THE PREVALENCE OF DRUG RESISTANT *SALMONELLA TYPHI* IN HOSPITALIZED TYPHOID FEVER PATIENTS IN LAGOS METROPOLIS

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ABSTRACT

Investigation was carried out on the prevalence of *Salmonella typhi* in stools of hospitalized typhoid fever patients in Lagos metropolis. Studies were carried out between January and December 2003. Four hundred and thirty four patients were hospitalized and treated for typhoid fever in the ten hospitals monitored during the period of this study. The incidence of typhoid fever was slightly higher in wet season than the dry season (51.25% and 48.75% respectively). Male patients hospitalized were 58.12% as against 41.88% females. There was a negative correlation with age ($r=-0.96$; $p<0.01$). Stool samples from patients were analyzed bacteriologically *S. typhi* was isolated from 42.5% of the stool samples. The isolated *S. typhi* showed a varied degree of resistance (10-90%) to 15 common antibiotics, but 100% susceptibility to chloramphenicol and amoxicillin. Other organisms isolated from the stool samples included *Escherichia coli* (23.8%), *Salmonella* species (12.3%), *Shigella* species (29.0%), *Klebsiella* species (5.5%) and *Proteus* species (11.5%).

Key words: Drug resistance, *Salmonella typhi*, Typhoid fever

INTRODUCTION

Typhoid fever is an enteric fever caused by *Salmonella typhi* (Brooks *et al.* 1991; Ojo 1993). Clinical picture of enteric fever could at times be produced by *Salmonella paratyphi* A, *S. schottemuelleri* (formerly *paratyphi* B) and *S. hirschifeldii* (formerly *paratyphi* C), thus difficult to diagnose correctly on clinical manifestations. But the enteric fever produced by these other three *Salmonella* serotypes is termed paratyphoid (Ojo, 1993).

Typhoid fever is of serious health importance in developing countries with most of such countries being endemic (Onile and Odugbemi 1989; Crump *et al.*, 2003). The route of infection is mainly the faecal-oral route, that is, by ingestion of contaminated food and water (Brooks *et al.*, 1991; Ojo 1993; Moro *et al.*, 2000).

Clinical manifestations of typhoid fever include malaise, frontal headache and occasionally, diarrhoea (Ojo, 1993). Other symptoms include constipation, bradycarda and myalgia (Brooks *et al.*, 1991). Diarrhoea is usually due to heavy dose of *S.typhi* or co-infection with other organisms (Ojo, 1993). Diagnosis of typhoid fever is based on clinical history, serology and bacteriology (Ojo, 1993). In Nigeria, the diagnosis is often based on clinical manifestation and Widal test (serological assay for detection of antibodies to O and H antigens of *S. typhi*) which is not so reliable (Famurewa & Moro, 1989). According to Ojo (1993) counter current immunoelectrophoresis (CIE) using lipopolysaccharide (LPS) is more specific than the Widal test in the serological diagnosis of typhoid fever.

Recovery from typhoid fever is usually complete on treatment, except in carrier cases, which is about 3 percent. In untreated cases, there could be complication, such as intestinal haemorrhages and perforation. In severe untreated cases, toxic myocarditis occurs and it is a significant cause of death in Africa and India (Ojo, 1993). Pneumonia and suppurative complication of bone, spleen and ovary may occur.

Bacteria resistance to antimicrobial agents is on the increase world wide (Descenclos and Guillemot, 2004). Chloramphenicol is considered as the drug of choice in symptomatic Salmonella infections especially typhoid

fever. Resistance of *Salmonella typhi* to chloramphenicol is on the increase, because the organisms can produce the enzyme-chloramphenicol acetyl transferase, which has the ability to destroy chloramphenicol. Chloramphenicol acetyl transferase is plasmid control (Brooks *et al*., 1991). Gram – negative rods usually develop resistance to b- lactam antibiotics because of their ability to produce b-lactamases, which include chromosome or plasmid encoded enzymes (Livermore, 1995; Muniesa & Jofre 1998). The likely consequences of such increasing antibiotic resistance by Salmonella and other enterics are increased duration of symptoms of illness, increased rate of hospitalization and increased risk of death (Descenclos & Guillemot, 2004).

