Original article

Chloramphenicol is re-emerging as an effective drug in the treatment of typhoid fever in Southern Benue state, Nigeria

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ABSTRACT

Background: Typhoid fever is an endemic disease in many developing countries with significant health implications that could sometimes, turn fatal. The study aimed to investigate the antimicrobial susceptibility of *Salmonella typhi* (*S. typhi*) isolates from stool samples of patients attending secondary health centres in Benue South geographical zone. **Methods:** One thousand and twenty-two (1022) stool samples were collected from 583 male and 439 female patients presumptively diagnosed with typhoid fever using Widal test. Isolation of *S.typhi* was according to standard procedure. The antimicrobial susceptibility of *S. typhi* isolates was tested against 10 different antibiotics, using the disc diffusion method. **Results:** A total of 447 (43.7%) *S. typhi* were isolated. Antibiotic resistance pattern showed that 64% (286/447) of the total isolates were resistant to at least two antibiotic classes. The isolates demonstrated the highest resistance to ciprofloxacin (55.6%; 159/286), and azithromycin (53.8%; 154/286). Resistance was highest to cephalosporin (ceftriaxone and ceftazidime) class of antibiotics (66.1%; 189/286). Isolates, however, showed susceptibility to the carbapenem (imipenem, 286/286), amphenicol (chloramphenicol, 286/286) and aminoglycoside (gentamicin 278/286). A total of 42.7% (122/286) isolates were resistant to three or more antibiotic classes, with different resistance patterns. **Conclusion:** These findings reveal multidrug resistance of *S. typhi* to antibiotics, with a possible positive reversing trend in the susceptibility characteristics of *S. typhi* to chloramphenicol in the study area, bringing to fore the need for adequate measures to control increasing resistance by this important pathogen and reconsideration for chloramphenicol in *S. typhi* treatment.

Introduction

Typhoid fever is a disease caused by the bacterium *Salmonella enterica* serotype typhi. The disease is endemic in many developing countries, including Nigeria and can affect different tissues and organs in an infected person [1]. It has been estimated to cause 21.6 million illnesses and 216, 000 deaths annually [2]. Transmission of the disease occurs via the faecal-oral route through

contaminated food and water. Hence, its presence in a population can be inversely related to adequate hygiene [3,4].

Infection can result in mild or severe illness, which could become fatal. Typhoid fever occurs in different parts of the world, with a higher prevalence in the tropics and subtropics, especially where sanitary conditions are inadequate [5]. The occurrence of typhoid fever in developed countries results from the acquisition of the organism from abroad or importation by emigrants [3]. Morbidity and mortality arising from the disease have been reported in Nigeria [3,4,6].

Typhoid fever is treated with antibiotics; unfortunately, the rise of multiple antibiotic-resistant bacteria has become a significant challenge of epidemiological importance [1]. Previously, traditional regimens applied to treat enteric fever included chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole but the rise in multidrug-resistant (MDR) salmonellae around the mid to late 1990s brought about the use of fluoroquinolones such as ciprofloxacin, in the treatment of infections caused by salmonellae [7]. Resistance by *Salmonella typhi* (*S. typhi*) to antibiotics such as ciprofloxacin, ampicillin, ceftriaxone, cotrimoxazole and others, has increased lately [8-10]. This trend has heightened the difficulties faced in managing and treating the disease in adults and infants [1,11]. **Akinyemi et al.** [12] has opined that, complications that arise while treating or managing enteric fever may not only lead to high death rate but has the potential to cause large scale outbreaks. Such outbreaks bear serious health consequences in countries with weak or inefficient healthcare systems and facilities.

One reason for antibiotic resistance in pathogenic bacteria such as *S. typhi* is antibiotic misuse. It is, therefore, important that adequate care is applied in antibiotic prescription and usage [1,6]. Regular investigation of susceptibility to antibiotics by pathogenic bacteria will promote efficient antibiotic prescription and reduce treatment time in patients.

