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ANTIBIOTIC SUSCEPTIBILITY OF SOME *SALMONELLA SPECIES* ISOLATED FROM DIARRHOEAL STOOLS OF HIV PATIENTS IN KADUNA, NIGERIA

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ABSTRACT

Antibiotic resistance is a growing phenomenom in contemporary medicine and has emerged as a serious public health concern of the 21st century in particular as it pertains to pathogenic organisms. A total of three hundred and ninety (390) stool samples of HIV seropositive individuals were screened for the presence of *Salmonella* using enrichment (selenite F broth) and selective (MCA,SSA and XLD) media. Biochemical identification tests were carried out using both the conventional and the Microgen Bioproduct identification system. Polyclonal antisera were employed for the slide agglutination tests. The standardized Kirby-Bauer technique was used for the antibiotic susceptibility testing where thirteen antibiotics were used. Three (3) *Salmonella typhi* and eleven (11) *Salmonella typhimurium* were isolated. Furthermore, 73% of *Salmonella typhimurium* isolated were resistant to ampicillin and Tetracycline respectively whereas all of the *Salmonella Typhi* were resistant to the antibiotics. Reduced susceptibility was observed with ciprofloxacin, pefloxacin, septrin and streptomycin for *Salmonella typhimurium* while no resistance was encountered for *Salmonella typhi*. An increased susceptibility of *Salmonella typhimurium* to chloramphenicol was observed but *Salmonella typhi* was completely susceptible. Ceftriaxone, Ofloxacin, Sparfloxacin and Amoxicillin showed effective antimicrobial activity against the *Salmonella serovars* and are therefore suggested as the drugs of choice for the treatment of Salmonellosis infections.

Keywords: Antimicrobial drugs, Chloramphenicol, Kirby-Bauer technique, *Salmonella serovars*, Susceptibility.

INTRODUCTION

Salmonellosis is an infection caused by *Salmonella* bacteria. Most persons infected with *Salmonella* develop diarrhoea, fever and abdominal cramps within 12 to 72 hours after infection. The illness usually lasts 4 to 7 days and most persons recover without treatment. The elderly, infants, and those with impaired immune systems are more likely to have a severe illness (Centre for disease control [CDC], 2009).

Salmonella can be found in food products such as raw poultry, eggs, beef and sometimes on unwashed fruits. Food prepared on surfaces that previously were in contact with raw meat or meat products can, in turn, become contaminated with the bacteria. This is called cross-contamination. In recent years, the Centre for Disease Control and Prevention has received reports of several cases of *Salmonella* from improper washing of hands prior to handling of food or after toilet use which is also an important source of transmission (National institute of allergy and infectious diseases [NIAID], 2010).

Antimicrobial resistance in *Salmonella spp.* is a serious health problem in human and veterinary medicine worldwide. In the last two decades, the emergence and spread of antimicrobial-resistant pathogens, among them *Salmonella*, has become a serious health hazard worldwide. The routine practice of giving antimicrobial agents to domestic livestock as a means of preventing and treating diseases, as well as promoting growth, is an important factor in the emergence of antibiotic-resistant bacteria that are subsequently transferred to humans by the food chain(Angulo *et al.*,2004). In the case of severe human *Salmonella* infections, resistance may limit the options for treatment, particularly when strains exhibit resistance to critically-important antibiotics (Collignon *et al*., 2009).

Three of the most common bacterial causes of diarrhoea in HIV-positive people are organisms belonging to the *Salmonella*, *Campylobacter*, and *Shigella* species. In HIV-positive people with suppressed immune systems, Salmonellosis, Campylobacteriosis, and Shigellosis can lead to severe diarrhea, while studies have demonstrated that HIV-positive people are at a higher risk for Salmonellosis between 20 to 100 times more so than HIV negative people (AIDSMED, 2008).

This study was conducted to evaluate the antibiotic susceptibility of *Salmonella serovars* isolated from diarrhoeal stools of HIV seropositive individuals. The results of the study would be useful in deciding the drugs of choice for the effective treatment of Salmonellosis infection among HIV patients.