The study was aimed at investigating the antibiotic sensitivity of *S typhi* isolates from stools of patients hospitalized and receiving treatment for typhoid fever in some hospital in Lagos metropolis.

MATERIALS AND METHODS

Study Subjects

The subjects studied were patients hospitalized and undergoing treatments for typhoid fever in two government owned hospitals and eight private clinics within the Lagos metropolis. The investigation was conducted between January and December, 2003. Sex of patients were determined and ages were obtained by personal interview after obtained consent.

Bacteriological investigations

Fresh stool samples were collected from the hospitalized patients into sterile closed cups. A sterile cotton swab was immersed in each stool sample. All swabs were squeezed in sterile buffered peptone and incubated at 37°C for 18-24 hours. One hundred microlitre (0.1ml) of all broth samples were placed on dexychocolate citrate agar, Salmonella-Shigella agar, Eosine-methylene blue agar and Mac-Conkey agar. These agars were incubated at 37°C for 18-24 hours. Pure colonies were subculture on nutrient agar and Mac-conkey agar. The isolates were subjected to biochemical tests, characterized and identified according to Cowan(1993).

Antibiotic sensitively test of each isolated organisms were determined by using the disc diffusion techniques. The antibiotics used and their concentrations are as follows: Ampicillin (10mg), Nitrofurantoin (20mg), penicillin (10mg), Nalidixic acid (30mg), Amoxicilin (20mg) Tetracyline (25mg), Kanamycin (30mg), Gentamycin (10mg), Colistin sulphate (25mg), Augmentin (30mg) Cloramphenicol (mg) Rocephine (30mg), ofloxacin (10mg), Ciprofloxacin (10mg). *E.coli* (NCTC 10418) served as control and results obtained were classified as resistant or sensitive.

Statistics

Pearson correlation coefficient was used to determine the association of incidence of typhoid fever with age.

RESULTS

A total of 434 patients were hospitalized and treated for typhoid fever in the 10 hospitals /clinics, during the period of our investigations. Two hundred and fifty two (58.25%) of the patients were males and 182 (49.75%) were females. The age distribution of the patients is as illustrated in Figure 1. The mean age was 24.23 ± 14.71 (mean ±S.D) years. The incidence of typhoid fever was negatively correlated with age ($r=-0.96$; $P < 0.01$). There were 222 (51.15%) cases between May and October 2003 (during the wet season) as against 212 (48.75%) patients who were hospitalized in the dry season.

Salmonella typhi was isolated from 42.5% of stool samples analyzed bacteriologically. The *S. typhi* isolates were 100% susceptible to chloramphenicol and Amoxicillin. The isolates of *S. typhi*, however, showed a varied degree of resistance (10-90%) to some of the commonly used antibiotics (Table 1).

Apart from *S. typhi*, other enteric bacteria isolated from the stool samples included *Escherichia coli*, *Salmonella* species, *Shigella* species, *Klebsiella* species and *Proteus* species. The incidence of occurrence of these organisms are illustrated in Figure 2 All these bacteria showed multiple-resistant to a variety of common antibiotics (Table 1)

Table 1: Antibiotic resistance pattern of Salmonella typhi and other enteric isolated from hospitalized typhoid patients.

Drug	Degree of resistance					
	Salmonella typhi	*Salmonella* species	*Escherichia Coli*	*Shigella* species	*Klebsiella* species	*Proteus* species
Ampicillin	10	20	30	65	100	75
Nitrofurantoin	90	0	80	60	100	90
Penicillin	90	100	100	100	100	100
Amoxicillin	0	50	50	100	100	100
Tetracycline	80	50	50	100	100	15
Kanamycin	90	30	15	60	0	100
Gentamycin	10	0	0	0	0	20
Streptomycin	30	20	35	10	40	90
Colistin sulphate	90	100	0	0	100	100
Augmentin	10	0	0	0	0	0
Chloramp -henicol	0	0	15	100	100	0
Peflacin	20	10	20	30	45	90
Rocephine	60	20	15	30	0	90
Ciprofloxacin	10	0	0	0	10	5
Ofloxacin	10	0	10	0	20	10
Ceftacidine	15	0	0	10	15	20

