Characterization and Antibiotic susceptibility Pattern of Gram-Negative Bacteria Isolates from Bloodstream infection at Sir Takhtsinhji Hospital, Bhavnagar

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Abstract

Background: Bacterial bloodstream infections constitute a significant public health problem in recent years. Sepsis is a leading cause of mortality and morbidity in Hospitals. Earlier identification of bacterial profiles and initiation of targeted antibiotic therapy is necessary for effective management of sepsis and preventing antibiotic resistance.**Material& Methods:** This study was conducted in the Bacteriology section of the Microbiology Laboratory at Sir Takhtsinhji Hospital Bhavnagar from January 2020 to November 2020. Samples received for blood culture were processed and species level identification for isolated Gram-Negative Bacteria by standard laboratory method and processed for antibiotic susceptibility test by modified Kirby Bauer disc diffusion method according to CLSI guideline 2020. Gram-negative bacterial Isolates and their antibiotic susceptibility pattern were recorded and analyzed.**Results:** There were 3643 blood culture samples, from which 574 (15.75%) showed bacterial growth. Out of 574 positive cultures, Gram Negative Bacteria were 407 (70.90%) and Gram-Positive Bacteria were 167 (29.09%). The most common Gram-negative isolate was Escherichia coli 56.51% followed by klebsiella pneumonia 28.25%. Escherichia coli showed the highest sensitivity to amikacin gentamicin. A high degree of resistance was found to cephalosporin and levofloxacin.**Conclusion:**The results indicate a high level of prevalence of Gram-negative bacteria among bloodstreaminfections and emerging resistance patterns among commonly used antibiotics. This study suggests continuous monitoring of antimicrobial susceptibility patterns through antibiogram so as to treat patient promptly and to build an effective hospital antibiotic policy.

Keywords: Blood Stream Infections, Gram-negative bacteria, Antimicrobial Susceptibility pattern.

Introduction

Bloodstream infection (BSI) is one of the most common causes of morbidity and mortality in the world. Around 200,000 cases of bacteremia occur annually with mortality rates of 20-50% worldwide[1].Bloodstream infections (BSI) refer to the presence of microorganisms in blood, which is one of the most serious situations among infectious diseases; because they are a threat to many organs in the body[2].Microbial invasion of the bloodstream can have serious immediate consequences such as shock, multiple organ failure, and DIC (disseminated intravascular coagulopathies) Therefore, timely detection of the causative agent is one of the most important goals of the microbiology laboratory [2].There are two major categories of bloodstream infections (BSIs): Intravascular and extravascular.

Risk Factors for the initiation of BSI are:

Immunosuppression

The use of broad-spectrum antimicrobial agents can suppress the normal flora; thus, allowing the emergence of resistant strains of bacteria[2].

Invasive procedures or extensive surgeries allow the bacteria to access the blood [2].

Bacteremia refers to the presence of bacteria in blood without any multiplication [2].Septicemia is a condition in which bacteria circulate and actively multiply in the bloodstream and produce their products (e.g., toxins) that cause harm to the host[2].

Studies of sub-Saharan countries including Ethiopia indicate that septicemia is an important cause of illness and death in children and the mortality rate approaches 53% which makes it a significant health problem in developing countries[3].

Types of Bacteremia

1. Transient bacteremia: It may occur spontaneously or with minor events such as instrumentation of contaminated mucosal site and surgery involving a non-sterile site [2].
2. Continuous bacteremia: The organisms are released into the bloodstream ata constant rate. It occurs in conditions such as Septic shock, endocarditis, and other endovascular infections [2].
3. Intermittent bacteremia: In some infections, bacteria are released into the bloodstream intermittently Sequestered focus of infection such as an undrained abscess meningitis, pneumonia, septic arthritis, and osteomyelitis[2]

In many studies revealed that a wide range of bacteria has been observed in febrile patients. One of the major causes of bloodstream infection is Gram-negative bacterial species such as Escherichia coli, Pseudomonas aeruginosa, Klebsiella species, Neisseria meningitides, and Haemophilus influenzae. Other gram-positive such as Coagulase-negative staphylococci (CONS), Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus payogenes, Streptococcus agalactiae, and Enterococcus aecium Daniel RK, et al. [4] Asrat D et al. [5] James AK, et al. [6] Rina K, et al. [7] Manjula M, et al. [8].Bloodstream infections increase the length of hospital stay, cost of care,rate of multidrug resistance and the situation further deteriorates.Early administration of proper antibiotics has been shown to decrease mortality and has a great impact on the outcome of septic conditions of patients and especially in view of the prevalence of infections caused by multi-drug resistant caused by gram-negative bacteria.

