Original Article

In vitro Assessment of Antimicrobial Susceptibility Patterns in Positive Blood Cultures of 22 Neonates with Neonatal Sepsis in a Children's Hospital in Baghdad

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Abstract

Introduction: neonatal sepsis (NS) is a serious disease in neonatology responsible for the higher percentage of neonatal deaths. It is a microbial disease caused by various types of bacterial and nonbacterial pathogens. **Objectives:** The objective is to determine the causative bacterial pathogens of NS for the specified period and their susceptibility toward the available antimicrobial drugs. **Patients and Methods:** Blood cultures of 67 neonates with clinical picture of NS were assessed for antimicrobial sensitivity. **Results:** Forty three cultures (64.2%) were negative, 4 cultures (5.9%) revealed *Candida* spp. and 20 (29.9%) cultures were positive. Gram-negative bacteria *Klebsiella* was the predominant pathogen causing NS (45.45%) followed be Gram-positive *Staphylococcus aureus* (18.2%). Vancomycin, ciprofloxacin, and amikacin were the most effective antimicrobials against Gram-positive isolates while imipenem was the most effective against Gram-negative bacteria. **Discussion:** *klebsiella* was the predominant microbe in NS in many studies, other pathogens were variable between countries. Vancomycin and imipenem approved their excellent effects reported in many others studies while penicillin approved high bacterial resistance recorded previously. **Conclusion:** Gram-negative bacteria were predominant and more resistant to the tested antimicrobials. Imipenem, vancomycin, ciprofloxacin were the best effective according to the isolated microbes.

Keywords: Antimicrobial, *Klebsiella*, neonatal sepsis, susceptibility

Introduction

Neonatal sepsis (NS) is one of the major challenges in pediatrics since it is the most common cause of neonatal mortality; it is responsible for about 30%–50% of the total neonatal deaths in developing countries.[1] NS is a clinical disorder of a systemic infection below 28 days of life, usually classified as early-onset (<72 h) and late-onset sepsis (more than 72 h), depending on the onset time of clinical manifestations of sepsis.[2] Sepsis-related morbidity and mortality carries increasing interests in Neonatal Care Units all over the world. NS is a clinical syndrome characterized by clinical picture of infection with or without associated bacteremia in the first 28 days of life. It includes different systemic infections of the newborn such as septicemia, meningitis, pneumonia, arthritis, osteomyelitis, and urinary tract infections.[3]

NS is a term refers to the presence of pathogenic microorganism in the bloodstream, cerebrospinal fluid, urine or any other sterile tissue. Bacterial and viral pathogens are the most common causes of NS; nevertheless, protozoa, and fungi may play a major role in this regard.[4]

Microbiologically, pathogens causing NS differ between countries especially high income and low income countries.[5] The most common microorganisms are group B streptococcus and *Escherichia coli*. The remaining cases of early onset NS are caused by *Staphylococcus aureus*, coagulase-negative staphylococci, *Listeria monocytogenes* and other Gram-negative bacteria.[6] In Late Onset NS (mainly in very-low-birth-weight infants), other important causative agents are *E. coli*, *Klebsiella*

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spp. and *Candida* spp. Less common agents of Late Onset NS include *S. aureus*, *Enterococcus* spp. and *Pseudomonas aeruginosa*.[7]

Although there are different regimes for the treatment of both early and late onset NS, penicillin and gentamicin are used worldwide for treating sepsis and suspected sepsis in the neonatal age group.[8]

Objectives

The objectives of this study are to determine the causative bacterial pathogens of NS for the specified period and evaluation of their susceptibility patterns toward the available antimicrobial drugs since the antimicrobial resistance is a continuous and growing problem and needs periodic reassessments of the used antimicrobials.

Patients and Methods

This prospective observational cohort study was conducted in the neonatal intensive care unit of Al-Elwiyah children's hospital in Baghdad-Iraq, between December 2018 and December 2020. Blood cultures of 67 neonates with clinical picture of NS were assessed for antimicrobial sensitivity.

