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Quinolone and Multidrug Resistant *Salmonella typhi* in Ibadan, Nigeria

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Abstract: Typhoid fever remains prevalent worldwide especially in a developing country like Nigeria. Many first line drugs such as chloramphenicol were discontinued due to Multi-Drug Resistant (MDR) *Salmonella Typhi* (*S. typhi*). Quinolones are now the recommended therapy but in spite of their usefulness there are several reports of failure of therapy due to quinolone resistance. This study sought to find the prevalence of quinolone resistant and MDR *S. typhi* in this environment. About 146 (4.6%) out of the 3184 blood culture samples collected for the study yielded *Salmonella typhi* disk diffusion antibiotic susceptibility testing was carried out for the following antibiotics: chloramphenicol, ampicillin, amoxicillin, amoxicillin-clavulanic acid, nalidixic acid, ciprofloxacin, azithromycin and ceftriaxone. The minimum inhibitory concentration of ciprofloxacin was determined against the isolates using broth macrodilution technique. Of the *Salmonella typhi* isolates 37.7, 32.2, 38.4 and 50.7% were susceptible to chloramphenicol, cotrimoxazole, ampicillin and amoxicillin, respectively while susceptibility to amoxicillin-clavulanic acid, nalidixic acid, ciprofloxacin, azithromycin and ceftriaxone were 87.7, 91.1, 95.9, 99.3 and 100%, respectively. The MIC_{50} and MIC_{90} of ciprofloxacin were 0.06 and 0.125 μg mL^{-1}, respectively. The prevalence of multidrug resistance was 56.2% while that of quinolone resistance was 8.9%. There is high prevalence of multidrug resistant *Salmonella typhi* therefore, the use of chloramphenicol and other previous first line antibiotics should be discouraged. Though, resistance appears to be emerging, quinolones remain useful in treating typhoid fever in this environment but surveillance should be continuous.

Key words: Typhoid fever, quinolones, multidrug resistant, *Salmonella typhi*, Nigeria

INTRODUCTION

Infections with *Salmonella enterica* ssp. *enterica* serovar Typhi (*Salmonella typhi*) continue to be a major problem worldwide causing typhoid fever in 21.6 million people and 216,500 deaths globally in year 2000 (Hasan *et al*., 2005). Typhoid fever is prevalent in developing countries of Africa, Southeast-Asia and also, Latin America as a result of poor sanitary conditions related to rapid population growth, increased urbanization, inadequate human waste treatment, limited water supply and overburdened health care systems (Lathi and Sudarsana, 2004). In Nigeria, the prevalence is between 12-16% in patients presenting with febrile illness (Akinyemi *et al*., 2005; Ogunleye *et al*., 2005).

The introduction of Chloramphenicol for the treatment of typhoid fever in 1948 transformed this severe disease into a readily treatable condition (Talawadekar *et al*., 1989). In developing countries, antibiotics most readily available for the treatment of typhoid fever are chloramphenicol, ampicillin and cotrimoxazole. Resistance to chloramphenicol and these other antibiotics has been a major setback resulting in Multi-Drug Resistant (MDR). *Salmonella typhi* with multiple resistance usually encoded by plasmids of the H1 incompatibility group (Rowe *et al*., 1997; Wain *et al*., 2003). These have rapidly assumed epidemic proportions accounting for almost 60-90% of all cases of typhoid in certain reports (Rowe *et al*., 1997; Kariuki *et al*., 2004). Typhoid outbreaks caused by MDR *S. typhi* in Asia and the Indian subcontinent have been well characterized. Though data from Africa is scarce there are reports of 70-78% in Kenya, 36-61% in Nigeria and 62% in Ghana (Akinyemi *et al*., 2005; Ogunleye *et al*., 2005; Kariuki *et al*., 2004; Newman *et al*., 2006).

This high prevalence of MDR *S. typhi* has led to a change in the choice of first line antibiotics for typhoid fever. Fluoroquinolones (usually Ciprofloxacin) have now become the first-line drugs for the treatment of typhoid fever and have proven to be effective for the treatment of typhoid fever caused by MDR strain (Rowe *et al*., 1991; Parry *et al*., 2002).

