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Research Article

Etiologies and antibiotic resistance patterns of acute bloodstream infections by gram-negative bacterial isolates in a tertiary care hospital, Sirajganj, Bangladesh

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ABSTRACT

Bloodstream infections (BSI) are serious, life-threatening, and critical clinical conditions with high global human morbidity and mortality rates. This study aimed to determine the Gram-negative bacterial profiles and antimicrobial resistance patterns of acute bloodstream infections in rural patients. Three hundred forty-six blood samples were collected and analyzed with the BD Bactec^{TM} FX40 automated culture system, selective media culture, and biochemical parameters. Finally, antimicrobial susceptibility testing was performed using the disk diffusion method. The most common age group affected out of 51 cases was 41-60 years, with 20 patients (39.22%), and 1-15 years, with 2 patients (3.92%). Male patients were more susceptible (66.67%) than female patients. Among the isolates, *E. coli* was the most common, with 23 cases (45.1%); Cephradine was the most resistant antibiotic, and Imipenem was the most sensitive. Multiple drug-resistant pathogens were one of the most notable findings in our work. Our study will surely provide the best guide for properly treating antibacterial-resistant bacterial diseases and minimizing their critical mortality and morbidity.

Introduction

Life-threatening bloodstream infections were linked to higher rates of mortality and morbidity, as well as higher medical expenses. (Blomberg et al., 2007). Blood culture is a vital tool for detecting common bacterial isolates that cause BSI and remains the gold standard for bacteremia detection (Vasudeva et al., 2016). The most well-known Gram-negative bacteria isolated from blood cultures are *E. coli, K. pneumoniae, P. aeruginosa,* and *S. paratyphi A* (Zakerin et al., 2021).

Since the early 1950s, there has been a striking expansion in the frequency of bacteremia brought about by members of Enterobacteriaceae and other Gram-negative bacilli (Arora and Devi, 2007). *E. coli,* which was reported to be shared in the past (Vasudeva et at., 2016; Arora and Devi, 2007), has been replaced by other bacteria that resist multiple drugs (Chaturvedi et al., 1989). Infections brought about by Gram-negative bacteria are the most challenging issue for healthcare professionals because of antibiotic resistance.

A crucial technique for active antibiotic resistance surveillance is examining clinical specimens for antibiotic susceptibility (Vernet et al., 2014). Bacterial antimicrobial susceptibility profiles typically differ between populations due to geographical differences, nearby antimicrobial prescribing, and the prevalence of resistant bacterial stains in a given area. Other than these, the accessibility of much less potent products, the utilization of antibiotics for veterinary products, a lack of standardized diagnostic facilities, and the expanded practice of antibiotic self-medication are

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straightforwardly related to improving antibiotic resistance (Manyi-Loh et al., 2018). Antibiotic resistance is spreading and becoming increasingly widespread globally (Aslam et al., 2020). Antimicrobial therapy is typically started empirically prior to the availability of the results of a blood culture (Ko and Hsueh, 2009). This research was done to identify the Gram-negative bacterial profiles of bacteremia and to evaluate the isolated pathogens' antibiotic resistance profiles. This study's main objective was to support clinicians in starting experimental antimicrobial therapy and creating effective antibiotic strategies.

Materials and Methods

Type of research

A cross-sectional investigation was carried out in a laboratory setting from January to June 2021. The study was approved by the ethical grant committee at Khwaja Yunus Ali University. At KYAMCH, 346 blood samples were collected from patients with fever, indoors or outdoors.

Blood sampling and laboratory investigation

All the samples were taken aseptically for culture in the automated blood culture system. Approximately 1-5 ml of blood collected from 1-12-year-old children were inoculated adequately into the BD Bactec^{TM} FX40 Peds Plus/F culture vial. Adults received an 8-10 ml blood sample inoculated into the BD Bactec^{TM} FX40 Aerobic/F culture vial. According to the manufacturer's guidelines, these vials were inserted quickly into the BD Bactec^{TM} FX40 System at 35±2°C for 5-7 days (Abedin et al., 2020a).

