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Antibiotic Susceptibility Pattern of Clinical Salmonella Isolates from Patients Attending Hospitals in Owerri Metropolis Nigeria.

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ABSTRACT

The wide spread of antibiotic-resistant Salmonella has been a serious global human and animal health problem. This study investigated the antibiotic susceptibility pattern of Salmonella isolated from stool specimens of patients attending five major hospitals in Owerri, South East Nigeria. A total of 144 non repetitive stool specimens were collected from the patients; cultured in MacConkey Agar and incubated overnight at 37 °C. Salmonella isolates were identified by adopting standard microbiological procedure while antibiotic susceptibility testing was done using Kirby-Bauer method. Sixty-seven (46.5%) specimens out of the total 144 were positive for Salmonella growth. The difference in the rate of isolation of Salmonella from the five hospitals was not statistically significant (P<0.05). Results of the susceptibility test showed that the isolates were highly resistant to erythromycin, chloramphenicol and ciprofloxacin while they were considerably susceptible to nitrofurantoin and gentamycin. The high antibiotic resistance observed in this study may be as a result of overdependence or uncontrolled use of the few available antibiotics and/or inaccurate or inconclusive diagnosis resulting in the development and spread of resistant strains of Salmonella. This study, therefore, highlights the need for a strong collaboration between the physicians and the laboratory in the choice of antibiotics for the treatment of bacterial diseases in order to discourage the development of resistant strain of bacterial pathogen.

***Key word**: Salmonella, Antibiotic Resistance, Nigeria.*

INTRODUCTION

Resistance to antibiotics is mainly driven by the selective pressure imposed by their inappropriate use. Especially in developing countries like Nigeria, people do not have the minimal awareness of resistance, antibiotics and infections. They want symptomatic relief to which the health professionals respond by prescribing antibiotics for quick recovery. Infections with drug-resistant microorganisms are associated with severity of the patient's illness, increased patient contact with healthcare personnel and length of stay in the hospital. Eventually it causes extra cost of health care, extended stay in the hospital, sudden or prolonged health complications including significant excess morbidity and mortality. Sometimes it reaches an extreme level through the cross-infection of hospitalized patients with such drug-resistant organism.

Typhoid fever (enteric fever) caused by the bacterium Salmonella enteric serovar typhi is an endemic disease in the tropics and subtropics. The disease is systemic and is often contracted by ingestion of food or water that is contaminated with the pathogen usually from a fecal-oral source. It may, therefore, be reasonable to conclude that the occurrence of typhoid fever is an indicator of poor personal and environmental hygiene. The illness may be mild or severe but sometimes fatal. It is encountered worldwide but is primarily found in developing countries where sanitary conditions are poor (WHO 2008). Typhoid fever is now uncommon in developed countries where most occurrences are either acquired abroad or imported by emigrants (Anderson and Smith 2002). With an estimated annual incidence of 540 per 100,000 or about 17 million cases worldwide (Neopane *et al.,* 2008), the disease is considered a major public health problem. In tropical countries including Nigeria where the disease is often encountered, they account for several cases of morbidities and mortalities (Ibekwe *et al.,* 2008).
Enteric fever has continued to be a major health problem despite the use of antibiotics and the development of newer antibacterial drugs. The causative organism Salmonella typhi has rapidly gained resistance to antibiotics like ampicillin, ceftriaxone, and cotrimaoxazole, and also to previously efficacious drugs like ciprofloxacin (Butt *et al.,* 2003). The emergence of antimicrobial resistance, especially the multidrug resistance to ampicillin, chloramphenicol, and cotrimaoxazole, has further complicated the treatment and management of enteric fever (Jesudason and John 1992). The resistance to well-known and trusted antimicrobial agents is widely recognized as one of the greatest challenges that physicians face in the management of adult and pediatric infections (Dajani , 2002).

Salmonella spp. particularly the multidrug resistant (MDR) strain is relatively ubiquitous and is the cause of many community endemic and epidemic typhoid fever infections. MDR strain of *Salmonella* is of concern not only because of its resistance to available antibiotics resulting in high death rate but also because of its potential for epidemic outbreaks, which may be difficult to manage. The consequence of such outbreak will no doubt be devastating especially in developing countries where health facilities are often inadequate.

