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(RESEARCH ARTICLE)

Characterization of multi-drug resistant *Salmonella typhi* from clinical specimens

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Abstract

Characterization of multidrug resistant *Salmonella typhi* from clinical specimens was investigated. Three hundred and sixty-four (364) clinical specimens of both stool and blood were collected from patients attending selected health facilities in Bauchi metropolis. The specimens were cultured using standard media, biochemical as well as serological tests for somatic H and O antigens were used to identify isolates. Antibiotics susceptibility test was performed using standard disk diffusion method, the isolates were screened for Multidrug resistance. The total of 9(2.4%) of the 364 specimens collected yielded positive for *Salmonella typhi*. of the Nine (9) positive *Salmonella typhi* 5(55.5%) were from females and 4(44.4%) from males. Typhoid fever was most frequent in children 5(55.5%) than in adults. There was no significant difference in isolates from the different health facilities with their p values >0.05. All isolates screened were resistant to more than three (3) antibiotics, of the total isolates, 88.8% were found to be resistant to ampicillin, 77.7% resistant to cotrimoxazole, and 88.8% resistant to chloramphenicol. However, resistance to ciprofloxacin by the isolates were not found. Therefore, ciprofloxacin remains the drug of choice for severe cases of typhoid fever, although caution should be exercised by clinicians in their prescription such that fluoroquinolones antibiotics therapy is used only in laboratory-proven cases of the disease and *Salmonella*-associated bacteremia to preserve its efficacy. To prevent occurrence of typhoid fever, good personal hygiene, provision of portable drinking water and environmental sanitation should be employed.

Keywords: *Salmonella typhi*; Antibiotics; Multi-drug resistance; Somatic Susceptibility; Blood; Stool

1. Introduction

In recent past, emergence of ever-increasing number of antibiotic resistant microbial strains has become a severe health threat to human-kind and one of the biggest challenges to global drug discovery programs [1]. The inappropriate and over-use of antibiotics and other antimicrobials to treat infections and consequent antibiotic selection pressure are thought to be the major causative factors contributing to the appearance of strains with reduced susceptibility to antibiotics [2].

Typhoid fever is largely a disease of developing nations due to their poor standard of hygiene and unavailability of clean water. It is transmitted faeco-orally through contaminated food and water. *Salmonella*, a primary inhabitant of the gastrointestinal tract, is recognized as one of the most common causes of diarrhea infections worldwide, resulting in millions of infection and significant human death annually [3]. People most at risk for serious complications due to *Salmonella* infections include older adults, pregnant women, infants, children, and people who have compromised immune systems [4].

Multidrug-resistant typhoid fever (MDRTF) is defined as typhoid fever caused by *Salmonella typhi* strains which are resistant to all the three first-line recommended drugs for treatment *i.e.* chloramphenicol; ampicillin and cotrimoxazole

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[5]. Although the global burden of typhoid fever has reduced, emergence of multidrug- resistant *Salmonella typhi* (MDRST) is still a threat to public health. Despite the emergence of newer antibacterial drugs, enteric fever has continued to be a major health problem [5]. *Salmonella typhi* gained resistance to antibiotics like ampicillin, ceftriaxone, and cotrimoxazole, besides developing resistance to efficacious drugs like ciprofloxacin. The aim of this study is to characterize multidrug resistance *Samonella typhi* from clinical specimen in selected health facilities.

2. Material and methods

2.1. Specimen collection

A total of 364 blood and stool samples was collected from the study areas to determine the presence of Salmonellae pathogens. Fluid Tetrathionate (8 ml) broth in Macartney bottles were used to collect 2ml of venous blood. About 1gram of stool sample was dispensed in nine (9 ml) of Selenite broth F (PART I and PART II) were used for pre enrichment and incubation was done at 370C for 48 hours and 24 hours respectively Turbidity was observed for both.

2.2. Isolation of *Salmonella typhi* from stool specimen

About 1 gram of faecal specimen was placed in Selenite F broth (Oxoid Limited, Hampshire, England) to allow for the multiplication of bacteria and incubated for 24 hrs at 37 °C, then sub cultured onto Salmonella-Shigella Agar, MacConkey agar and Brilliant Green agar, it was then incubated for 24 hrs at 37 °C. Typical colonies of Salmonella appear as pink colonies with or without black centers. Many cultures of *Salmonella* may produce colonies with large, glossy black centers or may appear as almost completely black colonies. The representative *Salmonella* colonies were characterized morphologically using Gram' staining method according standard protocols [6].