Information on the prevalence of typhoid fever in Benue state was very scanty at the time of this study. In our recently reported research, a high prevalence rate of 43.3% was observed [13]. An older study by **Umeh and Agbulu** [14] reported a prevalence of 35.8%. **Okwori et al.** [15] reported a prevalence rate of 56.8% in blood and stool of patients who attended General Hospital Otukpo between 1987 to 2000, with high resistance to chloramphenicol (49%), gentamicin (49%), ceftriaxone (25%) and ciprofloxacin (19%). Despite the increase in the prevalence of the disease in the study area, very little appears to be known about the susceptibility pattern of *S. typhi*. This study investigated the MDR status of *S. typhi* isolates from stool samples of patients who sought treatment at secondary health centres in Benue South geographical zone.

Materials and Methods

The study was undertaken in the Benue South geographical zone. The area lies within the Guinea Savannah region of Central Nigeria (Mid-belt region) and experiences a tropical climate with moderate rainfall. It has a population of about 1, 307, 647 [16]. The Benue South geographical zone has nine (9) Local government areas (LGAs) which are Ado, Agatu, Apa, Obi, Ogbadibo, Ohimini, Oju, Okpokwu and Otukpo.

The research was hospital-based prospective study and was conducted among patients suspected to have typhoid fever. The purpose and procedure of the study were explained to the patients (in and out-patients) and approval was obtained from the Ethical Committee on Research of the Benue State Hospitals Management Board.

One thousand and twenty-two (1022) stool samples collected from patients attending Government Secondary Health centres within the Nine (9) Local Government Areas of Benue South geographical zone, were examined at the Microbiology laboratory of Federal University of Agriculture Makurdi. Samples were collected between August 2016 and July 2017 at the various health centres.

Each sample was first inoculated into selenite broth base (Oxoid, CM 0395) and incubated for 18-24 hours at 37ºC for pre-enrichment. Loopfuls of the broth was streaked onto Salmonella Shigella agar (Oxoid, CM 0099) and xylose lysine deoxycholate agar (Oxoid, CM 0469). Inoculated plates were incubated at 37ºC for 24 hours; suspected colonies were sub-cultured onto bismuth sulfite agar (Oxoid, CM 0201) to obtain pure cultures.

Colonies were identified using morphological and biochemical characteristics. The shape, colour and elevation of bacterial colonies were observed visually. Gram reaction, motility,

catalase, indole, oxidase, citrate utilization and triple sugar iron tests were performed on isolates [17,18].

The *S. typhi* isolates were subjected to antimicrobial susceptibility test using the standard disc diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI). Antibiotic impregnated discs used were amoxicillin/clavulanic (30μg), ceftazidime (30μg), ceftriaxone (30μg), amoxicillin (10μg), sulphamethoxazole/trimethoprim (25μg), chloramphenicol (30μg), ciprofloxacin (5μg), azithromycin (15μg), gentamicin (30μg) and imipenem (10μg). Results were interpreted using the standard interpretative zones criteria of the Clinical Laboratory Standards Institute guidelines [19].

Statistical analysis

IBM SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA; 2013) was used to analyse results obtained. Simple frequencies and percentages were computed using cross-tabulation. Chi-square test was used to determine the significance of associations between variables. A *p*-value less than 0.05 was interpreted as statistically significant.

Results

A total of 447 *S. typhi* isolates were identified out of the 1022 samples tested. The resistance pattern showed that 64% (n=286) of the total isolates (n=447, data not shown) were resistant to one or more class of antibiotics **(Table 1)**. The isolates demonstrated the highest resistance of 55.6% (159/286) to ciprofloxacin, followed by 53.8% (154/286) resistance to azithromycin. Isolates, however, displayed complete susceptibility to chloramphenicol and imipenem. Resistance to gentamicin was also low (2.8%; 8/286).

The isolates displayed different patterns of MDR to the various antibiotics used **(Table 2)**. Out of the 286 isolates that showed resistance to more than one antibiotic, 42.7% (122/286) were resistant to three or more antibiotics, while 57.3% (325 (164/286) did not exhibit MDR. Five isolates (4.1%) were resistant to all of sulphamethoxazole/Trimethoprim, azithromycin, amoxycillin and ceftriaxone. The pattern of resistance to amoxycillin, azithromycin and amoxicillin-clavulanic acid occurred most frequently, (11; 9.0%). None showed resistance to all the antibiotics at once. The differences in the pattern of MDR among the *S. typhi* isolates was statistically significant (*p*<0.05).

