Studies on Extended Spectrum Beta Lactamase (ESBL) producing *salmonella* isolates from clinical samples of Nepal

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ABSTRACT

Present study was carried out among the patients of age 2-60 years from November 2009 to November 2011 to assess the production of Extended Spectrum Beta Lactamase (ESBL) by salmonella enterica serotype (*Typhi and paratyphi A*) at Kanti Children's Hospital (KCH), Nepal Medical College (NMC), Kathmandu Medical College (KMC),National Public Health Laboratory (NPHL) and Sankata Laboratory (SKL) of Nepal. Blood cultures were obtained from 4,820 patients with febrile illnesses. 400 strains of salmonella enterica were isolated. Antibiotic susceptibility testing was carried out using disk diffusion method by Kirby-Bauer technique and ESBL screening were done by Double Disk Synergy Test (DDST), following the Clinical and Laboratory Standard Institute (CLSI) recommendations for Escherichia coli. Male had higher infection (56%) than female (44%). Highest number of culture positive cases were (37%) in 10-19 years of age groups. Highest episodes of enteric fever cases occurred during April- June (45%) followed by July-September (35%).Among the total isolates 286(72%) were Multidrug resistanc(MDR). All the Multidrugs resistance salmonella, resistant to third generation Cephlospornis were ESBL producers.

Keywords: Multidrug resistance (MDR), Extended spectrum beta- lactamase (ESBL), *Typhi and Paratyphi A*.

INTRODUCTION

Enteric fever continues to be a major health problem in under-developed countries including South Asian nations. It afflicts local inhabitants as well as travelers to endemic areas. Increasing multidrug resistance in Salmonella enterica (S.enterica) serotype Typhi has been reported from various parts of the world.[1-4] Similarly, the causative agent a less severe variety of enteric fever, S.enterica serotype *paratyphi A,* has also been reported to have developed resistance to multiple antibiotics.[5] The World Health Organization (WHO) has estimated that annually typhoid fever accounts for 21.7 million illnesses (217,000deaths) and paratyphoid fever accounts for 5.4 million of these cases. The typhoidal salmonella, such as *Salmonella typhi (S.t.)* and *Salmonella paratyphi (S.p.)* only colonize human and are usually acquired by the consumption of food or water contaminated with human faecal material.

Enteric fever is endemic in Nepal. S. enterica serotype *Typhi* and S. enterica serotype *paratyphi A* have been reported as the most common culture isolates from patients with febrile illnesses during hospital admission.[7,8] Over the past decade, increasing antibiotic resistance in Salmonella has lead to shift in the antibiotics used against this organism from Chloramphenicol and ampicillin to trimethoprim-sulfamethoxazole, fluroquinolones (ofloxacin, ciprofloxacin) and ceftriaxone. Even with the use of these antibiotics, the positive response to treatment has only been in the range of 16-40% in Nepal.[8]

MATERIALS AND METHODS

This research work was conducted at Kanti Children Hospital (KCH), Maharajgunj. All together 4,820 blood samples from different tertiary care hospital and one private clinic were cultured for the identification of *salmonella* in laboratory, of which 320 samples were from Nepal Medical College (NMC), 4000 from KCH, 200 from Sankata Laboratory (SKL), 200 from National Public Health Laboratory (NPHL) and 100 from Kathmandu Medical College (KMC). All the samples received in Lab were processed following standard Microbiological techniques.

The blood samples were cultured using enrichment and selective plating methods. 5ml of blood samples was inoculated into 95 ml of Brain Heart Infusion (BHI) broth incubated for 24 hours at 37°C. From the growth positive BHI broth, one loopful of culture was further incubated on Mac Conkey Agar (MA), Chocolate Agar (CA) and Blood Agar (BA) plates. The isolated *salmonella* were subjected for biochemical test. The Confirmed colonies from biochemical reactions were further confirmed by serotyping.

After identifying the bacteria, sensitivity test was performed by Kirby Bauer disc diffusion technique. Next day Zone of Inhibition (ZOI) was noted and CLSI break

Table-1: Distribution of salmonella serotypes according to Gender

Male		Female		Total
No.	%	No.	%	
223	56	177	44	400

points were used to differentiate between susceptible and resistant isolates *E. coli*. ATCC 25922 was used as a reference strain for quality control purposes. If the isolates exhibit multidrug resistant properties, it was further subjected for ESBL test using both Ceftazidime and Cefotaxime discs (Becton, Dickinson and Company, USA) following the CLSI criteria for *E.coli*. The organisms showing ZOI equal to or less than 22mm and equal to or less than 27mm for Ceftazidime and Cefotaxime respectively, were also tested in combination with clavulanic acid. The organisms were phenotypically confirmed as ESBL producers when they showed an increase in ZOI by greater than or equal to 5mm when evaluated in combination with clavulanic acid.

RESULTS

Out of 400 isolated strains of salomonella 223 cases (56%) were from male and 177 (44%) from female attended in KCH, NMC, KMC, NPHL and SKL (Table-1). Male had higher percentage of infection rate than female.

Table-2 shows that maximum incidence of typhoid fever was seen in the summer month 180(45%) seen that and infection rate was very low in winter 30 (17%).

Table-3 shows the age wise distribution of salmonella spp. The age between 10-19 were mostly effected, 150 (37%).

Table-4 shows the distribution of MDR salmonella sps. KMC shows the highest prevalence of MDR salmonella sps.38 (86%) followed by KCH, 156 (78%).