It has been opined that the initial development of resistance by *Salmonella* and most other bacterial pathogens occurred as a result of indiscriminate use of antibacterial drugs hence the need for caution in antibiotic prescription and administration. Determining the antibiotic susceptibility pattern of isolates is necessary since it will guide the physicians in making the right choice of drugs when treating patients thus ensuring quick treatment of the infection without aggravating the illness and preventing antibiotic resistance. The present study was

undertaken to investigate the prevalence and antibiotic susceptibility of *Salmonella* isolates obtained from patients attending the five major hospitals in Owerri metropolis, Southeast Nigeria.

MATERIALS AND METHOD

Specimen Collection, Cultivation and Identification of *Salmonella*.

Stool specimens were collected in a wide mouth sterile container and immediately sent to the lab for microbiological analysis, one specimen was collected from each patient. The fecal specimens were streaked directly on MacConkey agar prepared according to the manufacturers directives, incubated overnight at 37 °C. The *salmonella* isolates were identified by adopting standard microbiological procedure which includes colony morphology, Gram stain reaction and biochemical reaction such as oxidase, catalase, sulfide indole motility test, citrate agar slant, methyl red test, Voges Proskauer test, triple sugar iron agar test and urease test. The sampling procedures were in accordance with guidelines of the National Health Research Ethics Committee, Nigeria (www.nhrec.net).

Antimicrobial susceptibility test

Salmonella isolates were subjected to *in-vitro* susceptibility test against commonly used antimicrobial agents using disk diffusion method following guidelines established by the Clinical and Laboratory Standards Institute (CLSI, 2012). In brief, by taking pure isolated colony, bacterial suspension was adjusted to 0.5McFarland turbidity standards. The diluted bacterial suspension was then transferred to Mueller-Hinton agar plate using a sterile cotton swab and the plate was seeded uniformly by rubbing the swab against the entire agar surface followed by 24 h incubation. After the inoculums were dried, antibiotic impregnated disks were applied to the surface of the inoculated plates using sterile forceps. The plates were then incubated aerobically at 37 °C for 24 h. *E. coli* (ATCC 25922), which was susceptible to all tested drugs, was used for quality control. Finally, the zone of inhibition was measured including the disk diameter. The susceptible, intermediate and resistant categories were assigned on the basis of the critical points recommended by the CLSI and according to the manufacturer's leaflet attached to the disks. Susceptibility was tested against the following antibiotics: chloramphenicol (30 µg), ciprofloxacin (5 µg), tetracycline (30 µg), Gentamicin (10 µg) and Nitrofurantoin (100 µg) (Oxoid, England).

Data analysis

Data generated was entered and analyzed using the statistical software SPSS version 20.0. Descriptive statistics was used to describe the frequency of *Salmonella* from different sampling hospitals and antimicrobial susceptibility pattern.

RESULTS

A total of 67 *Salmonella* isolates were isolated from 144 clinical samples showing a total prevalence rate of 46.5%. Out of this 67 isolates, 40 came from male patients while 27 came from female patients. When compare to the total number of males sampled to the total number of females sampled, the rate of isolation was not significantly different in both sexes (Table 1). The result of the in vitro antibiotic sensitivity test showed that isolates were generally resistant to chloramphenicol and ciprofloxacin which are the drugs of choice routinely used in the study area for the treatment of salmonella infection. However, the isolates were considerably sensitive to erythromycin, nitrofurantoin and gentamycin (Table 2).

DISCUSSION

The result of this study revealed a prevalence rate of 46.5% in the study area. This finding is similar to the 45.0% prevalence rate earlier reported (Adabara, *et al.,* 2012) in Mina, Nigeria, however both agree with the position of the World Health Organization (WHO, 2010) that the vast majority of typhoid fever cases occur in Asia, Africa and Latin America where water borne diseases are highly prevalent because of inadequate supply of potable water to the public with concomitant poor environmental and personal hygiene.