Figure 1 presents the resistance of *S. typhi* isolates to the different classes of antibiotics used. All isolates were susceptible to the carbapenem and amphenicol class of antibiotics (0/286), while the highest resistance rate was against the cephalosporin class (189/286).

Multidrug-resistant strains of *S. typhi* displayed various resistance patterns to classes of antibiotics used **(Figure 2)**. Eleven isolates were resistant to all antibiotics in the penicillin, macrolide, cephalosporin and β- lactamase inhibitor combination used in this study. The combination with the least resistant isolates was observed in the patternsulphonamide/macrolide/penicillin/cephalos porin (5 isolates). Resistance to the cephalosporin class of antibiotics appeared in 13 out of the 16 resistance patterns observed in this study, making it the highest occurring class, followed by the penicillin and fluoroquinolone classes which appeared in 11 and 10 resistance patterns each. Sulfonamide resistance appeared in only 4 of the patterns, while carbapenem, amphenicol and aminoglycoside classes did not appear in any of the patterns observed.

Table 1. Multiple antibiotic resistance pattern of *S. typhi* isolates to antibiotics.

Antimicrobials	Resistant isolates (n)	Rates (%)
Penicillins		
Amoxycillin (10 µg)	118	41.3
β-Lactam/β- lactamase inhibitor combinations		
Amoxicillin-clavulanic acid (30 µg)	35	12.2
Fluoroquinolones		
Ciprofloxacillin (5 µg)	159	55.6
Sulfonamides		
Sulphamethoxazole/Trimethoprim (25 µg)	65	22.7
Carbapenems		
Imipenem (10 µg)	0	0
Macrolides		
Azithromycin (15 µg)	154	53.8
Amphenicols		
Chloramphenicol (30 µg)	0	0
Aminoglycosides		
Gentamicin (30 µg)	08	2.8
Cephalosporins		
Ceftriaxone (30 µg)	107	37.4
Ceftazidime (30 µg)	82	28.7

Table 2. Pattern of multidrug resistance among the *S. typhi* isolates.

S/N	Antibiotics	Frequency(%)	Percentage (%)
1	SXT/AZM/AML/CFT	5	4.1
2	AML/CIP/CFT	6	4.9
3	CRO/AZM/CIP/CFT	6	4.9
4	AML/AZM/CIP/AMC/CFT	6	4.9
5	AML/CRO/CIP/AMC/CFT	6	4.9
6	CIP/AML/AZM	7	5.7
7	CRO/CIP/SXT	7	5.7
8	AML/CRO/SXT/AMC/CFT	7	5.7
9	CIP/AZM/CFT	8	6.6
10	SXT/CIP/AML	8	6.6
11	AML/AZM/CRO/CIP/CFT	8	6.6
12	AML/AZM/CRO/AMC/CFT	8	6.6
13	SXT/AMC/CFT	9	7.4
14	AML/AZM/CRO/CFT	10	8.2
15	AML/CRO/CIP	10	8.2
16	AML/AZM/AMC	11	9.0

$\chi^2 = 107.413$, $p<0.05$

AMC: amoxicillin/clavulanic; CFT: ceftazidime; CRO: ceftriaxone; AML: amoxicillin; SXT: sulphamethoxazole/trimethoprim; CIP: ciprofloxacin; AZM: azithromycin.

