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The Re-emergence of Chloramphenicol Sensitive *Salmonella* species among Typhoid Fever Patients in the Southern Geographical Zone of Nasarawa State, Nigeria

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Abstract

The emergence of drug resistant strains of *Salmonella typhi* and paratyphi has become the most challenging aspects of typhoid fever treatment due to resistance to the conventional antibiotic therapy. The objective of this study was to determine the prevalence and antibiotic susceptibility of *Salmonella* species among typhoid fever patients. A total of 400 stool samples were collected, processed and identified by standard microbiological method and biochemical tests. Antibiotic susceptibility was determined by disk diffusion method using 8 antibiotics. A total of 196(49.0%) *Salmonella* species were isolated. Of this, 78 (19.0%) and 118 (29.5%) were *Salmonella typhi* and *Salmonella paratyphi* respectively. The antibiotic susceptibility of *Salmonella* isolates in the study showed a high susceptibility of 85.9% for *S. typhi* and 86.4% for *S. paratyphi* to Chloramphenicol. All *S. typhi* isolates showed a low susceptibility of 20.5% to Amoxicillin, 14.1% to Amoxicillin/clavulinic acid, and 33.3% to ceftriaxone. 85.2%, 87.4% and 6.9% to Ciprofloxacin, Gentamycin and Tetracycline respectively. *Salmonella paratyphi* isolates on the other hand also showed higher susceptibility of 89.1%, 83.6%, 80.9%, 79.1% to Amikacin, Ciprofloxacin, Ceftriaxone, Gentamycin and lower susceptibility of 23.6%, 36.4% and 20.9% to Amoxicillin, Amoxicillin/clavulinic acid and Tetracycline respectively. The increasing susceptibility of *Salmonella* species to Chloramphenicol in this study therefore is a revelation that suggests the necessity for continuous surveillance of cases and re-evaluation of Chloramphenicol therapy in *Salmonella* infection in Nasarawa South and the state as a whole.

Key words:

Re-emergence; Chloramphenicol; *Salmonella* species; Nasarawa state

Introduction

Typhoid fever and paratyphoid fever are still serious public health problems in many geographic areas and are endemic in most developing countries. Typhoid fever is the most serious form of enteric fever and it was estimated that the global number of typhoid cases exceeded 21 000 000 with more than 2,00,000 deaths [1]. In cases of enteric fever, it is often necessary to commence treatment before the results of laboratory sensitivity testing become available and in this respect, Ciprofloxacin has become the first line drug for treatment, since the widespread emergence of *S. typhi* isolates that are multidrug resistant (MDRST) to the more traditional antimicrobial agents comprising chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole (cotrimoxazole). However, this switch to ciprofloxacin and selective pressures exerted by the irrational use of ciprofloxacin in human and veterinary therapeutics in a population endemic with nalidixic acid resistant *S. typhi* (NARST) strains has led to a subsequent increase in the occurrence of *S. typhi* isolates resistant to this antimicrobial agent and decline in MDRST, including in India [2,3]. For ciprofloxacin there has been an increase in minimum inhibitory concentration in strains imported into the UK, in Bangladesh, as well as in India.

Reports of typhoidal *salmonellae* with increasing MIC and resistance to newer quinolones raise the fear of potential treatment failures and necessitate the need for new, alternative antimicrobials. Extended-spectrum cephalosporins and azithromycin are the options available for treatment of enteric fever. The emergence of broad spectrum β-lactamases in typhoidal salmonellae also constitutes a new challenge.

In developing countries, ciprofloxacin continues to be the mainstay in the treatment of enteric fever as it is orally effective and economical. The emergence of *S. typhi* highly resistant to ciprofloxacin is a cause for worry for both clinicians and microbiologists as well as for patients. Though fluoroquinolone resistance is chromosomally mediated, selective pressures exerted by the overuse of these drugs may see such isolates becoming more common in the future. Of interest, though, is the possibility of turning to an older drug such as co-trimoxazole for treatment, in case of susceptible isolates. This study therefore, is a one year experience of antibiotic susceptibility pattern of *Salmonella typhi* and *Salmonella paratyphi* isolates from cases of typhoid fever in the Southern geographical zone of Nasarawa state, Nigeria.

