

Trends of Multiple Drug Resistance in *Salmonella Enterica* Serovar Typhi in Lagos, Nigeria.

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Background: The frequent treatment failures with empirical therapy observed in some hospitalized typhoid fever patients in the last decade is of great concern to both public and private physicians owing to the wide spread and circulation of antibiotic resistant strains of *S. typhi*

Methods: This study assessed the trends in antibiotic resistance in 235 *Salmonella typhi* stains isolated by standard procedures from blood and/stool samples of hospitalized patients from 1997 to 2003. All the isolates were subjected to antimicrobial susceptibility testing using the following antibiotics: chloramphenicol, Ampicillin, cotrimoxazole, tetracycline, nalidixic acid, ciprofloxacin and ofloxacin. Susceptibility and resistance were determined by standard methods.

Results: From 1997 through 2003, 188 (80%) of 235 isolates were multiple drug resistant (MDR), chloramphenicol being the most resisted antibiotic (83.0%) followed by Ampicillin (81.7%). Only one strain was resistant to both ciprofloxacin and ofloxacin. Also the prevalence of chloramphenicol resistant isolates increased gradually from 72.4% in 1997 to 89.2% in 2003. Similar trends were recorded for other four antibiotics tested, even for single drug resistance *S. typhi* isolates. Our study confirmed increased circulation of MDR-*S. typhi* isolates over relatively short period.

Conclusion: We hereby suggest for a while the immediate stoppage of prescription of chloramphenicol and other first line antibiotics used in the treatment of typhoid fever in Nigeria. The use of more effective drugs such as ofloxacin and ciprofloxacin would go a long way in stemming the prevalence of persons with chronic infections as well as reducing the widespread of MDR-*S. typhi* strains in our environment, but our fear is that resistance is likely to develop unless these valuable drugs are used prudently

Introduction

Typhoid fever remains an important public health problem particularly in developing countries with approximately 10 millions cases which result in 700,000 deaths annually^{1,2}. The situation is different in developed countries such as the USA and the UK where the incidence is low and most cases are found in international travelers³. The antibiotics that form the mainstay therapy for typhoid fever patients in developing countries are chloramphenicol, Ampicillin and cotrimoxazole. Resistance strains of *Salmonella typhi* to these antibiotics have emerged and continued to be of clinical significance. In Nigeria, typhoid fever is among the major widespread diseases affecting both young children and young adults in their productive years as a result of many interrelated factors such as increase urbanization, inadequate

supplies of potable water, regional movement of large numbers of immigrant workers, inadequate facilities for processing human wastes, overburdened health care delivery systems and indiscriminate use of antibiotics that contributed to the development of antibiotic resistant *S. typhi*^{4, 5}. Chloramphenicol has been employed successfully in the management of typhoid fever in Nigeria because *S. typhi* strains recovered from patients were routinely susceptible^{4,6}. The efficacy of this antibiotic and other first line antibiotics such as Ampicillin, cotrimoxazole and tetracycline became doubtful following unprecedented upsurge in enteric fever in early 1990s^{7,8,9}. From 1997 through 1998, study on the prevalence of multiple drug resistance (MDR) in *S. typhi* was conducted in Lagos, and most *S. typhi* strains isolated were resistant to these antibiotics¹⁰. However, once drug resistant *S. typhi* strain emerged, it quickly became a

significant public health problem, a pattern that has been repeated in many parts of the world where *S. typhi* is prevalent^{2,11,12,13,14}. Previous reports elsewhere have indicated either clonal spread and/or extra chromosomal genes to be the potential mechanisms for high level of reduced susceptibility¹⁵. In Nigeria, evidence of plasmid-mediated MDR *S. typhi* associated with typhoid fever complications has been documented recently⁵. Recently, increasing cases of typhoid fever complications among hospitalized patients has been speculated among public and private clinicians and health workers to be associated with the late reporting of patients to the hospitals at the on set of illness owing to the attitude of self medications among other factors. This situation is worrisome and has led to prolong stay of patients in hospitals because of frequent cases of treatment failures with the empirical therapy. It is on this premise that the current study was initiated with a view to assessing the trends in antibiotic resistance in *S. typhi* recovered from hospitalised typhoid fever patients from 1997 to 2003 and to provide information on the possible alternative antibiotics that could be used for the treatment of typhoid fever and its complications..