The aim of this study was to access the prevalence of Gram-negative bacteria in Bloodstream infections and rule out antibiotic sensitivity patterns as early as possible in Sir Takhtsinhji hospital Bhavnagar.

Material and Methods

This study was conducted in the Bacteriology section of the Microbiology Laboratory at Sir Takhtsinhji Hospital Bhavnagar from January 2020 to November 2020.A total of 3643 blood culture samples from clinically diagnosed cases of sepsis, were received in the Microbiology laboratory of Sir Takhtsinji hospital.

Inclusion criteria: Samples received from various wards, OPD, and ICU of Sir Takhtsinhji hospital for blood culture were included.

Exclusion criteria: Repeat samples of the same patient on the same day from the same site were excluded.

Blood samples were collected aseptically from patients for routine blood culture before taking any antibiotic treatment in the hospital [9].If the antimicrobial agent is already started, then the best time for collection is just before the next dose of the antimicrobial agent [9].The vein puncture site was disinfected with 70% alcohol and then a second antiseptic solution such as povidone-iodine, 2% tincture of iodine, or chlorhexidine should be applied before collection [2].Blood specimen is collected byusing a sterile syringe and needle. The higher the volume of blood, the greater the chance of isolation (yield increases by 3.2% per mL of blood cultured). At least 8–10 mL of blood per bottle for an adult and 1–3 mL of blood per paediatric bottle is recommended and inoculated into 50 ml and 25 ml of Brain Heart Infusion broth [9].Samples received for blood culture were labeled with laboratory identification number and location of a patient.

Samples received for blood culture were processed by the standard protocol. After overnight incubation in a blood culture bottle, positive blood cultures were processed for Gram stain[9].Subculture Blood culture broth onto solid media using blood agar, Mac-Conkey agar, chocolate agar, or BHI (Brain Heart Infusion media) [9].

Incubate the blood agar, MacConkey agar, and BHI plates aerobically and the chocolate agar plate in a carbon dioxide atmosphere (candle jar) and overnight incubation performed to see various morphology of organism and isolated colonies [9].Examine gram-stained colonies Depending on the bacteria seen, test the colonies further for various biochemical reactions like TSI (Triple Sugar Iron test), Citrate test, Urease test, Indole test, MR test, Oxidase test, coagulase, catalase, motility was for gram-negative bacteria following standard procedure [9].

Mueller Hinton agar wasprepared and sterilize as instructed by the manufacturer. Pour into 90 mm diameter sterile Petri dishes to a depth of 4 mm (about 25ml per plate) [9].Isolated colonies were then used to make standardized inoculum used for antibiotic susceptibility test performed on Mueller-Hinton agar plates using modified Kirby Bauer disc diffusion method as described by clinical and laboratory standards institute guidelines [10].Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC 25923), and Pseudomonas aeruginosa (ATCC 27853) were used as reference strains for culture and susceptibility testing [9].The antibiotic discs that were used to identify the susceptibility pattern of the gram-negative bacteria and their concentration include Ampicillin(10mcg), Amikacin(30mcg), Gentamicin(10mcg), Tobramycin(10mcg), Piperacillin (100mcg), ceftazidime(30mcg), ceftriaxone(30mcg), Cefazolin (30mcg), imipenem(10mcg), meropenem(10mcg), piperacillin+tazobactum(100/10mcg), levofloxacin(5mcg), cotrimoxazole (1.25/23.75) [10].

Gram-negative bacterial Isolates and their antibiotic susceptibility pattern were recorded and analyzed and interpreted according to CLSI guideline 2020.