Table-5 shows ESBL producing Salmonella spp. it shows very high in rate 180 (63%).

DISCUSSION

In modern medical practice, newer antimicrobial drugs have been used extensively resulting in emergence and rapid dissemination of resistant bacterial strains.Since one of the mechanisms of bacterial resistancte to beta lactam antibiotic is the production of beta lactamase enzyme that breaks down the structural beta lactam ring of penicillin and its synthetic derivatives.The property of stability to many bacterial beta lactamase was increased with the later generation of Cephalosporins. These enzymes are most commonly produced by *Klebsiella spp* and *E.coli*. but also occur in other gram negative bacteria including *Enterobacter Salmonella, Proteus*, *Citrobacter*, *Shigella dysentriae, Pseudomonas aeruginosa* etc.

The infection due to ESBL producing organism can cause the failure of treatment, if one of the above classes of drugs is used. Besides, ESBL producing bacteria are typically associated with multidrug resistance. Antibacterial choice is often complicated by multi-resistance. Thus infection due to ESBL producing bacteria can results in avoidable failure of treatment and increased cost in patients who have received inappropriate antibiotic treatment.[9]

Enteric fever continues to be a global health problem with an estimated 12-33 million cases and 6,00,000 deaths occuring world wide each year.[10,11] Only a minority of enteric fever case is traditionally associated with asymptomatic and mild clinical illness.[9,11] In this study the infection was more prevalent

Table-2: Seasonal occurance of *S. typhi* & *S. Paratyphi* serotypes

Summer season (April-Jun)		Monsoon Season (July-Sep.)		Pre. Monsoon (Oct.-Dec.)		Post Monsoon (Jan.--Mar.)	
No.	%	No.	%	No	%	No.	%
180	45	140	35	50	12	30	17

Table-3: Age wise Distribution of *Salmonella Sp*ecies at KCH, NMC, NPHL, KMC and SKL

Age group	KCH		NMC		NPHL		KMC		SKL	
	No.	%	No.	%	No.	%	N0	%	No.	%
0-9	50	12	24	6	10	2	26	6	18	4
10-19	150	37	12	3	12	3	12	3	24	6
20-29	-	-	6	1	4	1	-	-	2	0.25
30-39	-	-	6	1	-	0.25	4	1	4	1
40-49	-	-	4	1	-	-	-	-	6	1
50-59	-	-	8	2	-	-	2	0.5	6	1
60-69	-	-	8	2	-	-	-	-	-	-

Table-4: Hospitals wise distribution of MDR *Salmonella sps* isolates

(KCH)		(NMC)		(SKL)		(KMC)		(NPHL)		Total isolates
MDR isolates		MDR isolates		MDR isolates		MDR isolates		MDR isolates		286 (72%)
No.	%	No.	%	No.	%	No.	%	No.	%	
156	78	52	76	26	43	38	86	14	50	

Table-5: Prevalence of ESBL producing Salmonella sps. from blood samples according to hospitals

KCH		NMC		SKL		KMC		NPHL		Total ESBL positive isolates.	
No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
133	85	14	27	7	27	26	68	0	0	180	63

in male 223 (56%) and female was 177(44%). In accordance to this study, more prevalent rate of infection in male than females, which was done by Mohanty *et al* in all India Institute of Medical Science.[10] The one of the strong reason for a high rate is that males were usually work outside their homes & street food that are liable to contamination.

In this study it was seen that maximum incidence of typhoid fever was seen in the summer month, 180 (45%) and the least cases were in winter 30(7%). Recent studies from India and elsewhere have also noted increased isolation is particularly in the dry season. In this season, the water level gets progressively lower become more stagnant and potable quality deteriorate as the weather becomes hotter.[13]

The highest incidence of enteric fever occurs in the 5 -19 years of age. Three hundred thirty four (85%) positive culture seen among 400 total isolates. This finding is in accordance with the research finding done by Mohanty *et al*.[10] After the age 20, the incidence falls due to probably acquisition of immunity from clinical or subclinical infection.[14]

It observed that among the total isolates, 72% showed MDR. salmonella species. KMC shows the highest prevalent followed by KCH, NMC etc. This reflects the local practice of widespread use, and probably misuse and overuse of antibiotics. In addition, over-the-counter availability of these antibiotics, self-prescription by patients and incomplete courses of treatment are probable additional factors contributing to the development of MDR. All isolates, resistance to third generation Cephalosporins i.e. ceftriaxone and ceftazidime shows ESBL test positive. In our research work, ESBL producer salmonella are very high, 180 (63%). In contrast to this finding its prevalence in salmonella has been reported to be low so far by szych et al reported 0.3% occurrence of ESBL in Poland.[15] This is of concern for travelers visiting the region in the absence of an effective vaccine. A large outbreak of Salmonella enterica typhi from a contaminated drinking water supply in a small Nepali town has recently been described.

These findings are concerning for the natives of Nepal as well as travelers to the country, especially since the current typhoid vaccines do no provide protection against this serotype. Routine screening for ESBL production and surveillance for emergence of resistance are recommended. In the same way, if the patient is not responding to the third generation Cephalosporin the clinicians should think fro the ESBL producing organism and request for its test. Clinical studies to evaluate efficacy of newer antibiotics against S. enterica are also desirable. Beside these awareness and health education program can be an effective intervention.

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