Prevalence of *Salmonella* species in Clinical specimens and its antibiotic susceptibility profile

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Abstract

Typhoid fever is one of the endemic diseases in the tropic and sub-tropics. In this study, blood and stool specimens were collected from 200 participants comprising of 100 male and 100 female aged between 11 to 41 years and above. Of this, 168 (84%) of the participants had significant titre in their blood while 182 (91%) discharged the organisms in their stools with female having a higher prevalence than male. Age prevalence study showed a high prevalence of *Salmonella* species in people aged between 16 – 20 years, followed by people aged between 21 – 25 years and least prevalence in people aged 31 years and above. The result of the antibiotic susceptibility profile showed that the isolates were sensitive to Gentamycin (98.4%), Ciprofloxacin (97.8%), Sulfamethoxazole-trimethoprim (94.5%), Chloramphenicol (92.3%), Amikacin (89.0%) and Amoxycilin (56.0%) and resistance to Erythromycin (93.4%), Norfloxacin (91.2%) and Doxycyclin (77.5).

Keywords: *Antibiotics, Blood, Prevalence, Salmonella and Stool*

1. Introduction

Typhoid fever (enteric fever) is an endemic disease in the tropic and sub-tropics and has become a major public health problem in developing countries of the world with an estimated incidence of 540 per 100,000. The disease is caused by *Salmonella* species - a rod shaped, Gram negative, non-lactose fermenting and non-sporing bacteria with peritrichous flagella (Cheesbrough, 2006; Hardy, 2004). They are pathogenic to both man and animal (Okonkwo *et al*., 2010). The annual incidence of typhoid is estimated to be about 17 million cases worldwide (WHO, 2008). It is often encountered in tropical countries including Nigeria where it constitutes serious sources of morbidities and mortalities (Ibekwe *et al*., 2008). These diseases are attributed to poor sanitation and hygiene (Singh and Mcfeters, 1992) and Humans get infected by the oral route through ingestion of fecal contaminated food and water, unclean hands, flies and meat from infected animals (Carol *et al.*, 1989). Once the bacteria enter the person's body they multiply and spread from the intestines, into the bloodstream (WHO, 2008). The Widal agglutination test, a presumptive serological test for enteric or undulant fever was developed by Widal in 1896 to aid the diagnosis of typhoid fever via the agglutination of antibodies against Salmonella somatic (O) and flagella (H) antigens. This work therefore seeks to determine the titre of *Salmonella typhi* and *S.* paratyphi in some undergraduate students of the University of Uyo - Akwa Ibom State and its antibiotic susceptibility profile.

2.0 Materials and Methods

2.1 Sample collection

Following informed consent, Blood and stool samples were collected from 200 participants in EDTA and sterile universal containers respectively and conveyed to the laboratory within two hours

2.2 Sample Processing

Blood samples collected were analyzed within one hour for presence antibody against *Salmonella* species. The blood specimens were centrifuged at 5000 rpm for 20min to separate serum from plasma. Using Pasteur pipette, few drops of serum were placed in a white tile and each drop was mixed with *Salmonella* II serotype antigens (Atlas). The mixtures were tilted or rocked for about 20 mins. Then the antibody agglutination titre was observed and recorded. Any serum with antibody titre > $^1/_{80}$ for *Salmonella* species) somatic (O) antigen was considered positive for *Salmonella* infection. However, individual serum with titre > 1/40 but < $^1/_{80}$ was considered to possibly have trace infection (Cheesbrough, 2006; Andrews *et al*., 2005). Stool samples were inoculated directly on sterile *Salmonella-Shigella* Agar using a sterile swab stick. The plates were inverted and incubated at 37^o

2.3 Purification and Maintenance of Pure isolates

Discrete colonies on the Salmonella- Shigella plates were sub cultured unto freshly prepared nutrient agar plates. Colonies obtained from the sub-culture plates were maintained by inoculating them on Nutrient Agar slants using sterile wire loop. The slants were stored in the refrigerator at 4^0C until when needed.

2.4 Characterization of Bacterial Isolates

The bacterial isolates were characterized based on their morphological, biochemical and fermentative characteristics as described by Chessbrough, (2006). The obtained characteristics were compared with those given by Barrow and Feltham (2003) for identification.

2.5 Antibiotic susceptibility test

The antibiotics susceptibility profile of the isolates was determined against ten commercial antibiotics. A sterile wire loop was used to colonies of each isolates on the agar slants and emulsified in 4 ml of nutrient broth. The broth culture was incubated for few hours until it became slightly turbid and the turbidity of each suspension was then matched to standard turbidity (0.5 McFarland standards). A sterile cotton swab was dipped into the standardized bacterial test suspension and evenly inoculated on the entire surface of a sterile dry solidified agar plate. Excess fluid was removed by pressing and rotating the swab against the side of the tube above the level of the suspension before inoculation. After inoculation, the surface of the agar was dried for 5minutes with the petri dish lid in place after which the appropriate antibiotic disks, which have been allowed to attain room temperature about 1hour before use were aseptically placed evenly on each inoculated plates with sterilized forceps. Each disk was firmly pressed to ensure its constant with the agar surface. The plates were allowed for 30 minutes after applying the disks after which it was inverted and incubated aerobically at 37◦ C for 24 hours. *Salmonella* plate with no antibiotic disc inoculated was used as control. This procedure was repeated for each of the isolates. After 24 hours incubation, zones of inhibition exhibited by each isolate against tested antibiotics were measured and recorded in millimeter. These were then interpreted as either susceptible or resistant depending on the diameter of the halo (Cheesbrough, 2006; CLSI, 2007).