MATERIALS AND METHOD

Sample Size

Sample size was obtained using the equation described by Sarmukaddam and Garad (2006). The sample size obtained was three hundred and ninety (390).

Ethics Statement

Ethical clearance was obtained from the Ethical Committee of Kaduna State Ministry of Health.

Collection of samples

Three hundred and ninety (390) stool specimens were collected from patients attending the HIV clinic of selected State hospitals in Kaduna, which are tertiary care hospitals. The stool samples were collected in sterile stool containers and analyzed within two hours of collection in the microbiology laboratory of the hospitals.

Isolation, characterization and identification of bacterial isolates

Isolation of *Salmonella* spp.

All clinical samples collected were cultured aerobically for *Salmonella* spp. isolation and identification in the laboratory in a method described by Cheesebrough (2010) using enrichmentmedium (selenite F) and enriched media (MacConkey agar,Salmonella-Shigella agar and Xylose lysine deoxycholate agar) all of which were prepared following manufacturer's instruction. Suspected colonies with black doted centres were identified and sub-cultured on MacConkey agar plates for purity and subsequently sub-cultured into nutrient agar slants, which were thereafter stored at 4^0C prior to identification.

Morphological and biochemical identification of the pure isolates

Gram staining technique and the conventional biochemical identification described in Cheesebrough (2010) were carried out.

Biochemical test using Microgen™ GnA +B-ID system

The procedure for identification of Gram negative enteric organisms was carried out using the above kit following the manufacturer's instructions.

Serological Test

Serological test (slide agglutination) was carried out using polyclonal agglutinating antisera (Biorad diagnostics, California U.S.A).

Antibiotic susceptibility studies

This was carried out using a Kirby-Bauer technique that has been carefully standardized by the clinical and laboratory standards institute guidelines (CLSI) (CLSI 2014). At least three colonies were selected and transferred with a wire loop into 4-5 ml tryptic soy broth. This was incubated at 35^0C until it achieved turbidity of the 0.5 ml McFarland barium sulphate standard (a density of $1x10^8$ cells/ml). Within 15 minutes after adjusting the turbidity of the inoculums, sterile cotton swab was immersed into the inoculum and rotated firmly several times against the upper side wall of the tube to express excess fluid. The entire Muller-Hinton agar surface of the plate was inoculated to obtain even streaking before applying the antibiotic discs using a sterile forceps aseptically. This was followed by incubation at 35^0C for 24 hours. Growth within the apparent zone of inhibition was indicative of resistance while absence of growth was indicative of susceptibility to the antibiotics. The following oxoid (U.K) single discs were used: amoxycilin(10 µg), chloramphenicol (30 µg), pefloxacin (10 µg), ciprofloxacin (5 µg), ofloxacin (10 µg), ampicillin (10 µg) tetracycline (30 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg) streptomycin (10 µg) ceftriaxone (30 µg) augmentin (10 µg) gentamycin (10 µg) sparfloxacin (5 µg). The standard strain of *Escherichia coli* (ATCC 25922) was used as control.

RESULTS AND DISCUSSION

Morphological and biochemical characteristics of the presumptive isolates

This revealed that eighteen isolates were *Salmonella* isolates.

Biochemical test using Microgen™ GnA +B-ID system

Fourteen isolates were identified as *Salmonella spp* using the above kit.

Identification of *Salmonella enterica serovar Typhi* and *Salmonella enterica serovar Typhimurium* using serological test.

Out of a total of fourteen isolates (14), three (3) were identified as *Salmonella Typhi* while eleven (11) were identified as *Salmonella typhimurium*.

In vitro activity of antimicrobial agents against the Salmonella serovars.

