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#### **Research Article**

# Incidence of Bloodstream Infection in a Tertiary Care Hospital: A Laboratory-Based Analysis

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#### **ABSTRACT**

Bacteremia is considered one of the most important causes of mortality and morbidity worldwide, especially among sick people. This study aims to analyze bloodstream infection among indoor and outpatients with acute febrile illness at Khwaja Yunus Ali Medical College & Hospital (KYAMC&H), Enayetpur, Sirajganj. This study tracks and analyzes the culture results of 406 blood samples received in the department of microbiology at KYAMC&H throughout the past 4 months from suspected patients with bloodstream infection. The samples were prepared by exploiting the machine-controlled Automated Blood Culture System BACTEC<sup>TM</sup> FX40 (USA). All the isolated pathogens were identified using the biochemical method, except for Burkholder cepacia complex (BCC), which was identified using BD Phoenix<sup>TM</sup> M50 (USA). A total of 105/406 (25.9%) BACTEC 9120® system-positive samples were subculture on 7% sheep blood agar, MacConkey agar, and chocolate agar plates. The majority, 63 (60%) of those, were Gram-negative microorganisms, and only 42 (40%) isolates were Gram-positive pathogens. The most predominant organisms were Staphylococcus aureus, comprising 40 (38.1%), followed by Salmonella typhi 18(17.1%), E. coli 28(26.7%), Klebsiella pneumoniae 4(3.8%), Burkholder cepacia complex 5(4.8%) and others 10(9.5%). In antibiotic susceptibility tests, most of the isolated bacterial pathogens were found susceptible to Imipenem and Meropenem.

Keywords: Bloodstream Infections, Bacterial isolates, Antibiotics, Susceptibility

#### 1. Introduction

Blood is a fluid connective tissue and one of the most important components of life. It plays an important role in transporting gases, nutrients, and wastes, maintaining temperature and pH, clotting blood at the site of injury, and preventing the invasion of pathogens. Blood is normally a sterile environment, so the detection of microbes in the blood is always abnormal (Castillo *et al.*,2019). Acute febrile illness is a high fever that lasts for more than 4 days and does not subside with the usual dose of antibiotics or antivirals, with body temperatures constantly being above normal (El-Radhi *et al.*, 2018).

Bloodstream infections (BSI) are serious, life-threatening, and critical clinical conditions with high global human morbidity and mortality rates. Although BSIs require immediate antimicrobial treatment, their prevalence, etiology, and antimicrobial susceptibilities differ from one country to another. A laboratory-based analysis of the blood culture revealed 19.5% positive bacteremia patients in a tertiary

care hospital in Bangladesh with several suspected isolates *Staphylococcus aureus*, *Salmonella typhi*, *E. coli*, and *Acinetobacter spp*. (Abedin *et al.*,2020).

In Bangladesh, a study conducted in Chittagong district identified 9% of AFI cases as having undifferentiated febrile illness with 3.4% mortality (4.6% in children and 2.1% in adults) (Das *et al.*,2022). This investigation helped to distinguish between viral and bacterial infection in the blood, between the normal flora and the infectious bacteria.

Bloodstream infection can range from inapparent bacteremia to fulminant septic shock with high mortality. Microorganisms present in circulating blood whether continuously, intermittently, or transiently are a threat to every organ in the body. The culture of blood is a vital tool to diagnose such infections. Drug susceptibility patterns help in rationalizing therapy.

#### 2. Methodology:

#### **Study Area and Population**

The data was collected from Khwaja Yunus Ali Medical College & Hospital, Enayetpur, Sirajganj, which included indoor and outpatients with acute febrile illness between January 2022 and April 2022. The inclusion criteria were all the patients attending Khwaja Yunus Ali Medical College & Hospital with a history of fever, irrespective of their age. Blood samples (n = 406) were collected from suspected patients aseptically with bloodstream infection among the entire age group. Aseptic blood samples were drawn for culture by an automated system. A 1–5 ml and 8–10 ml amount of blood samples was inoculated into BD Bactec<sup>TM</sup> FX40 Peds Plus/F culture vials for 0–12-year-old children and BD Bactec<sup>TM</sup> FX40 Aerobic/F culture vials for adults more than 12 years old. Only aerobic blood cultures were used in this study.