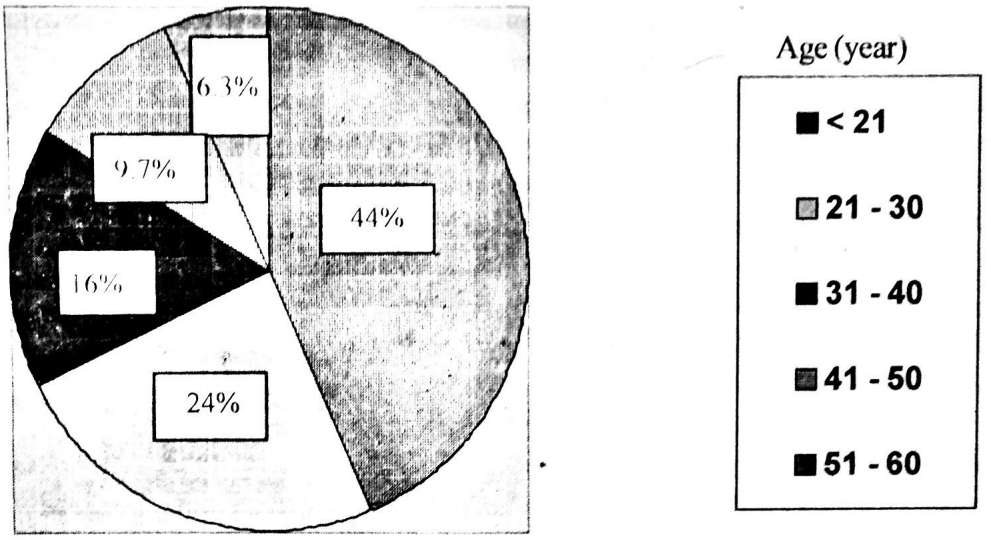

Figure 1: Age-distribution among hospittalized typhoid patients n = 434, r = -0.96, p<0.01

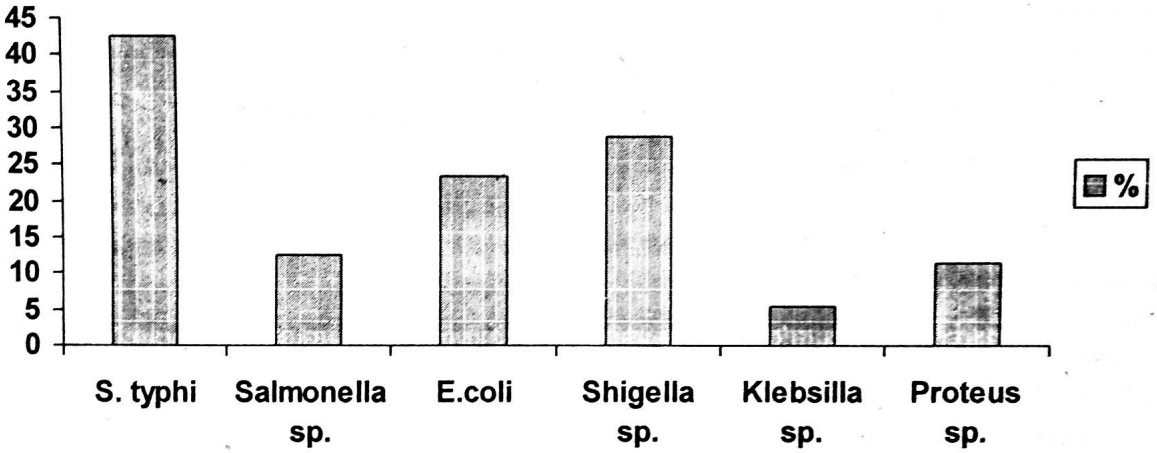

Figure 2: Incidence of enteric bacteria in stool of hospitalized

DISCUSSION

Development of resistance to antimicrobial agents and the emergence of multi-resistance pathogens have generated concern in the medical community. Infections caused by resistant bacteria are associated with higher rates of hospitalization, greater length of hospital stay and high rates of illness and death (Holmberg *et al.*, 1987).

