

Full Length Research Paper

Pattern of multi-drug resistant *Salmonella enterica* serovar typhi isolates in Nigeria

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Typhoid fever continues to remain a health problem as the causative organism, *Salmonella enterica* serovar typhi, has developed resistance to many antibiotics used. This study was undertaken to determine the current pattern of resistance to antimicrobial agents by *S. enterica* serovar typhi isolates from Akaraugo Hospital in Owerri, Imo state. Sensitivity to most common antimicrobial agents used in the management of typhoid fever was determined by disc diffusion according to National Committee for Clinical Laboratory (NCCL) standards. There was an increase in the number of isolates with decreased sensitivity to nalixidic acid and cotrimoxazole. All isolates had complete resistance to augmentin, tetracycline and amoxicillin commonly used for treatment of typhoid fever. Therefore physicians should be advised to conduct effective susceptibility test before prescribing an antibiotics to a patient. It is also important to step up awareness to individuals about indiscriminate use of antibiotics and the need to complete a regime once it is started.

Key word: Typhoid fever, antibiotic susceptibility, *Salmonella enterica* serovartyphi.

INTRODUCTION

Typhoid fever continues to be a major health problem causing approximately 16.6 million cases that result in 600,000 deaths each year, mostly in developing countries (Pang et al., 1998; Yeihun et al., 2007), despite the use of antibiotics and the development of newer antibacterial drugs like ofloxacin and nitrofurantoin. Typhoid fever is among the water borne infections characteristic of environment with poor sanitations and hygiene (Singh and Mcfeters, 1992). The causative agent *Salmonella enterica* serovar typhi is pathogenic both to man and animals with associable inflammatory reaction in the intestinal tract. Like other enteric pathogens, *S. enterica* serovar typhi is transmitted through food or water that has been contaminated with faeces from acutely infected person's persistent excretors (that is constant stooling or diarrhoea) or from chronic asymptomatic carriers (Rajiv et

al., 2007). *Salmonellae* are typically members of the enterobacteriaceae. The enterobacteriaceae are a large group of facultative anaerobic gram negative, catalase negative rods (Jawetz and Adelbergs, 1995).

Typhoid fever is an acute infection beginning in the lymphoid tissue of the small intestine but usually developing as a generalised infection of the body, particularly the lymphatic system. Intestinal perforation has been a major surgical complication of typhoid fever besides its severe symptoms of diarrhoea, vomiting, dehydration and convulsion in young children, the onset of abdominal pain and loss of appetite in adult (Khosta et al., 1977; Carol et al., 1989). The prevalence of typhoid fever has caused major economic and health impacts. As such, vaccines have been developed against strains of *Salmonella* (Myron et al., 1976). Based on the immunology of *Salmonella* antigens as a tentative evidence typhoid fever, a serological means of diagnosis have been developed; notably, the widal agglutination tests (Widal, 1996). However, with the knowledge of some draw backs in the use of vaccines to control typhoid fever, antibiotics thera-

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py remains a viable option in combating the infection. Some of the antibiotics used for typhoid fever treatment are chloramphenicol, ampicillin and other B-lactams, sulphadimidine, gentamicin and other aminoglycosides, nalidixic acid, ciprofloxacin and the fluoroquinolones (norfloxacin and ofloxacin) (Girgis et al., 1999; Kadhiraavan et al., 2005). In the early 1990's, multidrug resistant (MDR) strain of *S. enterica* serovar typhi, that were resistant to the first three line drugs (chloramphenicol, amoxicillin and cotrimoxazole), then in use emerged and become endemic in developing countries. The spread of MDR to the conventionally used antibiotics for treatment of typhoid fever has caused therapeutic and public health problem in the African continent, South East Asia and Middle East since 1987 (Samuel et al., 2000; Well, 2003).

The reliability of serologic test in solely diagnosing typhoid fever has suffered doubt. It has been reported to remain positive months after an effective therapy of the infection such that a positive test may not necessarily indicate active infection, making the test be of relevance in diagnosing post-infection complications. Also, the quality of *Salmonella* antigens and interpretations of results, specifically in the widal agglutination test have been identified as areas of controversy (Olopoenia and King, 2000), hence the suitability of stool culture in diagnosing active infection.

