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Burden of Enteric Fever and Antibiotic Sensitivity in Nepalese Children Prior to Typhoid Vaccine in National Immunization Program

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ABSTRACT

Background: Enteric fever is a major public health problem in developing and under developed countries. Case fatality rate without treatment is 10-30% and with appropriate treatment is only 1-4%. Gold standard for diagnosis is isolation of Salmonella enterica from blood or bone marrow. Antibiotics resistance is skyrocketing with emergence of multidrug resistance S. typhi and extensively drug resistant S. typhi.

Methods: The blood culture done in Kanti children hospital in last six years were taken from the data base and the culture positive cases were taken from which the salmonella species positive cases along with the drug sensitivity pattern were used in our study.

Results: The culture positivity rate was 2.8% and 7.6% (n=136) among the culture positive cases were Salmonella species. Salmonella typhi (121; 88.9%) was the most frequently isolated species, followed by Salmonella paratyphi A (13; 9.5%) and Salmonella paratyphi B (2;1.4%). Children with age 5-10 years was the most affected age group for infection with Salmonella, 50.0% (n=68). Nalidixic acid is resistant in 89.9% Salmonella typhi; followed by ciprofloxacin (31.8%), ofloxacin (18.2%), ampicillin (9.6%), azithromycin (8.4%), chloramphenicol (8.2%), cotrimoxazole (5.4%), cefixime (4%), ceftriaxone (2.5%) and cefotaxime (0.0%). Cefixime, ceftriaxone, cefotaxime are 100% sensitive to Salmonella paratyphi, followed by cotrimoxazole (92.9%), ofloxacin (81.8%), chloramphenicol (75%), azithromycin (66.7%), ampicillin (60%), ciprofloxacin (50%) and Nalidixic acid (23.1%).

Conclusions: Salmonella species culture isolatation are declining every year. Fluoroquinolones have more resistance than first line drugs of typhoid, azithromycin resistance is rising but 3rd generation cephalosporins are sensitive to Salmonella species.

Keywords: Drug sensitivity; enteric fever; salmonella paratyphi; salmonella typhi; typhoid vaccine

INTRODUCTION

Enteric fever is a major public health problem in developing and underdeveloped countries with estimated 21.6 million cases and 250,000 deaths annually worldwide.[1-3] The case fatality rate of enteric fever without treatment is 10-30% and with appropriate treatment is 1-4%.[2,4] Caused by Salmonella enterica serovar typhi, parathyphi A, B and C; enteric fever can cause acute and sometime life-threatening systemic febrile illness.[5,6]

The gold standard for the diagnosis of enteric fever is isolation of S. enterica from blood or bone marrow.[7] The rate of gaining resistance to antibiotics is skyrocketing evidenced with emergence of multidrug resistance S. typhi in late 1980s and extensively drug resistant including azithromycin resistant S. typhi in recent days.[7-9] Typhoid conjugate vaccine (TCV) was introduced in routine immunization of Nepal since April 2022.[10]

This study aims to know the positivity of Salmonella in blood culture, its yearly incidence pattern, and drug sensitivity pattern in last 6 years.

METHODS

The ethical committee clearance was taken from IRC

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of Kanti Children's Hospital (IRC ref no. 907). This retrospective descriptive study was possible with the help of secondary data that was preserved on the culture sensitivity pattern recorded in register of microbiology section of pathology department of Kanti Children's Hospital. The blood culture was done by both bactec and conventional method where the blood sample was taken by using standard collecting technique from the collecting center of Kanti children hospital and the culture sensitivity record from April 2016 to March 2022 was reviewed. The desired variables like age, sex, season, years, and organism isolated and sensitive/ resistant drugs were encrypted. The patient's identity was anonymized. Those culture sensitivity records were collected by MS Excel which later transcribed to statistical software IBM SPSS 20.0 and analyzed further. The frequency table was prepared and the percentage of different variables was calculated individually.

