Bayero Journal of Pure and Applied Sciences, 3(1): 202 - 206
Received: October, 2009
Accepted: January, 2010

INCIDENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *SALMONELLA SPECIES* IN CHILDREN ATTENDING SOME HOSPITALS IN KANO METROPOLIS, KANO STATE –NIGERIA

Abdullahi, M.
Medical Laboratory Science Department, School of Health Technology, Kano, Kano state, Nigeria.
Corresponding author: masadtof@yahoo.com

ABSTRACT
A study was carried out to investigate the incidence of Salmonella species among 300 children using stool samples from six hospitals in the metropolitan Kano. The organisms were investigated using cultural, serological biochemical characterization and sensitivity to some antimicrobial agents. The incidence of the bacteria was found to be highest among children of 20-24 months age and least in 5-9 months age group. Out of the total samples positive for Salmonella, 24 were from males and 17 from females. The species of Salmonellae isolated comprised of Salmonella typhimurium which was the most predominant followed by Salmonella typhi and Salmonella paratyphi. As at the time of the study, there was significance difference between males and females in the incidence at P≥ 0.05. Among the antimicrobials used ciprofloxacin was found to be more effective than the others.

Key word: Salmonellae, Incidence, Children, Antibiotics, susceptibility, Kano.

INTRODUCTION

Salmonellae are Gram negative, non-lactose fermenting and non-sporing bacteria. With exception of *Salmonella pullorum-gallinarum,* all salmonellae are actively motile. They are also, non-capsulated with the exception of *Salmonella typhi* belonging to the family *Enterobacteriaceae* (Cheesbrough, 2000; Perilla, 2003). The genus was named after Daniel Elmer Salmon, an American veterinary pathologist (FDA/CFSAN, 2009).

Salmonellae can be divided into two major groups of clinical importance: Group one, includes members of the genus that are involved as aetiologic agents of enteric fever (typhoidal salmonellosis): *Salmonella typhi* and *Salmonella paratyphi.* Group two, includes members of the genus that are involved as aetiologic agents of food poisoning (non typhoidal salmonellosis): *Salmonella typhimurium* and more recently serotype DT 104. Other members are *Salmonella enteritidis, Salmonella heidelberg, Salmonella agona, Salmonella newport, Salmonella hadar, and Salmonella dublin* (Arora, 2001; Adkins and Sandiago, 2006). Non-typhi Salmonellae (NTS) particularly *Salmonella typhimurium* and less frequently *Salmonella enteritidis* are common causes of bacteraemia and septicaemia in young children in developing countries (Cheesbrough, 2002; WHO, 2004).

Salmonellosis is an infection caused by ingesting salmonellae in food that is contaminated by faeces of animals or humans directly or indirectly. Common sources of infection include poultry meat and meat products, eggs and egg products. Some of the symptoms of salmonellosis are diarrhoea, vomiting fever, and abdominal pain these occur 12-36 hours after eating infected food, in acute infection, blood and mucous are present in faecal specimens (Al – jurayyan *et al.,* 2004). Salmonellae can be isolated from blood, stool, urine, bone marrow, duodenal aspirates and rose spots. From blood, the organisms can usually be detected in 75-90% of patients during the first ten days of infection, and in about 30% of patients during the third week (Cheesbrough, 2002).
The risk to salmonellosis is increased due to the following factors; absence of effective vaccines, modifying hand washing behaviour after defecating to control prolonged community out breaks and identifying high risk groups and targeting prevention measures ((Perilla, 2003).

The main Aims and objectives of the study are, to determine the incidence of *Salmonella* species serotypes in relation to age and sex among children, to isolate and characterize (Biochemically and Serologically) *Salmonella* species serotypes from stools of children in Kano metropolis and to determine the antimicrobial susceptibility patterns of the isolates.

