

BACTERIA ISOLATED FROM BLOOD, STOOL AND URINE OF TYPHOID PATIENTS IN A DEVELOPING COUNTRY

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Abstract. Blood, stool and urine samples were collected from 100 patients diagnosed as having typhoid in 5 hospitals in Akwa Ibom State and analyzed for the presence of *Salmonella* species and other bacteria. Of the 100 blood samples screened, 55 (55%) were positive with the Widal test and 39 (39%) were positive on blood culture. Thirteen (14.1%) out of 92 urine samples were positive for bacterial growth, while 22 (26.8%) of the stool cultures were positive out of the 82 samples screened. Those within the age range 11-20 years old were infected most frequently (33%), followed by the age range 21-30 (19%) and 41-50 (18%) years old. Those in the age range of 0-2 years old (4%) were least infected. Female subjects were more infected than males. The commonest organisms isolated from the blood samples were *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Streptococcus faecalis*, *Salmonella paratyphi* and *Salmonella typhi*. *S. aureus*, *S. epidermidis*, *E. coli*, *K. aerogenes*, *S. faecalis*, *Proteus mirabilis* and *P. aeruginosa* were isolated from urine, while those isolated from stool were *S. aureus*, *E. coli*, *S. typhi*, *S. paratyphi*, *Shigella* sp., *K. pneumoniae*, *P. vulgaris*, *P. aeruginosa* and *Vibrio cholerae* 01. The isolates were sensitive to peflacin, ceftazidime, ciprofloxacin, ceftriaxone, cefotaxime and chloramphenicol. These antibiotics are recommended as the drugs of choice in therapy. The results suggest the existence of symptomless carriers of enteric fever bacilli in the state. This is worrisome, since some of the *S. typhi* isolates exhibited multiple resistance to commonly used antibiotics.

INTRODUCTION

Typhoid (enteric) fever is a bacterial infection that may be transmitted through food, water, milk or shellfish that has been contaminated by *Salmonella typhi* or *S. paratyphi*. It is caused by several virulent serovars of *Salmonella typhi* which may be acquired by ingestion of food or water contaminated by feces from typhoid patients or carriers. The etiologic agents of paratyphoid fever are *Salmonella paratyphi* A, B and C (Inabo *et al*, 2000; Prescott *et al*, 2002).

Typhoid, and its complications, continue to pose major clinical problems, especially to developing countries of the world (Archampong, 1969). The disease is associated with socio-medical problems posed by poor standards of hygiene, particularly in overcrowded urban environments, according to Ogunbiyi and Onabowale (1997).

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The antibiogram of bacteria associated with the blood, stool and urine of enteric fever patients, and the prevalence of the disease in the Akwa Ibom State of Nigeria is reported here.

MATERIALS AND METHODS

Sources and sample collection

The blood, stool and urine of patients given the diagnosis of typhoid were collected from 5 hospitals in Akwa Ibom State, namely: St Luke's General Hospital, Anua; University of Uyo Teaching Hospital, General Hospital Iquita Oron; General Hospital, Ikot Ekpene; and Emmanuel Hospital, Eket. Venous blood samples were aseptically collected into blood culture bottles using sterile needles and syringes. Ten mls of venous blood were collected from adult patients into 50 mls tryptone soy broth (Oxoid, England) in blood culture bottles, while 5 mls were taken in the case of children. The processed samples were incubated at 37°C overnight after which they were subcultured onto McConkey agar (Oxoid, England), blood agar (Oxoid, England) and Salmo-

nella-Shigella agar and incubated at 37°C for 24-48 hours.

Patients were given commercial urine bottles (Oxoid, England) containing boric acid and instructed to collect fresh, clean midstream urine samples of about 10-20 ml, preferably the first urine excreted by the patient early in the morning. The urine samples were immediately inoculated onto McConkey agar, Salmonella-Shigella agar (including a subculture of Selenite-F broth) and Cystein-Lactose-Electrolyte Deficient (CLED) medium on arrival at the laboratory, then the plates were incubated at 37°C for 24-48 hours.

Stool samples were collected in clean bottles and processed for microbiological analysis as soon as they arrived at the laboratory. A loopful of each stool sample was inoculated onto Salmonella-Shigella agar (including a subculture of Selenite-F broth), McConkey agar (including a subculture from nutrient broth) and TCBS Cholera Medium (including a subculture of alkaline peptone water). The cultures were incubated at 37°C for 24-48 hours.