Unloading positive and negative vials

A red and green light on the outside of the drawer indicated that positive and negative vials were waiting for removal. These positive vials were taken for further analysis, and negative vials were considered culture-negative.

Bacteriological culture

A drop of blood from a positive culture vial was placed onto blood agar and MacConkey agar culture plates and then incubated at 37^{0}C for 24 hours. Bacterial growth on the MacConkey agar plates indicated Gram-negative bacteria. Blood agar cultures were only used to determine whether or not bacteria were Gram-negative.

Identification of bacteria using biochemical assays

Gram-negative bacterial isolates were identified using biochemical indicators. These indicators included alkaline reactions, acidic reactions, hydrogen sulfide (H_2S) production, gas production, motility tests, indole production, and urea hydrolysis. Anti-sera were used only for *Salmonella* identification and gram staining techniques were performed to differentiate between Gram-positive and Gram-negative bacteria (Abedin et al., 2020a).

In-vitro antibiotic sensitivity test

According to CLSI recommendations, all bacterial isolates underwent antimicrobial susceptibility testing using the Kirby-Bauer disc diffusion method on Mueller Hinton agar (MHA) (CSLI, 2019). The antibiotic discs (6 mm, HiMedia, India) were placed in different positions on the plate. Finally, the plates were closed and incubated at 37°C for 24 hours. The zone sizes were interpreted as per the CLSI guidelines. Amoxicillin (10 µg), cefoxitin (30 µg), ceftriaxone (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), levofloxacin (5 µg), cephradine (30 µg), imipenem (10 µg), and imipenem (10 µg) were the antibiotics utilized in the test. A control strain of *Salmonella typhi* (ATCC 14028) was used for all culture and antibiotic susceptibility tests throughout the investigation (Abedin et al., 2020b).

Data Analysis

Using Excel 2016 and SPSS version 20, the data was examined. To verify the statistical analysis, descriptive statistics and chi-square tests were used. The significant p-value was determined to be <0.5.

Results and Discussion

The present study was conducted with 346 outdoor and indoor patients who were suffering from bloodstream infections during the period. There were 51 (14.7%) blood culture-positive cases with Gram-negative bacilli among them, and the remaining 295 (85.3%) were reported as negative results. When the prevalence of isolated Gram-negative bacteria was compared between genders, it was found that Gram-negative bacteria were significantly higher in males (34/51) (66.7%) than in females (17/51) (33.3%). Bloodstream infections are standard worldwide, and Gram-negative bacteria predominantly cause them. The distribution and frequency of BSIs caused by Gram-negative bacteria vary according to age and gender. It is critical to evaluate resistant Gram-negative bacteria using a standardized, simple, and reproducible in vitro assay to determine antimicrobial drug activity against isolated strains. Only a few studies have been done in Bangladesh regarding the prevalence of BSIs caused by Gram-negative bacteria and the antibacterial susceptibility of BSI-causing Gram-negative bacteria.

Among 346 total isolates, 51 were tested by BD Bactec^{TM} FX 40 and found 14.7% positive for Gram-negative bacteria. The negative results of the automated system were 85.3% due to the presence of Gram-positive bacteria and the contamination of isolates with non-pathogenic microbes (Fig. 1).