Results

There were 3643 blood culture samples, from which 574 (15.75%) showed bacterial growth.Out of 574 positive cultures, Gram Negative Bacteria were 407 (70.90%) and Gram-Positive Bacteria were 167 (29.09%).The most common Gram-negative isolate was Escherichia coli 56.51% followed by klebsiella pneumonia 28.25%.Escherichia coli showed highest sensitivity to Imipenem (100%), Meropenem (98%), Amikacin (90%),Levofloxacin(75%), ceftriaxone (55%), cotrimoxazole(52%) and resistance to cefazolin(40%), Ampicillin (10%). Klebsiella pneumonia showed highest sensitivity to Imipenem (100%), Meropenem (100%), Amikacin (95%), ceftriaxone (60%), and resistance to cefazolin(45%),Levofloxacin(45%), Ampicillin (08%). Among Escherichia coli and Klebsiella species Imipenem sensitivity was seen at 100%.Sensitivity to piperacillin-tazobactam among Escherichia coli was 56% and among Klebsiella pneumonia was 52%.Among non-fermenters Pseudomonas aeruginosa and Acinetobacter species showed the highest sensitivity to Imipenem and Meropenem.Sensitivity to Piperacillin Tazobactam was 89% in Pseudomonas aeruginosa and 95% in Acinetobacter species.Sex-wise distribution of gram-negative isolates were as follows E.coli 86 in male, 74 in female, Klebsiella 65 in male,41 in female, Pseudomonas aeruginosa 18 in male, 16 in female, Proteus 0 in male,5 in female.

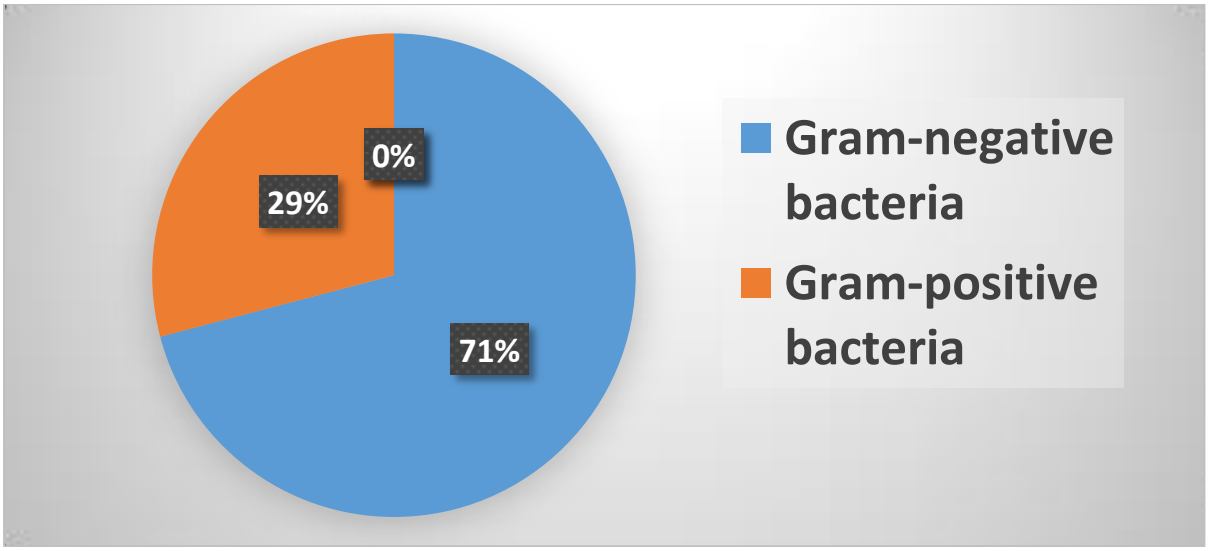

Fig.1: Prevalence of gram-negative bacteria among blood culture of blood stream infection

Table 1: gram-negative bacteria

Bacteria	Total Isolate	Prevalence
Escherichia coli	230	56.51%
Klebsiella spp.	115	28.25%
Pseudomonas aeruginosa	34	8.3%
Acinetobacter spp.	19	4.6%
Proteus spp.	5	1.22%
Salmonella typhi	2	0.49%
Providencia	1	0.24%
Citrobacter spp.	1	0.24%