Widal test

This was carried out as described previously (Itah and Akpan, 2004). Briefly, sera for the screening test were collected from fresh blood samples by centrifugation. Eight drops of each serum sample were carefully transferred onto eight rings on a white tile using a Pasteur's pipette. The *Salmonella* antigen reagent was also dropped into the rings. Both were thoroughly mixed with the help of an applicator stick and the tile gently swirled for one minute for observable agglutination. Reacting antigens were classified as positive (+) while non-reactive antigens were classified as negative (-). Reactive titers of 1:80 and above were classified as positive (+), while titers less than 1:80 were classified as negative (-). All sera with negative slide test were confirmed by a tube test.

Identification of isolates

Following incubation, at least 10 representative growth colonies from each culture plate were subcultured on appropriate media by streak plating technique. Purified colonies were characterized and identified using standard micro-

biological and biochemical schemes (Cowan 1985; Holt *et al*, 1994). The tests included Gram's reaction, nitrate reduction, catalase, oxidase, coagulase, gelatin, starch hydrolysis, sulfite reduction, urease test and sugar fermentation profile. Some isolates needed further confirmation. For instance, *Salmonella typhi* were further confirmed on Bismuth sulfite agar (form black colonies with metallic sheen), *S. aureus* on Staphylococcus Medium 110, *E. coli* on EMB-agar followed by IMViC and Eijkman's test at an elevated temperature of 44±0.5°C for 24-48 hours. *Pseudomonas* species were confirmed on cefrimide agar.

Antibiotics susceptibility test

The agar disc diffusion technique was adopted (Cruickshank *et al*, 1975; Itah and Opara 1994; Itah, 1999b) using iso-sensitest agar (Oxoid, England), commercial antibiotics discs (Jireh Laboratories, Nigeria) and a loopful of 6-8 hours old tryptone soy broth culture of test organisms. The antibiotics and their concentrations were chloramphenicol (25 µg), ceftriaxone (30 µg), nitrofurantoin (20 µg), cefotaxime (30 µg), cefuroxime (30 µg), tetracycline (25 µg), gentamicin (10 µg), ceftazidime (30 µg), cotrimoxazole (25 µg), amoxicillin (10 µg), ciprofloxacin (10 µg), rifampicin (10 µg), penicillin (1 unit), and peflaxine (30 µg). Sensitivity plates were examined after 24 hours incubation at 37°C for zones of growth inhibition. The test organisms were classified as sensitive or resistant to the antibiotics in each disc based on the presence or absence of clear inhibition zones. The results were interpreted as moderately sensitive (+); sensitive (++) , very sensitive (+++) and resistant (R).

RESULTS

Incidence rate in relation to the three samples

Out of the 100 blood samples investigated, 55 (55%) were positive with the Widal test and 39 (39%) were positive on blood culture. Thirteen (14.1%) out of 97 urine samples yielded growth of bacteria, while 22 (26.8%) of the stool cultures were positive out of the 82 samples studied.

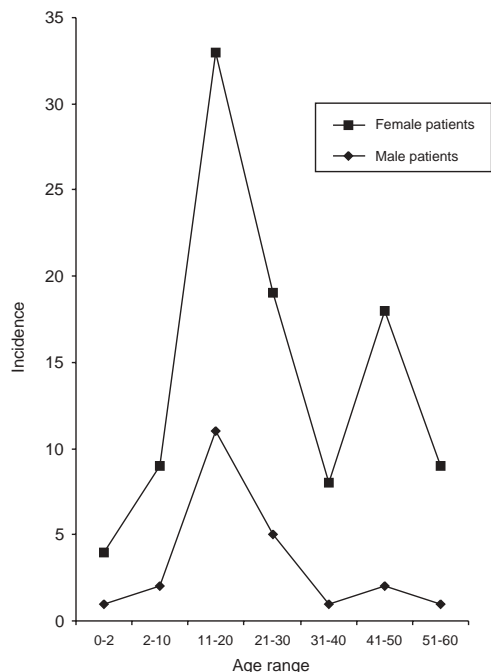


Fig 1–Distribution of typhoid infection according to ages and sexes of patients.