A total of three *Salmonella typhi* and eleven *Salmonella typhimurium* were isolated. *Salmonella typhi* was highly susceptible unlike *Salmonella typhimurium*. However, all of the *Salmonella serovars* isolated were susceptible to ceftriaxone, sparfloxacin, ofloxacin and amoxicillin. *Salmonella typhi* isolates were found to be susceptible to all of the antibiotics used in this study with the exception of tetracycline and ampicillin. *Salmonella typhimurium* isolates gave a reduced susceptibility to pefloxacin, ciprofloxacin, trimethoprim-sulfamethoxazole and augmentin while an increased susceptibility (72.7%) was observed with chloramphenicol (Table 1).

Table 2, shows a low percentage of *Salmonella Typhimurium* (9.1%) was resistant to Pefloxacin, Ciprofloxacin, augmentin and gentamicin. Whereas 27.3% were resistant to Streptomycin. In the case of ampicillin and tetracycline a total of 72.7% was resistant.

Table 3, summarises the antibiotic resistance and susceptibility profile of the *Salmonella* serovars.With Chloramphenicol, the serovars gave a64.3% susceptibility whereas with streptomycin and trimethoprim-sulfamethoxazole they were 78.6% susceptible. A low resistance of 7.1%, 21.4%, and 35.7% were observed for pefloxacin, streptomycin and cotrimoxazole. Whereas, ciprofloxacin, augmentin and gentamicin were slightly resistant at 7.1%.

Table 1. Antibiotic susceptibility profile of *Salmonella serovars*.

Antibiotic	*Salmonella spp* (%)	
	*S.Typhi*n=3	*S.Typhimurium*n=11
PEF	3(100)	10(90.9)
OFL	3(100)	11(100)
S	3(100)	8(72.7)
CXT	3(100)	8(72.7)
C	3(100)	8(72.7)
SP	3(100)	11(100)
CIP	3(100)	10(90.0)
AMX	3(100)	11(100)
AU	3(100)	10(90.9)
GN	3(100)	10(90.0)
CEF	3(100)	11(100)
TET	0(0.0)	3(27.3)
AMP	0(0.0)	3(27.3)

Key: SP = Sparfloxacin, N=no of isolates, PEF = Pefloxacin, SP = Sparfloxacin, CEF= Ceftriaxone,
OFL = Ofloxacin, CIP= Ciprofloxacin, S = Streptomycin, AMX = Amoxicillin, CXT = Trimethoprim-Sulfamethoxazole, TET=Tetracycline, AMP=Ampicillin, AU = Augmentin, C = Chloramphenicol,
GN = Gentamycin

Table 2. Antibiotic resistance profile of *Salmonella serovars*

Antibiotic	***Salmonella spp*(%)**	
	***S.Typhi*n=3**	***S.Typhimurium*n =11**
PEF	0(0.0)	1(9.1)
OFL	0(0.0)	0(0.0)
S	0(0.0)	3(27.3)
CXT	0(0.0)	3(27.3)
C	0(0.0)	3(27.3)
SP	0(0.0)	0(0.0)
CIP	0(0.0)	1(9.1)
AMX	0(0.0)	0(0.0)
AU	0(0.0)	1(9.1)
GN	0(0.0)	1(9.1)
CEF	0(0.0)	0(0.0)
TET	3(100)	8(72.7)
AMP	3(100)	8(72.7)

Key: SP = Sparfloxacin, PEF = Pefloxacin CEF= Ceftriaxone OFL = Ofloxacin, CIP= Ciprofloxacin, S = Streptomycin, AMX Amoxicillin,
CXT=Trimethoprim-Sulfamethoxazole, TET=Tetracycline AMP=Ampicillin,
AU = Augmentin C = Chloramphenicol, n = no of isolates,

Table 3. Overall distribution of antibiotic susceptibility/resistance profile of *Salmonella* isolates