RESULTS

Retrospectively analyzing the data of past 6 years, the culture positivity rate was 2.8% (1778 positive case among 62,643 samples sent for blood culture) and 7.6% (n=136) among the culture positive cases were Salmonella species in overall. Among the total 136 Salmonella positive cases, 56.6% (n=77) were male, 43.4% (n= 59) were female. The children in age group less than 5 years were 28.6% (n=39), 5-10 years were 50% (n=68), more than 10 years were 21.4% (n=29). Number of blood culture requisition, blood culture positivity rate and proportion of Salmonella typhi in yearly pattern is presented in the figure 1.

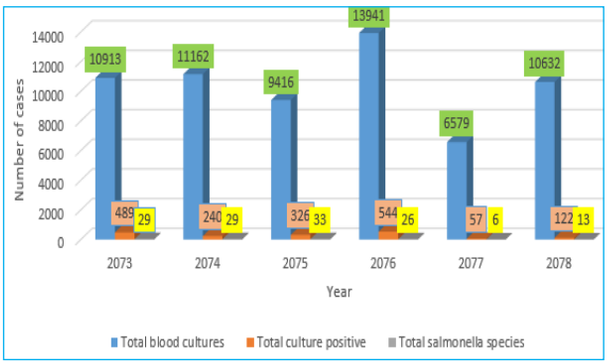

Figure1. Year wise culture positivity and isolation of Salmonella species.

We looked the positive salmonella and the sensitivity pattern of antibiotics and burden of different Salmonella species isolates over past 6 years beginning from April 2016 to March 2022. A total of 136 Salmonella species were isolated over the duration of 6 years among them Salmonella typhi (121; 88.9%) was the most frequently isolated species, followed by Salmonella paratyphi A (13; 9.5%) and Salmonella paratyphi B (2;1.4%). The yearly pattern of isolation of different Salmonella species is presented on bar diagram in figure 2.

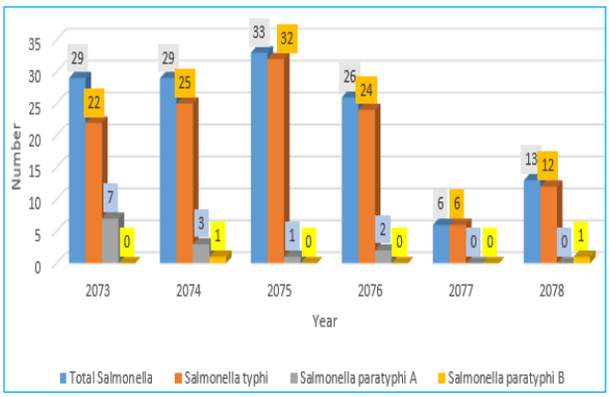

Figure 2. Yearly pattern of isolation of different Salmonella species.

Children with age 5-10 years was the most vulnerable age group for infection with Salmonella species accounting 50.0% (n=68) while the prevalence among age group <5 years and ≥10 years was comparable (28.6% vs 21.3%). which is shown in figure 3. In all age group Salmonella typhi was the most common isolate of Salmonella species.

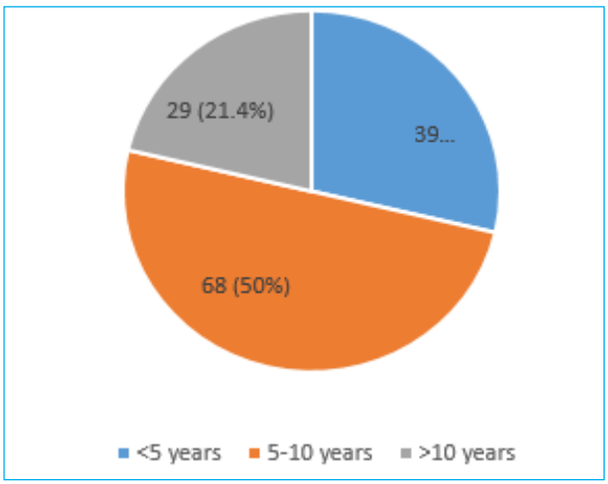

Figure 3. Age wise prevalence of Salmonella typhi.