Methods

Patient population and study Centre: - A retrospective study of 235 *S. typhi* isolates recovered from patients whose blood and/or stool samples were brought to the Central Public Health Laboratory Services (CPHLS), Yaba was conducted in order to assess antimicrobial resistance patterns of the isolates. CHPLS is a referral laboratory that serves an estimated population of 12 million people of Lagos State and had been established for the evaluation and processing of laboratory samples from patients admitted to hospitals and/or undergoing treatment of infectious diseases in children and adults. The subjects in this study were patients who presented with two or more of the following symptoms, diarrhoea, vomiting, headache, loss of appetite, malaise and abdominal pain and had been diagnosed for typhoid fever based on Widal agglutination test (from the diagnostic centres) and had been on at least one of the first line antibiotics but did not respond to treatment before reported and/or admitted to the hospital.

Bacteriology –

Fresh aliquot of each of the patients blood collected aseptically was directly inoculated into brain heart infusion (BHI) broth (Oxoid U.K) in 1:9 blood broth ratio while stool specimens collected in Selenite F broth (Oxoid, U.K) incubated at 37°C aerobically for 18-24hrs. Subcultures were made from the broths into *Salmonella- Shigella* agar and Desoxycholate citrate agar (Oxoid, U.K.) and plates further incubated at 37°C for 18-24 hrs. In negative cases, subcultures were repeated from the infusion broth daily for 7 consecutive days before discarded. Bacterial colonies were identified using standard procedures¹⁶.

Antimicrobial susceptibility testing

Susceptibility to Ampicillin (25ug) chloramphenicol (30ug), co-trimoxazole (25ug), tetracycline (50ug) and nalidixic acid (30ug), ciprofloxacin (20ug) and Ofloxacin (20ug) was determined for all isolates by the disk diffusion Kirby Bauer method³⁰. After, 1999, susceptibility testing was performed using the reference broth micro dilution methods recommended by National Committee for Clinical Laboratory Standard (NCCLS)³¹. Susceptibility was determined using the established NCCLS break points. For Ampicillin and chloramphenicol, break points 8.0 and 16.0ug/ml were used for intermediate and resistant respectively. Tetracycline and cotrimoxazole ≥ 4.0 ug/ml for intermediate and ≥ 8.0 ug/ml for resistant, while nalidixic acid ≥ 4.0 ug/ml for resistant was used. The minimum inhibitory concentration (MIC) of ≤ 0.03 ug/ml was taken to be fully sensitive for ciprofloxacin and ofloxacin. For analysis in this study, both intermediate and resistant categories were considered to be resistant. *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853 were used as controls in susceptibility testing. MDR *S. typhi* infection in this study was defined as an infection due to *S. typhi* isolate that was resistant to three or more antibiotics.

Results

From 1997 through 2003, a total of 235 *S. typhi* isolates were identified in blood and/stool cultures. Of these, 188 were resistant to at least

three antibiotics given a prevalence of 80% MDR- *S. typhi* in this study. The highest number of *S. typhi* (16.1%) was recorded in 2000. Our study showed that for *S. typhi* isolates in aggregate, the highest percentage of resistance was recorded in chloramphenicol (83.0% of isolates) followed closely by Ampicillin (81.7% of isolates) and cotrimoxazole (80.4% of isolates) (Table 1). However, resistant to individuals antibiotics varied slightly during the periods under study. For instance, the prevalence of chloramphenicol resistant isolates which was

72.4% in 1997 increased gradually to 89.2% by year 2003. Similar trend was also observed for Ampicillin, cotrimoxazole and tetracycline. Only one strain of *S. typhi* was found to be resistant to ciprofloxacin and ofloxacin throughout the periods. Also, the prevalence of MDR-*S. typhi* isolates was similar to that of *S. typhi* isolates with single drug resistance (including nalidixic acid) with gradual increase from 70.0% in 1997 to 88.6% in 2002 but later decreased in 2003.