There was no significant difference in clinical presentation shown by the subjects as against what has been reported (Neil *et al.,* 2009) in the editorial commentary of the February edition of Clinical Infectious Diseases from Uganda where intestinal perforation was observed among children aged between 1–5 years. In the study area, access to medical care is available which makes it easy for subjects to seek medical attention without the needless delay that often account for the pathologic damages seen in cases where access to healthcare services is constrained such as intestinal perforation.

High level of antibiotic resistance by isolates was observed to the routinely used antibiotics in the study area. This in agreement with the earlier report of a worldwide occurrence of multidrug resistance strains of *Salmonella.* This development as observed may be traceable to wrong and inaccurate diagnosis and abusive use of the available antibiotics resulting in the development and spread of multidrug resistant strains of *Salmonella*.

In the last decade, there have been some reports of ciprofloxacin resistance in *Salmonella* (Nath *et al.,* 2003). It is believed that nalidixic acid resistance is a surrogate marker for ciprofloxacin resistance, as clinical failures have been documented in cases where ciprofloxacin has been used (based on susceptibility) for nalidixic acid resistant strains (Mandal *et al.,* 2012). In our study, 36% of isolates displayed reduced susceptibility to ciprofloxacin. Kirby-Bauer disc diffusion assay using currently recommended breakpoints to ciprofloxacin may not be a reliable method, E-test should be the preferred method of choice to determine ciprofloxacin MIC (Crump *et al.,* 2003; Harish *et al.,* 2011). Routine investigation and reporting of ciprofloxacin and azitromycin MICs in patients presenting with invasive *Salmonella* infections, like typhoid fever have been suggested Parry et al., 2010; Ray *et al.,* 2006).

Since its introduction in 1948, chloramphenicol has been the treatment of choice for typhoid fever and remains the standard against which newer antimicrobials are compared. Treatment with chloramphenicol reduces mortality due to typhoid fever from about 20 to 1 per cent and the duration of fever from 14-28 days to 3-5 days

(Mirza *et al.,* 1996). However, chloramphenicol therapy has been associated with the emergence of resistance to chloramphenicol, a high relapse rate, bone marrow toxicity and high mortality rates in a recent study reported from the developing world (Nagshetty *et al.*, 2010). In our study *Salmonella* sp. were resistant to chloramphenicol, alternative drug should be used for the treatment of salmonella infection in our region.

In conclusion, the result of this study has further accentuated the growing concern about the presence of and the spread of multidrug resistant Salmonella thereby underscoring the need for rational application of antibiotics and other necessary interventions that will help to control the menace of antibiotic resistance. Provision of potable water, accurate laboratory diagnosis, public education, and so forth, are, therefore, recommended. Surveillance programs to monitor antimicrobial resistance patterns in other parts of the state and the entire country in general are also recommended.

Table 1. The rate of Isolation of Salmonella from the various sample sources

Source of Sample	MALES		FEMALES	
	No. Sampled	No. Positive (%)	No. Sampled	No. Positive (%)
FMC Owerri	15	8 (57.2)	15	6(42.9)
Gen. Hospital Owerri	19	8(42.1)	11	5(45.5)
St. David Hospital Oweri	17	10(58.8)	13	7(46.1)
Holy Family Hospital	12	6(50)	12	4(33.3)
Ezem Hospital Owerri	19	8(42.1)	11	5(45.5)
Total	82	40(48.8)	62	27(43.5)

Table 2. The Antibiotics Susceptibility Pattern of *Salmonella* Isolated from Various Hospitals.

Sample Source	AE		NP		NB		CI		GI	
	%R	%S	%R	%S	%R	%S	%R	%S	%R	%S
Federal Medical Center, Owerri	40	60	30	70	30	70	70	30	40	60
Ezem Medical Center, Owerri	30	70	40	60	20	80	80	20	10	90
Holy Family Hospital	40	60	40	60	30	70	60	40	30	70
St. David Hospital, Owerri	40	60	20	80	40	60	70	30	10	90
General Hospital, Owerri	10	90	50	50	40	60	90	10	40	60

Key: N=Nitrofurantoin, C=Chloramphenicol, E=Erythromycin, G=Gentamycin. P= Ciprofloxacin

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