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Research Article

ANTIBIOTIC RESISTANCE PATTERN OF BLOODSTREAM BACTERIAL ISOLATES FROM ENTERIC FEVER PATIENTS: A PROSPECTIVE CROSS-SECTIONAL STUDY

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ABSTRACT

Background: Enteric fever continues to remain a major public health problem, especially in regions of South-East Asia due to poor sanitation and personal hygiene. Bangladesh open prone to enteric fever outbreaks and is an endemic region of typhoid fever. Enteric fever caused by *Salmonella typhi* has not yet been adequately explored in this region. The emergence of antimicrobial resistance has complicated the treatment and management of enteric fever.**Aims:**To describe the pattern of bacterial isolates from the blood cultures and determine their antibiotic resistance in cases of bacteremia due to enteric fever. **Methodology:** This was a laboratory based prospective cross sectional study. A total of 62 isolates of *Salmonella typhi*&*Salmonella paratyphi* were found in the blood C/S report. Blood cultures were performed to isolate bacteria and susceptibility to antibiotics was assessed as per standard procedures of microbiological methods. The data was analyzed using SPSS for windows version 22.0 software.**Results:** Among the total isolates studied, 20% was *S. typhi* were found multidrug resistant (defined as resistance to ampicillin, chloramphenicol and cotrimoxazole). There was a decrease in the susceptibility to ciprofloxacin and azithromycin. Ceftriaxone and cefixime showed 100 % sensitivity. **Conclusion:**MDR *Salmonella typhi* continues to be important public health issue. Presence ciprofloxacin and azithromycin resistance is a concern and requires further study.

Keywords: Enteric fever, Antibiotic resistance, Bacteremia, MDR**.**

INTRODUCTION:

Bacterial resistance to antibiotics has been a recognized reality almost since the dawn of the antibiotic era, but only within the past twenty years has the emergence of dangerous, resistant strains occurred with a disturbing regularity.[1]

Factors such as unregulated dispensing and manufacture of antimicrobials, truncated antimicrobial therapy, inadequate access to effective drugs and sometimes drugs of questionable quality and poverty are likely to be contributing to antimicrobial resistance.[2]

Bloodstream infections cause significant morbidity and mortality worldwide and are among the most common healthcare-associated infections.[3] Illness associated with blood stream infection ranges from self-limiting infections to life threatening sepsis that require rapid and aggressive antimicrobial treatment.[4]

Enteric fever is a systemic infection caused by the human adapted pathogens *Salmonella enteric* serotype Typhi (*S. typhi*) and *S. paratyphi* A, B, and C. These organisms are important causes of febrile illness among crowded and impoverished populations with inadequate sanitation who are exposed to unsafe water and food, and also pose a risk to traveler's visiting endemic countries.[5]

Outbreaks of typhoid fever are frequently reported from sub- Saharan Africa often with large numbers of patients presenting with intestinal perforations leaving open important questions

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about the epidemiology of enteric fever in the region.[6] Simultaneously, *S. Paratyphi* A appears to be responsible for a growing proportion of enteric fever in a number of Asian countries, sometimes accounting for 50% of Salmonella bloodstream isolates among enteric fever patients. This trend raises important concerns about the impact of typhoid fever vaccine on enteric fever rates.[7, 8]

Today enteric fever prevention focuses on improving sanitation, ensuring the safety of food and water supplies, identification and management of chronic carriers of *S. Typhi*, and the use of typhoid vaccines to reduce the susceptibility of hosts to infection.[9]

Antimicrobial resistance is a major public health problem in both *S. Typhi* and *S. Paratyphi* and timely treatment with appropriate antimicrobial agents is important for reducing the mortality of enteric fever.[10]Optimal antimicrobial management of patients with enteric fever depends on an understanding of local patterns of antimicrobial resistance and is enhanced by the results of antimicrobial susceptibility testing of the *Salmonella*isolated from the individual patient.[11]

In Bangladesh rational use of antibiotics is becoming a challenge due to flexible law enforcement and public awareness. So this study was undertaken in spite of having various limitations with a view to find the current situation of antibiotic resistance in bloodstream infections due to salmonella. The reports may come handy to bring a change in overall circumstance.

