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A study on the prevalence of Salmonella species in Yola North Local Govt. area of Adamawa State, Nigeria.

***Midala, T., J.; **Agina, S.A. and !*De, N.**
* Department of Microbiology
Federal University of Technology, Yola.
Adamawa State.
** Department of Botany University of Jos
Plateau State.
! * Corresponding author.

Abstract
A total of three hundred stool samples collected from one hundred and twenty six patients (attending Federal Medical Centre ; Bamaiyi Hospital, Peace hospital, Yola Biomedics Laboratory and Beta Laboratory) and one hundred and seventy four volunteers (students of Daubeli Secondary School) were examined. Twenty of the samples analyzed were non-lactose fermenters and were subjected to serotyping. The results of the serotyping identified 11 S. typhi isolates (3.7%), 3 S. paratyphi A isolates (1.0%) and 2 S. paratyphi B isolates (0.67%) and 4 non- Salmonella isolates. Antibiotic sensitivity test on the eleven S. typhi isolates shows that gentamicin has the highest efficacy (90.9%) followed by ofloxacin (63.6%) and colistin sulfate (27.3%) respectively. The efficacies of streptomycin, chloramphenicol and tetracycline against these isolates were around 9.1%.

Key words: Non-lactose fermenter, Salmonella species, and Antibiotic sensitivity.

Introduction

Typhoid fever is an acute systemic bacterial disease caused by Salmonella typhi and Salmonella paratyphi A, B and C. The enteric fever caused by S. typhi leads to a series of severe pathogenic conditions while that by S. paratyphi A, B or C is usually milder (Chessbrough, 1984).

In developing countries where adequate hygienic practice, potable drinking water proper sanitary measures are lacking, fatality rate cases due to enteric fever caused by S. typhi varies between 12% and 32% while in developed countries, the rate has been reduced to less than 2% (Benoit et al., 2003; Anita et al., 2002 and Pang et al., 1995).

Typhoid fever can be transmitted by contamination of water, milk and foods by a convalescent or chronic carrier and mechanically from feces to food by flies. The disease can also be transmitted by the contamination of bodies of water used for cultivation of fish, fruits and vegetables (Ibrahim et al., 1998). Large epidemics are most often related to fecal contamination of water supplies or street vended foods. A chronic carrier state-excretion of the organism for more than a year- occurs in approximately 5% of infected persons. The chronic carrier remains the chief problem in the elimination of typhoid fever in the human race (Wheeler, 2004).

In Nigeria, incidence of typhoid fever is however higher in slums than in the modern part of cities, because of poor hygienic and sanitary status of such areas (Boosma, 1988). Recent studies in some parts of Nigeria e.g. city of Zaria showed that high prevalence of typhoid exists (Ibrahim et al., 1998). The aims and objectives of this study was to

determine the prevalence of typhoid fever in Yola North Local Govt. area so as to provide basis for recommendation to the policy makers on health matters for planning.

Materials and Methods

Study area
The study area was Yola North Local Govt. area of Adamawa State.

Samples Collection
Stool samples were collected between the months of August and November 2001 from volunteers (students of Daubeli Secondary School) and from patients (attending Federal Medical Centre, Bamaiyi Hospital, Peace hospital, Yola Biomedics Laboratory and Beta Laboratory). The samples were cultured within two hours of collection using Selenite F broth.

Isolation of *Salmonella* species
About 1 ml of each watery stool sample was inoculated into a screw capped universal bottle containing 9mls of Selenite F broth and incubated at 37oC for 18 hours. For each of formed or semi formed stool specimens, a thick suspension was made in 1 ml of sterile peptone water and was inoculated into a screw capped universal bottle containing 9mls of Selenite F broth and incubated at 37oC for 18 hours.
A loopful of the 18-hour-old culture in Selenite F broth was then streaked on *Salmonella Shigella* agar as described by Baron et al. (1994). The pale colonies with black centers on *Salmonella Shigella* agar were further sub cultured on MacConkey agar to obtain discrete pure colonies and were kept in the refrigerator at 4oC for further use.

Identification of isolates
Gram staining procedure was done according to Jenson's Method (CruickShank et al., 1975 and Cheessbrough, 1984).
Hanging drop method was employed to determine the motility of the test organisms (CruickShank et al., 1975 and Cheessbrough, 1984).
The colonies that were motile, non lactose fermenters and gram negative bacilli were further subjected to Indole test, Methyl red test, Voges Proskauer test, Citrate utilization test as described by Cheessbrough (1984).
Each of the isolated colonies above were now subjected to TSI test (CruickShank et al., 1975).

Serotyping
Commercial polyvalent *Salmonella and Salmonella typhi* antisera obtained from Biotech. Laboratories Ltd. was used for serotyping the presumptive *Salmonella* isolates according to manufacturer's instructions.

Sensitivity tests
Eleven labeled seropositive isolates (*Salmonella typhi*) obtained were used for sensitivity testing (Bauer et al. (1966). The commercial disc strengths used were Gentamicin - 25µg/ml; Augmentin-30µg/ml; Chloramphenicol-30µg/ml; Tetracycline-30µg/ml;

Ofloxacin-30µg/ml; Colistin sulfate- 10µg/ml; Cotrimoxazole- 25µg/ml; Ampicillin- 25 µg/ml; Streptomycin-25µg/ml; Amoxicillin- 25µg/ml. The antibiogram (radius of zones of inhibition) was recorded. In this case, a zone radius that was 3 mm or above reported sensitive and a zone of radius 2 mm or less was recorded as resistant (Cheessbrough, 1984).

Results

Of the three hundred stool samples analyzed, forty-four samples produced colorless colonies and two hundred and fifty six samples produced pink colonies.