In Nigeria, the harsh economic climate has encouraged a cancerous rate of household production of various food products with the attendant risk to public health. As a matter of fact, the unreported cases of waterborne infections, particularly typhoid fever has been more than those reported to hospitals for treatment. This study was aimed at investigating the current pattern of resistance to antimicrobial agents of *S. enterica* serovar typhi isolates among patients attending Akaraugo Hospital in Owerri.

MATERIALS AND METHODS

Sample collection

Early morning stool samples from 150 adult patients with clinical manifestation of typhoid fever, over a period of two (2) months, were collected into wide-mouthed sterile plastic containers with screw cap for bacterial culture to detect *S. enterica* serovar typhi. The point of sample collection was Akaraugo Hospital, Ikenegbu, Owerri, Imo state. All the samples were packed well on ice pack and taken to the Nigeria Institute of Medical Research (NIMR), Yaba, Lagos state for isolation, antimicrobial susceptibility test and plasmid DNA isolation.

Isolation

A loop full of each stool sample was homogenized in normal saline and then inoculated into selenite-f broth at room temperature and incubated at 37°C for 24 h. The resultant enriched culture was then sub-cultured onto salmonella/shigella agar (SSA) plate and incubated

at 37°C for 24 h. The resultant culture was sub-cultured into MacConkey's agar plate and incubated similarly manner. Thereafter, the isolated non-lactose fermenter colonies were screened through biochemical tests (Cowan and Steel, 1993) for *S. enterica* serovar typhi.

Antibiotic sensitive test

Disk-diffusion technique was employed in determining the sensitivity of pure culture of *S. enterica* serovar typhi. Muller-Hinton agar medium is the antimicrobial test medium used, as has been validated by National Committee for Clinical Laboratory (NCCL) standard using *Escherichia coli* (ATCC 25922). The 0.5 Macfarland turbidity standards were used to adjust the turbidity of the inocula and antimicrobial disc was applied to the plate 15 min after inoculation. All isolates were subjected to antimicrobial susceptibility testing using 8 different antibiotics. The antibiotic discs used were as follows: Cotrimoxazole (25 g), Nitrofurantoin (30 g), Gentamicin (10 g), Nalidixic acid (30 g), Ofloxacin (30 g), Augmentin (30 g), Tetracycline (30 g) and Amoxicillin (25 g).

Plasmid DNA isolation

The plasmid isolation was done according to the method described by Birboin and Doly (1979).

RESULTS AND DISCUSSION

A total of 150 samples were tested and out of these, 84 (56%) were suspected to be *Salmonella* species, while 27 (32.14%) were identified and confirmed as *S. enterica* serovar typhi (Cowan and Steel, 1993). All the *S. enterica* serovar typhi isolated recorded resistance to two (2) or more of all the antibiotics tested particularly the three most commonly used antibiotics (augmentin, tetracycline and amoxicillin) in the treatment of typhoid fever in this part of Nigeria. However, ofloxacin (88.9%) and nitrofurantoin (88.9%) showed appreciable inhibition to most of the isolates giving a clear edge above other antibiotics. Remarkably augmentin, tetracycline and amoxicillin recorded no activity against all the isolates (Table 1). Three of the isolates recorded resistance to all the antibiotics tested, therefore all the isolates were subjected to plasmid DNA isolation. Remarkably only three of the isolates, resistant to all the antibiotics, harboured plasmid DNA (figure not shown).

This study revealed the high prevalence of multi-drug resistance among *S. enterica* serovar typhi isolates from Owerri, Imo State, Nigeria. The injudicious administration and rampant use of antibiotics in Nigeria probably contributed to the reduced susceptibility and the emergence of very high level or complete resistance of isolates of *S. enterica* serovar typhi to many antibiotics especially the first line drugs. The rapid spread of MDR

Table 1. Antibiogram of *S. enterica* serovar typhi isolates in Owerri.

