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Antimicrobial Susceptibility Pattern of Some Recently Isolated Serovars of Salmonellae in Jos, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author SOO designed the study, performed the statistical analysis and wrote the protocol. Author YTK-O wrote the first draft of the manuscript. Authors EBB, DZE and JDM managed the analyses of the study. Authors YTK-O and SCC managed the literature searches. Author SCC wrote the final draft of the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: To determine the salmonellae serovars circulating in North Central Nigeria and their treatability with commonly used antimicrobial agents.
Study Design: Investigative
Place and Duration of Study: Samples were collected and processed at the Jos University Teaching Hospital (JUTH), Plateau State, Nigeria between 2006 and 2011.
Methodology: Standard microbiological tests were used for isolation, identification and serotyping of salmonellae from 3509 blood and 5426 stool samples collected from patients attending Jos University Teaching Hospital (JUTH), Jos between 2006 and 2011. Identified serovars were subjected to antimicrobial susceptibility testing using disc diffusion method.
Results: 89 *Salmonella* isolates were obtained from 8935 samples. Blood and stool cultures yielded 1.4% and 0.70% salmonellae respectively. The highest number of

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isolates was from age group 0-9 years 30(33.7%) while patients aged 70 and above accounted for the least number of isolates 1(1.1%). Males accounted for more isolates 49(55.1%) than females 40(44.9%) ($p<0.05$). The 89 isolates encountered comprised 39 serovars of which 74(83.1%) were non-typhoidal. Frequently isolated serovars were *S. typhimurium* and *S. bargny* 11(12.3%) each, *S. typhi* 7(7.7%) and *S. paratyphi* B and S. *saint* Paul 6(6.7%) each. Rare serovars isolated included *S.* Lagos, *S. aba*, *S. kisii*, S. *okerara* and *S. aminatu* 1(1.1) each. All isolates were susceptible to ciprofloxacin and ceftriaxone(MIC≤ 1µg/mL) while more than 50% of the frequently isolated serovars were resistant to chloramphenicol, amoxicillin and cotrimoxazole.

Conclusions: This study reveals a high occurrence of invasive non-typhoidal, multi-drug resistant *Salmonella* serovars. However, susceptibility to ciprofloxacin and ceftriaxone is completely preserved and can be used for empirical treatment of salmonellosis.

Keywords: Salmonella; serovars; Jos; Nigeria; antimicrobial susceptibility.

1. INTRODUCTION

The salmonellae are a group of gram-negative bacilli which belong to the family Enterobacteriaceae. Most salmonellae that cause significant human disease are classified as *Salmonella enterica*, which currently consists of approximately 2,587 serotypes [1]. They produce three main types of disease in humans; enteric fevers (typhoid/ paratyphoid fevers), bacteremia with focal lesions and gastroenteritis [2]. Generally, the majority of human infections are caused by a limited number of serovars which may vary from country to country and over time [3]. Gastroenteritis caused by salmonellae is usually self-limiting but may lead to systemic infections in children, the elderly and immunocompromised [4]. The enteric fever serovars are pathogens restricted to humans, while the non-typhiodal serovars can be isolated from a wide range of animal hosts. Enteric fever and non-typhiodal salmonellosis (NTS) are endemic in Sub-Saharan Africa and have posed major public health problems in these countries, Nigeria inclusive [3,5,6].

The incidence of invasive NTS is reported to be high in Sub-Saharan Africa especially among infants, young children and young adults suffering from Human Immunodifficiency Virus(HIV)infections, malaria and malnutrition [3,4).

The emergence of antibiotic resistant *Salmonella* strains is increasing and multi-drug resistant (MDR) serovars with substantial resistance to the traditional first-line antibiotics ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole have been reported [3,4,5].

This has further complicated the health problems due to *Salmonella* in Sub-Saharan Africa, necessitating usage of expensive antimicrobials like ciprofloxacin and ceftriaxone, that are less widely available, or inconvenient to use in resource-limited settings [4].