## Blood culture through the BD BACTEC<sup>TM</sup> FX40 (Becton, Dickinson, and Company, Spark, USA) method

According to the maker's directions, culture bottles were stacked into the BD BACTEC<sup>TM</sup> FX40 (Becton, Dickinson, and Company, Spark, USA) system with inoculated blood samples at 35±20C. Three days' incubation time was maintained in the complete examination. Every culture vial contained advanced Soybean-Casein Digest broth with CO2 and resin (nonionic adsorbing resin and cationic exchange resin) to neutralize an enormous number of antibacterial agents. At the bottom level, each vial has a synthetic sensing element that might observe a rise in carbon dioxide (CO2) created by development organisms. This sensing element was checked by the machine every 10 minutes for a rise in its fluorescence units that corresponded to the measure of CO2 created. A positive perusal demonstrates the hypothetical nearness of feasible microorganisms within the vials. At whatever point there was a signal of microorganism development, the location time was reported by the BD Bactec<sup>TM</sup> FX40 system programming bundle. Days were determined as full 24-hour time frames. For instance, disconnects were recognized at 24, 48, and 72 h, thought of as distinguished on the very first moment, two and three severally.

#### **Bacterial subculture**

All machine-signal-positive case bottles were subculture on MacConkey agar, blood agar, and chocolate agar media, followed by CLSI (2021) routine microbiological techniques. Also, we conducted Gram staining on all of the machine-sensing positive cases, and then the primary results were shared with a medical practitioner. Standard biochemical methods were used for identification from sub-culture growth.

#### Biochemical and serological analysis and Gram staining

Simon citrate agar tubes, MIU, Klignar Iron Agar (KIA), and oxidase tests were done for biochemical analysis to identify pathogens. Specific antisera (Becton, Dickinson, and Company, Spark, USA) were

used for confirmation of *Salmonella* spp. To distinguish between Gram-positive and Gram-negative bacteria, Gram staining methods were used (Abedin *et al.*,2022).

#### Antibiotic sensitivity test

The Kirby Bauer method of disc diffusion on Mueller Hinton agar was used for susceptibility testing of in vitro antimicrobials for all the bacterial isolates. The antibiotics used in the test were Amoxycillin (10µg), Ceftriaxone (30µg), Cefixime (5µg), Ceftazidime (10µg), Cefuroxime (30µg), Ciprofloxacin (5µg), Azithromycin (10µg), Nalidixic acid, Chloramphenicol (30µg), Gentamicin (10µg), and Imepenem (10µg). In this research, we used *Staphylococcus aureus* (ATCC 25923) and *Salmonella typhi* (ATCC 14028) as controls for the culture and sensitivity analyses.

#### 3. Results

In this study, a total of 406 clinical blood samples were analyzed by BD BACTEC<sup>TM</sup> FX40 from hospitalized and outdoor patients. Among them, 105 (26%) were blood culture-positive cases **Table 1.** Out of 105 positive cases, 72 (69%) were male and 33 (31%) were female **Table 2**.

Table 1: Analysis results from BD BACTECTM FX40 culture system & conventional culture methods						
Overall	Number	Frequency (%)				
Growth	105	26				
No growth	301	74				

Table 2: Number of Mae and Female						
Gender	Number	Frequency (%)				
Female	33	31.4				
Male	72	68.6				
Total	105	100				

#### Subculture of clinically vital microorganisms

A total of 105 positive vials were sub-cultured; out of that, 42 (40%) were gram-positive bacteria and 63 (60%) were gram-negative bacteria (Table 3). In this research, the most prominent bacteria were *S. aureus* (38.1%), *Salmonella typhi* (17.1%), *K. pneumoniae* (3.8%), *E. coli* (26.7%), and *B. cepacia complex* (4.8%) (Table 4).

Table 4: Bacterial isolated according to age groups

<b>Isolated Pathogens</b>	Age Group							
	0-12	13-24	25-36	>37	Total	Frequency (%)		
	years	years	years	years				
Gram Positive:								
S. aureus	3	5	7	25	40	38.1		
Gram Negative:								
S. typhi	0	2	5	11	18	17.1		
K. pneumoniae	0	0	1	3	4	3.8		
E. coli	2	4	4	18	28	26.7		
B. cepacia complex	0	0	0	5	5	4.8		
Others	0	1	3	6	10	9.5		
Total	5	12	20	68	105	100.0		

#### **Antimicrobial Susceptibility Profiles**

Antibiotic susceptibility tests for *S. aureus*, *S. typhi*, *K. pneumoniae*, *E coli*, and *B. acepacia complex* by the Kirbey-Bauer disc diffusion method are shown in **Figure 1-6.** It shows that *Staphylococcus aureus* is most sensitive to Meropenem and Imepenem (92.5%) and resistant to Cefixime (67.5%); *Salmonella typhi is* most sensitive to Ciprofloxacin and Levofloxacin (94.4%) and resistant to Clindamycin, (88.9%); *Klebsiella pneumonia is* sensitive to Azithromycin, Cefixime, Ceftriaxone, and Amoxicillin +clavulanic acid (100%); and resistant to Amoxycillin, Clindamycin, and Colistin (100%); *E. coli* is most sensitive to Imepenem (92.9%) and resistant to Clindamycin (100%); *Burkholder cepacia complex* is sensitive to Azithromycin, Doxycycline, Levofloxacin, Meropenem, and Gentamicin (100%) and resistant to Amoxycillin, Clindamycin, Colistin, Gentamicin, Aztreonam, Cefaclor, Cefotaxime, Amoxicillin +clavulanic acid (100%); and others are sensitive to Amikacin, Meropenem, and Imepenem (100%) and resistant to Amoxycillin and Cefaclor (90%).