Table 1. Antibiotic Resistant *Salmonella Typhi* Isolated From Hospitalized Patients From 1997-2004 In Lagos.

Year	Number of isolates	Number of Isolates resistant to each antimicrobials (%)							Number of MDR
		Amp	Chl	Tet	Cot	Nal	Cip	Ofi	
1997	29	20 (70.0)	21 (72.4)	21 (72.4)	20 (70.0)	14 (42.3)	0 (0.0)	0 (0.0)	20 (70.0)
1998	37	28 (75.7)	30 (81.1)	26 (70.3)	30 (81.1)	16 (43.2)	0 (0.0)	0 (0.0)	28 (75.7)
1999	26	21 (80.0)	20 (76.9)	20 (76.9)	21 (80.0)	11 (42.3)	0 (0.0)	0 (0.0)	20 (76.9)
2000	38	30 (79.0)	31 (81.6)	29 (76.3)	31 (81.6)	17 (47.4)	0 (0.0)	0 (0.0)	30 (79.0)
2001	33	28 (84.8)	29 (87.9)	28 (84.8)	27 (81.8)	17 (51.5)	0 (0.0)	0 (0.0)	28 (84.8)
2002	35	31 (88.6)	31 (88.6)	30 (85.7)	30 (85.7)	20 (57.1)	0 (0.0)	0 (0.0)	31 (88.6)
2003	37	32 (86.5)	33 (89.2)	31 (83.8)	30 (81.1)	20 (56.8)	0 (0.0)	0 (0.0)	31 (83.8)
2004	39	34 (87.2)	34 (87.2)	33 (84.6)	34 (87.2)	23 (59.0)	1 (2.5)	1 (2.5)	33 (84.6)
Total	274	224 (81.8)	229 (83.6)	218 (79.6)	223 (81.4)	140 (51.1)	1 (0.4)	1 (0.4)	221 (80.7)

Amp = ampicillin, Chl = chloramphenicol, Tet = tetracycline, Cot = cotrimoxazole, Nal = nalidixic acid, Cip = ciprofloxacin, Ofi = ofloxacin

Discussion

The frequent treatment failures with empirical therapy observed in some hospitalized typhoid fever patients in the last decade is of great concern to both public and private physicians owing to the wide spread and circulation of antibiotic resistant strains of *S. typhi*. Our study

showed that 70%, 72.4%, 70% and 72.4% of *S. typhi* isolates examined in 1997 were resistant to Ampicillin, chloramphenicol, tetracycline and cotrimoxazole respectively. There was increase in the resistant recorded to all the aforementioned antibiotics in the subsequent years. For example 79.0%, 81.6%, 76.3% and 81.6% as well as 86.5%, 89.2%, 83.8% and 81.1% of the isolates were resistant to Ampicillin, chloramphenicol, tetracycline and cotrimoxazole in 2000 and 2003 respectively

(Table 1). The prevalence of MDR- *S. typhi* increased from 70.0% in 1997 to 83.3% in 2003. The increasing trends of MDR- *S. typhi* recorded might have arisen from drug abused. This is because therapeutic intervention in suspected cases of typhoid fever due to attitude of self medications usually prevents early reporting of patients to the hospitals at the onset of the disease symptoms except where complications had occurred. This is because our present study was hospital-based and close observations revealed that most of the patients had been on antibiotic self medications (at least one of the first line antibiotics) and only untreated cases of typhoid fever by self medications were brought to the hospitals. Severe, refractory or complicated infections have been attributed to increase chloramphenicol resistance in strains of *S. typhi* in some parts of the world¹⁷, a situation that seems to have come to stay in our environment in recent time. However, this study is in contrast to some reports from African countries such as Senegal and Egypt, where significant shift in the antibiotics susceptibility to the first line antibiotics have been recorded. For instance in Egypt $\geq 60\%$ prevalence of MDR- *S. typhi* was recorded in 1987 but gradually decreased to only 5% in 2000¹⁸. Also in Senegal, there was a shift in resistant patterns of *S. typhi* isolates to the first line antibiotics from over 50% recorded in 1997 to about 3% resistant recorded in 2002¹⁹ (Similar observations of increased susceptibility of *S. typhi* to the first line antibiotics particularly chloramphenicol had since been made in India and Bangladesh^{20,21} and were attributed to the restricted use of chloramphenicol for few years. The implication of high prevalence of MDR-*S. typhi* recorded in our study is that efficacy of the relatively cheap empirical therapy for typhoid fever patients in Nigeria is now doubtful and thus call for urgent attention. More importantly, the increasing trends of MDR -*S. typhi* may spread to neighbouring countries of Africa that recorded low prevalence of MDR-*S. typhi* recently and as well to other parts of the world particularly among the travellers returning from this region if the unprecedented upsurge remains unchecked.