In addition, decreased susceptibilities to the fluoroquinolones have also been recorded in the United States [4], United Kingdom [7], India [8], some African countries [9,10] and some parts of Nigeria [3,11]

Studies from a few areas in Nigeria have reported isolation of certain serotypes from humans, poultry and cattle which include *S. typhi*, *S. paratyphi*, *S. enteriditis*, *S. typhimurium*, *S. dublin*, *S. hadar*, *S. kentucky* and *S. hiduddify* [3,11,12]. There is however, paucity of data on *Salmonella* in Nigeria as a whole. Due to globalisation and international trade in animal food products *Salmonella* serovars within countries are constantly changing [13]

This study was therefore undertaken to determine the prevalent *Salmonella enteric* serovars in the study area (Jos, Nigeria) and evaluate their antimicrobial susceptibilities to routinely used antibiotics with the ultimate aim of establishing which antibiotics can be used for empiric treatment of *Salmonella* infections in this locality.

2. METHODOLOGY

2.1 Study Site and Ethical Issues

Sample collection was carried out at Jos University Teaching Hospital (JUTH), Jos. The hospital is located in Jos, Plateau state, north central Nigeria. JUTH is a referral hospital for four states: Plateau, Bauchi, Nasarawa and Benue States. The consent of the target patient population was sought after they were fully educated on the scope of the research and its potential benefits. Consented patients were subsequently enrolled into the study

2.2 Sample Source, Collection and Isolation

Blood and stool samples were collected from patients presenting with pyrexia between 2006 and 2011. A total of 8935 samples comprising 3509 blood and 5426 stool were analysed for *Salmonella*. Stool specimens were collected and pre-treated as described by Baird-Parker [14]. Selenit F was used for enrichment purpose for all stool samples after which they were sub-cultured onto Deoxycholate citrate bile salt agar (DCA) (FLUKA, Switzerland) and Xylose lysine decarboxylase agar (XLD) (FLUKA, Switzerland).

For blood samples, each 2 mL of blood sample was inoculated onto 18 mL each of thioglycolate and brain-heart infusion broths (FLUKA, Switzerland). The inoculated media were incubated at 37°C for up to 10 days. Blood cultures with growth were sub-cultured onto DCA and XLD agar and incubated as stool cultures above. Phenotypic identification was done as earlier described [15].

2.3 Serotyping of Isolates

This was carried out as earlier described by Agada et al. [16] according to the Kauffmann-White scheme.

2.4 Antimicrobial Susceptibility

Susceptibility of *Salmonella* isolates to antibiotics (ciprofloxacin 5µg, ceftriaxone 30µg, amoxicillin+clavulanic acid 30µg, chloramphenicol 25µg, amoxicillin 25µg, cotrimoxazole 25µg) was determined on Mueller-Hinton agar (FLUKA, Switzerland) using the disk diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI) [17]. Diameter zone of inhibition for each test antibiotic was measured and sensitivity or resistance estimated by comparing with zone-diameter interpretive standard according to that recommended by CLSI. Minimum inhibitory concentration (MIC) values were determined by using the E-test macro-method (Biomerieux, Sweden).

2.5 Statistical Analysis

Data obtained was analysed using a Chi-squared test and a probability of p=0.05 as the level of significance. (software: SPSS version 16 Command Syntax Reference, SPSS Inc., Chicago).

3. RESULTS

3.1 Bacterial Isolates

8935 samples were collected and analysed. 4549 were obtained from males while 4386 were from females. 89 (1%) of the samples yielded Salmonellae of different serotypes while 8846 were salmonellae-free. Isolation rate from blood cultures was slightly higher 49 (1.4%) than from stool cultures 40 (0.7%) (Table 1). The highest number of isolates 30 (33.7%) was obtained from age group 0-9years while the 1(1.1%) was from age group 70 years and above (Table 2). The number of isolates for both sexes decreased with increasing age from 50-70 years and above. Overall, more isolates were obtained from males 49(55.1%) than females 40(44.9%) although there is no statistical difference between the number of isolates from males and females (Table 2).