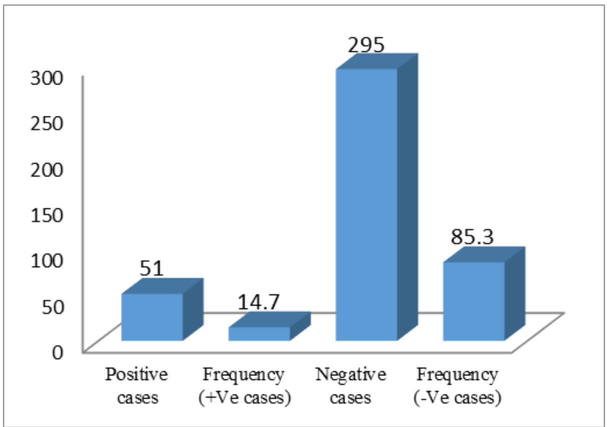

Fig. 1. Number of positive and negative cases of Gram-negative BSIs patient

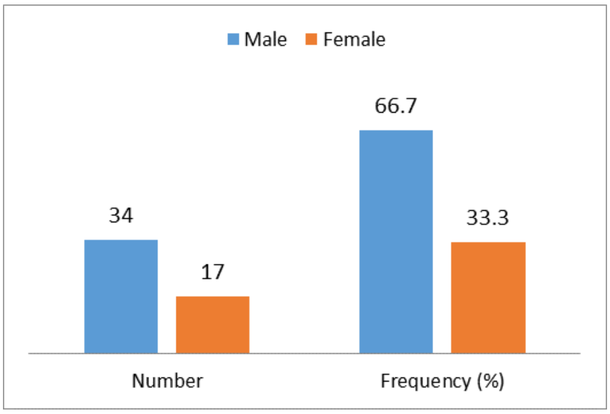

Fig. 2. Prevalence of Gram-negative BSIs patients on gender distribution

Table 1. Age distribution of Gram-negative BSIs patient

Age Range (Years)	Frequency (%)
0-15	2(3.9)
16-25	5(9.8)
26-40	9(17.7)
41-60	20(39.2)
61-Above	15(29.4)

Table 2. Distribution pattern of isolated Gram-negative bacterial species of bloodstream infection (n=51)

Gram-negative bacterial isolates	Total number	Percentage (%)
E. coli	23	45.1
K. pneumoniae	03	5.9
P. aeruginosa	03	5.9
S. typhi	16	31.4
Achromobacter spp.	02	3.9
B. cepacian complex	04	7.8
Total	51	100%

Out of 51 cases, the most common age group affected was 41–60 years with 20 cases (39.2%), followed by 61 and above years with 15 cases (29.4%), 26–40 years with 9 cases (17.7%), and 16–25 years with 5 cases (9.8%), respectively. Besides these, children aged 1–15 years with 2 cases (3.9%) were the least affected group (Table 1), and males 34/51 (66.7%) were more commonly attended than females 17/51 (33.3%) (Fig.2). This finding is nearly correlated with a previous study showing that being elderly and male are risk factors for acquiring BSI (Uslan et al., 2007; Khan et al., 2019).

In this study, out of 51 Gram-negative isolates, *E. coli* was the most common, with 23 cases (45.1%), and *Salmonella typhi* had 16 cases (31.34%). *K. pneumoniae, P. aeruginosa, Achromobacter spp., and Burkholderia cepacian* complex were also found with 3 cases (5.9%), 3 cases (5.9%), 2 cases (3.9%), and 4 cases (7.8%), respectively (Table2). These results contradict those of a recent report on resistance trends in BSI from China (surveillance study 1998–2017), which identified *E. coli* as the most common BSI-causing pathogen (Musicha et al.,2017) and a study conducted in Australia (Tian et al., 2019). However, a previous study in China's Hubei Province identified gram-negative bacteria, such as *E. coli* and *K. pneumoniae*, as common BSI-causing pathogens. Reports from Malawi in Africa (Uslan et al., 2007) revealed *S. typhi* and *K. pneumoniae* as BSI-causing pathogens, whereas *P. aeruginosa* was more common in Iran (Kreidl et al., 2019). Similarly, Japan has shown a varying pathogenic profile of BSIs, with Gram-negative bacteria, including *E. coli* and *Klebsiella spp.*, as the most common organisms (Keihanian et al., 2018). These varying accounts of BSI-causing pathogens account for regional variances. Our findings closely matched those of a Saudi Arabian investigation that found *E. coli* and *K. pneumoniae* to be the most prevalent generic Enterobacteriaceae isolates (Hattori et al., 2018).