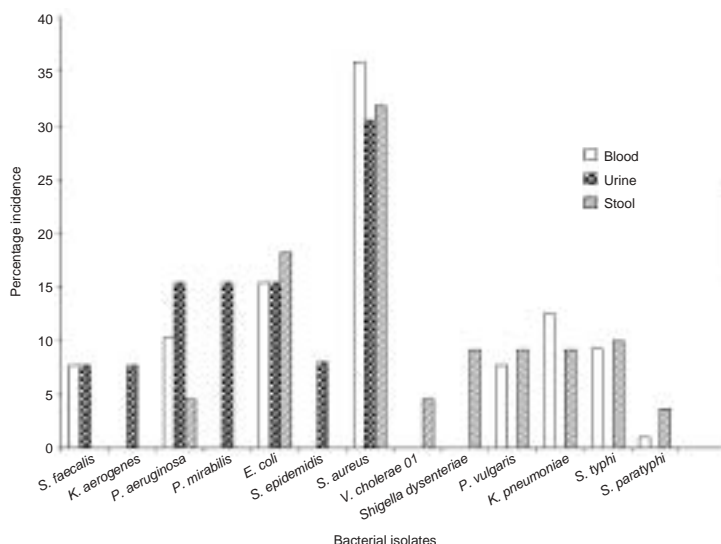


Fig 2–Sources and percentage incidence of isolates from typhoid patients.

Typhoid infections in relation to sex and age group

Those within the age range of 11-20 years were the most infected (33%), out of which 22% were females and 11% were males. This was followed by the age range 21-30 years (19%),

out of which 14% were females and 5% were males. The age groups of 2-10 and 51-60 years had 9% infection rates in each case, while subjects within the age range of 0-2 years were the least infected (4%) with three females and one male (Fig 1).

Isolates identified

The organisms identified in the blood were *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus faecalis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Salmonella typhi*. *S. aureus*, *E. coli*, *S. faecalis*, *Klebsiella aerogenes*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* were isolated from the urine. *S. aureus*, *E. coli*, *S. typhi*, *S. paratyphi*, *Shigella* sp, *K. pneumoniae*, *P. vulgaris* and *Vibrio cholerae* 01 were isolated from the stool samples. The distribution of the isolates and their sources are presented in Fig 2. The most frequently occurring organism in blood samples was *S. aureus* (35.9%) followed by *E. coli* (15.4%), while *S. paratyphi* was the least. In the urine and stool cultures, *S. aureus* and *E. coli* maintained their former positions as the highest, 30.5% and 31.8% and second highest, 15.4% and 18.2%, respectively, in their percentages of occurrence. All the organisms were not, however, encountered in all the samples.

Antibiogram of isolates

The *S. aureus* isolates were sensitive to all the antibiotics tested (92.9%) except nitrofurantoin (7.1%) while *E. coli* isolates were sensitive to all the drugs (78.6%) except cotrimoxazole, cefuroxime and penicillin, with a percentage resistance of 21.4. *Klebsiella pneumoniae* exhibited 28.5% resistance, particularly to nitrofurantoin, cotrimoxazole, cefuroxime, and penicillin (28.6%). The resistance patterns of other isolates were: cefotaxime, cotrimoxazole, and penicillin for *S. typhi*; ceftriaxone, nitrofurantoin, cotrimoxazole, ciprofloxacin, and penicillin for *P. vulgaris*; ceftriaxone, cotrimoxazole, and

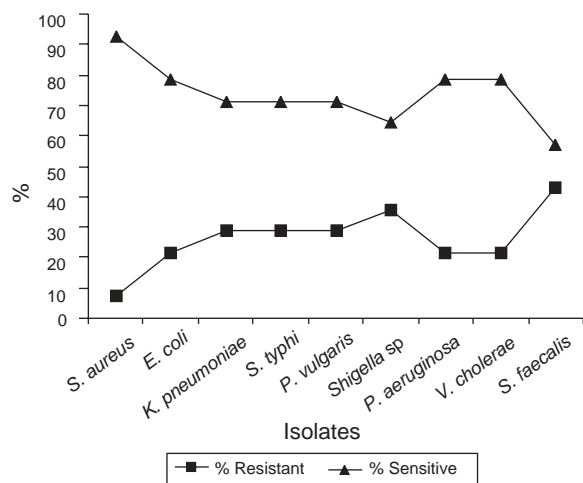


Fig 3—Antibiogram of named isolates to drugs tested.