3.2 Serotyping of Isolates

The 89 isolates consisted of 39 different serovars of which 74(83.1%) were non-typhoidal *Salmonella*. The most commonly isolated serovars were *Typhimurium* and *Bargny* 11(12.3%) each, *Typhi* 7(7.7%), Paratyphi B and St. Paul 6(6.7%) each. The 5 frequently isolated serotypes constituted 41(46.0%) of the total isolates. Which represented 25(51.0%) of the total blood isolates and 16(40%) of the total stool isolates (Table 3). Several uncommon serovars were among the infrequently isolated *Salmonella*, such as Lagos, Aba, Kisii, Okerara and Aminatu 1(1.1%) each. There were 2(2.2%) sub spp *Salame* and 6(6.7%) non-typable *Salmonella*. These accounted for 24(49%) of the total blood isolates, 24(60%) of the total stool isolates and 48(53.9%) of the total isolates (Table 4).

3.3 Antimicrobial Susceptibility

All *Salmonella* isolates were susceptible to ciprofloxacin and ceftriaxone (MIC≤ 1µg/mL). However, varying degrees of susceptibilities were recorded for the other antimicrobial agents: 55% were susceptible to amoxicillin+clavulanic acid, 37% to chloramphenicol, 25.8% to amoxicillin and 14.6% to cotrimoxazole (Fig. 1). Figures for the 5 frequently isolated serotypes were; 5(83.3%) Saint Paul, 9(81.8%) Bargny, 4(66.7%) Paratyphi B, 7(63.6%) Typhimurium, and 4(57.1%) Typhi, were sensitive to amoxicillin+clavulanic acid. Six (55.5%) Bargny, 3(50%) Saint Paul, 5(45.4%) *Typhimurium*, 2(33.3%) Paratyphi B and 2(28.6%) Typhi were sensitive to chloramphenicol. Three (50%) Saint Paul, 4(36.3%) Bargny, 2(33.3%) Paratyphi B, 2(28.6%) Typhi and 3(27.2%) *Typhimurium* were sensitive to amoxicillin. Three (27.2%) Bargny, 2(18.1%) *Typhimurium*, 1(16.7%) Saint Paul and 1(14.2%) Typhi were sensitive to cotrimoxazole. All Paratyphi B isolates were resistant to cotrimoxazole. More than 50% of the 5 commonly isolated serovars were resistant to chloramphenicol, amoxicillin and cotrimoxazole (Table 3).

Table 1. Distribution of Salmonella isolates in Jos in relation to sex and type of specimen

Specimen Type	Male	(% Positivity)	Female	(% Positivity)	Total % positivity
Blood *n=3509*	1689	26 (1.5)	1820	23 (1.2)	49 (1.4)
Stool *n=5426*	2860	23 (0.8)	2566	17 (0.7)	40 (0.7)
Total *N*=8935	4549	49 (1.0)	4386	40 (0.9)	89 (1.0)

Table 2. Distribution of Salmonella isolates in Jos in relation to age and sex of patients

Age (years)	Sex/No (%) Tested		No. (%) isolates detected	No. (%) found in males	No. (%) found in females
	M	F			
0 – 9	1205 (26.5)	1807 (41.2)	30 (33.7)	12 (24.4)	18 (45.0)
10 – 19	713 (15.7)	592 (13.5)	13 (14.6)	9 (18.3)	4 (10.0)
20 – 29	602(13.2)	703 (16.0)	13 (14.6)	6 (12.2)	7 (17.5)
30 – 39	904(19.9)	702 (16.0)	16 (17.9)	10 (20.4)	6 (15.0)
40 – 49	603(13.3)	100 (2.3)	7 (7.8)	6 (12.2)	1 (2.5)
50 – 59	301 (6.6)	201 (4.6)	5 (5.6)	3 (6.1)	2 (5.0)
60 – 69	151 (3.3)	251(5.7)	4 (4.5)	2 (4.0)	2 (5.0)
≥ 70	70 (1.5)	30 (0.7)	1 (1.1)	1 (2.0)	0 (0.0)
Total	4,549 (50.9)	4,386 (49.1)	89 (100)	49 (55.1)	40 (44.9)