In the current investigation, 51 isolates were evaluated against regularly used antibiotics using the disk diffusion method. Out of 26 isolates of *E. coli* (Table 3), it was observed that Cephradine, Cefuroxime, and Amoxicillin were resistant with 95.7%, 82.6%, and 73.9%, respectively. Other antibiotics showed more than 50% resistance, namely Amoxiclav (69.6%), Ampicillin (60.9%), Azithromycin (56.5%), Gentamicin (60.9%), and Cefixime (60.9%). This study nearly correlated with another in which *E. coli* displayed the highest resistance patterns (Tian et al., 2019).

In the case of *Salmonella typhi,* Cefixime was resistant at 62.5%, followed by Ampicillin (56.3%) and Cephradine (56.3%) (Table 3). The rest of the drug-resistant patterns were higher than 50%, which is correlated with Abedin et al. (2020a).

Levofloxacin, Ceftazidime, and Imipenem were completely sensitive in all three cases of *Pseudomonas spp.* Aside from that, cephradine was utterly resistant. In *Achromobacter* spp., there was 100% resistance to Ampicillin, Azithromycin, Cefixime, Cefuroxime, Cephradine, Ceftazidime, and Gentamicin. In the case of *Klebsiella pneumoniae,* all commonly used antibiotics are 66.7% resistant, namely Ampicillin, Cefuroxime, Cephradine, Ciprofloxacin, and Gentamicin. In the *B. cepacia* complex, Ceftazidime was most sensitive at 75%, while Cefixime, Azithromycin, and Ampicillin were resistant at 100%. Imipenam was the most effective in this study against Gram-negative bacteria that caused BSIs. This study nearly correlated with another study where indicated that significant levels of resistance were reported against Ampicillin, Amoxicillin, Ceftriaxone, and co-Trimoxazole with a pooled resistance range of 52.8-85.7% in Gram-negative isolates (Tianet al., 2019). The current study found that 84.3% and 54.9% of Ampicillin and Amoxicillin were resistant to gram-negative bacteria.

The disk diffusion method is the most simple, reliable, and economical way to perform simultaneous experiments on many organisms. It can be used in clinical laboratories for routine antibacterial susceptibility testing of Gram-negative bacteria isolated from BSI patients.

Table 3. Antimicrobial sensitivity profile of *E. coli* and *Salmonella typhi*

Antibiotics	*E. coli* (23)		*Salmonella typhi*(16)	
	R (%)	S (%)	R (%)	S (%)
Amikacin	7(30.4)	16(69.6)	8 (50.0)	8 (50.0)
Amoxicillin	17(73.9)	6(26.1)	8 (50.0)	8 (50.0)
Amoxiclav	16(69.6)	7(30.4)	8 (50.0)	8 (50.0)
Ampicillin	14(60.9)	9(39.1)	9 (56.3)	7 (43.8)
Azithromycin	13(56.5)	10(43.5)	6 (37.5)	10 (62.5)
Cefixime	14(60.9)	9(39.1)	10 (62.5)	6 (37.5)
Cefuroxime	19(82.6)	4(17.4)	6 (37.5)	10 (62.5)
Cephradine	22(95.7)	1(4.3)	9 (56.3)	7 (43.8)
Ceftazidime	9(39.1)	14(60.9)	6 (37.5)	10 (62.5)
Ciprofloxacin	7(30.4)	16(69.6)	2 (12.5)	14 (87.5)
Gentamicin	14(60.9)	9(39.1)	8 (50.0)	8 (50.0)
Imipenam	2(8.7)	21(91.3)	2 (12.5)	14 (87.5)
Levofloxacin	6(26.1)	17(73.9)	3 (18.8)	13 (81.2)
Meropenem	1(4.3)	22(95.7)	2 (12.5)	14 (87.5)