Materials and Methods

A total of 400 stool samples were collected from consented clinically diagnosed typhoid fever patients and parents or guardians of children that presented themselves for typhoid fever treatment in the Primary Health clinics (PHCs) in the southern geographical zone of Nasarawa state into sterile plastic universal containers. All samples were

aseptically labelled appropriately and packed well in an ice pack and taken to the Microbiology laboratory of Nasarawa State University Keffi, Nasarawa State for isolation and other microbiological analyses. A constructed questionnaire was also prepared to collect all relevant socio-demographic information of patients.

Isolation

A loop full of each stool sample was homogenized in normal saline and then inoculated into Tetrathionate broth (Oxoid Ltd USA) at room temperature and incubated at 37°C for 24 hours. The resultant enriched culture was then sub-cultured onto Bismuth sulphite agar (Oxoid Ltd USA) and incubated at 37°C for 24 hours. The isolated non-lactose fermenter colonies were again subculture on Bismuth sulphite each time to obtain pure culture of isolates. All the isolates were also subjected to Biochemical tests.

Drug sensitivity testing

The antibiotic susceptibility of all the *Salmonella* isolates was done using Kirby-Bauer disc diffusion method [4,5] against 8 antibacterial agents. These antibiotics discs were commercial discs (Oxoid Ltd USA) namely: Amikacin (30 µg), Amoxycillin (25 µg), Amoxycillin/ Clauvulinic acid (30 µg), Ceftiaxone (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (5 µg), Gemtamycin (10 µg) and Tetramycin (30 µg). Results were interpreted using the criteria of the National Committee for Clinical Laboratory Standards (NCCLS) [6]. Briefly, 2 ml of overnight culture of each *Salmonella* species isolates in nutrient broth was used. Its turbidity was adjusted to the optical density of 0.5 Mc Farland turbidity standards and flooded over the prepared Mueller-Hinton (Oxoid Ltd USA) agar plates. Excess were drained off and allowed to dry in a warm incubator for about 15-20 minutes.

With the aid of sterile forceps, a set of antibiotic discs were gently placed on the surface of the Mueller-Hinton agar and pressed onto the medium surface to ensure firm contact. These set ups were then incubated at 37°C for 24 hours. Diameters of zone of inhibition around the antibiotic disc were measured to the nearest millimetre using a ruler. Results were interpreted using the criteria of the National Committee for Clinical Laboratory Standards (NCCLS) (NCCLS, 2002).

Statistical method used

To achieve the objective of this research work, SPSS version 10.00 (2003) was used for descriptive and inferential analysis (Chi-Square). A difference was taken as significant at a P-value less than 0.05.

Results

A total of 196(49.0%) *Salmonella* species (*S. typhi* and *S. paratyphi*) were isolated from 400 stool samples of clinically diagnosed typhoid fever patients. Of this, 78(19.0%) were *Salmonella typhi* and 118(29.5%) were *Salmonella paratyphi*.

Figure 1 shows the antibiotic susceptibility pattern of *Salmonella typhi* and *Salmonella paratyphi*. In this study, a high susceptibility of 85.9% and 86.4% for *S. typhi* and *S. paratyphi* to Chloramphenicol respectively was observed. *Salmonella typhi* isolates further showed a high susceptibility of 96.1%, 93.6%, and 84.6% to Amikacin, Ciprofloxacin, and Gentamycin respectively and a low susceptibility of 20.5% to Amoxicillin, 14.1% to Amoxicillin/clavulinic acid, and 33.3% to ceftriaxone. *Salmonella paratyphi* isolates however, also showed a high susceptibility of 89.1%, 80.9%, 83.6%, 79.1%, to Amikacin, Ceftriaxone, Ciprofloxacin, Gentamycin respectively while a low susceptibility of 23.6%, 36.4%, and 20.9% to Amoxicillin, Amoxicillin/ clavulinic acid, and Tetracycline respectively (Figure 1).