MATERIALS AND METHODS

a). Study population

Faecal samples were collected from patients (children of less than 24 months) presenting with clinical symptoms of gastroenteritis such as diarrhoea, fever, dysentery (with mucus or blood) and abdominal pains attending five selected study hospitals.

b). Sample size

$$n = \frac{Pq}{(E/Z)^2}$$

Where
P = prevalence of previous studies = 17.50% (Liverworth, 2005)
Q =100-p =100-17.50 = 82.50%
E = allowable error = 5%
n= number of sample to be collected
Therefore, when the values are substituted

$$n = \frac{17.50 \times 82.50}{(5/1.96)^2} = \frac{1443.75}{(2.55)^2}$$

$$= \frac{1443.75}{6.5} = 222.12 \text{ samples}$$

Approximately = 222 samples

However, for more accurate results in this research work, a total of 300 stool samples were collected from children of less than 24 months attending five hospitals in Kano metropolis.

c. Sample collection

A total of 300 faecal specimens were collected from children (children less than 24 months) by their mothers in clean, wide- mouthed containers, without disinfectant or detergent residue and tight-fitting leak-proof lids. When delay was unavoidable (more than 2 hours), faecal samples were placed in stuart's transport medium and refrigerated immediately.

d. Culture of the faecal specimens:

The faecal specimens were cultured into Pre-enrichment broths (Selenite F) to allow the multiplication of bacteria; these were then subsequently sub-cultured onto a MacConkey agar (MCA) and Deoxycholate citrate agar (DCA) and then incubated aerobically at 37^0C for 24 hours (Cheesbrough, 2002).

e. Biochemical screening and serological tests for Salmonellae

Identification of *Salmonella* species was done biochemically. Triple sugar Iron (TSI) agar motility, urease and citrate utilization tests were also used to screen the isolates before serologic testing was performed (Cheesbrough, 2002; Perilla, 2003).

Triple sugar iron agar (TSI) test

At least one of each colony type of the well-isolated colonies was selected on plate using a sterile straight wire loop. The center of the colony was lightly touched and prepared TSI medium were inoculated by stabbing the butt and streaking the slants. These were then incubated at 37^oc for 24 hours (Cowan and Steel, 2002).

A yellow butt (acid) and red or pink (alkaline) slope indicates the fermenting of glucose only. Cracks and bubbles in the medium indicate gas production from glucose fermentation. A yellow (acid) butt indicates the fermentation of lactose. A red or pink (alkaline) slope and butt indicates no fermentation of glucose or lactose. Blackening along the stab line or throughout the slant indicates hydrogen Sulphide (H_2S) production. *Salmonella* forms a red slope (alkaline) and yellow (acid) butt with/out gas or H_2S production (Cheesbrough, 2002).

Urease test (Christensen's (modified) urea broth):-

Urea agars were inoculated heavily over the entire surfaces of the slants in bijou bottles. The cap were loosened and then incubated at 37^oC for 3-12 hours.

A urease-positive culture produces an alkaline reaction in the medium, evidenced by pinkish-red colour of the Medium. Urease-negative organisms do not change the colour of the medium, which is pale yellow-pink. *Salmonella* is always urease negative (Cowan and Steel, 2002).

Citrate utilization test using Simmon's citrate agar:-

Simmon's citrate slopes were prepared in bijou bottles as recommended by the manufacturer (stored at 2-8^{o}C). And the slopes were then stabbed and incubated at 37^oC aerobically for 48 hours (Cheesbrough, 2000).

Salmonella is citrate negative as such Simmon's citrate agar slopes remained as green in colour. And blue colour indicates a positive reaction (Bello, 2002).

Motility Test (using motility agars):-

Motility agar were prepared and inoculated with a straight inoculating needle making a single stab about 1-2cm down into the medium. The motility was examined after 35-37^{o}C for 24 hour

Motility was indicated by the presence of diffuse growth (appearing as colouring of the medium) away from the line of inoculation. With exception of *Salmonella pullorum-gallinarum,* all *Salmonella* species are motile (Cheesbrough, 2002; Perilla, 2003).

f. Serological identification of *Salmonella* species

Serologic identification of *Salmonella* species was performed by slide agglutination test with (Cheesbrough, 2000; Andrews *et al.,* 2005). A Commercial kit was used to agglutinate and serogroup salmonellae by their O antigens: A,B,C, D, and E. When positive agglutination reaction was obtained in one of the antisera, the *Salmonella* subgroup was identified, and no further testing with antisera needed to be conducted (Andrews *et al.,* 2005).