Further biochemical tests produced twenty presumptive *Salmonella* isolates. These 20 isolates were then subjected to serotyping test.

The results of serological tests of the 20 isolates (Table 1) reveals that 11 (55%) of them were *Salmonella typhi*, 5(22%) were *Salmonella paratyphi* while 4(20%) were not *Salmonella sp*

Table1: Results of serotyping of presumptive Salmonella isolates

Sites	A	B	C	D	E
Federal Medical Centre	9	4	1	2	2
Doubeli Secondary School	11	7	2	-	2
Total	20	11	3	2	4

A- Total number; B- *S typhi*; C- *S paratyphi* A, D- *S paratyphi* B; E- Non-*Salmonella* isolates

The results of antibiotic sensitivity test are as shown in Table 2. Of the eleven isolates, 4; A1, A8, A10 and A11 showed radius of zone of inhibition ranging from 3.0 mm to 7.0 mm against Gentamicin and 6 of the isolates; A2,A4, A6, A7-A9 and A11 showed radius of zone of inhibition ranging from 3.0 mm 7.0 mm against ofloxacin respectively. Most of the isolates showed radius of zone of inhibition below 3.0 mm against the other tested antibiotics.

Table 2: Zones of inhibition in mm (radius) of each of *Salmonella* isolates to standard antibiotics

Isolate	Gen	Aug	Chl	Tet	Ofl	Col	Cot	Amp	Str	Amx
A1	6.5	1.5	1.0	-	2.0	-	-	-	-	-
A2	5.0	-	-	2.0	7.0	-	-	-	-	-
A3	7.0	-	-	-	2.5	6.5	-	-	-	-
A4	6.5	-	1.0	7.5	3.0	2.0	-	-	-	-
A5	3.5	-	-	1.0	1.5	-	-	-	-	-
A6	4.5	-	1.5	1.5	6.5	-	-	-	-	-
A7	6.5	-	-	-	3.0	6.0	-	-	-	-
A8	4.0	-	-	1.0	3.0	2.0	-	1.0	1.5	-
A9	1.5	-	-	-	4.0	3.0	-	-	6.5	1.0
A10	6.5	-	7.0	-	2.0	-	1.0	1.5	-	-
A11	6.0	-	-	-	3.0	-	-	-	1.5	-

Gen- Gentamicin ; Aug- Augmentin; Chl- Chloramphenicol; Tet- Tetracycline; Ofl- Ofloxacin; Col- Colistin sulfate; Cot- Cotrimoxazole; Amp – Ampicillin; Str-streptomycin; Amx- Amoxicillin.

Percentage efficacies of various antibiotics tested against the isolates are as shown in Table 3. Gentamicin showed highest efficacy (90.9%) followed by ofloxacin (63.6%) and colinstin sulfate (27.3%).

Table 3: Percentage efficacies of various antibiotics against the *S.typhi* isolates

Antibiotic	No. of sensitive isolates	Percentage efficacy
Gentamicin	10	90.9
Ofloxacin	7	63.6
Colistin sulfate	3	27.3
Tetracycline	1	9.1
Chloramphenicol	1	9.1
Streptomycin	1	9.1
Ampicillin	-	-
Augmentin	-	-
Cotrimoxazole	-	-
Amoxicillin.	-	-

Discussion

From the three hundred stool samples analyzed, twenty of that were presumptive as *Salmonella* sp. were serotyped and eleven (11) of them (3.7%) were positive for *S. typhi*. The isolation rate among patients was 1.33% whereas the isolation rate for volunteers was 2.33%. This result agrees with a case study in Zaria, which reveals that approximately 2% of the patients that visited the clinics or hospitals were diagnosed and treated for typhoid fever (Ibrahim et al., 1998). The low isolation rate could be attributed to the fact that most of the stool specimens collected were not at their peak time of isolation (third week of infection), and also, the patients were not in the hospitals or clinics for typhoid infection cases! Most came with complains of gastroenteritis, fever, diarrhea and some are already on antibiotic therapy. Most of the patients had only 4 to7 days infection period, this could lead to the low rate of isolation.

The isolation rate from volunteers (2.33%) was slightly higher than that of patients (1.33%) even though they were apparently healthy. This could be attributed to the habits of students (the volunteers) feeding on the cheap cafeterias, eating unwashed fruits and local salad that could harbor the pathogens. Also they are exposed to contaminate drinking water sold as packaged water. These group of people (infected volunteers) may have the tendency to excrete a very high numbers of *Salmonella typhi* for as short as three weeks to many years, thereby causing a lot of hazards to the Community (Ikeme and Anan, 1996; Olubuyide, 1992). These carriers can cause an epidemic in a community as was the case of Typhoid Mary in United States of America (Madigan et al., 2000).

The antibiotics used here for the sensitivity tests are conventional antibiotics used for gram-negative organisms at the recommended concentrations. Ofloxacin and Gentamicin exhibited the highest antimicrobial activities against the *S. typhi* isolates, with percentage efficacy ranging from 63.6%- 90.9% as was the case observed in California and Boston in U.S.A. (Bissett et al., 1974; Barros et al., 1977). Colistin sulfate was less effective (27.3%) and chloramphenicol (the drug of choice for typhoid fever reatment), tetracycline and streptomycin in this study showed lower efficacy against the *S. typhi* isolates (9.1%). Four of the 11 isolates showed resistance to ampicillin, augmentin and amoxicillin. According to Patricia and Collins, (1984), an organism is sensitive to a particular agent implies that the infection should respond to treatment with normal doses under normal circumstances and also, an organism which is resistant to a particular agent is unlikely to show any clinical response at all. The choice of antibiotics to used for the treatment of typhoid fever should be accurate, because of the increasing rates of antibiotic resistance by Bacteria (Encyclopedia, 2003; Alawode, 2003).

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