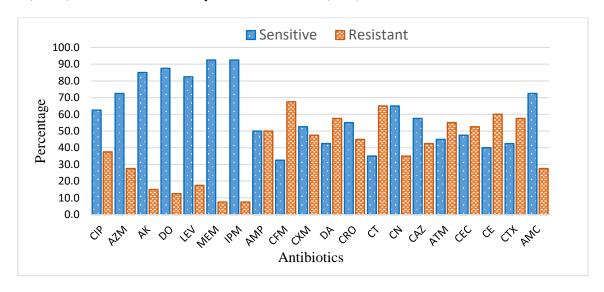


Figure 1: Antibiotic susceptibility pattern of Staphylococcus. Aureus

(CIP= Ciprofloxacin, AZM= Azithromycin, AK= Amikacin, DO=Doxycycline, LEV= Levofloxacin, MEM=Meropenem, IPM=Imepenem, AMP=Amoxycillin, CFM=Cefixime, CXM= Cefuroxime, DA= Clindamycin, CRO=Ceftriaxone, CT=Colistin, CN=Gentamicin, CAZ=Ceftazidime, ATM=Aztreonam, CE=Cefradine, CEC= Cefaclor, CTX= Cefotaxime, AMC= Amoxicillin +clavulanic acid).

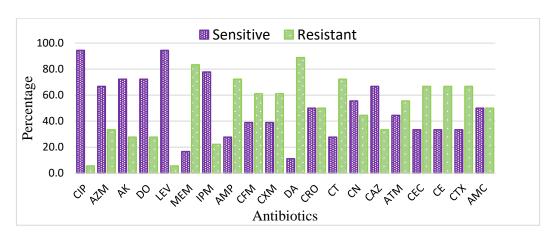


Figure 2: Antibiotic susceptibility pattern of Salmonella. typhi

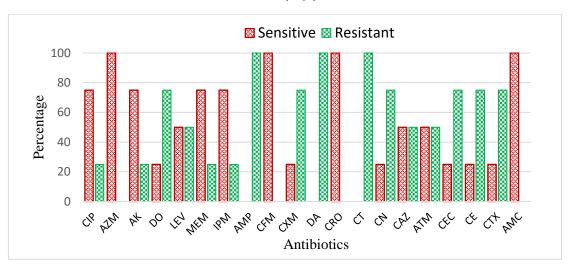


Figure 3: Antibiotic susceptibility pattern of *K. pneumoniae* 

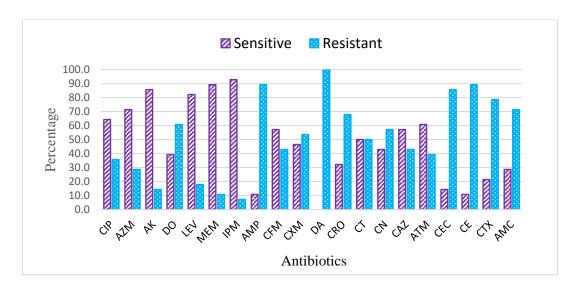


Figure 4: Antibiotic susceptibility pattern of E. coli

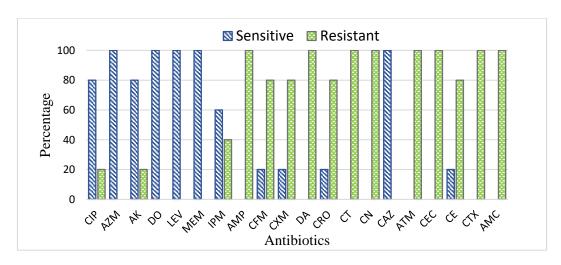
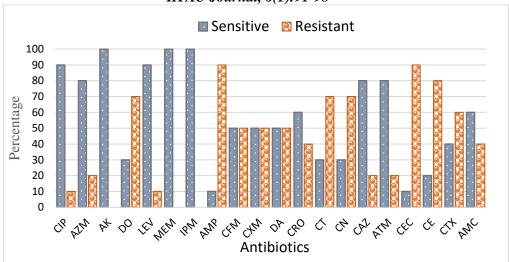


Figure 5: Antibiotic susceptibility pattern of *Burkholderia cepacia*.

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**Figure 5:** Antibiotic susceptibility pattern of others bacteria.