The age distribution of typhoid patients observed in this study is consistent with earlier reports that typhoid fever is predominantly a disease of school age children and young adults (Famurewa & Moro 1989; Ojo, 1993). The lower incidence of typhoid fever among females than males reported in this study is similar to that reported by Crump *et al.* (2003) in Egypt with 26.3% incidence in females with typhoid fever. The higher incidence of typhoid fever among males may be due to the outdoor feeding habit of males as compared to females that carefully prepare their foods at home. Food vendors have been reported as carriers of *S. typhi* (as well as other enterics) and could play a significant role in the epidemiology of typhoid fever in any community (Moro *et al.*, 2000). The higher incidence of typhoid fever during the wet season observed in this study, may suggest a higher unhygienic practices resulting in higher contamination of food, and water, during the wet season, among people living in the ever congested Lagos metropolis.

Salmonella typhi was isolated from 42.5% of the stool samples analyzed bacteriologically in this study. The low percentage of *S. typhi* isolation may be attributed to the fact that the patients were at different stages of the illness and have been subjected to different levels of drug administration prior to stool sampling. This may have effect on the number and the sensitivity of isolated of *S. typhi*. Similarly the low percentage of *S. typhi* isolated from patients stools could be due to wrong diagnosis of typhoid fever by health workers as clinical symptoms of most bacterial pathogens overlap. Crump *et al* (2003) observed that 26 (87.1%) of 31 patients with brucellosis were diagnosed with and treated for typhoid fever. They demonstrated that brucellosis was as important as typhoid fever as a cause of prolonged fever in Bilbeis district of Egypt. In Nigeria, many cases of prolonged fever probably due to non-typhoidal Salmonella, other bacterial pathogens or chronic malaria, may often be wrongly diagnosed as and treated for typhoid fever.

Salmonella typhi isolates, from this study showed a varied degree of resistance to some common antibiotics but 100% susceptibility to chloramphenicol and amoxicillin (Table 2). Brooks *et al* (1991), however, reported isolates of *S.typhi* that were 5% resistant to chloramphenicol. The resistance of *S. typhi* to a variety of drugs observed in this study, may be attributed to the fact that the patients might have abused such drugs, as they were likely hospitalized only when the illness became very serious. A sentinel surveillance in Egypt by Crump *et al* (2003) revealed very high community use of antimicrobial agents among patients with typhoid fever and other febrile illness in Bilbeis district. Their findings were consistent with an epidemic of community based antibiotics abuse reported earlier by Okeke *et al* (1999).

Apart from *S.typhi* ; *E.coli*, *Salmonella* sp, *Shigella* sp, *Klebsiella* sp and *Proteus* sp were isolated from the stool samples of the patients (Table 1). When normal host defenses are inadequate, all these enterics can reach the blood stream producing sepsis. The enteric bacteria possess endotoxins –lipopolysaccharrides in their cell walls, which are potent producers of fever (Brooks *et al.*, 1991; Ojo 1993). All the isolated Gram-negative bacteria in this study are common isolates from previously reported cases of diarrheal patients (Famurewa & Moro 1989; Ojo 1993; Moro *et al.*, 2000). All the enteropathogens isolated were resistant to a variety of antibiotics (Table 2). The isolated organisms showed more, resistance to penicillin, tetracycline, Amoxicillin (with exception of *S.typhi*) and colistin sulphate, suggesting drugs that are likely to be commonly abused. There was more susceptibility to newer quinolenes (ofloxacin and ciprofloxacin than the old quinolene (nalidixic acid) which may be due to abuse of nalixidic acid. Among the ciphalosporins, ceftacidine is more potent than rocephine. Antibiotic resistance pattern varies from one study to another as resistance can be either chromosomally and plasmid mediated.