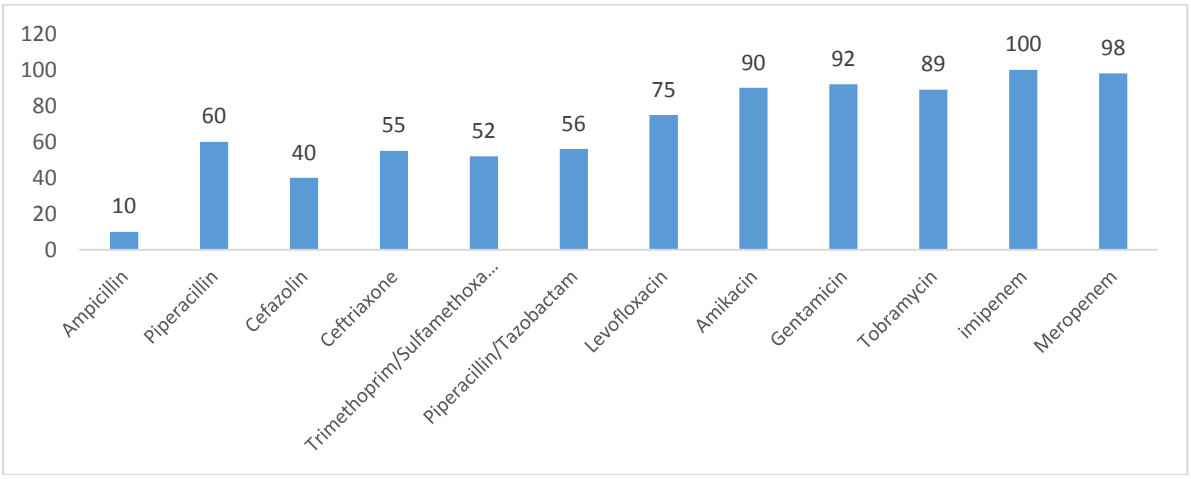

Fig.2: Antibiotic sensitivity pattern of Escherichia coli isolates percentage sensitivity

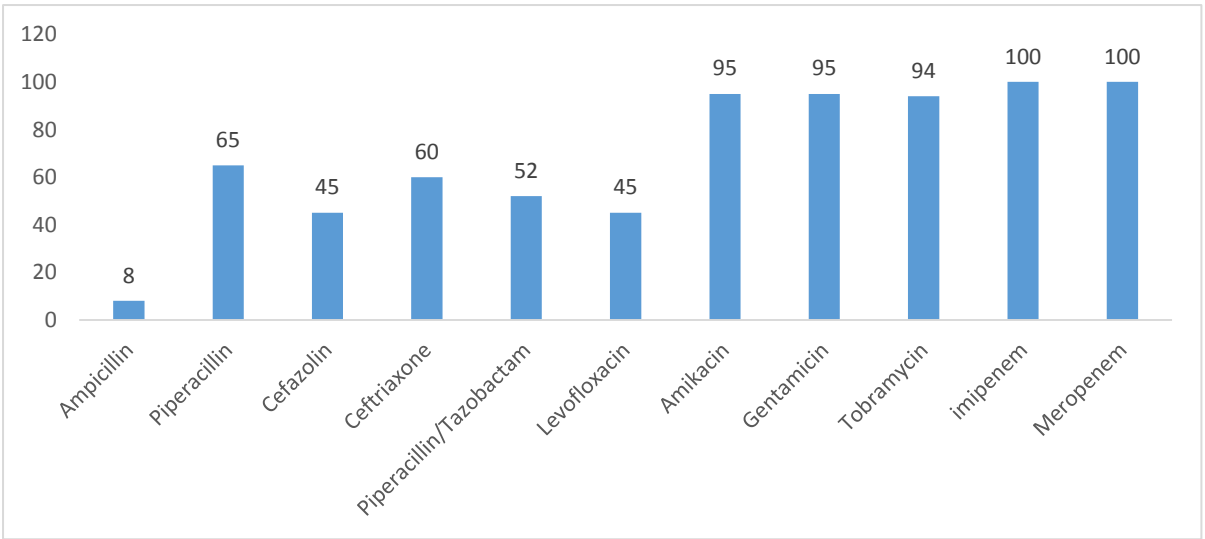

Fig.3: Antibiotic sensitivity pattern of Klebsiella isolates percentage sensitivity

