*, 2011).

Determination of Multiple Antibiotic Resistance (MAR) Index: The multiple antibiotic resistance index $\frac{a}{b}$ was calculated, where a represents the number of antibiotics to which the isolates were resistant and b represents the total number of antibiotics to which the isolate was exposed (Mishra *et al.*, 2013).

RESULTS AND DISCUSSION

Salmonella spp. were obtained from soil samples of all locations and from all the dumpsites that were sampled (Tables 1). The antibiotic susceptibility pattern of 21 isolates of *Salmonella* spp. to ten commonly used antibiotics is presented in Table 2. The result indicated that 90% *Salmonella* spp. isolates were susceptible to Nitrofurantoin (30µg), 100% to Gentamicin (30µg) and Chloramphenicol (30µg), 76% susceptible to Ciprofloxacin (10µg), 62% to Ofloxacin (10µg) and 57% to Ceftriaxone (30µg) (Table3). On the other hand, 90% were resistant to Ampicillin, followed by Tetracycline (81%), Amoxicillin-clavulanic acid (71%), and Sulphamethoxazole-trimethoprim (52%).

The antibiotic resistant pattern of the *Salmonella* isolates is presented in Table 3. The results showed that the isolates produced 10 phenotypic resistance profiles and were resistant to 2-7 antibiotics. The isolates with the highest multidrug resistance were obtained from Zaria city. One isolate obtained from Sabon-Gari was found to be resistant to six antibiotics. Most of the resistant isolates in this study showed the Amp-Aml-Cef-Sxt-Te -resistance pattern. These antibiotics belong to the β-lactams, Sulfonamides and Tetracycline classes of antibiotics.

Table 1: Distribution of *Salmonella* spp. isolated from waste dumpsites in various locations

Sampling Location	Dump Location	Number of Sample Collected	<i>Salmonella</i> spp. N (%)	Number of isolates/location N (%)
Sabon-Gari	Army children school	7	1(14.3)	6(21.4)
	Chikaji	7	2(28.6)	
	Independent cinema	7	1(14.3)	
	Rail way Yan Katako	7	2(28.6)	
Samaru	Hayin-Dogo	7	2(28.6)	4(14.3)
	Kwangila	7	1(14.3)	
	Danra Estate	7	0(0.0)	
	Palladan	7	1(14.3)	
Tudun-Wada	Agolo	7	3(42.9)	7(25.0)
	Kwarin	7	2(28.6)	
	PZ	7	0(0.0)	
	Total Filling Station	7	2(28.6)	
Zaria City	Al-Hudahuda	7	2(28.6)	11(39.3)
	Dan-Gama	7	3(42.9)	
	Kusfa-Zaria	7	4(57.1)	
	Limancikwana	7	2(28.6)	

N-Number of isolates

Table 2: Antimicrobial Susceptibility Patterns of *Salmonella* spp. Isolates from Waste Dump Samples

<i>Salmonella</i> spp. (N=21)				
Antibiotics Tested	Symbol (µg)	R (%)	I (%)	S (%)
Ampicillin	AMP (10)	19(90.5)	1(4.8)	1(4.8)
Amoxicillin-clavulanic acid	AMC (30)	15(71.4)	4(19.1)	2(9.5)
Chloramphenicol	CHL (30)	0(0.0)	0(0.0)	21(100)
Ceftriaxone	CEF (30)	8(38.1)	1(4.8)	12(57.1)
Ciprofloxacin	CIP (10)	2(9.5)	3(14.3)	16(76.2)
Gentamicin	CN (30)	0(0.0)	0(0.0)	21(100)
Nitrofurantoin	F (30)	2(9.5)	0(0.0)	19(90.4)
Ofloxacin	OFX (10)	4(19.0)	4(19.0)	13(62.0)
Sulphamethoxazole/trimethoprim	SXT (25)	11(52.4)	1(4.8)	9(42.9)
Tetracycline	TE (30)	17(81.0)	0(0.0)	4(19.0)

Key: N-Number of isolates tested, R- Resistant, I-Intermediate, S-Susceptible

Table 3: Antibiotic Resistance Patterns of *Salmonella* spp. Isolates

Number of Antibiotics	Number of Resistant isolates (%)	Isolate code	Dumpsite Location	Resistant Pattern
2	3(14.3)	ZC ₁₀₁	Kusfa-Zaria	Amp-Te
		SA ₁₂	Hayin-Dogo	
		TW ₆₇	Agolo	
		TW ₉₂	Kwarin	
3	4(19.0)	ZC ₈₄	Al-Hudahuda	Amp-Amc-Cef
		SA ₄₆	Kwangila	Amp-Amc-Ofx
		ZC ₃₃	Dan-Gama	Amp-Cip-Te
4	6(28.6)	SG ₄₉	RailwayYan	Amp-Amc-Sxt-Te
		SA ₂₈	Katako,	
		ZC ₅₇	Hayin-Dogo	
		SG ₃₆	Chikaji,	
		TW ₁₄ ,TW ₃	Agolo, Total	
5	3(14.3)	ZC ₆ , ZC ₄₃	Kusfa-Zaria	Amp-Amc-Cef-Sxt-Te
		TW ₃₁	Kwarin	
6	1(4.8)	SG ₅₂	Chikaji	Amp-Amc-Cef-Sxt-Ofx-Te
7	2(9.5)	ZC ₄₇	Limancikwana	Amp-Amc-Ofx-Sxt-Cef-F-Te
		ZC ₂₄	Dan-Gama	

