

**SUSCEPTIBILITY OF SALMONELLA SPECIES ISOLATED FROM PATIENTS' STOOL
TO COMMONLY USED ANTIBIOTICS****Oguamanam Okezie Enwere^{1*} and Kelechi Ihechinyerem Osuagwu²**¹Department of Medicine, Faculty of Medicine, Imo State University, Imo State, Nigeria.²Department of Obstetrics & Gynecology, Madonna University Elele, Rivers State, Nigeria.***Corresponding Author: Dr. Oguamanam Okezie Enwere**

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ABSTRACT

Background: Salmonella infection among humans is an important public health challenge. While commonly available antibiotics are regularly used for its treatment, a changing antibiotics susceptibility pattern has been observed over the years. We assessed antibiotic susceptibility to salmonella isolated from patients' stool. **Methodology:** Patients had a stool sample collected on clinical assessment of typhoid fever. Stools were then tested for presence of salmonella species in addition to other pathogenic parasites. Isolated cases of salmonella species was then subjected to antibiotic sensitivity using the disc diffusion method. **Results:** A total number of 105 samples were analyzed and 60 (57.1%) were positive for salmonella species. Salmonella was least resistant to streptomycin (18.3%). The fluoroquinolones, ofloxacin and ciprofloxacin had 33.3% and 35% resistance respectively. Chloramphenicol also had 35% resistance, while pefloxacin had the most resistance of the fluoroquinolones at 41.7%. Nalidixic acid had the highest resistance (76.6%). **Conclusion:** The fluoroquinolones remain the first line drug treatment for salmonella infection. However, we observed a rising level of resistance level compared to previous years. Streptomycin had the least resistance but available only as injection.

KEYWORDS: susceptibility, salmonella, typhi, antibiotics, patients' stool, common.**INTRODUCTION**

Salmonella typhi, a Gram-negative, motile, aerobic, non-spore, intracellular *Bacillus* is responsible for causing typhoid fever which can be a life threatening systemic infection.^[1,2,3] It occurs worldwide and is considered to be a disease of global public health concern with estimates of 21.6 million new cases of typhoid fever and 216,500 deaths recorded globally in 2000.^[2,4] The burden of typhoid disease is particularly worse in developing countries where it is associated with higher levels of morbidity and mortality.^[5]

In Nigeria, Salmonella infection and intestinal helminthes infestation are widespread among the population.^[6] Poor environmental and personal hygiene, poor waste disposal practices as well as consumption of food prepared by commercial vendors are major factors that encourage stable transmission as the typhoid fever is transmitted mainly via the faeco-oral route.^[7,8]

Typhoid fever, in most individuals, presents with nonspecific generalized symptoms like fever, headache, rigors, joint pain, nausea, vomiting, constipation, and diarrhea, similar and sometimes indistinguishable from other causes of fever such as malaria.^[3,9] A definitive diagnosis of typhoid fever involves isolation of the causative organism, salmonella typhi from blood

especially in the first week of illness.^[3,10,11,12] This remains the gold standard for diagnosis. However, the presence of clinical symptoms characteristic of typhoid fever and the detection of a specific antibody response is regarded as being only suggestive of typhoid fever.^[13,14] The widal agglutination test is widely employed in making a presumptive diagnosis of typhoid fever in Nigeria.^[15] Also, isolation of the organism in stool is commonly done in various hospitals in Nigeria, and in the presence of clinical symptoms can be used to diagnose typhoid fever presumptively.^[3,16]

The prevalence in Nigerian communities of salmonella infection is difficult to establish as many studies are hospital based. Among children with septicemia in Ibadan, salmonella specie was responsible in 56% of cases,^[17] while among adults, hospital prevalence were as high as 80% to as low as 9%.^[11,15,18]

Typhoid fever has been treated with various antibiotics over the years. The fluoroquinolones are considered to be the first line optimum treatment option for typhoid fever recently.^[19] Other antibiotics with less efficacy include the macrolides, penicillins, aminoglycosides, cotrimoxazole and chloramphenicol.^[11,17]

Commonly available fluoroquinolones in Nigeria like ofloxacin, ciprofloxacin, and pefloxacin are highly active and equivalent in efficacy (except norfloxacin which has inadequate oral bioavailability and should not be used in typhoid fever) and have generally proved effective in treating typhoid fever.^[3] There is no evidence of the superiority of any particular fluoroquinolone.^[3] In recent years, however, there have been many reports of reduced susceptibility and treatment failure for ciprofloxacin.^[20] Changing antibiotic susceptibility of salmonella species to various antibiotics in different parts of the world has also been observed.^[3]

A stool culture for pathogenic organisms including salmonella species is commonly conducted in many hospitals in Nigeria as part of a sepsis work up. The isolation of salmonella species in the presence of symptoms suggestive of typhoid fever is many times used for a presumptive diagnosis. Carrier states for salmonella are also assessed by using stool isolates of salmonella species in Nigeria. The scope of this study was to observe the current antibiotic susceptibility pattern to isolated salmonella species from stools of patients with suspected typhoid fever at a prominent private hospital in Owerri, Imo State, Nigeria.

MATERIALS AND METHODS

This was a cross sectional study of patients seen at a private hospital in Owerri, Imo State, Nigeria who were being investigated as part of a sepsis work-up had their stool samples collected and tested for pathogenic organisms including salmonella species. Imo state is located in the southeastern part of Nigeria.