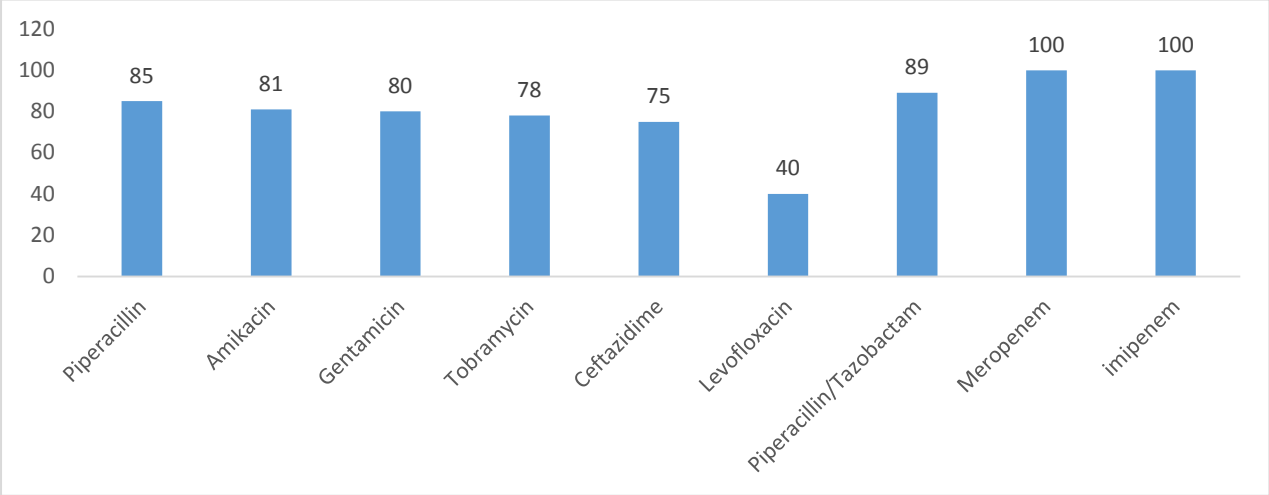

Fig.4: Antibiotic sensitivity pattern of Pseudomonasaeruginosa isolates percentage sensitivity

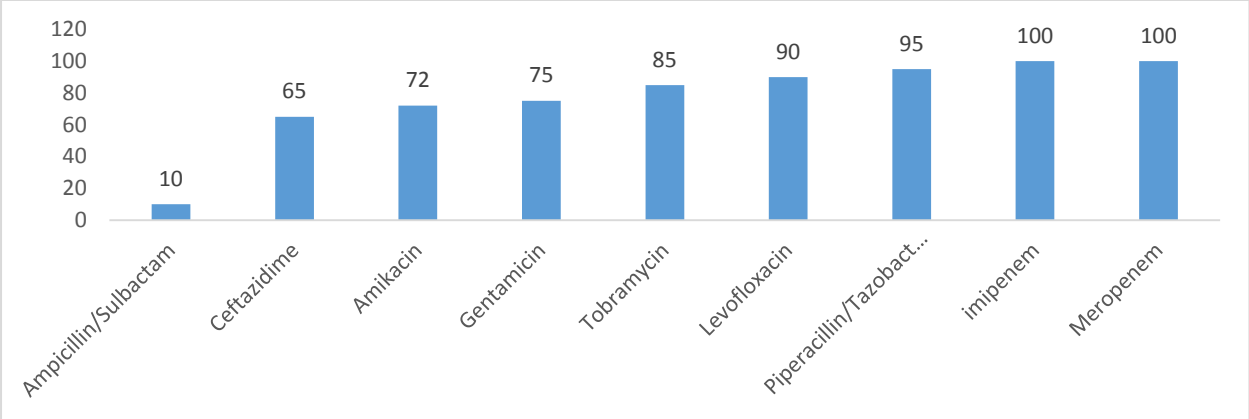

Fig.5: Antibiotic sensitivity pattern of Acinetobacter isolates percentage sensitivity.