2.3. Isolation of *Salmonella typhi* from blood specimen

About 2 ml of venous blood will be drawn aseptically from each patient by cleaning the skin using tincture of iodine, and placed into 8 ml of tetrathionate broth (Oxoid Limited, Hampshire, England). Blood culture broths were incubated at 37 ^{o}C and checked for signs of bacterial growth daily for up to ten days. Bottles which showed signs of growth were sub-cultured onto Salmonella-Shigella Agar, Brilliant Green agar and MacConkey agar. Blood culture broths with no bacterial growth after seven days were also subcultured before being reported as Negative.

2.4. Biochemical screening

Biochemical test such as Sugar Fermentation, Urease, Hydrogen Sulphide production were carried out using standard procedures.

2.5. Serotyping of identified Salmonella species

Colonies considered to be of *Salmonella* spp. were further tested for somatic (O) and flagella (H) antigens with polyvalent antisera (OXOID), [6], [7].

2.6. Antimicrobial sensitivity pattern and multi-drug resistance screening

The susceptibility testing was carried out by disc diffusion method using Mueller Hinton agar and it was tested *in vitro* for susceptibility to the following antibiotics (OXOID Ltd., UK) suggested by WHO,2010, ampicillin (A,10 µg), amoxicillin (AMC,30 µg), augmentin (AUG,15 µg), amikacin (AK, 10 µg), ceftriaxone (CFX,30 µg), ceftazidine (CTX, 30 µg),ciprofloxacin (CFX, 10 µg), ,neomycin (N, 30 µg), erythromycin (E, 25 µg),chloramphenicol (C, 30 µg), naliixic acid (NA, 30µg), and imipenem (I,10 µg) this is based on [6]

This was conducted using the method of [7]. 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 were used to place the antimicrobial discs on the inoculated plates. Within 30 minutes after applying the disc, the plates were incubated at 35^{0}C for 16-18 hours. 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 [6], [2].

3. Results and discussion

Table 1 Distribution of specimens collected and percentage of *Salmonella typhi* isolated from blood and stool according to different health facilities in the study area

Health facilities	Number of specimens collected		*Salmonella typhi* isolated (%)		P value (x^2)
	Blood	Stool	Blood	Stool	0.86
ATBUTH	72	37	02(2.7)	01(2.7)	
SHB	55	48	01(1.8)	01(2.0)	
TBC	83	69	01(1.2)	03(4.3)	
Total	210	154	04(1.9)	05(3.2)	

Key: ATBUTH- Abubakar Tafawa Balewa University Teaching Hospital, SHB- Specialist Hospital Bauchi, TBC- Tashan Babiye Clinic, (%)- P

Table 2 Gender and patient group distribution pattern of specimens collected and *S. typhi* isolated in the study area

	Number of specimens collected	Number of *S. typhi* isolated (%)
Sex		
Male	191	5 (2.6)
Female	173	4 (2.3)
Group		
In patient	107	2 (1.8)
Out patient	257	7 (2.7)
Total	364	9 (2.47)

P value (x^2) > 0.85

Table 1 indicates the distribution of specimens and percentages of *Salmonella typhi from* blood and stool in different health facilities. The table shows that there was no significant difference (p>0.05) between blood and stool samples. Stool had the highest number of positive samples 5(55.5%) and blood had the least 4(44.4%). The isolation of *S. typhi* from stools and bloods is suggestive of typhoid fever. Widal test which is a serological test is the most widely requested by clinicians. The test has only moderate sensitivity and specificity. It can be negative in up to 30% of culture-proven cases of typhoid fever. This may be because of prior antibiotic therapy that has blunted the antibody response. On the other hand, *S. typhi* shares O and H antigens with other *Salmonella* serotypes and has cross-reacting epitopes with other Enterobacteriacae, and this can lead to false-positive results. Hence, the gold standard for *Salmonella typhi* identification is in blood and stool cultures [2].