Key: SA-Samaru, SG-Sabon-Gari, TW-Tudun-Wada, ZC-Zaria city, AMP-Ampicillin, AMC- Amoxicillin-clavulanic acid, CHL-Chloramphenicol, CIP-Ciprofloxacin, CEF-Ceftriazone, GN-Gentamicin, SXT-Sulphamethoxazole/trimethoprim, F-Nitrofurantoin, TE-Tetracycline

Fifty seven (57%) of the isolates tested were multidrug resistant (MDR). Multidrug resistance was taken as

resistance of isolate to at list four different antibiotics (Ezekiel *et al.*, 2011). Multiple resistance was

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observed with 9.5%, 28.6%, 4.8% and 14.3% resistant to 7, 4, 6 and 5 antibiotics respectively (Figure 1). The occurrence of Multiple Antibiotics Resistance Index (MARI) ranged from 0.2 – 0.7. Isolates with 0.4 had the highest occurrence of 28.6%, followed by isolates with 0.3 (19.0%), 0.5(14.3%), 0.2(14.3%) and 0.7(9.5%). Isolates with 0.6 MARI had the least occurrence of 4.8% (Figure 2). Isolation of *Salmonella* spp. from refuse dumpsites showed that waste management is generally poor in the locations that were sampled. *Salmonella* is the most common causative agent of a wide range of human diseases. The *Salmonellae* are constantly found in environmental samples because they are excreted by humans, pets, farm animals and wild life. Their presence in waste directly confirms the presence of faecal wastes at various dumpsites since it was common practice to dump human excreta in the sites which could also be used as latrines at night. The organisms have also been isolated by other researchers who worked with waste dumps in other parts of the country like (Ikpeme *et al.*, 2011; Odeyemi *et al.*, 2011; Adekanle *et al.*, 2014). The variation in the susceptibility and resistance level of the *Salmonella* isolates to different classes of antibiotics in this study could be due to differences in strains from location to location thus the rate of resistance to antibiotics varies among them. High incidence resistance was evident against Ampicillin (90.5%), Amoxicillin-clavulanic acid (71.4%) and Tetracycline (81%). Resistance of the *Salmonella* isolates to various antibiotics has been demonstrated in previous studies (Boyen *et al.*, 2008; Duru *et al.*, 2014; Titilawo, 2015). The marked resistance of species of *Salmonella* to β -lactams and Tetracycline classes of antibiotics as shown in the present study agrees with the findings of Ash *et al.* (2002) and Gregory *et al.* (2013) working on rivers in the United States. Similarities in antibiograms among isolates from both environmental sources indicate a possible infiltration of pathogens from dumpsite soils to water sources. The high resistance observed to Tetracycline may be attributed to it being the most commonly available antibiotic used as growth promoter and routine chemoprophylaxis among livestock in Nigeria (Olatoye, 2010). This is worrisome considering the fact that Tetracycline is a first line drug in Nigeria, and as in most developing countries, people with gastrointestinal infections readily purchase it across the counter for self-medication (Chigor *et al.*, 2010). Results of several studies have also revealed bacterial resistance to the penicillin family (Ampicillin and Amoxicillin-clavulanic acid in this study) [Ikpeme *et al.*, 2011; Odeyemi, 2012; Adekanle *et al.*, 2014; Nyandjou *et al.*, 2017]. These could be because these antimicrobial agents are inexpensive and affordable. The

inexpensive drugs in developing countries are widely available across the counter without prescription from authorised health institutions and pharmacies, as well as from unauthorised patent medicine shop and other distributors (Sharma and Rai, 2012). The observed high resistance of the isolates to some of the antibiotics in this study is not surprising as factors that could promote or encourage development of antibiotic resistance such as non-adherence to treatment strategies, sub-standard drugs, over-the-counter sale of potent antibiotics and self-medication are present in developing countries including Nigeria. Over use/miss-use and the use of antibiotics at low concentration is a potentially dangerous practice that can encourage the production of antibiotic resistant organisms.