Most of the cases were isolated during spring season (March- May) 39.7% (n=54) followed by Summer (June-August) 26.4% (n= 36). Winter season (December-February) has slight higher number of isolates 20.6% (n=28) compared to autumn (September- November) 13.2% (n=18).

Boys were slightly more affected 56.6 % (n=77) compared to girls 43.4 % (n= 59) by Salmonella species in overall although small proportion contributing (1.5%; n=2)

Salmonella paratyphi B affects the girls only.

Nalidixic acid is ineffective to kill 89.9% Salmonella typhi; followed by ciprofloxacin (31.8%), ofloxacin (18.2%), ampicillin (9.6%), azithromycin (8.4%), chloramphenicol (8.2%), cotrimoxazole (5.4%), cefixime (4%), ceftriaxone (2.5%) while cefotaxime had 100% sensitivity for isolates. The drug sensitivity pattern of Salmonella typhi is presented on table 1.

Sensitivity testing for Azithromycin was found since the year 2019/20 only. Since then, sensitivity testing was done in 36 isolates of Salmonella typhi (80% of the isolates since the year 2019/20) among which 91.6% (n=33) were sensitive and 8.4% (n=3) were resistant.

Cefixime, ceftriaxone, cefotaxime are 100% sensitive to Salmonella paratyphi, followed by cotrimoxazole (92.9%), ofloxacin (81.8%), chloramphenicol (75%), azithromycin (66.7%), ampicillin (60%), ciprofloxacin (50%) and Nalidixic acid (23.1%). This pattern is shown in table 2.

Table1. Drug sensitivity pattern of Salmonella typhi.

Salmonella typhi (n=121)				
Common antibiotics	Sensitive n(%)	Resistant n(%)	Inter-mediate n(%)	Total tested n(%)
Azithro	33 (91.6)	3 (8.4)	0 (0.0)	36 (29.7)
Ampicillin	63 (86.3)	7 (9.6)	3 (4.1)	73 (60.3)
Cotrimoxazole	106 (94.6)	6 (5.4)	0 (0.0)	112 (92.6)
Ciprofloxacin	65 (60.7)	34 (31.8)	8 (7.5)	107 (88.4)
Ofloxacin	89 (80.9)	20 (18.2)	1 (0.9)	110 (90.9)
Cefixime	95 (95.0)	4 (4.0)	1 (1.0)	100 (82.6)
Ceftriaxone	78 (97.5)	2 (2.5)	0 (0.0)	80 (66.1)
Cefotaxime	13 (100.0)	0 (0.0)	0 (0.0)	13 (10.7)
Chloramphenicol	44 (89.8)	4 (8.2)	1 (2.0)	49 (40.5)
Nalidixic acid	7 (10.1)	62 (89.9)	0 (0.0)	69 (57.0)

Table 2. Drug sensitivity pattern of Salmonella paratyphi.

Salmonella paratyphi (n=15)				
Antibiotics	Sensitive n (%)	Resistant n (%)	Intermediate n (%)	Total tested n (%)
Azithro	2 (66.7)	1 (33.3)	0 (0.0)	3 (20.0)
Ampicillin	6 (60.0)	3 (30.0)	1 (10.0)	10 ((66.7)
Cotrimoxazole	13 (92.9)	1 (7.1)	0 (0.0)	14 (93.3)
Ciprofloxacin	7 (50.0)	6 (42.9)	1 (7.1)	14 (93.3)
Ofloxacin	9 (81.8)	1 (9.1)	1 (9.1)	11 (73.3)
Cefixime	12 (100.0)	0 (0.0)	0 (0.0)	12 (80.0)
Ceftriaxone	9 (100.0)	0 (0.0)	0 (0.0)	9 (60.0)
Cefotaxime	1 (100.0)	0 (0.0)	0 (0.0)	1 (6.7)
Chloramphenicol	3 (75.0)	0 (0.0)	1 (25.0)	4 (26.7)
Nalidixic acid	3 (23.1)	10 (76.9)	0 (0.0)	13 (86.7)

There was no any specific pattern (increasing and decreasing) in sensitivity or resistance of specific drugs used in treatment of Salmonella over the course of time from 2016/17 to 2021/22.