Antibiotic sensitivity test is important in the treatment of every case of febrile illness. The test result normally takes between 48 to 72 hours to be available. As labouratory test is being awaited, it is suggested that the patient should be placed on drugs (that are more potent on all the isolated organisms in this study) such as getamycin, augmentin, ciprofloxacin, ofloxacin and ceftacidine. According to Brooks *et al.* (1991) gentamycin when used along with penicillins produce better result. Penicillins may precipitate gentamycin in vitro (and thus must not be mixed), but in vivo they may facilitate the aminoglycoside entrace into streptococci and Gram –negative rods thus result in bactericidal synergism, beneficial in sepsis and endocarditis. The synergistic benefits of combining two antibiotics in the treatment of bacterial diseases should always be exploited. The high rate of antimicrobial resistance in this study suggest the possibility of genetic transfer of antimicrobial factors between these isolated gram-negative bacteria. Therefore, the presence of these drug resistant enteric bacteria could play a significant role in the epidemiology of multiple-drug resistant *S.typhi* infections.

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CONCURRENT INFECTIONS OF SOME PATHOGENIC BACTERIA AND PARASITES IN PATIENTS DIAGNOSED OF GASTROENTERITIS IN YABA, LAGOS, NIGERIA

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ABSTRACT

Bacteriological and parasitological analyses were carried out on 200 stool samples from patients diagnosed of gastroenteritis who were referred to the clinical diagnostic unit of Nigerian Institute of Medical Research from hospitals/clinics in Yaba, Lagos. The ages of the subjects ranged between 1 and 60 years and the age group with the highest frequency was the 21-30 years (40%). Bacteriological studies showed that 86% of the stool samples had six different bacterial genera. The isolates and their incidence were *Escherichia coli* (51%), *Pseudomonas* spp (10%), *Proteus* spp (9%), and *Klebsilla* spp, (8%) *Salmonella* spp (6%) and *Shigella* spp, (2%). Of the 174 that had parasites, 70 (40.2%) had *Ascaris lumbricoides*, 20(11.5%) had ova of *Trichuris trichiura*, 12(6.9%)had *Ancylostoma duodenale*, 4(2.3%) had *Taenia* spp, while 68 (39.1%) had trophozoites and / or cysts of *Entamoeba histolytica*. Thirteen percent of the stool samples had concurrent infection of *E. coli* and *Ascaris lumbricoides*, 11% had *E. coli* and *Entamoeba histolytica*, and 4% had *Salmonella* spp and *Ancylostoma duodenale* while only 2% had *Proteus* spp and *Ascaris lumbricoides*. Similarly, 5.75% of the 174 with intestinal parasites had mixed infection of *Ascaris lumbricoides* and *Trichuris trichiura*, 19% had both *Ascaris lumbricoides* and *Entamoeba histolytica* while others had one parasite each. Measures aimed at effective diagnosis and treatments of cases of gastroenteritis are recommended.

Keyword: Concurrent infections, Bacterial infections, Intestinal parasites, Dr. D.D. Moro, Corresponding author

INTRODUCTION

Bacterial and parasitic infections are common in the tropics (Moro et al., 2000). Several bacteria of the family; Enterobacteriaceae have been implicated in intestinal infections, but only four have been documented as enteric pathogens which include *Escherichia, Samonella, Shigella, and Yersinia* species (Ewing, 1986, Karmali, 1989, Farmer and Kelly, 1990). Despite reports that *Escherichia coli* is a member of healthy intestinal flora of both humans and animals, several workers have implicated *E. coli* as a causative agent of both intestinal and extra intestinal infections (Eisenstein, 1990, Abbot and Janda, 1992, Raj, 1993). Moro (2003) reported 25.4% incidence of *E. coli* in diarrhoeal patients while Olasupo *et al.*, (1999) reported a prevalence of 37% *E. coli* of all isolates and Iwalokun 2000 reported as high as 86.5% prevalence of diarrhoeal patients in Lagos.

Olasupo *et al* (1999) further reported that 23.9%, 12%, 3.3% and 1.1% of their bacterial isolates were *Samonella typhi, Shigella spp, Klebsiella spp and Proteus spp* respectively.