Note: R=Resistant, S=Sensitive, %= Percentage

Table 4. Antimicrobial sensitivity profiles of other Gram negative isolates

Antibiotics	*B.cepacia complex (4)*		*K. pneumonia (3)*		*Achromobacter spp.(2)*		*Pseudomonas spp. (3)*	
	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Amikacin	3 (75.0)	1 (25.0)	1 (33.3)	2 (66.7)	1 (50.0)	1 (50.0)	2 (66.7)	1 (33.3)
Amoxicillin	2 (50.0)	2 (50.0)	1 (33.3)	2 (66.7)	0	2 (100)	1 (33.3)	2 (66.7)
Amoxiclav	2 (50.0)	2 (50.0)	0	2 (66.7)	0	2 (100)	1 (33.3)	2 (66.7)
Ampicillin	4 (100)	0 (0.0)	2 (66.7)	1 (33.3)	2 (100)	0	2 (66.7)	1 (33.3)
Azithromycin	3 (75.0)	1 (25.0)	0	3 (100)	2 (100)	0	2 (66.7)	1 (33.3)
Cefixime	4 (100)	0	0	3 (100)	2 (100)	0 (0.0)	1 (33.3)	2 (66.7)
Cefuroxime	3 (75.0)	1 (25.0)	2 (66.7)	1 (33.3)	2 (100)	0	2 (66.7)	1 (33.3)
Cephradine	3 (75.0)	1 (25.0)	2 (66.7)	1 (33.3)	2 (100)	0	3 (100)	0 (0.0)
Ceftazidime	1 (25.0)	3 (75.0)	1 (33.3)	2 (66.7)	2 (100)	0	0 (0.0)	3 (100)
Ciprofloxacin	3 (75.0)	1 (25.0)	2 (66.7)	1 (33.3)	0	2 (100)	1 (33.3)	2 (66.7)
Gentamicin	3 (75.0)	1 (25.0)	2 (66.7)	1 (33.3)	2 (100)	0	2 (66.7)	1 (33.3)
Imipenam	2 (50.0)	2 (50.0)	0	3 (100)	0	2 (100)	1 (33.3)	2 (66.7)
Levofloxacin	2 (50.0)	2 (50.0)	0	3 (100)	1 (50.0)	1 (50.0)	0	3 (100)
Meropenem	2 (50.0)	2 (50.0)	0	3 (100)	0	2 (100)	0	3 (100)

Note: R=Resistant, and S=Sensitive

Conclusion

E.coli and *Salmonella typhi* were the most common Gram-negative bacteria isolated from bloodstream infections. Bloodstream infection was predominant among male patients, and the 41–60 age groups were the riskiest. According to a descriptive study of our research, men and older people should be more cautious about bacterial bloodstream infections. During our study, Imipenem and Imipenem were the most effective antibacterial drugs against all isolates except for *Burkholderia cepacia* complex (ceftazidime). Except for *Achromobacter spp.* (cefixime), most bacteria, including *E. coli* (cephradine) and *Salmonella typhi* (cefixime) were Clindamycin-resistant. A multidrug-resistant pathogen was found in our research, which is one of the most remarkable points. We believe that combining imipenem with other drugs can effectively control multidrug-resistant bloodstream infections caused by the subject pathogens. Bacterial infection has emerged daily in developing countries because of the lack of awareness, inappropriate lifestyle, and improper hygiene of the masses. In addition, antibioticresistance is also increasing the problem to a great extent. Our research identified the most potent and prevalent infectious bacterial agents in different age groups and also assessed the responsiveness of currently used antibiotics. This will help physicians to choose appropriate medicinal therapy and guide scientists to screen for multidrug-resistant pathogens.

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Conflict of Interests

All authors agreed before submitting the article, and there were no conflicts of interest.

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