In this study we observed an increased in the emergence of nalidixic acid resistant *S. typhi* from 42.0% in 1997 to 57.1% in 2002 and

decreased slightly to 56.8% in 2003. This result is surprising as nalidixic acid is not frequently prescribed by physicians in most clinics and hospitals in Nigeria. However, the increasing emergence of nalidixic resistant *S. typhi* may probably be attributed to the use of quinolone antibiotics in animal feeds in the country. Several workers have reported from elsewhere that the use of quinolones in food animals have led to the rapid emergence of resistant *Salmonella* infections to humans^{22,23,24,25,26,27}. Studies have also revealed that antimicrobial agents used in agriculture and closely related agents used in human medicine have been exerting selective pressure on their target bacteria particularly *Salmonella*, *Campylobacter* and *Escherichia coli*^{24,28,29}, a situation that might have arisen in Nigeria.

It is interesting to note that MDR- *S. typhi* strains recovered from patients from 1997 to date were all sensitive to ciprofloxacin and ofloxacin except one ($1/26$) MDR- *S. typhi* isolated in 1999 which was resistant to both ciprofloxacin and ofloxacin. Clinical history of the patient revealed an earlier diagnosis of human immunodeficiency virus – associated acquired immune deficiency syndrome. This unusual isolation of fluoroquinolone resistant *S. typhi* was first of its kind and thus calls for urgent attention. In spite of this unusual observation, these two antibiotics seem to offer new hope for the treatment of typhoid fever and its complications. To date fluoroquinolone antibiotics are still under strict prescription of clinicians and are less abused due to high cost of their procurement in Nigeria. Although, in other parts of the world such as South-east Asia and the United Kingdom^{3,15} there have been rapid emergence of fluoroquinolones resistance in *S. typhi* isolates but no such previous observation has been reported in Nigeria.

Conclusion

This study recorded high prevalence as well as increased in the trend of MDR-*S. typhi* throughout the 7 year-period. Chloramphenicol, ampicillin cotrimoxazole and tetracycline are no longer effective for treatment of typhoid fever and its complications. The use of more effective antibiotics such as ofloxacin and ciprofloxacin would go a long way in stemming the

prevalence of persons with chronic infections as well as decreasing the circulation and widespread of MDR- *S. typhi* strains, thus prevent the spread to other African countries. But our fear is that resistance is likely to develop unless these valuable drugs are used prudently. It is on this note that we suggest the followings:

1. Comprehensive enlightenment campaigns on early reporting of patients to the hospital once the symptoms of typhoid fever are noticed, and the implication of engaging in antibiotics self medication.
2. Enhancement and prudent use of laboratory services in order to back up clinical diagnosis is essential.
3. Restriction and/or immediate stoppage for a while, the use of chloramphenicol, ampicillin cotrimoxazole and tetracycline for the treatment of typhoid fever and its complications in Lagos , Nigeria..
4. Enforcement of existing laws relating to drug sales that will only permit qualified medical and other authorized personnel to handle antimicrobial drugs in developing countries and
5. Constant collection and monitoring of data that document antibiotic resistance patterns is equally advocated.

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