DISCUSSION

In this study, Salmonella typhi (121; 88.9%) was the most frequently isolated species, followed by Salmonella paratyphi A (13; 9.5%) and Salmonella paratyphi B (2;1.4%). Prajapati et al. reported 235 isolates of Salmonella species, out of which 83% (n=195) were Salmonella typhi and 17% (n=40) were Salmonella paratyphi A[11]. Rai GK et al. found only 60 Salmonella species among them 72% (n=43) were Salmonella typhi and 28% (n=17) were Salmonella paratyphi A.[12] Maharjan et al. isolated 40 salmonella species among which 29 (72.5%) isolates were S. Typhi and 11 (27.5%) isolates were S. Paratyphi A.[13] and Subedi et al. in 2020 with data of 2 years recovered 104 isolates of *Salmonella enterica*,

of which *Salmonella enterica* serovar Typhi were 77.9%, while *Salmonella enterica* serovars Paratyphi A and B were 21.1% and 1% respectively.[14] Fida et al. in 2019, presented 52 cases of Salmonella among them 90.4% (n=47) were Salmonella typhi and 9.6% (n=5) were Salmonella paratyphi.[15] Contrarily, Kirtika Gautam isolated 200 salmonella species out of the isolates 90% were Salmonella typhi and 10% were Salmonella paratyphi. [16]

Though some recent studies showed high number of Salmonella cases, the overall the prevalence of Salmonella species infection in children is falling and this falling trend is consistent with our study too. This falling trend could be explained by increased awareness of sanitation, healthy eating behavior, piloting immunization campaign for typhoid, access of typhoid vaccine to affording parents as well as use of antibiotics prior to the collection of blood for culture and sensitivity.

Among the isolates of Salmonella species, typhi is more prevalent than paratyphi (88.9% vs 11.1%) which is supported by Prajapati et al.[11] (83% vs 17%), Rai GK et al.[12](72% vs 28%), Ali et al.[17] (78.7% vs 21.3%)[17], Fida et al.[15] (90.4% vs 9.6%). A different prevalent pattern was shown by Ohanu et al[18] in adult population with 63.2% (n=72) of Salmonella paratyphi and 36.8% (n=42) of Salmonella typhi among 114 typhoid species isolated blood cultures.

Children with age 5-10 years was the most vulnerable age group for infection with Salmonella species accounting 50.0% (n=68) which is a comparable finding of Sharma AK et. al[19], Rai GK et. al[12]and contradicts with Joshi et al.[20] which favors >10 years and Prajapati et al.[11] which favors the under 7 years. The age group 5-10 years are poor in immunity against typhoid but more vulnerable to contaminated foods and water.

Most of the cases were isolated during spring season 39.7% (n=54) followed by Summer 26.4% (n= 36). Winter season has slight higher number of isolates 20.6% (n=28) compared to autumn 13.2% (n=18). There is more chances of contamination of food and water in rainy season and flies which are the media of spread of disease are less abundant in winter.

Boys were slightly more affected 56.6 % (n=77) compared to girls 43.4 % (n= 59) by Salmonella species in overall probably due to nature of entertaining out door plays by boys as this makes them vulnerable to be in contact with contaminated water and waste.

The first line therapeutic drugs for enteric fever viz. ampicillin, cotrimoxazole, and chloramphenicol were found to be sensitive in 86.3% (n=63), 94.6% (n=106), 89.8% (n=44) Salmonella typhi isolates. This is way better scenario compared to Ali et al.[17] which demonstrated multidrug resistant Salmonella typhi and paratyphi in 37.5% (n=74) of overall isolates. Fida et al.[15] showing 7.7% (n=4) sensitive to first line antibiotics for enteric fever (non-resistant), 21.2% (n=11) were multidrug resistant, 71.2% (n=37 were extensive drug resistant). Siddiqui et al.[21] demonstrated 77.7% Salmonella isolates being resistant to ampicillin and 11.1% to cotrimoxazole and 100% typhoidal Salmonella enterica being sensitive to chloramphenicol, ceftriaxone, cefixime, nalidixic acid and ofloxacin. The sensitivity pattern of first line drugs of enteric fever of this study is similar to Prajapati et al.[11] who had looked on isolates that are highly sensitive to cotrimoxazole (93.5%), chloramphenicol (93.2%) but lacks the data on ampicillin. Joshi et al.[20] had even better results demonstrating no resistance to cotrimoxazole and chloramphenicol. The sensitivity pattern of Rai GK et al.[12] had mixed picture on first line typhoid drugs as they found 100% resistant strain for ampicillin, strain with low resistance for chloramphenicol (2.4%) and 100% sensitive strain for cotrimoxazole. Sharma AK et al.'s[19] finding were also close to current study with resistance to cotrimoxazole and chloramphenicol being 5.8% and 6.1% respectively.