Antibiotic Agent	No. tested	µg	Susceptible (%)	Resistance (%)
PEF	14	10	92.9	7.1
OFL	14	10	100	0.0
S	14	10	78.6	21.4
CXT	14	25	78.6	21.4
C	14	30	64.3	35.7
SP	14	10	100	0.0
CIP	14	10	92.9	7.1
AMX	14	30	100	0.0
AU	14	10	92.9	7.1
GN	14	10	92.9	7.1
CEF	14	30	100	0.0
TET	14	10	21.4	78.6
AMP	14	10	21.4	78.6

Key: SP = Sparfloxacin, n=no of isolates, PEF =Pefloxacin, CEF= Ceftriaxone, OFL =Ofloxacin,, CIP=Ciprofloxacin, S = Streptomycin, AMX=Amoxicillin, CXT=Trimethoprim-Sulfamethoxazole, TET=Tetracycline, AMP=Ampicillin, AU = Augmentin, C = Chloramphenicol.

In this study, resistance to quinolones was observed to be low (9.1%) as only two (2) isolates out of a total of fourteen (14) *Salmonella* serovars were found to be resistant to ciprofloxacin and pefloxacin. These two isolates were identified as *Salmonella enterica typhimurium*. Flouroquinolone resistance may therefore be said to be gradually emerging within the study population (HIV infected). Interestingly however, all the *Salmonella Typhi* isolated was susceptible to ciprofloxacin, ofloxacin and pefloxacin (100%). This is similar to the findings of Abdullahi *et al.* (2014) in Katsina, North-east Nigeria, where all the clinical *Salmonella typhi* isolated in their study were susceptible to similar fluoroqunolones used in this study. Comparable findings of ciprofloxacin resistance although lower in the present study when compared to (18.4%) in the studies of Agada *et al.* (2014) in Jos, Plateau North central Nigeria. Although, same drug concentration was used in both studies, maybe differences in the number of isolates and study population when compared to this study may have a role to play. However, the present study disputes the findings of Olufunmilola *et al.* (2012) in Ibadan where a 4.1% fluoroquinolone resistance was observed for *Salmonella typhi*. The disparity to the present study may be due to the low concentration (5 µg) of ciprofloxacin, source of isolation and study population used in their study. The emergence of fluoroquinolone resistance *Salmonella typhimurium* from humans and poultry had been documented by *Fasure et al. (*2012). In Nigeria, *Salmonella* serotypes with reduced fluoroquinolone susceptibility from humans had been documented Akinyemi (as cited by Fasure *et al.,* 2012). Prescription pattern, availability and cost effectiveness of quinolones as drugs that are usually prescribed in the management of most resistant bacterial infections were suggested as factors that could be responsible for continued rapid evolution of fluoroquinolone resistant bacteria in Nigeria Lamikara (as cited by Yemisi *et al.,* 2014). It is therefore imperative that necessary steps be taken to circumvent fluoroquinolone resistance in order to curb future threat this may pose particularly to the study (HIV- infected) population.

In addition, all the *Salmonella serovars* were found to be susceptible (100%) to ceftriaxone. Hence, implies that ceftriaxone is an excellent drug that can be used for effective treatment of salmonellosis infection. This is in agreement with the findings of Akyala *et al*. (2013) in Nasarawa, North central Nigeria. Similarly, the findings of Agidigbi *et al*., 2016 in South western Nigeria, as well as that of Ifeanyi *et al*. (2013) in Ibadan South western Nigeria all of which clearly demonstrated an excellent antibacterial activity of ceftriaxone on all of their *Salmonella enterica* strains.

Cephalosporins are known to exert bacterial activity by interfering with bacterial cell wall synthesis, inhibiting cross linking of peptidoglycan as well as activation of autolysins which may contribute to cell lysis. All of these combined with their pharmacodynamics and pharmacokinetics makes them an effective drug in treatment of bacterial infections (Cephalosporin, n.d.). To the best of knowledge ceftriaxone resistance is yet to be reported in the study area.

Augmentin and amoxicillin were observed to also have an effective (100%) antibacterial activity against all the *Salmonella* serovars in this study. Hence may be deduced to be a good choice of penicillin to be used in the treatment of Salmonellosis infection. This finding is in agreement with that of Iroha *et al.* (2007) in Abakaliki, wherein bacterial isolates from stool were all susceptible to augmentin.