Figure 1. Antibiotic resistance of *S. typhi* isolates to different classes of antibiotics.
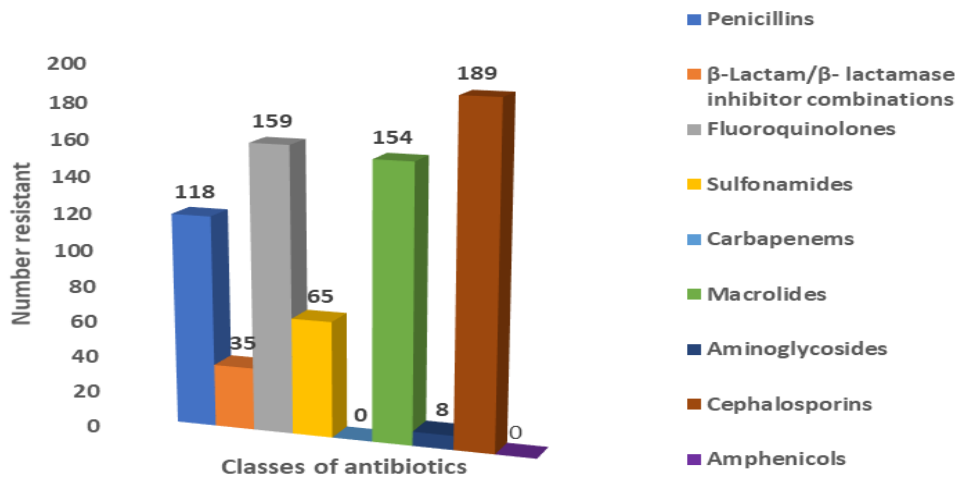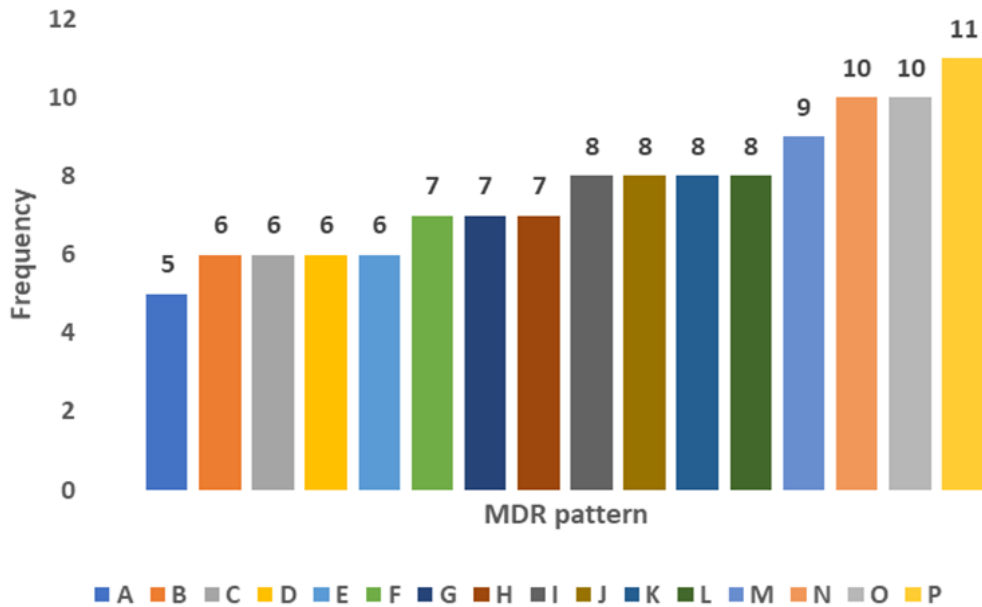

Figure 2. Multidrug resistance pattern of *S. typhi* isolates to three or more different classes of antibiotics.