#### 4. Discussion:

Bloodstream infection (BSI) is associated with major morbidity and mortality. Blood culture has been the most effective test with high affectability and explicitness to identify the etiologic agents in bloodstream infection among all other relevant tests. In our analysis, the blood culture positivity rate was 26% which is consistent with the results of other studies led by (Abedin *et al.* 2020) and that was 19.50%. The limited number of positive cases of blood culture might be clarified by the way that a significant number of the patients likely got antimicrobial medications before they sought treatment to tertiary healthcare facilities (Ahmed *et al.*, 2017).

In the study, among all positive cases, 68.6% were males and 31.4% were females having a slight predominance of males than females. This outcome was predictable with the investigation done by (Ambroz Singh *et al*, 2015) and (Abedin *et al*. 2020) where 60.2% were male and 36.7% were female. A comparative report was finished that revealed a high culture-positive rate of 61.4% in men (Gandra *et al.*, 2016, and Akter *et al*. 2021). Also, the study reported the presence of medically important Gram-positive and Gram-negative bacteria in the bloodstream with 87.27% and 12.73 % respectively; the same isolation rates accounted by (Akter *et al*, 2021) were found higher reports of bacteremia caused by Gram-positive bacteria.

However, in some investigations as announced by (Oza *et al.*,2023), Gram-negative microorganisms were found as high as (70.90%) as compared to 29.09% of Gram-positive microbes. *Staphylococcus aureus* was the most predominant Gram-positive bacteria (38.1%) found in our analysis similar results were found by (Abedin *et al.*, 2020). This is overwhelming in all age groups being most elevated in adults. This perception is in non-concordance with different examinations led by (Sweta *et al.*, 2016). *Escherichia coli* (26.7%) was the second most commonly isolated pathogen found in adults in our study that result was predictable (Akova, 2016) but the opposite findings were by Abedin, Ahmed et.al 2020 where the second most prevalent bacteria was *Salmonella typhi* (7.30%).

This study finding was *Staphylococcus aureus* is most sensitive to Meropenem and Imepenem (92.5%) and resistant to Cefixime (67.5%) where, whereas Ghadiri *et al.*, (2012) found *S.aureus is* mostly resistant to penicillin. *Salmonella typhi is* most sensitive to Ciprofloxacin and Levofloxacin (94.4%) and resistant to Clindamycin, (88.9%) in our study and another study found 100% resistant to Ciprofloxacin Arora, (Usha *et al.* 2007); *Klebsiella pneumonia is* sensitive to Azithromycin, Cefixime, Ceftriaxone, and

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Amoxicillin +clavulanic acid (100%); and resistant to Amoxycillin, Clindamycin, and Colistin (100%) Similar findings analyzed by Qiu, Yue, *et al.* 2021 that *Klebsiella pneumonia* is highly resistant to Clindamycin; As *E. coli* is the second most prevalent bacteria, *E. coli* is most sensitive to Imepenem (92.9%) Ghadiri, Hamed, *et al* 2012 also found the lowest level (15.6%) of resistant against Imipenem & meropenem. However, it showed resistance to Clindamycin (100%) in our study.

*Burkholderia cepacia* complex (BCC) is an emerging pathogen causing nosocomial bloodstream infections (BSIs) and its treatment is challenging due to its multidrug resistance Siddiqui, Tasneem, *et al.* 2022 it is also a clear scenario that *BCC* is resistant to Amoxycillin, Clindamycin, Colistin, Gentamicin, Aztreonam, Cefaclor, Cefotaxime, Amoxicillin +clavulanic acid (100%) in this study. As well as *BCC* is sensitive to Azithromycin, Doxycycline, Levofloxacin, Meropenem, and Gentamicin (100%).

#### 5. Conclusions

In conclusion, we have performed blood culture by automated BD BactecTM FX40 and in vitro analysis of antibiogram patterns from patients with acute febrile illness seeking treatment at KYAMC&H. The blood culture analysis revealed 26% (105/406) positive bacteremia patients and several suspected isolates were *S. aureus*, *S. typhi*, *E. coli*, *K. pneumoniae*, BCC, and others. The presence of antibiotic-resistant isolates imposes a serious concern about the drug of choice for the treatment of bacteremia in patients. Meropenem, Imepenem, Ciprofloxacin, Levofloxacin, Azithromycin, Cefixime, Ceftriaxone, Amoxicillin +clavulanic acid, Doxycycline, Gentamicin, and Amikacin showed higher susceptibility to almost all organisms, whereas Clindamycin, Colistin, Aztreonam, Cefaclor, Cefotaxime, and Amoxycillin showed the least sensitivity. Careful consideration ought to be taken before deciding the empirical antibiotic for treatment to prevent the emergence of anti-microbial resistance and the significant mortality and morbidity associated with the illness.

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