METHODOLOGY

Study design and ethical clearance: This was a laboratory based prospective cross sectional study. Blood culture and sensitivity report was collected from 15th November 2015 to 14th April 2016. Ethical clearance was taken from the authority of North South University, Dhaka. Letter of support was also obtained from Medinova Medical Service Ltd. Written informed consent was secured from each participant. Privacy and confidentiality regarding the study was maintained. All the patients who submitted their blood samples in the department of microbiology of Medinova Medical Service Ltd. for blood culture and sensitivity examination, during the study period was included in the study. Then culture negative reports were excluded from the study. The following functional definitions are defined by the investigators for the study purpose.

Bacterial profile: refers to the presence of bacteria and their prevalence.

Enteric fever: Any of a group of febrile illness with enteric symptoms caused by species salmonella. Also known as typhoid and paratyphoid fever.

MDR Salmonella: Species of salmonella resistant to ampicillin, chloramphenicol and Cotrimoxazole.

Data collection and laboratory procedure: The study used purposive sampling to identify the study subjects. Collection of blood samples, performing blood culture and sensitivity test was performed by trained microbiologist with aseptic measures. All blood samples were collected in BacT/ALERT@FA and BacT/ALERT@PF bottle irrespective of antibiotics administration. Quantity of blood sample from adult and children was 5-10 ml and 1-5 ml respectively. Samples were incubated in the automated BacT/ALERT 3D system for 72 hours. The preliminary signal of bacterial growth bacterial growth in BacT/ALERT bottle was detected and displayed on the 3D monitor of BacT/ALERT system. Specific identification of all culture positive samples was accomplished by sub-culture on Blood agar, Chocolate agar and MacConkey agar media. Inoculated Blood agar and MacConkey agar plates were incubated aerobically at 37^{0}C. The Chocolate agar plates were incubated at 37^{0}C under 5-10% CO_2 condition and examined after 18-24 hours of incubation. The microbial isolates was examined under microscope after Gram staining. Oxidase test, coagulase test, and other biochemical test was performed to identify the species of bacteria.[12]Antimicrobial susceptibility testing for all blood culture isolates was done on Mueller-Hinton agar plates following the standardized Kirby Bauer disc diffusion technique according to the criteria of the Clinical and Laboratory Standards Institute (CLSI) of 2011.[13]

The antibiotic disks used in this study was Ampicillin (10µg), Gentamycin (10µg), Cotrimoxazole (1.25µg+ 23.7510µg), Cefixime (5µg), Ceftriaxone (30µg), Ciprofloxacin (5µg), Amoxiclav (5µg), Chloramphenicol (30µg), Azithromycin (15µg) and Nalidexic acid (30µg). CLSI guideline was followed. After collection of

data, subsequent analysis was done by using SPSS 22.

RESULTS

Demographic data: Out of 1650 patients, who did their blood C/S, 67 reports showed positive growth. 92.5% sample was positive to salmonella.

Table: 1. Summary of blood C/S reports.

Organism	Frequency	Percentage
Salmonella typhi	44	65.8 %
Salmonella paratyphi	18	26.67 %
Staphylococcus aureus	2	3.3 %
Escherichia coli	1	1.67 %
Acinetobacter	1	1.67 %
Candida	1	1.67 %

Patients having positive blood culture for salmonella had a median age of 20 years. Minimum and maximum age at which positive results were found 3 and 59 years respectively. Male and female constituted 51.6% & 48.4% of positive *Salmonella* samples. The percentage of *Salmonella typhi* and *Salmonella paratyphi* was 71% and 29% respectively.

Table: 2. Distribution of positive culture by age group.

Age group (years)	Frequency
<20	30
20-40	30
>40	2

Antibiotic Sensitivity: Among the 9 antibiotics used in sensitivity test, Ceftriaxone and cefixime showed 100% sensitivity followed by gentamycin (98.2%), Co-trimoxazole (85.6%), Chloramphenicol (85.6%), and Ampicillin (74.8%). Highest resistant was found in Nalidexic acid (96.4%) followed by azithromycin (27.9%). Ciprofloxacin and azithromycin showed intermediate sensitivity of 92.8% and 70.3 respectively.