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Quinolones target the DNA gyrase enzyme of bacteria and inactivate it (Hooper, 2000). Despite their efficacy there have been increasing reports of delayed response or failure of therapy with quinolones (Threlfall and Ward, 2001; Nkemngu *et al*., 2005). This resistance is associated with chromosomal mutations in the *gyrA* gene resulting in substitutions at the Serine-83 position often to tyrosine, phenylalanine or alanine and aspartate-87 substitutions to asparagine, glycine or tyrosine (Brown *et al*., 1996; Phung *et al*., 2002).

Such isolates are usually nalidixic-acid resistant and have elevated fluoroquinolone (ciprofloxacin) Minimal Inhibitory Concentrations (MIC) with associated treatment failure (Wain *et al*., 1997). These quinolone resistant strains have become a major problem in endemic regions of South-East Asia accounting for up to 90% of infections and seriously jeopardizing their relevance in treating typhoid fever in those regions with attendant problems including choice of alternative therapy (Lathi and Sudarsana, 2004). In Nigeria, out of the MDR *S. typhi* tested by Akinyemi *et al.* (2000) no isolate showed resistance to ofloxacin and ciprofloxacin however by 2005 a study in Ibadan on childhood septicaemia showed a 10.5% quinolone resistance in Salmonella (Ogunleye *et al*., 2005; Akinyemi *et al*., 2000).

As a result of reports suggesting that quinolone resistant strains are in circulation in this environment this study therefore, sought to determine the prevalence of Quinolone resistant *Salmonella typhi* to estimate the prevalence of multi-drug resistant *Salmonella typhi* in this environment and to establish the antimicrobial susceptibility pattern of *Salmonella typhi* in Ibadan.

MATERIALS AND METHODS

The study was carried out in Ibadan which has witnessed rapid population growth and urbanization in recent years associated with human and environmental poverty, the declining quality of life, inadequate housing and facilities like water and electricity all of which increase the risk of infectious disease including water borne diseases such as typhoid fever.

About 146 isolates of *Salmonella typhi* were included in this study. Isolates were obtained from blood samples collected from patients with a clinical impression of typhoid fever attending the general out-patient clinic or admitted on the ward of five hospitals and three health care centres serving the metropolis including the University College Hospital, Catholic Hospital Oluyoro, Adeoyo Maternity Teaching Hospital, General Hospital Moniya, Ring Road State Hospital and Health Centres at Sango. Samples were collected from March 2008 to January 2009.

Ethical approval for research was sought and obtained from the Joint Ethical Committee of the University of Ibadan/University College Hospital from the Oyo State Ethical and Research Review Committee and also from the Catholic Hospital Oluyoro.

All participants had blood samples collected, 5-10 mL of peripheral venous blood was collected from adults while 2-3 mL was collected from children aseptically into blood culture bottles containing brain-heart infusion broth (Oxoid, UK) then transferred to the laboratory for further processing.

Blood culture bottles were incubated aerobically at 37°C and observed daily for a maximum of 7 days. Initial subculture was done on day 2, subculture was repeated if there were signs of bacterial growth or on day 7. Samples were subcultured onto blood agar and MacConkey agar and also incubated aerobically at 37°C for 24-48 h.

Suspected isolates were initially identified on the basis of colonial morphology of non-lactose fermenting, non-haemolytic colonies, gram stain of gram negative bacilli and oxidase negative test. They were then subjected to biochemical tests using urease agar, citrate agar and Kliger Iron Agar slants (Oxoid, UK).

Serological identification of isolates that were citrate negative, urease negative and fermented only glucose on KIA with hydrogen sulphide production was done with slide agglutination test using Salmonella polyvalent O antiserum then monovalent Group D factor 9 antiserum (Pro-Lab diagnostics, UK). *Salmonella typhi* NCTC 10787 was used as positive control for the above procedures.

Isolates identified as *Salmonella typhi* were subjected to antibiotic susceptibility testing following the CLSI guidelines on the Kirby-Bauer disc diffusion technique. Antibiotics discs used were those containing chloramphenicol (30 μg), amoxicillin (25 μg), augmentin (30 μg), ampicillin (30 μg), cotrimoxazole (25 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg), azithromycin (15 μg) and ceftriaxone (30 μg) (Oxoid, UK).

Also the minimum inhibitory concentration of ciprofloxacin against the isolates was determined by the broth macrodilution technique using ciprofloxacin concentrations of 0.016, 0.0312, 0.0625, 0.125, 0.25, 0.5, 1, 2, 4 and 8 μg mL^{-1} (VS International, India). This was performed according to guidelines for carrying out sensitivity testing (Andrews, 2001). *Escherichia coli* ATCC 25922 was used as control organism for antibiotic susceptibility testing. Data was analyzed using the Statistical Package for the Social Sciences (SPSS) Version 15.0 Software.