Table 3 Frequency of occurrence of *Salmonella typhi* across age group and selected health facilities in the study area

AGE (years)	ATBUTH	Isolates (%)	SHB	Isolates (%)	TBC	Isolates (%)	Total	Isolates (%)	P value (x^2)
0-9	31	2(6.4)	28	1(3.5)	37	2(5.4)	96	5(5.2)	0.92
10-19	17	0(0)	13	0(0)	21	0(0)	51	0(0)	
20-29	15	0(0)	17	1(5.8)	25	1(4.0)	57	2(3.5)	
29-39	19	1(5.2)	18	0(0)	24	1(4.1)	61	2(3.2)	
40-49	9	0(0)	10	0(0)	19	0(0)	38	0(0)	
50-59	7	0(0)	8	0(0)	16	0(0)	31	0(0)	
60-69	8	0(0)	9	0(0)	5	0(0)	22	0(0)	
>70	3	0(0)	0	0(0)	5	0(0)	8	0(0)	

ATBUTH- Abubakar Tafawa Balewa Teaching Hospital, SHB- Specialist Hospital Bauchi, TBC- Tashan Babiye Clinic and (%)- Percentage

This present study indicates that Females had the highest percentage (55.5%) while males had the least (44.4%) Table 2. This is in agreement with report of [8] which could be attributed to the fact that females are closer to edible items than their male counterparts. It also indicated that outpatients (77.7%) had the most percentage of *Salmonella typhi* isolated than the in patients (22.2%). The frequency of occurrence of *S. typhi* across age group in selected health facilities is represented in table 3. Age group 0-9 had the highest frequency of occurrence and age group 60-69 had the least. There was no significant difference between the age group and the number of isolates as p>0.05. [9] showed that prevalence of typhoid fever in endemic areas is considered high in n school aged children and young adults. Older adults are presumably relatively resistant due to frequent boosting of immunity.

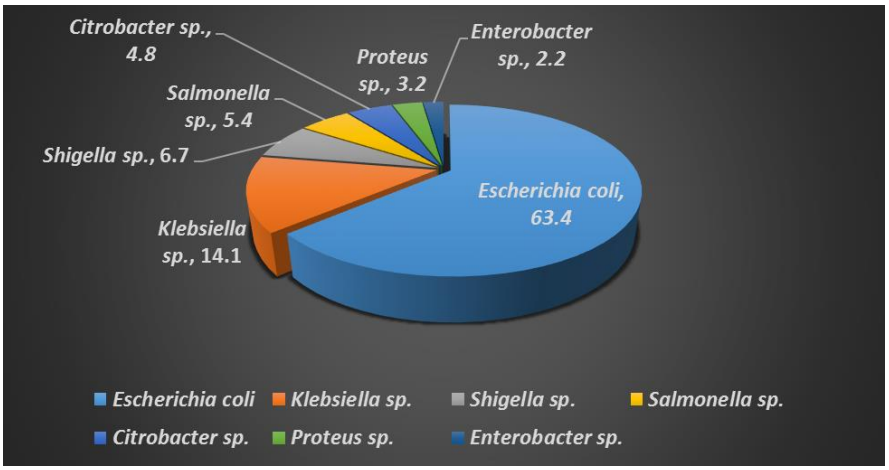

Figure 1 Frequency of occurrence of different isolates

Figure 1 shows frequency of different enteric bacteria isolates with *Escherichia coli* having the highest occurrence at 63.4% and the least was *Enterobacter* at 2.2%. *S. typhi* was found to be 5.5% out of the total number of samples collected.

Table 4 Antibiotics susceptibility pattern of *S typhi* isolated from the study area

Antibiotics	Disc potency (µg)	Number (%) of sensitive	Number (%) of resistant	P value (x^2)
Amoxicillin	25	2(22.2)	7 (77.7)	0.62
Ampicillin	10	1(11.1)	8 (88.8)	
Augmentin	30	0(0)	9 (100)	
Amikacin	30	0(0)	9(100)	
Ceftriaxone	30	2(22.2)	7(77.7)	
Cotrimoxazole	25	1(11.1)	8(88.8)	
Ceftazidine	30	0(0)	9(100)	
Chloramphenicol	30	1(11.1)	8(88.8)	
Ciprofloxacin	5	9(100)	0(0)	
Erythromycin	25	0(0)	9(100)	
Imipenen	10	0(0)	9(100)	
Nalidixic Acid	5	1(11.1)	8(88.8)	