In this study, high levels of resistance were encountered with ampicillin and tetracycline. This elevated resistance levels may be tied to the fact that these drugs have been observed to be among the first line drugs self prescribed by majority of people within the study area for treating diarrhoea cases as they are easily available over the counter due to low cost, hence easily misused (I.Abdulrazaq, personal communication, October 2015). This may therefore explain the high resistance observed for these drugs in this study. Similarly high resistance (86.9% – 92.3%) to ampicillin and tetracycline on *Salmonella* serotypes have also been observed in the studies of Abdullahi *et al*., 2015 in Kano, North-west Nigeria, Olowe *et al*., 2007 in Osogbo, South western Nigeria and Benacer *et al*. (2012) in Malaysia.

Sulfamethoxazole resistance was also encountered in this study. This resistance is particularly worrisome as it may affect the study group (HIV infected), considering the fact that the drug is clinically being used in a long term prophylaxis management of opportunistic infections like *Pneumocystic carinii* pneumonia in them. Previous studies by Abdullahi *et al*. (2014) in Katsina have also encountered resistance to sulfamethoxazole among non-typhoidal *Salmonella* isolates but higher (31.8%) than the value in this study (27.3%). However, sulfamethoxazole resistance is said to have been documented, and is reported to be widespread among fecal *E. coli*, which can be easily transferred to *Salmonella* as well as other Enteriobacteriacea through a high degree of rapid plasma-mediated transfer of CTX (cotrimoxazole) resistance in Uganda (Murray and Resimer, 1983; Mermin *et al*., 2004).

Among the first-line drugs historically used for the treatment of Salmonellosis was Chloramphenicol. However an increased side effect such as aplastic anaemia and widespread documentation of chloramphenicol resistant strains has discouraged its use in treatment. In this study however, increased susceptibility of *Salmonella typhimurium* (72.7%) to chloramphenicol was encountered. In addition all the *Salmonella typhi* isolates were susceptible to the drug, however the explanation for this observation is not easy to make, therefore deserves further study. The studies of Bobai *et al*. (2015) recorded a resistance of 12.5% to chloramphenicol on *Salmonella typhi* isolates which is contrary to this study. 'The recent decline in prevalence of chloramphenicol resistance in many endemic areas has led to reconsideration of its use as an alternative to newer-generation fluoroquinolones or azithromycin. There are also widespread concerns about aplastic anaemia with chloramphenicol use" Beeching (as cited by Salmonellosis treatment, n.d.).

In treatment of non-typhoidal salmonellosis antimicrobial therapy is not recommended for mild or moderate cases in healthy individuals. The use of antibiotics in such cases may select for resistant strains, which leads to ineffectiveness of the drugs. However, health risk groups may receive antimicrobial therapy or if the infection spreads from intestine to other parts (WHO, 2013). Furthermore, some variants of Salmonella have developed multidrug-resistance as an integral part of the genetic material of the organism, and are therefore 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 Centre for Diseases Control, (CDC) (as cited by Olowe *et al*., 2007).

In addition, there are numerous literatures that have documented on the affects of antimicrobial misuse without medical guidance of which is the risk of the selection of resistant bacteria and concomitant spread of antimicrobial drug resistance which has dire effect on treatment alternatives for infectious diseases.

CONCLUSION

The results of the antibiotic susceptibility of this study, therefore strongly suggests Ceftriaxone, as an alternative therapy in the event of fluoroquinolone resistant *Salmonella.* Augmentin and amoxicillin can as well serve as drugs of choice for the effective treatment of Salmonellosis infection based on their effective antimicrobial activity against the *Salmonella* serovars in this study. Chloramphenicol having shown an increased antimicrobial activity against *Salmonella typhimurium* but completely susceptible against *Salmonella typhi*, deserves further study.

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