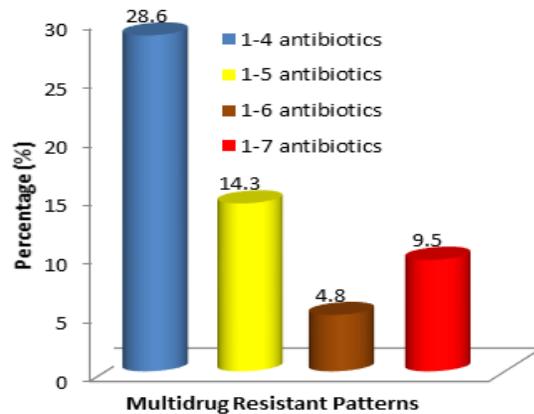


Fig 1: Percentage Multidrug Resistance of *Salmonella* spp. Isolates

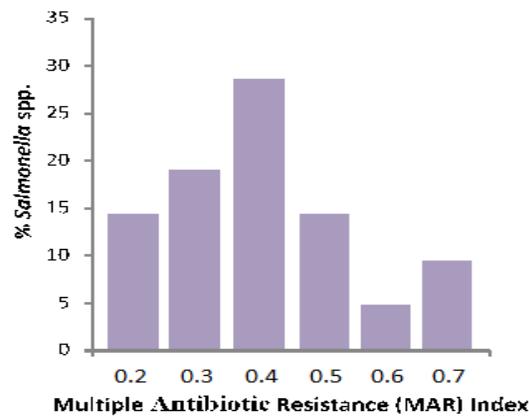


Fig 2: Percentage *Salmonella* spp. and their Multiple Antibiotic Resistance Indices

When people use sub-therapeutic doses of antibiotics, as obtained when the dosage for the drugs are not strictly adhered to, highly resistant strains of the organisms are selected sequentially. Poor quality drugs can provide sub-inhibitory selective pressure, of which neither the patient nor the prescriber may be aware. There have been numerous reports of sub-standard drugs in Nigeria. These reports describe drug

preparations containing between 0-80% of the stated label claim. Some of them contain such low concentrations that they can only be considered counterfeit, meaning that they were deliberately manufactured with low or no active drug content. Others may have complied with pharmacopoeia standards at some time but have, in the course of distribution and display, been degraded by heat and humidity (Okeke and Sosa, 2003).

The isolates were susceptible to Chloramphenicol, Gentamicin followed by Ciprofloxacin and ceftriazone. This agrees with the work of Tesfaw *et al.* (2013), Jasil *et al.* (2016) who reported high susceptibility of *Salmonella* spp. isolates to fluoroquinolone and third-generation cephalosporin (Ciprofloxacin and Ceftriazone in this study). High susceptibility levels to the newer, more valuable antimicrobial compounds, such as phenicols, aminoglycosides, as well as fluoroquinolones and third-generation cephalosporin could yield good therapeutic results in treating infections caused by MDR *Salmonella* spp. and probably Enterobacteriaceae in general. Multidrug resistance has become a common feature of many microorganisms especially the human pathogens. Data obtained in this study showed higher percentage MDR (57.2%) than those earlier reported (Ezekiel *et al.*, 2011; Tesfaw *et al.*, 2013; Jasil *et al.*, 2016) in which about 50% of the isolates tested exhibited multidrug resistance, suggesting the existence of higher frequency of MDR species in our study area. Multiple antibiotic resistance (MAR) among bacterial isolates from the various study locations is frightening because such organisms can become endemic within the environment and pose serious public health threats. Bacterial antibiotic resistance is transferable. If the resistant bacteria are allowed to distribute in the environment, they will most likely transfer resistance gene to other bacteria of same species or different. Dissemination of these resistant bacteria will not be restricted to a particular geographical area; drug resistance can be expected to spread steadily to all parts of the world. The scenario is plausible, but the risks cannot be estimated because of the insufficient understanding and of limited data availability. The isolation of antibiotic resistant bacteria in waste dump samples therefore is of public health concern because of the possibility of transferring multiple antibiotic resistances to the microbial flora and a potential for development of resistance in these microorganisms. They may also act as reservoirs which will contribute to the maintenance and spread of antibiotic resistance genes (Guardabassi *et al.*, 2004). Many of the *Salmonella* spp. isolates from this study were observed to have patterns of resistance that indicate that the

genes responsible were acquired from other organisms, such as resistances to antibiotics of different classes, and resistance that show that they produce extended spectrum beta-lactamases. Multidrug resistance (MDR) is a cause for concern in both clinical and veterinary medicine because it limits the therapeutic options available for treatment. Multiple antibiotic resistance (MAR) index is a measure of the extent of the isolates' resistance to antimicrobial agents within the group of antibiotics studied. According to Mishra *et al.* (2013), MAR index of 0.2 and above indicates high risk sources of contamination, MAR index of 0.4 and above is associated with human faecal source of contamination. Thenmozhi *et al.* (2014) opined that MAR index values > 0.2 indicate existence of isolate from high risk contaminated source with frequent use of antibiotics while values ≤ 0.2 show bacteria from source with less antibiotic usage. High MAR indices calls for vigilant surveillance and remedial measures. MAR index of 0.2 and above by 76% of *Salmonella* spp. isolates in this study is worrisome. Sensitivity patterns and treatment must be guided by laboratory investigations.

Conclusion: Results from this study showed that all parts of Zaria Metropolis were generally poor in sanitation and waste management and were reservoirs of antibiotic resistance. It is therefore advisable that, wastes should be removed regularly and the waste stream effectively managed. Persistent multiple drug resistance of most isolates to appropriate drugs of choice are of great public health concern and calls for periodic monitoring of antibiograms to detect possible changing patterns.

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