Table: 3. Antibiotic sensitivity pattern

Antibiotics	Sensitive (%)	Intermediate (%)	Resistant (%)
Ampicillin	74.8	9.0	16.2
Azithromycin	1.8	70.3	27.9
Cefixime	100	0	0
Ciprofloxacin	5.4	92.8	1.8
Cotrimoxazole	85.6	0	14.4
Gentamycin	98.2	0	1.8
Ceftriaxone	100	0	0
Chloramphenicol	85.6	0	14.4
Nalidexic Acid	1.8	1.8	96.4

Table: 4. Sesitivity of *S. typhi* and *S. paratyphi* to individual antibiotics:

Antibiotics	*S. typhi*			*S. paratyphi*		
	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Ampicillin	79.7	0	20.3	62.5	31.2	6.2
Azithromycin	2.5	74.7	22.8	0	59.4	40.6
Cefixime	100	0	0	100	0	0
Ciprofloxacin	7.6	89.9	2.5	0	100	0
Cotrimoxazole	79.7	0	20.3	100	0	0
Gentamycin	97.5	0	2.5	100	0	0
Ceftriaxone	100	0	0	100	0	0
Chloramphenicol	79.7	0	20.3	100	0	0
Nalidexic Acid	2.5	2.5	94.9	0	0	100

According to this table, sensitivity of Ampicillin was 79.7% and 62.5% sensitive for S. typhi and S. paratyphi respectively. Cotrimoxazole and chloramphenicol showed 100 % sensitivity for S. paratyphi while they are each 79.7% sensitive for S. typhi. Among the nine dugs used in C/S test, Ceftriaxone (100%), Cefixime (100%) and gentamycin (97.5%) showed almost full sensitivity for both the organisms. S. typhi were found in 44 patients among them 9 were Multidrug resistant *S. typhi*(20.45%).

DISCUSSION

Typhoid fever is endemic in Bangladeshand most documented typhoid fever cases involve school aged children and young adults. Previous studies suggest that Salmonella infection is common in urban areas and is commonly transmitted in foods from street vendors.[14, 15] In our study we have found, that 92.5% blood culture was positive for salmonella among the total positive cultures. A study held (2009) in ShaheedSuhrawardy Medical College, Dhaka found 88% positive samples for salmonella.[16]By age group, we have found most of our culture positive patients fall in the categories of less than 20 years and 20 to 40 year age group. We could not draw any inference regarding age and sex because of the small sample size. Further studies can be done in a bigger scale to find if there is any association of enteric fever and the particular age group people and their lifestyle.

Disease caused by *Salmonella typhi* is 10 times more common than caused by S. paratyphi.[17]This study showed that S. typhi (71%) contributes to more positive samples than S. paratyphi (29%). Here the ratio is almost 7:3. So future studies need to focus if there is any increasing trends of S. paratyphi incidence.

Between 1999 and 2006, 13% of salmonella typhi isolates collected in the United States were multidrug resistant.[18]In India during the year 2006-2007 the presence of MDR S. typhi was 10%.[19]The data in this study revealed that the existence of almost 20% MDR S. typhi. It is needed to find out the actual scenario of MDR Salmonella species because they are a real threat for developing future resistance to newer antibiotics.

This study highlighted that ciprofloxacin was 92.8% intermediate sensitive for Salmonella. A study conducted in 2015 in Bangladesh found that Ciprofloxacin is intermediate sensitive to *Salmonella typhi*.[20]Ciprofloxacin was the drug of choice for ten years following the emergence of resistance to chloramphenicol, ampicillin, and trimethoprim (multidrug resistant typhoid). Over 80% *S. typhi* and 70% *S. paratyphi* have reduced susceptibility towards ciprofloxacin.Unnecessary use of ciprofloxacin should be halted and an alternative fluroquinolones could be a potent option after clinical trial.[21]

In case of azithromycin, the study showed that it was 71% intermediate resistant to Salmonella. Back in 2011, a research conducted in Rajshahi Medical College found out that azithromycin was 88% sensitive for salmonella.[22]This study had contradicted with previous result. It could be due to sample size limitation. Chloramphenicol and cotrimoxazole showed 79.5% sensitivity for Salmonella which is a good indication and these drugs could once again be used in the treatment of enteric fever. The third generation cephalosporins in our study are the 2nd line of drug choice for enteric fever is 100% sensitive.

CONCLUSION

We need use our current drugs better and use the best and most affordable drugs available in order to prevent further resistance. Moreover, the genetic analysis of resistant bacteria that could help finding the actual cause behind the emerging drug resistance. It could be concluded by stating a very worrying possibility that there might be a time when we will have a lot of antibiotics in the market but not a single one sensitive enough to treat our disease.

CONFLICT OF INTREST:

The authors hereby declare that, there no conflict of interest in the conducted study

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