Method:-
An agglutination test was performed on a clean glass slide. The slide was divided into sections with a wax pencil and one small drop of physiological saline was placed in each test section on the slide. By using a sterile inoculating loop a portion of growth from the surface of TSI agar was removed and emulsified in each drop of physiological Saline on the slide. It was then mixed thoroughly to create a moderately milky suspension. A bent inoculating loop was used to pick a small drop of antiserum and transferred to one of the suspensions; the second suspension served as the control (usually approximately equal volume of antiserum and growth suspension was mixed). The suspension and antiserum was mixed very well and then the slide was rocked to observe for autoagglutination (agglutination is more visible if the slides is observed under a bright light and over a black background).
If the reaction is positive, clumping will appear within 30 to 60 seconds. The saline suspension (control) was examined carefully to ensure that it was even and did not show clumping resulting from auto agglutination. If autoagglutination occurs, the culture is termed "rough" and cannot be serotyped. Also, cultures that reacted serologically and showed non conflicting results in the biochemical screening tests were reported as positive for *Salmonella*.

g. Susceptibility Testing of *Salmonella* species Isolates
The susceptibility testing was carried out using Mueller Hinton agar and were tested in vitro for susceptibility to five (5) different antimicrobial agents suggested by WHO (i.e Ampicillin, Trimethoprim-sulfamethoxole (cotrimoxazole), chloramphericol, Nalidixic acid and Ciprofloxacin) the following procedures were followed (Perilla, 2003).

Method:-
Using a sterile wire loop, 3-5 well isolated colonies were picked and emulsified in nutrient broth. The prepared turbidity was matched with a turbidity standard (0.5 McFarland) to have an equivalent suspension. Sterile swab was used to inoculate the suspension by streaking on the prepared and dried Mueller Hinton agar plate evenly. It was then allowed to stay for 3-5 minutes. Sterile forceps was used to place the antimicrobial discs on the inoculated plates. Within 30 minutes after applying the disc, the plate was incubated at $35^{O}C$ for 16-18 hours. By using Meter rule on the underside of plate, the diameter of each zone of inhibition was measured in millimeter. Zone diameter for ATCC 25922 was compared with NCCLS Published Limits; Interpretative chart was then used to interpret the zone sizes of Inhibition.
Result was recorded as susceptible, intermediate susceptible, or resistant based on the Zones sizes of each antimicrobial disc used (NCCLS 2003; WHO, 2004; Andrews *et al.,* 2005).

RESULTS
From Table 1, the frequency of salmonellosis was higher in 20-24 months age groups with 20(6.66%) followed by 15-19 (11), 10-14 (7), and 5-9 (3) with (3.66%), (2.33%) and (1.00%), respectively. However, there is no infection among children with age group 0-4 months. In the two sexes, males children were more infected than their female counter parts in the same age groups with 24(8.00%) and 17(5.67%) respectively.

Table 1: Age and sex distribution of Salmonellae isolates among children in Kano metropolis.

Age group (months)	Sex		Total
	Males	Females	
0-4	0(0.0)	0(0.0)	0(0.0)
5-9	2(0.67)	1(0.33)	3(1.00)
10-14	4(1.33)	3(1.00)	7(2.33)
15-19	6(2.49)	5(1.17)	11(3.66)
20-24	12(3.99)	8(2.67)	20(6.66)
Total	**24(8.00)**	**17(5.5)**	**41(13.50)**

From Table 2, the species of *Salmonella species* isolated comprised of *Salmonella typhimurium* (predominant) with 4.33%, followed by *Salmonella typhi* and *Salmonella paratyphi* with 4.00%, and 2.00% respectively. Out of the total number of *Salmonella species* isolated 41(13.67%), twenty four (8.00%) were from males and seventeen (5.67%) were from females patients (males where more infected than females). Also, there is a significant difference between the rate of infection among the two sexes at $P \geq 0.05$ and 3 degree of freedom, because, the calculated chi-square (x^2) value was less than the tabulated value (7.815) in the hospitals studied.

Table 2: the distribution of Salmonellae isolates in children in relation to sex in Kano metropolis.