Discussion

The present study gives information about the distribution of bacterial isolates causing bloodstream infections along with their antibiotic susceptibility pattern in Sir Takhtsinhji Hospital Bhavnagar. This information plays a major role in the effective management of septicemiccases.In our study Blood culture positivity rate among clinically suspected septicemia cases was 15.75% which was approximately similar to the studies which have shown blood culture positivity rates between 9.94% to 11.2% Mehta Manjula, et al. [11]Arora Usha, et al. [12]Shalini S, et al. [13]Ghadiri Hamed, et al. [14]. This is in contrast to other studies by Venkatesh, et al. [21] which showed a positivity of 27.16%, Wasihun, et al. [22] showed 28%, Ali, et al. [23] showed 24.2%, Nikita Vasudeva, et al. [24] showed 31.2% positivity which shown higher positivity rate.Such differences in the prevalence of BSI could be due to the different methodology used in the blood culture system, the study design, geographical location, nature of the patient population, an epidemiological difference in the etiological agents, and differences inantibiotic susceptibility test method and infection control policies Gohel, Kalpesh, et al.[25]Zenebe, Tizazu, et al. [26]Dagnew, Mulat, et al. [27].In our study, Gram-negative and Gram-positive bacteria constituted 71% and 29% respectively. This finding was in accordance with other studiesMehta, Manjula, et al. [11]Prabhash, Kumar, et al. [19] Chen, Chien-Yuan, et al. [20]were Gram-negative bacilli have taken over the Gram-positive organisms.In the present study, predominant gram-negative isolates were Escherichia coli (56.51%) followed by Klebsiella species (28.25%), Pseudomonas aeruginosa (8.3%), and Acinetobacter species (4.6%) which was in concordance with other studies Moghnieh, Rima, et al. [28]Irfan, Seema, et al. [29]Al-Otaibi, Fawzia E., et al. [30]Gustinetti, Giulia, et al. [31] Bansal, S, et al. [32]. In contrast to this finding, a study from Mumbai revealed that

Pseudomonas species was the most common cause (30.37%) and Escherichia coli and Klebsiella species accounted for 16.06% and 10.61%respectivelyPrabhash, Kumar, et al. [19].Both Escherichia coli and Klebsiella showed the highest sensitivity to Imipenem 100% and sensitivity toamikacin, 90%, and 95% respectively. Sensitivity to β-lactam/β-lactamase inhibitors (piperacillin+tazobactam) among Escherichia coli was 56% and among Klebsiella species was 52% respectively. This is similar to a study from Mumbai where the susceptibility of β-lactam/β-lactam inhibitors (piperacillin+tazobactam) was 56.5% Singhal, T., et al. [33].In our study both Escherichia coli and Klebsiella showed higher sensitivity to amikacin 90% and 95% respectively which was higher in comparison to 83.60% and 61.53% in Radha Rani et al. [35].A high degree of resistance to Ampicillin and low sensitivity to first generation (cefazoline) among Enterobacteriaceae could be due to the fact that cephalosporins are one of the most commonly used antibiotics for inpatients as well as for outpatients in developing countries and other reason is that in most of the cases self-medication is very common as the medicines are available at the counter NathisuwanSurakit, et al. [34].

Conclusion:

The present study brings attention to local scenario and various bacterial etiology of bloodstream infections in this study group. The findings of the study suggest that gram negative bacteria contributes more towards blood stream infection. This studyshown susceptibility to higher antibiotics and more resistance to commonly prescribed antimicrobials. Whereas carbapenems and aminoglycosides showed better response. So, the timely isolation with the information of susceptibility pattern will help the clinician to select proper antibiotics and to prevent drug resistance, reducing mortality rates and rational use of antibiotics. This study suggests continuous monitoring of antimicrobial susceptibility patterns so as to reduce various types of bloodstream infections.

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Conflict of Interest:The authors declare no conflict of interest.

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