Table 3. Characteristics of 5 frequently isolated Salmonella enteric serovars from patients attending Jos University Teaching Hospital Jos, Nigeria

Serotypes	No (%) from 49 blood Isolates	No (%) from 40 Stool Isolates	Total (%) of N=89	No (%) Sensitivity to Antibiotics					
				CI	TX	AUG	CHL	AC	SXT
Typhimurium	7 (14.2)	4 (10.0)	11(12.3)	11 (100)	11 (100)	7 (63.6)	5 (45.4)	3 (27.2)	2 (18.1)
Bargny	5 (10.2)	6 (15.0)	11(12.3)	11 (100)	11 (100)	9 (81.8)	6 (55.5)	4 (35.3)	3 (27.2)
Typhi	4 (8.1)	3 (7.5)	7 (7.7)	7 (100)	7(100)	4 (57.1)	2 (28.6)	2 (28.6)	1 (14.2)
Paratyphi B	4 (8.1)	2 (5.0)	6 (6.7)	6 (100)	6 (100)	4 (66.7)	2 (33.3)	2 (33.3)	0 (0.0)
Saint Paul	5 (10.2)	1 (2.5)	6 (6.7)	6 (100)	6 (100)	5 (83.3)	3 (50.0)	3 (50.0)	1 (16.7)
Total	25 (51.0)	16 (40.0)	41(46.0)	41 (100)	41 (100)	29 (70.7)	18 (43.9)	14 (34.1)	7(17.0)

CI = Ciprofloxacin; TX = Ceftriaxone; AUG = Amoxicillin+ Clavulanic acid; CHL = Chloramphenicol; AC = Amoxicillin; SXT = Cotrimoxazole

Table 4. Infrequently isolated Salmonella serotypes from patients attending Jos University Teaching Hospital Jos, Nigeria

Serotype	No (%) in 49 Blood samples	No (%) in 40 Stool samples	Total (N=89)
Rissen	0 (0.0)	2 (5.0)	2(2.2)
Korbol	1 (2.0)	1 (2.5)	2 (2.2)
Lomita	2 (4.0)	0 (0.0)	2 (2.2)
Brazaville	1 (2.0)	1 (2.5)	2 (2.2)
Enteritidis	1 (2.0)	1 (2.5)	2 (2.2)
Lamberhaust	2 (4.0)	0 (0.0)	2 (2.2)
Paratyphi A	0 (0.0)	1 (2.5)	1 (1.1)
Paratyphi C	1 (2.0)	0 (0.0)	1 (1.1)
Koessen	1 (2.0)	0 (0.0)	1 (1.1)
Glassgow	0 (0.0)	1 (2.5)	1 (1.1)
Kingston	0 (0.0)	1 (2.5)	1 (1.1)
Chester	1 (2.0)	0 (0.0)	1 (1.1)
Abony	0 (0.0)	1 (2.5)	1 (1.1)
Stanleyville	0 (0.0)	1 (2.5)	1 (1.1)
Schwarzangra	0 (0.0)	1 (2.5)	1 (1.1)
Lagos	1 (2.0)	0 (0.0)	1 (1.1)
Mono	0 (0.0)	1 (2.5)	1 (1.1)
Agona	1 (2.0)	0 (0.0)	1 (1.1)
Eppendorf	1 (2.0)	0 (0.0)	1 (1.1)
Newport	0 (0.0)	1 (2.5)	1 (1.1)
Kentucky	0 (0.0)	1 (2.5)	1 (1.1)
Larochele	0 (0.0)	1 (2.5)	1 (1.1)
Djeifa	1 (2.0)	0 (0.0)	1 (1.1)
Aba	1 (2.0)	0 (0.0)	1 (1.1)
Kastrup	0 (0.0)	1 (2.5)	1 (1.1)
Kisii	0 (0.0)	1 (2.5)	1 (1.1)
Othmarschen	1 (2.0)	0 (0.0)	1 (1.1)
Margherafelt	0 (0.0)	1 (2.5)	1 (1.1)
Isreal	1(2.0)	0 (0.0)	1 (1.1)
Okerara	1 (2.0)	0 (0.0)	1 (1.1)
Ngor	0 (0.0)	1 (2.5)	1 (1.1)
Masembe	0 (0.0)	1 (2.5)	1 (1.1)
Aminatu	0 (0.0)	1 (2.5)	1 (1.1)
Anatum	1 (2.0)	0 (0.0)	1 (1.1)
Sub spp *Salome*	1 (2.0)	1 (2.5)	2 (2.2)
Non typable	4 (8.1)	2 (5.0)	6 (6.7)
Total	24 (49.0)	24 (60.0)	48 (53.9)

