

Study of Some Gram-Negative Bacteria from Blood Cultures Among Neonatal Iraqi Patients and their Antibiotics Resistance Pattern

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Abstract

Background and Objective: Neonatal sepsis is a serious condition that can result in death if treatment is delayed. The current study looked at the prevalence of Gram-negative bacteria species that are implicated in bloodstream infection (BSI) among neonates admitted at the Medical city, Baghdad, as well as their antibiotic resistance patterns. **Materials and Methods:** The Bact/ALERT 3D device (Bio-Merieux, France) was used to culture all the blood samples while the API system was used to identify the pathogenic organisms at the species level. The Kirby-Bauer method was used to determine the antibiotic susceptibility of the identified microbes. The study considered a total of 125 blood cultures collected from newborns who were suspected to be born with bloodstream bacteremia. **Results:** Among the 23 positive cases with Gram-negative bacteria, the rate was higher in the males [15 (65.2%)] compared to the females [8 (34.7%)]. *Klebsiella pneumoniae* was found to be the most identified Gram-negative bacteria in the studied blood samples [10 (43.4%)]. **Conclusion:** Tigecyclin was the most potent antibiotic against the identified Gram-negative bacteria.

Keywords: Blood stream infection, Neonatal septicemia, Blood culture, Anti-microbial susceptibility, Bacterial isolates.

1. Introduction

Sepsis neonatorum refers to any systemic infection of bacterial origin that presents a positive blood culture within the first month of life [1]. The pathogen implicated in neonatal septicemia might differ across countries and regions, as well as from one hospital to the other within the same country [2]. Bloodstream infection (BSI) is a major disease that needs to be addressed and treated right away. It is a leading cause of death, especially when multidrug-resistant bacteria are involved [3, 4] because they are life-threatening and requires quick identification of the causal agent as well as their antibiotic susceptibility pattern to permit precise antimicrobial treatment [5].

Bacteriological culture for the identification of the implicated bacteria, as well as the knowledge of the isolates' sensitivity patterns, are still the mainstays of conclusive diagnosis and treatment of BSI [3, 6]. A full cycle of bacteriological cultures and antibiotic sensitivity testing take 3-4 days to get complete. Being that early and proper antibiotic therapy is a significant determinant in the eventual fate of individuals with sepsis, it is normal practice to place the patient on broad-spectrum antibiotics during the early treatment phase upon suspicion of the clinical characteristics that imply bacteremia [6]. Some of the symptoms of untreated and clinically severe bacteremia are sepsis, septic shock, systemic inflammatory response syndrome (SIRS), as well as multiple organ dysfunction syndrome (MODS) [7]. One of the most essential bacteriological tests with clinical and therapeutic implications is blood culture; hence, it should be

requested in all cases with symptoms of septicemia, severe infection, or endocarditis [8].

Bacteremia necessitates the use of immediate and suitable antibiotics, as any delay in treatment may result to death [9, 10]. In underdeveloped countries, more than 14 million children below age 5 die during their childhood [11], with infections causing about 70% of the overall death rate [12]. This study is aimed at investigating the prevalence of bacteria isolated from neonatal blood cultures in one Baghdad hospital, as well as their antibiotic susceptibility pattern.

2. Materials and Methods

Clinical specimens

A total of 125 blood specimens were collected (collected in duplicate) from 125 neonatal patients suspected with blood stream infections.

Period of study

This study was done during the period from 1 July till 31 October 2021.

Collection of blood specimens

The samples were collected in duplicates from admitted neonates that showed signs of BSI before the commencement of antibiotics treatment. For each blood culture, 1-2 mL of blood was obtained from the neonates (≥ 0.5 mL for those below 1 month old, and ≥ 1 mL for those aged 1-30 months of age) [13].

Isolation and identification of bacterial isolates

Blood specimens were cultured using the Bact/ALERT 3D device

(Bio-Merieux, France) while the isolation was done using standard microbiological methods [14]. The microorganisms were identified at species level using the API system (Bio-Merieux, France).

Antimicrobial susceptibility test

The Kirby-Bauer method was followed as described by the World Health Organization to carry out the antibiotics susceptibility test for different antibiotics used in this study [15]. The antibiotics used in this work include Levofloxacin, Piperacillin/Tazobactam, Ceftriaxon, Gentamicin, Imipenem, Amikacin, Meropenim, Piperacillin, Cefotaxime, Amikacin, Tigecycline, and Trimethoprim- Sulphamethoxazole. The zone of inhibition formed around each antibiotic was measured using a metric ruler and recorded in millimeter (mm) as recommended by the Clinical Laboratories Standards Institute [16]. The sensitivity pattern of the microbes was interpreted by comparing the zones of inhibition produced against each antibiotic with that of standards.

3. Results

Study patients

Blood specimens of 125 neonates suspected with BSI were collected upon admission to the Medical city; the sample was collected for a period of three months (from July 1 to 31 October 2020). The age of neonates ranged from 1 day to one month.