RESULTS AND DISCUSSION

About 146 isolates of *Salmonella typhi* were obtained from blood culture out of the three thousand one hundred and eighty four samples collected. Table 1 shows the antibiotic susceptibility pattern of the isolates of *Salmonella typhi* to the nine tested antibiotics. The highest level of resistance was to cotrimoxazole as 67.8% of all isolates were either resistant or had intermediate level of susceptibility to this antibiotic. The next was chloramphenicol with a 62.3% level of resistance and that was closely followed by Ampicillin resistance of 61.6%.

About half of all isolates, 50.7% (74) were sensitive to amoxicillin while up to 87.7% (128) were sensitive to augmentin. One hundred and thirty three isolates (91.1%) were sensitive to nalidixic acid while 140 (95.9%) were sensitive to ciprofloxacin. Only one isolate (0.7%) was resistant to azithromycin while all the 146 isolates were susceptible to ceftriaxone. Table 2 shows the resistance profile of the isolates. There was a high prevalence of MDR *S. typhi* as eighty two isolates (56.2%) were multidrug resistant whereas only 13 (8.9%) were quinolone resistant. The minimum inhibitory concentration of ciprofloxacin against the isolates is as shown in Table 3. The highest number of isolates, 63 (43.2%) were inhibited by a ciprofloxacin concentration of 0.063 μg mL^{-1}. Only one isolate (0.7%) displayed high level of resistance to ciprofloxacin with MICs of 4 μg mL^{-1}. The MIC_{50} was 0.063 μg mL^{-1} while the MIC_{90} was 0.125 μg mL^{-1}.

Table 1: Antibiotic susceptibility pattern of *Salmonella typhi*

Disc diffusion for all antibiotics N = 146

	Susceptible		Resistant	
Antibiotic	Freq. (n)	Percent	Freq. (n)	Percent
Chloramphenicol	55	(37.7)	91	(62.3)
Cotrimoxazole	47	(32.2)	99	(67.8)
Ampicillin	56	(38.4)	90	(61.6)
Amoxicilin	74	(50.7)	72	(49.3)
Augumentin	128	(87.7)	18	(12.3)
Nalidixic acid	133	(91.1)	13	(8.9)
Ciprofloxacin	140	(95.9)	6	(4.1)
Ceftriaxone	146	(100.0)	0	(0.0)
Azithromycin	145	(99.3)	1	(0.7)

Table 2: Prevalence of drug resistance in the *S. typhi* isolates

Drug resistance	Yes (%)	No (%)
Multidrug-resistant *S. typhi*	56.2	43.8
Quinlone resistant *S. typhi*	8.9	91.1

Table 3: Minimum inhibitory concentration of ciprofloxacin

Ciprofloxacin concentration (μg mL^{-1})	Frequency (n)	Percentage
0.016	17	11.6
0.03	51	34.9
0.06	63	43.2
0.125	3	2.1
0.25	6	4.1
0.50	2	1.4
1.00	3	2.1
2.00	0	0.0
4.00	1	0.7
8.00	0	0.0
Total	146	100.0

This study demonstrated that there is some level of resistance to quinolones inthe environment as 6 out of the 146 isolates were either fully resistant or had intermediate level resistance to ciprofloxacin, the prototype fluoroquinolone used in this study, giving a prevalence of ciprofloxacin resistance of 4.1%. Quinolone resistance in *Salmonella typhi* appears to be an emerging problem in this environment as none was detected by Akinyemi in the study carried out about a decade ago and also Wariso found their *S. typhi* isolates to be highly susceptible to quinolones (Akinyemi *et al*., 2000).

Although, quinolone resistant *S. typhi* was found in this study, the prevalence is quite low compared to the prevalence of 10.5% that was found by Ogunleye in the same city in 2005 among children seen in University College Hospital. This disparity might be due to the selected population of patients, i.e., children seen in the outpatient department of a tertiary care facility included in their study and also the small sample size of the study. The finding from this study is also in contrast with reports from the Asian continent and Europe where quinolone resistant strains account for 20-30% of their isolates.