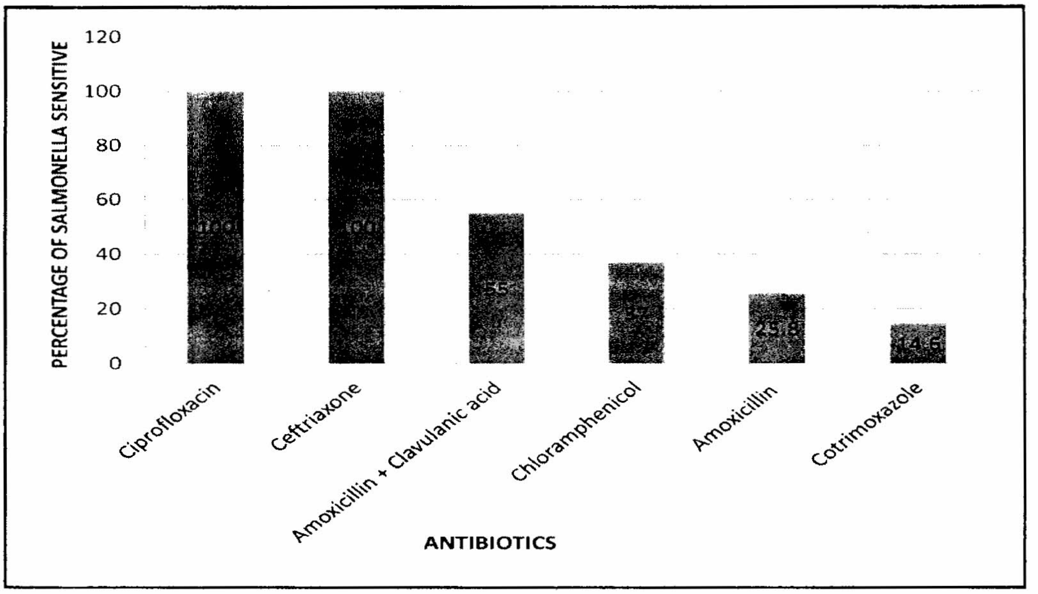

Fig. 1. Invitro antimicrobial susceptibility profile of the 89 Salmonella isolates from patients attending Jos University Teaching Hospital Jos, Nigeria

4. DISCUSSION

The prevalence rate of 1% for Salmonellae in this study is much lower than the 17.5% obtained by Akinyemi et al. [18] from Lagos, Nigeria. It is also slightly lower than the 2.3% reported by Cajetan *et al* [19] from Abuja, Nigeria. The decrease in prevalence rate could be due to the fact that the study site being a referral centre hence uncomplicated cases are naturally exempted as they are effectively handled at most primary health centres without the need for referrals. However, the isolation rate of salmonellae from blood specimens (1.4%) compares well with that from Kenya (1%) [20] and Mozambique (2%) [21].