Antibiotic classes combination in MDR patterns
A. Sulfonamides/Macrolides/Penicillins/Cephalosporins
B. Penicillins/Floroquinolones/Cephalosporins `
C. Cephalosporin/Macrolides/Fluoroquinolone/β- lactamase inhibitor combination/Cephalosporin
D. Peniccilin/Macrolide/Fluoroquinolone/β- lactamase inhibitor combination/Cephalosporin
E. Penicillin/Cephalosporin/Fluoroquinolone/β- lactamase inhibitor combination/Cephalosporin
F. Fluoroquinolone/Penicillin/Macrolide
G. Cephalosporin/Fluoroquinolone/Sulfonamide
H. Penicillin/Cephalosporin/Sulfonamide/β- lactamase inhibitor combination/Cephalosporin
I. Fluoroquinolone/Macrolide/Cephalosporin
J. Sulphonamide/Fluoroquinolone/Penicillin
K. Penicillin/Macrolide/Cephalosporin/Fluoroquinolone/Cephalosporin
L. Penicillin/Macrolide/Cephalosporin/β- lactamase inhibitor combination/Cephalosporin
M. Sulfonamide/β- lactamase inhibitor combination/Cephalosporin
N. Penicillin/Macrolide/Cephalosporin/Cephalosporin
O. Penicillin/Cephalosporin/Fluoroquinolone
P. Penicillin/Macrolide/β- lactamase inhibitor combination

Discussion

Antimicrobial susceptibility data for several medically important bacteria are not well documented in many parts of Africa [20]. To effectively combat the threat to efficient medical care posed by global trends in MDR by pathogens, proper documentation of susceptibility data is unavoidable. High level of resistance to amoxicillin, ciprofloxacin, azithromycin, ceftazidime and sulphamethoxazole/trimethoprim was observed in this present study. This agrees with reports by **Adabara et al.** [3], **Baya et al.** [10], **Ayalu et al.** [8] and **Bulbu et al.** [21] in Nigeria, Ghana, Ethiopia and Dhaka respectively. **Harriet and Nandita** [1] opined that the incidence of MDR *S. typhi* to commonly or routinely used antibiotics could result from misdiagnosis of diseases and antibiotics abuse. Inaccurate diagnosis leads to the inappropriate prescription of antibiotics by physicians which in turn lead to the development of resistance by organisms. Also, it has been opined that increasing resistance of *Salmonella* to antibiotics could have arisen due to infections with a mixed population of *Salmonella*, or new mutations arising from selective pressure due to the use of broad-spectrum antibiotics [22]. Susceptibility to imipenem and chloramphenicol was however observed in all isolates.

The resistance pattern showed that 64% (286/447) of the isolates displayed multiple antibiotic resistance. Isolates demonstrated the highest resistance to the cephalosporin class of antibiotics, followed by fluoroquinolone and macrolide antibiotic classes. Most of the isolates were, however, susceptible to the carbapenem, amphenicol and aminoglycoside antibiotic classes. Similar findings were reported by **Saana et al.** [11], **Lucky et al.** [23] and **Khanal et al.,** [24] in Ghana, Indonesia and Nepal respectively. Fluoroquinolones (such as ciprofloxacin) and extended-spectrum cephalosporins (such as ceftriaxone) have been recommended as replacements to traditional first-line antibiotics such as ampicillin, chloramphenicol, and trimethoprim in the treatment of *Salmonella* infections [25,26]. Azithromycin has also been reported to be more effective than fluoroquinolones and extended-spectrum cephalosporins [27,28]. Our findings, however, reveal that ciprofloxacin (a fluoroquinolone) and azithromycin (a macrolide) were unsuitable antibiotics for treatment of typhoid fever, compared to the other antibiotics used in this study. Imipenem, gentamicin and chloramphenicol

proved to be the most effective of all the antibiotics used. Resistance to ciprofloxacin in the study area may be because of its widespread use in the treatment of typhoid fever over the years. It appears to be the most frequently prescribed antibiotic for typhoid fever treatment in most communities within the zone. This trend of over-usage has been associated with resistance [22]. Resistance to antibiotics by *S. typhi* is also mediated by antibiotic-destroying enzymes produced by the bacterium and the activation of efflux pumps that actively remove antibiotics from within the cell environment [29]. The level of resistance exhibited by *S. typhi* isolates to ciprofloxacin observed in this study may mean that the antibiotic which has been previously very effective in treating typhoid is fast becoming ineffective. An alternative antibiotic may need to be identified in order to reduce morbidity and mortality associated with typhoid fever.