However, this finding is similar to recent publications from Ghana and Senegal where quinolone resistance is not yet a problem among their *S. typhi* isolates. In Cameroun as well, resistance is just beginning to emerge (Nkemngu *et al*., 2005; Dromigny and Perrier-Gros-Claude, 2003). The MIC_{90} is used to infer the usefulness of an antibiotic and in this study for ciprofloxacin it was 0.125 μg mL^{-1} which is regarded as susceptible.

It is well known that isolates which are nalidixic acid resistant may not respond to treatment with fluoroquinolones despite *in vitro* susceptibility (Nkemngu *et al*., 2005). Nalidixic acid resistant isolates usually have single chromosomal mutations which confer decreased susceptibility to fluoroquinolones. It is therefore important to screen isolates for nalidixic acid resistance. The prevalence of nalidixic acid resistance was 8.9% compared with 4.1% for ciprofloxacin resistance.

The overall prevalence of quinolone resistance in view of nalidixic acid resistance is therefore 8.9%. It is therefore inferred from this study that although, quinolone resistance inthe *Salmonella typhi* isolates is emerging, the level is relatively low compared with findings from several other regions of the world, especially the Indian subcontinent where quinolones are rapidly loosing their usefulness in managing typhoid

fever. It should be borne in mind however that in regions where quinolone resistance has become a major problem, the occurrence has been attributed to injudicious administration and rampant use of quinolones for a wide variety of infections due to their ready availability.

Multidrug resistant *Salmonella typhi* has long been identified as a problem and since, 1989 strains of *Salmonella typhi* resistant to chloramphenicol, ampicillin and trimethoprim (Multidrug-Resistant (MDR) strains) have been responsible for numerous outbreaks in countries in the Indian subcontinent, Southeast Asia and Africa as well (Rowe *et al*., 1997). It was this widespread occurrence of MDR *Salmonella typhi* that led to the recommendation of fluoroquinolones for treating typhoid fever.

The prevalence of Multi-Drug Resistant (MDR) *Salmonella typhi* was 56.2% as organisms that were resistant to chloramphenicol, ampicillin and cotrimoxazole accounted for 82 out of the 146 isolates. This prevalence is in keeping with numerous reports of circulation of MDR *S. typhi* which account for 60-90% of infections which is also similar to the finding of 61% prevalence by Akinyemi *et al.* (2005) in Lagos, Nigeria (Kariuki *et al*., 2004; Mirza *et al*., 2000). It is however, remarkably higher than the report of 36% by Ogunleye in Ibadan and reflects the high percentage of multi-drug resistant *Salmonella typhi* (Ogunleye *et al*., 2005).

Close to 88% of the isolates were sensitive to augumentin (amoxicillin-clavulanic acid) which is most likely to be due to the beta-lactamase stability of clavulanic acid. This makes it a relatively good choice if penicillin is to be used for the treatment of this infection. Many studies have found that typhoid fever that did not respond to quinolones usually had good response to third generation cephalosporins including ceftriaxone, cefixime and cefotaxime and also the macrolide antibiotic, azithromycin which has made them the suggested alternative therapies in cases of quinolone resistant *Salmonella typhi* (Newman *et al*., 2006; Saha *et al*., 2006; Mandal *et al*., 2003). The present study also corroborated this finding as there was no resistance to ceftriaxone and also virtually all isolates were sensitive to azithromycin.

However, these alternative drugs are quite expensive and not as readily available as ciprofloxacin. Also, a drug like ceftriaxone is available only in the parenteral form impairing ease of administration.

CONCLUSION

The use of former first line therapy such as chloramphenicol and amoxicillin should be discouraged as resistance to them is high. Although, resistance appears to be on the increase, quinolones remain relevant in the management of typhoid fever in this environment as majority of isolates in this environment are still susceptible to quinolones and also, augmentin also could be used with caution. Ceftriaxone and azithromycin appear effective as alternatives in cases of quinolone resistance due to their remarkable *in vitro* activity against *Salmonella typhi*. Misuse of antibiotics due to their ready availability over the counter, aid in selection of resistant organisms and should therefore be discouraged to ensure that they remain efficacious for long.

Also, vaccination of high risk groups, especially young children is highly recommended until the disease is no longer of public health importance in this region. Routine surveillance of this infection should be carried out so as to be aware of the trends in prevalence of infection and antibiotic susceptibility in order to take appropriate actions.

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