Salmonellae isolates	Sex		Total
	Males	Females	
Salmonella typhimurium **[23(7.67)]**	13(4.33)	10(3.33)	23(7.67)
Salmonella typhi **[12(4.00)]**	7(2.33)	5(1.67)	12 (4.00)
Salmonella paratyphi **[6(2.00)]**	4 (1.33)	2(0.67)	6(2.00)
TOTAL	**24(8.00)**	**17(5.67)**	**41(13.67)**

Table 3, An *in vitro* – antimicrobial sensitivity testing on these isolates against five antimicrobial agents demonstrated that ciproflaxacin was the most effective agent with 31(34.07%) followed by cotrimoxazole, choloramphenicol, nalidixic acid and ampicillin with 25(27.47%), 18(19.78%), 10(10.99%) and 7(7.69%) susceptibilities respectively.

Table 3: Antimicrobial susceptibility patterns of *Salmonella species* among children in Kano metropolis

ANTIMICROBIALS	SXT	CH	CPX	NA	AMP
POTENCY (µg)	30	30	5	30	10
SALMONELLAE ISOLATES	SUSCEPTIBILITY OF THE ISOLATES TO ANTIMICROBIAL AGENTS				
Salmonella typhimurium **[23(4.33%)]**	15(16.48%)	11(12.09%)	18(19.78%)	5(5.49%)	4(4.39%)
Salmonella typhi **[12 (4.00%)]**	7(7.69%)	5(5.49%)	9(9.89%)	3(3.30%)	3(3.30%)
Salmonella Paratyphi **[26(2.00%)]**	3(3.30%)	2(2.20%)	4(4.39%)	2(2.20%)	0(0.00%)
Total [41(13.67%)]	**25(27.47%)**	**18(19.78%)**	**31(34.07%)**	**10(10.99%)**	**7(7.69%)**

Key: % = Percent, SXT= COTRIMOXAZOLE, CH= CHOLORAMPHENICOL, CPX= CIPROFLOXACIN, NA= NALIDIXIC ACID, AMP= AMPICILLIN

DISCUSSION

Various studies have been conducted by many researchers in different parts of the world establishing the significance of *Salmonella typhimurium*, *Salmonella typhi* and *Salmonella paratyphi* in the causation of salmonellosis. The above three species of *Salmonella* species were encountered in the present study. Among the *Salmonella species* isolated *Salmonella typhimurium* was predominant, followed by *Salmonella typhi* and *Salmonella paratyphi*. Male children were more infected than the female children in the same age group. These agree with the work of Adkins and Santiago (2006); Al-jurayyan (2004) and Ngozi and Onyenekwe (2003). In the study area male children were less protected than their female counter parts. This would probably increase the level of infection among the male children. However, the high prevalence observed in 20-24 months age group at the hospitals studied could be due to inadequate treatment of salmonellosis leading to severe illness or due to the immune state (immunity) of the children screened. In addition, many hormones, growth factors and bioactive substances present in the maternal organs are now known to pass into the colostrums often exceeding concentrations occurring in maternal plasma (Zubairu, 2002). As baby is growing, the concentration of breast milk reduce, also the concentrations of most essential factors reduce (Zubairu, 2002). Change in diet from solely breast feeding to a combination of foods may affect the immune status of the children. Most mothers now-a-days are advised to feed their babies with breast milk only and should not give their babies even water until after the age of 6 months. This could be a reason why children in the age group of 0-4 months were not found to present with salmonellosis in this work (Gallies, 2007). The low infective dose of ten to one hundred (10-100) bacilli only needed to initiate infection makes exposed children easily infected (Gallies, 2007; Kotloff *et al.*, 2005).

Although antibiotic therapy is important in the treatment of Salmonellae, in most endemic countries, antibiotics, such as; ampicillin, trimethoprim-sulphamethoxole and nalidixic acid, have been banned in Asia and sub Saharan Africa (Amyes, 2002).

Not all antimicrobials, at the concentration required to be effective are completely non-toxic to human cells. However, most antimicrobials show sufficient selective toxicity to be of value in the treatment of microbial diseases. In addition, susceptibility testing help on providing guidance and monitor of treatment, narrower the spectrum of its antimicrobial (the more proffered is it's use when one knows specifically the organism being treated), degree of susceptibility of organism can assist in determining the length of therapy (but not the only factor) and choice of cheaper antimicrobial agents with less side effects (Ngozi and Onyenekwe, 2003; Edward and Ewing, 2003).