Quinolones and fluoroquinolones though being second line drugs for typhoid have disappointing sensitivity pattern as Nalidixic acid is ineffective to kill 89.9% Salmonella typhi; followed by ciprofloxacin (31.8%) and ofloxacin (18.2%). These findings are somehow comparable to Rai GK et al.[12] that had comparable but slight high ciprofloxacin resistance (46.4%) but low nalidixic acid (59.4%) and ofloxacin (2.6%) resistance. But Prajapati et al.[11] findings on fluoroquinolones sensitivity was somehow reassuring and contrast from the current study as they found sensitivity of ofloxacin (93.5%), ciprofloxacin (86.6%). Sharma AK et al.[19] findings were comparable to Prajapati et al.[11] with resistance of both ciprofloxacin and ofloxacin being 14%. Joshi et al.[20] had even better findings on fluoroquinolones with 12.5% ciprofloxacin resistant and 5.0% ofloxacin resistant Salmonella species but resistance with Nalidixic acid was massive (57.5%). Ohanu et al.[18] showed 100% sensitivity with ciprofloxacin and levofloxacin. Maharjan et. al in 2019 showed 12.5% susceptibility to nalidixic acid 15% susceptibility to ofloxacin, 20% susceptibility to levofloxacin, respectively, 0.0% ciprofloxacin.[13] Subedi et. al explored high resistance of Salmonella species towards Nalidixic acid (97.1%) and Ciprofloxacin

(91.3%).[14]

Third generation cephalosporin were promising agents for treatment of enteric fever evidenced by low resistance with cefixime (4%), ceftriaxone (2.5%) and cefotaxime (0.0%). Similar high sensitivity was explored by Rai GK et al.[12] with 100% sensitive to ceftriaxone and cefotaxime but sensitivity testing with cefixime was disappointing as 19% had resistance with this particular drug. Prajapati et al.[11] didn't look for cefixime sensitivity but the sensitivity with ceftriaxone (98.6%) and cefotaxime (100%) was quite impressive. Finding of Sharma AK et al.[19] was very close to Prajapati et al demonstrating 1.4% resistance with ceftriaxone and no resistance with cefotaxime but they also lack to see the sensitivity pattern of cefixime. Joshi et al.[20] didn't found any resistance against cefixime, ceftriaxone and cefotaxime in their study. Ohanu et al.[18] had isolates with 95.6% sensitivity with ceftriaxone, Deksissa et al.[22] reported up to 80% resistance to cefotaxime.

This retrospective study was done using the secondary data retrieved from microbiology lab record register. Volume of blood sent for culture might not be unanimous as it is beyond the scope of microbiology lab technologist/technician. Antibiotic disc supply from hospital logistics had been breached on several occasion limiting the lab technologist to give sensitivity reports without testing the mandatory minimum disc for Salmonella.

CONCLUSIONS

The number of Salmonella species culture isolatation is declining every year. Salmonella paratyphi isolation rate is even falling. Not having adequate volume of blood sample and children already being in some sorts of antibiotics could have contributed in low yield in culture positive rate. Sensitivity of fluoroquinolones category second line typhoid drugs have more resistance than first line. However, 3rd generation cephalosporins are promising in killing Salmonella. Azithromycin resistant species are also abundant in recent years.

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CONFLICT OF INTEREST

None

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