Freshly passed stool was collected in a sterile container and 1g of the stool sample was transferred and pre enriched into prepared buffered peptone water (1part sample + 9 part buffer) in test tube. Mixed and incubated at 37° C overnight (16-20 hours). Then 1ml of pre-enriched sample was transferred and cultured on a selective media and differential media (Salmonella -

Shigella Agar and Maconkey agar). After 48hrs, the growth was subjected to biochemical and sensitivity tests for confirmation of the organism according to Cheesbrough (2004/2006).^[21] A portion of the 24hr growth was examined microscopically to determine Gram reaction.

Antibiotic susceptibility was performed by using the disk diffusion method, and the zone of inhibition was measured in millimeters. The panel of antibiotics assessed is as follows: ofloxacin, pefloxacin, ciprofloxacin, chloramphenicol, Nalidixic acid, streptomycin, septrin (cotrimoxazole), augmentin (co-amoxiclav), ceporex (cephalexin) and amoxicillin.

RESULTS

Over a period of 10 months in 2017, 105 samples of patients being investigated for typhoid fever was analyzed. Females were 66 (62.9%) and males were 39 (37.1%). The age of the patients ranged from 3years to 81years. All were being investigated for sepsis and had a stool sample collected and examined for pathogenic organisms including salmonella species.

Sixty (60) samples were positive for salmonella species, being 57.1% of the total number of patients analyzed. The prevalence among the sexes are as follows: females had 42 (63.6%) positive samples out of 66 total number of females tested; while males had 18 (46.2%) positive samples out of 39.

Susceptibility pattern of the salmonella isolates are as shown in table 1. Streptomycin, an aminoglycoside, had the least resistance (18.3%). The fluoroquinolones, ofloxacin and ciprofloxacin had 33.3% and 35% resistance respectively. Chloramphenicol also had 35% resistance, while pefloxacin had the most resistance of the fluoroquinolones at 41.7%. Other antibiotics demonstrated higher levels of resistance: augmentin (56.7%), septrin, ceporex and ampicillin (66.7% each), and nalidixic acid had the highest resistance (76.6%).

Table 1: Antibiotic sensitivity of salmonella isolates from patients' stool.

Antibiotic	Sensitivity (%)	Resistance (%)
Ofloxacin	40 (66.7%)	20 (33.3%)
Pefloxacin	35 (58.3%)	25 (41.7%)
Ciprofloxacin	39 (65%)	21 (35%)
Chloramphenicol	39 (65%)	21 (35%)
Nalidixic acid	14 (23.3%)	46 (76.7%)
Streptomycin	49 (81.7%)	11 (18.3%)
Septrin	20 (33.3%)	40 (66.7%)
Augmentin	26 (43.3%)	34 (56.7%)
Ceporex	20 (33.3%)	40 (66.7%)
Ampicillin	20 (33.3%)	40 (66.7%)

DISCUSSION

Salmonella infection remains a public health challenge in both developed and developing countries alike. It can present in a varied pattern of symptoms from a high

carrier, none-symptomatic state, to frank symptoms of gastrointestinal disturbance and sepsis.^[18]

We observed a higher prevalence of salmonella infection amongst the female patients (63.6%) compared to a rate

of 46.2% amongst the males. Patient susceptibility to salmonella can be varied depending on the location; either with a higher prevalence among women to the reverse, or no predilection at all in relation to age and sex.^[18,22]

We observed the lowest resistance of the isolates to gentamycin, an aminoglycoside. Gentamycin, not commonly used by the general population, can therefore be a treatment option but its use will be limited by its availability as an injection. However, high salmonella resistance to streptomycin, another aminoglycoside, has been observed in other parts of the world.^[23] The fluoroquinolones are recommended as first line drug treatment for salmonella infections.^[3] We observed that ofloxacin was the most sensitive of the quinolones against salmonella, followed by ciprofloxacin. But our observed level of sensitivity for ofloxacin, ciprofloxacin and pefloxacin to salmonella were far less compared to observation by Ogunleye VO et al in 2005. Perhaps there is a growing resistance of salmonella to the fluoroquinolones.

Resistance to the fluoroquinolones may be total or partial and, demonstration of Nalidixic-acid-resistant *S. typhi* (NARST) is a marker of reduced susceptibility to fluoroquinolones.^[3] Resistance to Nalidixic acid, which is never used for treatment of salmonella infections, we observed was 76.7%. The clinical response to treatment with fluoroquinolones of nalidixic-acid-resistant strains is significantly worse than with nalidixic-acid-sensitive strains.^[3] An important fact to be borne in mind when testing susceptibility.

Chloramphenicol, we observed, had similar sensitivity pattern to the fluoroquinolones. This drug until a few years ago was the first line drug treatment, but still a popular choice in some African countries.^[3,24] A similar sensitivity pattern of 63% sensitivity was also observed by Ogunleye VO et al in 2005 compared to 65% sensitivity we observed. It is possible response to chloramphenicol has remained stable over the years as its use is now restricted by a relatively high rate of relapse, long treatment courses (14 days) and the frequent development of a carrier state in adults.^[3]

The other antibiotics, augmentin, septrin, ceporex and ampicillin exhibited high levels of ineffectiveness against the isolated salmonella organism and ideally, should not be considered as a treatment option.

CONCLUSION

Gentamycin was the most effective antibiotic against salmonella, and can be considered a treatment option. While the fluoroquinolones, the recommended first line treatment, remain effective against salmonella though with higher rates of resistance compared to previous studies demonstrating a rising antibiotic resistance pattern. Other antibiotics were not as effective for treatment. The need for continuous regional antibiotic

susceptibility testing of salmonella is important to enable demonstration of the most appropriate antibiotic treatment.

Conflict of interest: None.

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