Resistance to ampicillin, chloramphenicol, cotrimoxazole and ciprofloxacin was observed in this study, the resistance could be as a result of indiscriminate use of antibiotics. The inclusion of preventive doses of antimicrobial agents in poultry feed as growth promoters is often associated with the development of resistance in enteric bacteria. During this study, of the total isolates, 88.8% were found to be resistant to Ampicillin, 77.7% resistant to Cotrimoxazole, and 88.8% resistant to Chloramphenicol. However, resistance to Ciprofloxacin by the isolates were not found. The use of antimicrobials for growth-promotion, prophylaxis and treatment of animal of animal's food increases the prevalence of resistance in human pathogens. High susceptibility of *Salmonella typhi* was observed against fluoroquinolones (ciprofloxacin). This finding is similar to a report by [10]. However, in Lagos, Nigeria, [11] reported 18% reduced susceptibility of *Salmonella* spp. To ofloxacin and ciprofloxacin. The high susceptibility of enteric bacteria to the

fluoroquilones recorded in this study may be connected to relatively high cost of ciprofloxacin [11]. Therefore, fluoroquinolones are not used indiscriminately because not many could afford them.

4. Conclusion

Multidrug resistant *Salmonella typhi* (MDR) is progressively increasing compared to earlier reports in the country. Children account for the highest percentage of *Salmonella typhi* isolated from the study group. The high incidence rate suggest that challenges of portable water availability exist in the community. Apart from contaminated water, faecal-oral route of transmission may also be a reason for the preponderance in children of *Salmonella* infection in children. Resistance to first line antibiotics (Ampicillin, Chloramphenicol and Cotrimozaxole) suggest possible abuse of drugs and consequent high resistance in the community. Resistance to Ciprofloxacin was not recorded which may be due to less frequent use in infection treatment because of their costs. Ciprofloxacin still remain the drug of choice but caution should be taken by clinicians to avoid resistance of *Salmonella typhi* to the antibiotic.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

There's no what so ever any conflict of interest we are giving the outfit full right to publication of this work.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

References

[1] Alanis AD, Calzada F, Cervantes JA, Torres J and Ceballos GM. (2005). Alanis, A. D., Calzada, F., Cervantes, J. A., Torres, J., & Ceballos, G. M. (2005). Antibacterial properties of some plants used in Mexican traditional medicine for the treatment of gastrointestinal disorders. Journal of Ethnopharmacology, 100(1-2), 153-157.

[2] World Health Organization, (WHO). (2011). Economic cost of typhoid fever. Salmonella control program report. http://who.typhoid-fever/accessed on 1-5-2012.

[3] Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL and Griffin PM. (2011). Foodborne illness acquired in the United States—major pathogens. Emerging infectious diseases, 17(1), 7.

[4] Dworkin RH, Nagasako EM, Johnson RW and Griffin DR. (2001). Acute pain in herpes zoster: the famciclovir database project. Pain, 94(1), 113-119.

[5] Zaki SA and Karande S. (2011). Multidrug-resistant typhoid fever: a review. The Journal of Infection in Developing Countries, 5(05), 324-337.

[6] Cheesbrough M. (2006). Salmonella species. In District Laboratory Practice in Tropical Countries. Low-priced ed. Cambridge, 182-186.

[7] Perilla MJ, Bopp C, Elliott J, Facklam R, Popovic T, Wells J and World Health Organization. (2003). Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world: *Haemophilus influenzae*, *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Neisseria gonorrhoea*, *Salmonella serotype Typhi*, *Shigella*, and *Vibrio cholerae*.

[8] Ogunbiyi TA and Onabowale BO. (1997). Typhoid enteritis in Lagos, Nigeria. Nigerian Med J, 6, 505-11.

[9] Parry CM. (2005). Epidemiologalcal and clinical aspects of human typhoid fever: Insalnonelly Infection: Clinicas, ed. Mastroen: P, Maskell D. Cambridge University Press, 1-10.

[10] Compus J and Macnab RM. (1990). Flagellar assembly in *Salmonella typhimurium*: analysis with temperature-sensitive mutants. Journal of bacteriology, 172(3), 1327-1339.

[11] Akinyemi KO, Bamiro BS and Coker AO. (2007). Salmonellosis in Lagos, Nigeria: incidence of Plasmodium falciparum-associated co-infection, patterns of antimicrobial resistance, and emergence of reduced susceptibility to fluoroquinolones. Journal of Health, Population and Nutrition, 351-358.

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