Antibiotic	Number of isolates sensitive	Number of isolates resistant	Percentage
Cotrimoxazole	15	12	55.5%
Nitrofuratoin	24	3	88.9%
Gentamicin	12	15	44.4%
Nalixidic acid	12	9	66.7%
Ofloxacin	24	3	88.9%
Augmentin	*	27	—
Tetracycline	*	27	—
Amoxicillin	*	27	—

*No zones of inhibition observed.

observed in this town is of epidemiological concern as reported by Samuel et al. (2000) and Well (2003), and this would restrict the choices of antibiotics in the treatment of typhoid fever to a few compounds. The emergence of MDR *S. enterica* serovar typhi as discovered in this study is closely related to the irrational/ indiscriminate use of antibiotics in treating human infections or in cases where they are likely to be of little or no therapeutic value. It could be due to the presence of plasmid DNA with the gene for resistance which according to Pelczar et al. (1993), could be as a result of transmission of antibiotic resistance through conjugation from other organisms like *E. coli* resident in a patient's intestinal tracts.

However, antimicrobial agents may be life saving for malnourished and other immuno-compromised patients who have typhoid fever. In developing countries, the identification of risk factors and relevant route of transmission for disease such as typhoid fever is essential for the development of rational control strategies. Therefore special considerations are needed to encourage patients to procure and consume a complete regime of antibiotics. Proper sanitation, public health education and vaccination are long-term preventive measures that would improve this situation.

In conclusion, the findings of the present study indicated that Augmentin, Tetracycline and Amoxicillin may not have a role in the treatment of typhoid fever in this part of Nigeria. A simple disk diffusion test can rapidly provide information that can predict treatment failures.

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REFERENCES

- Birboin HC, Doly N (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Research* 7: 15-18.
- Carol A, Joseph OA, Palmer RS (1989). Out break of Salmonella infection in hospitals in England and Wales. *Br. Med. J.* 289: 1161-1164.
- Cowman ST, Steel S (1993). Manual for the identification of medical bacteria. 3rd edition, Cambridge university press, pp. 140-144.
- Girgis NI, Buttler T, French RW, Suitan Y, Brown FM (1999). Azithromycin versus ciprofloxacin for treatment of uncomplicated typhoid fever in a randomized trial in Egypt that included patients with multi-drug resistance. *Antimicrob. Agents. Chemother.* 43(6): 14441-14444.
- Jawetz M, Adelberg S (1995). Medical microbiology, 20th edition. Prentice Hall International Inc. pp. 214-217.
- Kadhiravan T, Wig N, Kapil A, Kabra SK, Renuka K, Misra A (2005). Clinical outcomes in typhoid fever: adverse impact of infection with nalixidic acid – resistant Salmonella typhi. 5(37): 1471-1481.
- Khosta SN, Sniraslava SC, Gupta S (1977). Nero-psychiatric manifestation of typhoid fever. *J. Trop. Med. Hyg.* 8: 895-898.
- Myron M, Herbert LD, Richard BH (1976). Attenuated streptomycin dependent *Salmonella typhi* oral vaccine. *J. Infect. Dis.* 133: 4.
- Olopoenia LA, King AL (2000). Widal agglutination test – 100 years later: still plagued by controversy. *Postgrad. Med. J.* 76: 80-84.
- Pelczar MJ, Chan ECS, Krieg NR (1993). Antibiotics and other Chemotherapeutic Agents In: *Microbiology Concepts and Applications.* McGraw – Hill, Inc. New York, pp. 556-588.
- Pang T, Levine MM, Ivanoff B, Wang J, Finlay BB (1998). Typhoid fever: important issues still remain. *Trends Microbial* 6: 131-133.
- Rajiv K, Nomeeta G, Shalin S (2007). Multi-drug resistant typhoid fever. *Ind. J. Ped.* 74(1): 39-42.
- Samuel SC, Gutura R, Anthony CH (2000). Genotypic analysis of multi-drug resistant Salmonella enterica serovar typhi, in Kenya. *Emerg. Infect. Dis.* 6: 641-651.
- Singh A, Mcfeters G (1992). Detection methods of water borne pathogens, in: Mitchell R (Ed) *Environmental Microbiology*, Wiley-liss, New York. pp. 125-189.
- Well J (2003). Manuel for laboratory identification and antimicrobial susceptibility testing of bacteria and pathogens of public health impor-

tance in the developing world. WHO, pp. 103-119.

Widal F (1996). Serodiagnostic de la fièvre typhoïde. Bull. Mem. Mem. Hop. Paris. 14: 561-566.