3.0 Results and Discussion

The results of the morphological and biochemical characteristics of *Salmonella* species isolated from the various clinical specimens is as given in Table I below

Table I: Morphological and biochemical characteristics of *Salmonella* species isolated from clinical samples.

Test	Shape	GS	Mot	Cit	Cat	U	MR	Oxi	Ind	VP	G	M	L	S
Observation	Rod	-	+	+	-	-	+	+	-	-	AG	A	-	-

Key: GS, Grams Staining; Mot, Motility; Cit, Citrate; Cat, Catalase; U, Urease; MR, Methyl Red; Oxi, Oxidase; Ind, Indole; VP, Vogues Proskauer; G, Glucose; M, Maltose; L, Lactose; S, Sucrose; "+"; Positive; "-"; Negative; A: Acid

Analysis of the gender distribution and prevalence of *Salmonella* species in the specimens collected showed that of the 200 (100 male and 100 female) participants recruited for the study, 168 (84%) of the participants had significant titre in their blood while 182 (91%) discharged the organisms in their stools. Of the 168 (84%) participant that had significant titre, 76 (45.2%) were male while 92 (54.8%) were female. This high prevalence in female tend to agree with Itah and Akpan (2005) who showed that female subjects were more infected with *Salmonella* species than their male counterparts and this may be attributed to the fact that females are exposed to various ways of contracting these infections thereby making them more vulnerable to this disease. A summary of this is given in Table II below

Table II: Gender Distribution and Prevalence of *Salmonella* species in Samples collected

Gender	Total No. Screened	No. of positive Blood Samples (%)	No. of Positive Stool samples (%)
Male	100 (50)	76 (45.2)	86 (47.3)
Female	100 (50)	92 (54.8)	96 (52.7)
Total	200 (100)	168 (84)	182 (91)

Age distribution samples and Gender prevalence of the *Salmonella* species in the samples collected showed that there was a high prevalence of *Salmonella* species in people aged between 16 – 20 years. This high prevalence is followed by people aged between 21 – 25 years. The least prevalence was seen in people aged between 36 years and above although all the participants within this age range were still discharging the organism in their stools. A summary of this result is given in Table III below;

Table III: Age Distribution and Prevalence of *Salmonella* species in Samples collected

Age Range	Total Number Sampled (%)	No. of positive Blood Samples (%)	No. of Positive Stool samples (%)
11 - 15	14 (7)	10 (6.0)	12 (6.6)
16 – 20	67 (33.5)	61 (36.3)	64 (35.2)
21 – 25	52 (26)	49 (29.2)	50 (27.5)
26 – 30	48 (24)	36 (21.4)	38 (20.9)
31 – 35	11 (5.5)	8 (4.8)	10 (5.5)

36 – 40	6 (3)	3 (1.9)	6 (3.3)
41 and above	2 (1)	1 (0.6)	2 (1.1)
Total	200 (100)	168 (84)	182 (91)

The result of the antibiotic susceptibility profile of the isolates according to NCCLS (2000) showed that a greater percentage of the isolates were sensitive to Gentamycin (98.4%) followed by ciprofloxacin, Sulfamethoxazole-trimethoprim, Chloramphenicol, Amikacin and Amoxycilin with percentage 97.8%, 94.5%, 92.3%, 89.0% and 56.0% respectively. The isolates were resistance to Erythromycin (93.4%), Norfloxacin (91.2%) and Doxycyclin (77.5). A summary of this result is given in Table 5 below. These results tend to agree with the results of Frost (1996), Dhanashree (2007) and Mijovic *et al.*, *(*2012).

Table 5: Antibiotic Susceptibility Profile of *Salmonella* species isolated from the Stool Specimens

	Nos. of Isolates Screened (n = 182)		
Antibiotics	Sensitive (%)	Intermediate (%)	Resistance (%)
Neomycin	82 (45.1)	87 (47.8)	13 (7.1)
Erythromycin	0 (0)	12 (6.6)	170 (93.4)
Gentamycin	179 (98.4)	3 (1.6)	0 (0)
Ciprofloxacin	178 (97.8)	4 (2.2)	0 (0)
Doxycyclin	18 (9.9)	23(12.6)	141(77.5)
Amoxycilin	102 (56.0)	68 (37.4)	12 (6.6)
Amikacin	162 (89.0)	12 (6.6)	8 (4.4)
Norfloxacin	2 (1.1)	14 (7.7)	166 (91.2)
Chloramphenicol	168 (92.3)	12 (6.6)	2 (1.1)
Sulfamethoxazole - trimethoprim	172 (94.5)	8 (4.4)	2 (1.1)

4.0 Conclusion and Recommendation

The result of this study has provided information on the gender and age prevalence of *Salmonella* species in clinical specimens and its antibiotic susceptibility profile. Based on the result of this study, it is therefore important to adhere to and maintain hygienic and aseptic protocols while handling clinical specimen as well as in food processing. Overuse and abuse of antibiotics should be discouraged as this increases the chances of the pathogen acquiring resistant to such drugs. It is also recommended that antibiotic susceptibility profile screening be carried out on patients before commencing any antibiotic treatment.

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