Conclusion and Recommendations

The children in the older age bracket of 20-24 months were found to be most susceptible to salmonellosis. The risk of infection reduces in lower age group. The *Salmonella typhimurium* remains the prominent causative agent in the population under study and *Salmonella paratyphi* was the least. The infection is more in males than females. Ciprofloxacin was the most effective antimicrobials while ampicillin was the least susceptible antimicrobial agent in the found in the present study. To prevent salmonellosis in children observation of personnel hygiene and environmental sanitation, cleaning hand with soap/detergent immediately after defecation or after washing child's bottom after defecation and Oral hydration should be encouraged also Vaccination\ Immunization for salmonellosis should be given good attention (Barrow *et al.,* 2007).

To reduce this antimicrobial resistance, all suspected cases should be properly diagnosed in well equipped reference laboratories. This will contribute towards successful management of all cases. Laboratories should have the power and responsibility to contribute to the shaping of local policy for prevention and treatment of disease by Community setting.

REFERENCES

Adkins, H.J. and Santiago, L.T (2006). Increased recovery of enteric pathogens by use of both stool and rectal swab specimen .*Journal.of Clinical. Microbiology* **25**:58 –9.

Al – jurayyan, A.M., AI-Rasheedi, N. A. M., Mohammad, N., Alnasser, M.M., Mugeiren, A.T. and Abdullahi, S. A. (2004). Childhood bacterial Diarrhoea in regional hospital in Saudi Arabia. *Journal of Tropical Medicine Hygiene* **97:** 87 – 90.

Amyes, P, G.K., and Gupta, S.P. (2002). *Salmonella* Serotypes in utter pradesh. *Indian.Journal of Medicine* **52**: 235- 240.

Andrews, G.P., Hromockyi, A.E., Coker, C.and Maurelli, A.T (2005). Two novel virulence loci, MxiA and MxiB, in *Shigella flexneri* facilitate excretion of invasion plasmid antigens. *Infecion and.Immunity* **59**: 19-25.

Arora, D.R. (2001). *Text book of Microbiology*. Second edition. New York, United .Kindom. Pp. 294-300.

Barrow, D.K.and Boyd, J.S.K. (2004). Laboratory finding in chemical dysentery. *Journal of Pathology and Bacteriology* **58**: 237-241.

Bello, C.S.S. (2002). *Laboratory manual for students of medical microbiology*. Second edition. Satohgraphics press. Jos Pp. 80-85.

Cheesbrough, M. (2000). *District laboratory practice in tropical countries.* E.C.B.S.Cambridge University Press edition **2**:256-267.

Cheesbrough, M. (2002). *District laboratory practice in tropical countries.* E.C.B.S edition Combridge University Press **2:**97-182.

Cowan, S.T., and steel, K.J. (2002). *Manual for the identification of medical bacteria*. 2nd edition. Cambridge University press Pp. 51 – 120.

Edward, P.R. and Ewing, W.H. (2003). *Identification of Enterobacteriaceace.* 3rd edition, Burges, Minneapolis Pp. 10 – 15.

FDA/CFSAN, (2009): food safety A to Z Reference Guide – Salmonella (http:// www. Cfsan.fda.gov/dms.) (4/24/2009).

Gallies, R.R. (2007):- *Lecture notes on Medical Microbiology*; 2nd edition Black well scientific publication. Churchill living stone, New York P.p 114.

Kotloff, K., Winkoff, J.P., Invaoff, B., Clemens, J.D., and Swed, W.W.O. (2005). Global burden of *Shigella* infections. *Implication for vaccine development and implementation.* WHO **77 (8)**: 650 – 655

National Committee for Clinical Laboratory Standard (NCCLS) (2003). Diagnostics and treatment of serious antimicrobial resistance of *Shigella species. Infectious Diseases* **IV (4)**:183-200.

Ngozi, F. and Onyenekwe, B.C. (2003). Enteropathogens in food handlers, Enugu, Nigeria. *Nigerian Medical Practice* **Vol. 25 (6)**:90-95.

Perilla, M. J. (2003). *Manual for the laboratory identification and antimicrobial testing of bacterial pathogens of public health importance in the developing world*. World health organization. Atlanta Georges U.S.A Pp. 133 – 284.

WHO (2004). *Manual for laboratory investigations of acute enteric infections.* WHO. CDO/83.

Zubairu, S. M. (2002). Paediatric articles on Breast Feeding. *Journal of National Issue of Muslim Health Student*s. The healing message Pp 23-25.