Blood culture

The frequency of positive blood culture that indicates true bacteremia in the studied patients was 23 (18.4%) cases out of 125 blood specimens. The incidence of positive blood cultures was higher among males 15 (65.2 %) than the females 8 (34.7%).

Frequency of bacteria isolated from blood culture

The prevalence of Gram-negative bacteria implicated in true bacteremia among the studied neonates was summarized in Tables 1 and 2.

Table 1. Frequency of Gram-negative bacteria isolated from blood cultures of males

Gram negative bacteria	No.	%
Klebsiella pneumoniae	8	53.3
Acinetobacter baumannii	2	13.3
E. coli	2	13.3
Serratia marcescens	1	6.6
Enterobacter cloacae	2	13.3
Total	15	100

As shown in Tables 1 and 2, K. pneumoniae was the most frequently isolated Gram-negative bacteria in the blood culture of the neonates 10 (34.4 %).

Table 2. Frequency of Gram-negative bacteria isolated from blood cultures of females

Gram negative bacteria	No.	%
Klebsiella pneumoniae	2	25
Acinetobacter baumannii	1	12.5
Enterobacter cloacae	2	25
E.coli	3	37.5
Total	8	100

Antimicrobial susceptibility test

Table 3 showed the antibiotics susceptibility pattern of the identified Gram-negative microbes isolated from the blood cultures in this study. The Gram-negative microbes was more sensitive to Tigecyclin as it showed 100 % sensitivity. However, the rest of the isolates were completely resistant to Piperacillin, Piperacillin + Tazobactam, Cefotaxime, Ceftriaxon, Trimethoprim + Sulphomethaxazol, and differed in resistance to Levofloxacin, Amikacin, Imipenime, Meropenim, Gentamicin, and Doxycycline.

Table 3. Susceptibility rate of Gram-negative bacteria under study towards various antibiotics.

Antibiotics	Klebsiella pneumoniae n=10	Acinetobacter baumannii n =	E. coli n = 5	Serratia marcescens n=1	Enterobacter-cloacae n = 4	Resistance from total 23 isolates %
	No. of Sensitive isolates (S)	No. of Sensitive isolates (S)	No. of Sensitive isolates (S)	No. of Sensitive isolates (S)	No. of Sensitive isolates (S)	
Levofloxacin	4	0	5	1	3	(10)43.4%
Amikacin	0	0	3	1	3	(16)69.5%
Pipracillin	0	0	0	0	0	(23)100%
Pipracillin +Tazobactum	0	0	0	0	0	(23)100%
Ceftriaxon	0	0	0	0	0	(23)100%

Cefotaxime	0	0	0	0	0	(23)100%
Imipenim	4	0	5	1	4	(9)39.1
Meropenim	5	0	5	1	4	(8)34.7
Tigecyclin	10	3	5	1	4	0%
Gentamicin	0	0	1	1	2	(19)82.6%
Doxycycline	8	0	1	1	2	(11)47.8
Trimethoprim + Sulphamethaxazol	0	0	0	0	0	(23)100%

4. Discussion

Rapid diagnosis and treatment are necessary for the prevention of BSI related complications and mortality [6]. In this study, out of 125 neonatal blood specimens, the frequency of positive blood culture that indicated true bacteremia in the studied patients was 23 cases with gram negative bacteria isolates. In a study done in India, the positivity of blood culture in cases of BSI was 42 % (770/1828) [17]. The most common Gram-negative organism implicated in neonatal sepsis was *K. pneumonia* [18]. The prevalence of *K. pneumoniae* was 15.3% as it was detected in samples from 17 males and 7 females suspected to be cases of neonatal sepsis [19]. The Gram-negative microbes accounted for most of the isolates encountered in cases of neonatal septicemia, with *Enterobacter* species being the most predominant organism (78.1%) [20].

Gram-negative bacilli (GNB) are well-known causes of human infections, especially among neonates admitted to intensive care units (ICU). Antibiotics are regularly administered such infants in ICU even though there is a significant variance in practice between institutions. Exposure to antibiotics has been linked to alterations in infection susceptibility and microbial ecology which can have unanticipated negative implications. The mortality rate is significantly higher among neonates with multidrug-resistant GNB bacteremia, especially those that are inadequately exposed to antibiotic at the early treatment. These findings imply that promoting antimicrobial stewardship and reducing needless antibiotic usage in the NICU population is crucial.

Being that neonatal infections might present with nonspecific signs and may be difficult to differentiate from other conditions with a non-infectious origin, it is difficult to reduce the use of broad-spectrum antibiotics in the neonatal ICU. Nonetheless, there are chances for stewardship. For instance [21], suggested that antibiotic resistance can be minimized through institutional procedures for narrow-spectrum empiric therapy for early-onset sepsis. As the causative agents of neonatal early onset sepsis that occurs in the first week of life, as well as those for late onset sepsis that occurs in the 7th to 3rd month of life, are frequently transferred from mother to child, it is necessary to perform vaginal cultures on all expectant mothers as a form of pre-natal screening and positive cases

should be appropriately treated before delivery [20]. Current findings provide conclusive evidence that the positive blood cultures were higher among males than of females. The most effective antibiotic was tigecycline.

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