Moro *et al* (2000) reported that 11% of *S. typhi, 9% of E. coli, 6% of Proteus mirabilis and 2.6% of Shigella* spp in asymptomatic food handlers in Lagos.

Agbonlahor (1983), also reported an incidence 1.3% (14 out of 1082 bacteria studied were of *Yersinia* species. Other members of the Enterobacteriaceae especially in the genera *Halma, Citrobacter, Morganella, Proteus, Klebsiella, Enterobacter,* and *Serratia* have been reported as causes of intestinal infections in humans (Wadstrom *et al.*, 1976). Parasitic diseases are important causes of morbidity and mortality in many parts of the world and

are especially problematic when they occur in individuals weakened by malnutrition and other diseases (Fritsche and Smith, 1995). The occurrence of nematode infections such as ascariasis, trichuiriasis, and hookworm infections have decreased greatly while other parasitic diseases mostly protozoal infections like giardiasis, cryptosporadiasis have come too prominent in the United States. Imported cases of non-indigenous parasitic infections have also been reported in individuals who are often asymptomatic for months before diseases develop or relapses occur (Fritsche and Smith, 1995). Moro *et al.* (2000) also reported a high prevalence of intestinal worms with *Ascaris lumbricoides* constituting 75%, *Trichuris trichuira* 44%, *Schistosoma mansoni* 35% and 27% of *Fasciola hepatica*. A significant association between *Salmonella* and intestinal worms infections have been reported in Ado-Ekiti and in Lagos, Nigeria. (Famurewa and Moro, 1989, Moro *et al.*, 2000).

The objective of this study is therefore to isolate and characterize bacterial pathogens among patients diagnosed of gastroenteritis and the prevalence of concurrent bacterial and parasitic infections among the subjects.

MATERIALS AND METHODS

Study population

Patients diagnosed clinically of gastroenteritis and those who had not had any antibiotics or antihelmintic therapy in the preceding two weeks were enrolled into this study. Patients who were on a recent co wise of antibiotic and or antihelmintic therapy were excluded from the study. The patients were those referred to the Central Public laboratory and the Clinical Diagnostic Unit of Nigerian Institute of Medical Research from Hospitals and Clinic in Yaba area.

Sample collection

A total of 200 early mornings freshly voided stool samples were collected from the subjects into sterile universal bottles. The subjects were enlightened on the purpose of the study .The stool samples were taken to the laboratory promptly for bacteriological and parasitological analyses.

Bacteriological studies

A small portion of each stool sample was inoculated into buffered peptone water (BPW) and incubated at 37°C for 18 hours .The stool samples were then sub cultured onto MacConkey agar, Eosin methylene blue (EMB) and *Salmonella-Shigella* agar (SSA). All bacterial isolates were identified based on their morphological, cultural and biochemical characteristics (Cheesbrough, 1993; Cowan, 1993).

Parasitological studies

Three different methods were used to study the intestinal parasites.

(a) Direct saline smear: A small portion of each stool sample was emulsified in a drop of normal saline and a cover slip applied and examined under the microscope with X10 and X40 objectives.

(b) Iodine preparation: A portion of each stool specimens was emulsified in two drops of iodine solution and a cover slip applied .It was then examined under the microscope with X10 and X40 objectives to identify and differentiate cyst of *Entamoeba* species.

(c) Concentration method: The method of Washington (1981) was used .A small portion of each stool sample was put into a tube and emulsified thoroughly in 8ml of 10% normal-saline .The emulsion was strained through wire sieve of 40 meshes to the itch into a centrifuge tube. About 3ml of ether was added to the tube and shaken thoroughly. The tube was centrifuged at 2000rpm for 2 minutes. The layer of the debris between the ether layer and faecal emulsion was loosened gently with a swab stick and all the supernant fluid and the debris was poured into a disinfectant jar. The inner side of the tube was wiped with swab. The drop of fluid and the remaining deposit in the tube was shaken up and poured into a glass slide. A cover slip was applied and examined under the