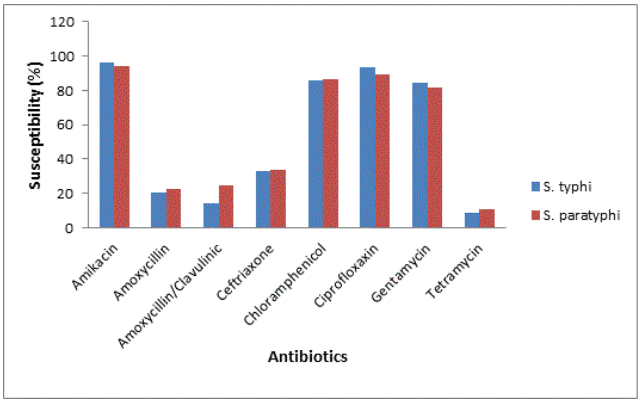

Figure 1: Antibiotic susceptibility pattern of *Salmonella typhi* and *Salmonella paratyphi* among typhoid fever patients.

The study further revealed that males had higher burden of infection with 50.7% and 59.5% for *S. typhi* and *S. paratyphi* respectively (Table 1). With respect to age, *S. typhi* infection was higher (23.1%) among ages 31-40 followed by ages 21-30 years. Patients of ages 21-30 were also observed to have higher prevalence in terms of *S. paratyphi* infection (Table 2).

Sex	No. Sampled	*S. typhi* (%)	*S. paratyphi* (%)	Total (%)
Male	207	40(50.7)	70(59.5)	110(53.1)
Female	193	38(49.4)	48(40.6)	86(44.1)
Total	400	78(19.5)	118(29.5)	196(49.0)
X^2=1.2327, P<0.05				

Table 1: Prevalence of *Salmonella* species among typhoid fever patients in Nasarawa South Senatorial zone with respect to sex.

Age group	No. Sampled	*S. typhi* (%)	*S. paratyphi* (%)	Total (%)
<10	50	10(20.0)	15(30.0)	25(50.0)
11-20	72	15(20.8)	13(18.1)	28(38.9)
21-30	89	20(22.5)	37(41.6)	57(64.0)

31-40	65	15(23.1)	20(30.8)	35(53.8)
41-50	55	11(20.0)	16(29.1)	27(49.1)
51-60	44	07(15.9)	08(18.2)	15(34.1)
60>	25	-- --	04(16.0)	4(16.0)
X^2=5.6976, P<0.05				

Table 2: Prevalence of *Salmonella* spp among typhoid fever patients in Nasarawa southern Senatorial zone with respect to age.

Discussion

Typhoid fever is an endemic disease in the tropic and sub-tropic regions of the world and has become a major public health problem in developing countries including Nigeria. The disease is a water-borne infection and the incidence of infection is compounded by poor sanitation and hygiene, overcrowded and unhealthy urban conditions were inadequate sanitary infrastructure prevail, and the emergence of resistant pathogens to antibiotics.

Chloramphenicol has been the mainstay of treatment for typhoid fever until the 1990s when resistance to this drug emerged [7]. Fortunately the emergence of other first line drugs like trimethoprim, sulfamethaxazole, and Ampiciline and even Ciprofloxacin provided good alternatives for the multidrug resistant enteric fever pathogens [8].

Increasing resistance of *Salmonella* species to antibiotics in other parts of Nigeria have been reported by [9-13]. This study however showed an 85.9% susceptibility to Chloramphenicol in *S. typhi* and 86.4% susceptibility in *S. paratyphi*. The result of this study in Nasarawa state, Nigeria favourably agreed with what reported in India [2,3,14].

The outcome of this study could probably be as a result of the withdrawal of Chloramphenicol for treatment of typhoid fever which led to the withdrawal of selective pressure. Thus resistant bacteria no longer have the advantage of survival in such settings. The re-emergence of Chloramphenicol sensitive *Salmonella* species in Nasarawa state is a revelation that may suggests the necessity for continuous surveillance of *Salmonella* cases and re-evaluation of Chloramphenicol therapy in *Salmonella* infection in the state. It is possible that Chloramphenicol may re-emerge as the antibiotic of choice.

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