The highest number of isolates was recovered from children 0-9 years. The high susceptibility of young children to Salmonella infections has been documented [19; 22; 23]. Gastric hypoacidity or gut immaturity may be a contributing factor for this susceptibility [22]. The lowest number of isolate was from male patient above 70 years. The low isolation rate of salmonellae within this age group could be attributed to small sample size rather than actual occurrence since high prevalence of Salmonella infections and carrier rates have been observed in individuals older than 60 years [24; 25]. Findings from this study indicate that Salmonella isolation rates were generally higher in males than females. The high isolation rates of salmonellae and the high prevalence of salmonellosis among males have been observed by several researchers [3; 19; 22]. Among the reasons which have been given to account for differences of Salmonella infections by sex include host factors, such as higher male susceptibility to certain infections [26] and behavioural factors which include the propensity of males to participate in more high-risk food handling, preparation and consumption [27]. It is believed that consumption behaviours of males within the study area may play a major role in their acquisition of salmonellae. The gizzards of chickens, for instance, are considered a delicacy and should be eaten mostly by men [28; 29; 30]. A study from Meknes, Morocco reported the highest isolation of Salmonella from chicken gizzards [31]. Also, from the study area the second highest isolation rate of salmonellae was obtained from chicken gizzards and many of the serovars isolated from chickens were also isolated from humans [32].

Data from this study revealed the presence of multiple Salmonella serovars which were exceptional because of their large diversity. This finding may be related to the collection centre being a referral hospital for 4 states. The most prevalent serotypes in this study were *Salmonella* Typhimurium and Bargny. Typhimurium is one of the most frequently isolated serotype worldwide [33]. *Salmonella* Bargny, a very rare "exotic" serotype [34] was first isolated from a horse in Senegal [35]. There have been only 2 other reported isolation of this serotype (to the authors' knowledge) one from cattle in Guinea Bissau [34] and the other from human in Poland [36]. It's isolation in this study appears to be the first documented one from Nigeria.

The relatively high frequency of occurrence of *S.* Bargny in this study signifies an emerging trend where rare *Salmonella* serotypes are becoming prevalent in West Africa. This is because *S.* Hiduddify, a hitherto rare serovar has now been isolated from chickens in Maiduguri, Nigeria [12]; *S.* Colindale from chickens in N' Djamena, Chad [10] and *S.* Eko from poultry in Cameroon [37].

The presence of enteric fever serotypes (Typhi and Paratyphi B) among the 5 frequently isolated ones is in order, since enteric fever is endemic in Nigeria [6]. The representation of *Salmonella* St. Paul among the prevalent ones is unexpected. However, globalisation and international trade in animal food products have made possible the introduction of new serotypes into importing countries [13] *S.* Saint Paul was first isolated in 1940 [38] in the United States of America and was once a common serotype in the United Kingdom. This old serotype has been implicated in outbreaks associated with consumption of bean sprouts in the United Kingdom [39]. The other serovars reported in this study occurred infrequently. However, there is cause for concern because a large percentage of both the prevalent and infrequently occurring serovars were found in the blood. In addition, most salmonellae isolated were non-typhoidal. This presents a dilemma since the Widal test is widely used for the diagnosis of *Salmonella* infections in Nigeria. Most hospitals, primary health care centres and clinics do not have facilities or trained personnel to culture and identify these organisms.

Most Salmonella isolates were resistant to amoxicillin+ clavulanic acid, chloramphenicol, amoxicillin and co-trimoxazole. Resistance to one or more of these traditional first-line and relatively inexpensive antibiotics have been reported from Lagos [18], Ibadan [3] and Abuja [19] (all in Nigeria) as well as from Kenya [5] and the Democratic Republic of Congo [9].

None of the isolates in this study were resistant to ciprofloxacin and ceftriaxone. This is in agreement with the findings of Cajetan et al. [19] from Abuja, Nigeria where all Salmonella isolates were susceptible to the 2 antimicrobial agents. However, several studies [3,4,7,8,9,10,11] have reported Salmonella serovars showing reduced susceptibility to ciprofloxacin which is contrary to findings in this study. Ciprofloxacin and ceftriaxone can therefore be used for empiric treatment of Salmonella infections in the study area. These antibiotics however, are relatively expensive and may not be within the reach of the most Nigerians. Also, indiscriminate use of these antibiotics will undoubtedly lead to the development and spread of resistance.

5. CONCLUSION

It is clear from the available data that Salmonellosis is prevalent in Jos, Nigeria, especially with the emergence of strange and hitherto uncharacterized serotypes. It is worthy of note however from the susceptibility pattern that with proper diagnosis and treatment, the disease can easily be eradicated.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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