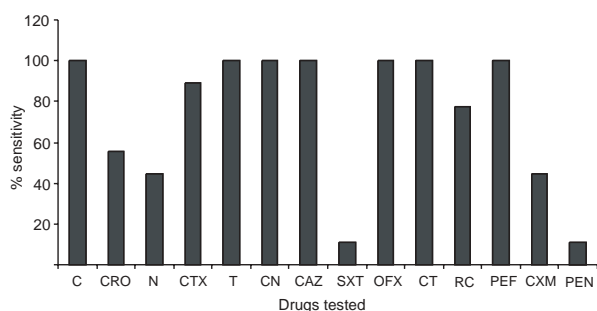


Fig 4—Percentage of total isolates sensitivity to tested drugs.

C = chloramphenicol; CRO = cefotaxime; N = nitrofurantoin; CTX = ceftriaxone; T = tetracycline; CN = gentamicin; CAZ = ceftazidime; SXT = cotrimoxazole; OFX = ciprofloxacin; CT = amoxicillin; RC = rifampicin; PEF = peflacin; CXM = cefuroxime; PEN = penicillin

penicillin for *Shigella* species; ceftriaxone, cotrimoxazole, and penicillin for *P. aeruginosa*; nitrofurantoin, cotrimoxazole, and penicillin for *V. cholerae*; and ceftriaxone, nitrofurantoin, cotrimoxazole, ciprofloxacin, cefuroxime, and penicillin for *S. faecalis*. *Streptococcus faecalis* exhibited 42.9% resistance to drugs, while *S. aureus* exhibited the lowest resistance to all the drugs tested, 7.1% (Fig 3). An overview of the antibiogram reveals 100% susceptibility of all the isolates to chloramphenicol, tetracycline, gentamicin, ceftazidime, amoxicillin, ciprofloxacin,

and peflacin but only 12% were sensitive to cotrimoxazole and penicillin (Fig 4).

DISCUSSION

Typhoid fever, a major cause of morbidity and mortality in developed and developing countries of the world is indeed greatly under-reported in the tropics. This may be due to indiscriminate use of antibiotics, inadequate laboratory and research facilities (Black *et al*, 1982; Ogunbiyi and Onabowale, 1997).

Our results reveal the presence of bacteria in 39 (39%) blood samples, 13 (14.1%) of 92 urine samples, and 22 (26.8%) of 82 stool samples from patients with clinical symptoms of enteric fever. This is at variance with an earlier report by Ogunbiyi and Onabowale (1997) who observed a higher incidence: 49 (47.1%) blood and 33 (31.7%) stool samples after examining 104 samples of each. They reported a low incidence of bacterial infection (1.9%) in the urine compared to 22 (26.8%) samples encountered in our investigation. The isolation of *S. typhi* and *S. paratyphi* in some cultures that yielded negative Widal results in patients clearly exhibiting clinical symptoms of enteric fever underscores the fact that the Widal test alone is unreliable as a tool in the diagnosis of typhoid fever and should be backed up with culture samples. These views have been shared by Egah and Sule (2001).

We found that 10.3% of the blood cultures yielded *S. typhi* on Bismuth sulfite agar as black colonies with a metallic sheen due to sulfite reduction. This is at variance with earlier report by Alausa and Onile (1984) who encountered enteric fever bacilli in 43.7% of the blood cultures studied. This may suggest that community acquired infections are rare in Akwa Ibom State and that more patients have access to antibiotics for self medication. The practice by some medical doctors and para-medical staff who presume any fever as typhoid fever may support this assertion. Our findings show that typhoid fever is more prevalent among females than males. This may be attributed to the fact that females are closer to edible items than their male counterparts. There may also be a sex-linked factor responsible for the higher incidence of

enteric fever in females than in males of all age ranges in our study. Ogunbiyi and Onabowale (1997) reported the incidence was higher in females in some age ranges than in males. In our study, a higher incidence rate were observed with respect to sex in the age group 11-20 followed by 21-30, 41-50, 2-10, 51-61, 31-40, and finally age less than 2 years.

Bacteria isolated from all the samples exhibited multiple antibiotic resistance to common antibiotics used in therapy. This suggests they all possess resistant factors. Multiple antibiotics resistance amongst members of the enterobacteriaceae and *Staphylococcus aureus* has been well documented (Cruickshank *et al*, 1975; Itah and Opara, 1994, Itah, 1997, 1999a,b). The isolates were resistant to antibiotics in varying degrees, hence there is the need to intensify the public health campaign against drug abuse if the present generation of antibiotics is to remain efficacious against targeted bacteria. Chloramphenicol, tetracycline, gentamicin, ceftazidime, ceftriaxone and peflacin are recommended for use in therapy, as all the isolates exhibited 100% sensitivity to these drugs.

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