Historically, chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole were the most preferred choices for treating typhoid fever [30]. A very important finding in the present study is the re-emergence of chloramphenicol from the amphenicol class of antibiotics [31,32], with a higher inhibitory activity than those currently applied to treat the disease. This agrees with the findings of **Adabara et al.** [3] in Nigeria, **Poudel et al.** [9] and **Raza et al.** [33] both in Nepal. Also, **Islam et al.** [34] reported in a study conducted in Bangladesh that only 28 (32.5%) out of 86 *S. typhi* isolates showed resistance to chloramphenicol, suggesting more susceptibility than resistance. Similarly, in a review of 6 different studies conducted in different countries of the world including Bangladesh, **Ahasan et al.** [35] reported increasing susceptibility of salmonella isolates to the first line antibiotics chloramphenicol, ampicillin and cotrimoxazole. **Ayalu et al.** [8] and **Baya et al.** [10] however, reported resistance to chloramphenicol in their findings. Our findings revealed that chloramphenicol is currently not commonly prescribed in the study area. Suspending the use of an antibiotic to which resistance has developed over a period, could reverse the trend, and restore its effectiveness by the elimination of selective pressure [1,35]. Also, the increase in susceptibility of *S. typhi* to chloramphenicol as observed in this study could have resulted from the emergence of new susceptible strains and the loss of self-transmissible, high molecular weight plasmids by the bacterium [36]. The resurgence of susceptibility

to chloramphenicol as has been observed in this study may suggest that a return to the use of previously discontinued traditional, historically useful drugs may be advantageous to health care and particularly in the management of *S. typhi* infection, although this may require careful consideration. Such drugs are cheaper and more accessible in developing countries than some of the currently prescribed regimens for treating *S. typhi* infections [36].

Multidrug resistance is mediated by resistance-bearing plasmids in bacteria [36]. However, genes responsible for MDR can also be chromosome-borne. **Chiou et al.** [37] reported that over 50% of the gene responsible for MDR *S. typhi* in Bangladesh were borne on the chromosome. The most dominant pattern of MDR observed in their study was Ampicillin/chloramphenicol/streptomycin/sulfamet hoxazole/trimethoprim [37]. A MDR rate similar to that observed in this study (42.7%) was reported by **Mthembu et al.** [38] (43%) in a survey of MDR salmonella in livestock in South Africa. In many farms, macrolides, beta-lactams, tetracyclines, fluoroquinolones and sulfonamides are administered to animals as growth promoters. Hence, the resistance of *S. typhi* isolates to macrolides, beta-lactams, sulfonamides and fluoroquinolones observed in this study may not be unconnected to this practice [38].

Resistance to cephalosporins and fluoroquinolones as observed in this study is a reason for public health concern, considering their importance in treating salmonella infections [36]. Salmonellosis is treated using antibiotics such as ciprofloxacin, trimethoprim-sulfamethoxazole, tetracycline, ceftriaxone, azithromycin, chloramphenicol, ampicillin and amoxicillin-clavulanate [38]. In this study, resistance to ciprofloxacin, azithromycin, ceftriaxone, trimethoprim-sulfamethoxazole and amoxicillin-clavulanate by *S. typhi* isolates was observed with some isolates showing resistance to more than two of these antibiotics simultaneously. One limitation of this study is the absence of data for minimum inhibitory concentration of the antibiotics and molecular detection of resistance genes in MDR isolates. These could reveal more information regarding the pattern of resistance exhibited by *S. typhi* isolates in this study.

Conclusion

This study observed increasing resistance to antibiotics used for the treatment of typhoid fever in the study area. Interestingly, chloramphenicol, imipenem and gentamicin inhibited the growth of *S. typhi*, demonstrating a positive reversal trend for susceptibility of *S. typhi* to chloramphenicol. Our findings lend credence to the campaign for effective antibiotic stewardship in disease treatment, to stem the increasing trend in antibiotic resistant pathogenic bacteria such as *S. typhi*. Since the disease is inversely related to hygiene, access to safe drinking water and enlightenment of the public towards keeping neater living environments could mitigate its spread. Keeping a tab on the antibiotic resistance pattern of pathogenic bacteria is necessary for effective and efficient treatment and management of diseases.

Authors contribution

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