The antimicrobial susceptibility tests for the bacterial isolates of the 20 positive culture of 20 cases were carried out for the blood samples of neonates against number of antimicrobials by using disc diffusion method.[9] Samples were collected by a standard technique in sterile containers and cultured on blood and MacConkey agar media to know if there is growth of bacteria, then incubated aerobically at 37°C for 24 h and extended up to 48 h in cases of G-ve. The identification of isolates was done by standard method depending on observation of colony characteristics and antimicrobial sensitivity test was performed by disc diffusion method using Muller-Hinton agar and choose of antibiotic disks according to type of isolated bacteria. If there is growth of bacteria around antibiotics disc in inhibition zone this means that the bacteria are sensitive to this antibiotic and vice versa if there is no growth.

Data were represented as numbers and percentages. Microbial susceptibility was tabulated according to their Gram stain into Gram-negative and Gram-positive groups. Later, the main microbial species isolated were specified to assess their susceptibility to each of the selected antimicrobials.

Results

In this study, 67 male and female neonates suspected to have NS were subjected to blood culture and susceptibility tests. Forty three cultures (64.2%) were negative, 4 cultures (5.9%) revealed *Candida* spp. and 20 (29.9%) cultures were positive revealing 22 isolates for the 20 cases (2 cases of mixed infection). It was shown that the Gram-negative bacteria *Klebsiella* was the predominant pathogen causing NS (45.45%) followed be Gram-positive *S. aureus* (18.2%), there were 2 cases of mixed bacterial infection. Table 1 shows these isolated pathogens species.

Bacterial sensitivity towards the used antimicrobials

The results have revealed both bacterial sensitivity and resistance toward different antimicrobial drugs. The sensitivity was variable according to the species of microbes. Since the use of antimicrobial disc was determined according to the type of microorganism, drug antimicrobial effects were studied against the Gram-positive and Gram-negative bacteria separately. Tables 2 and 3 list the sensitivity patterns in each bacterial group.

Regarding Gram-positive bacteria, vancomycin, ciprofloxacin and amikacin were the most susceptibility causing antimicrobial drugs (100%) [Figure 1], while imipenem was the most effective antimicrobial against the Gram-negative pathogens (80%) [Figure 2].

Regarding the most common isolated pathogens their susceptibility patterns have shown that imipenem was the most effective antimicrobial which eradicated *E. coli* (100%) and *Klebsiella* (90%). Vancomycin, ciprofloxacin, and amikacin have shown the higher susceptibility of the Gram-positive *S. aureus*. Pipracillin was resisted totally by both Gram-negative species as well as penicillin with *S. aureus*. Results are recorded in Table 4.

Discussion

Regarding the causative pathogens of NS, it was found in this study that *Klebsiella* was the most frequent isolated pathogen

Table 1: Percentage of isolated bacterial species

Type of microorganism	Number of isolates	Percentage
Klebsiella	10	45.45
S. aureus	5	22.7
E. coli	3	13.6
A. baumanni	2	9.09
Enterobacter species	1	4.54
S. epidermidis	1	4.54
Total	22	100

S. aureus: Staphyllococcus aureus, E. coli: Escherichia coli, A. baumanni: Acinetobacter baumanni, S. epidermidis: Staphyllococcus epidermidis

Table 2: Gram positive bacteria sensitivity and resistance towards the used antimicrobials

Antimicrobials	Number of antimicrobial use	Sensitivity, *n* (%)	Resistance, *n* (%)
Vancomycin	6	6 (100)	0
Penicillin	6	0	6 (100)
Ciprofloxacin	6	6 (100)	0
Cotrimoxazole	6	1 (16.6)	5 (83.4)
Erythromycin	6	3 (50)	3 (50)
Gentamicin	6	4 (66.6)	2 (33.4)
Amikacin	6	6 (100)	0
Tobramycin	6	0	6 (100)
Clindamycin	6	6 (100)	0

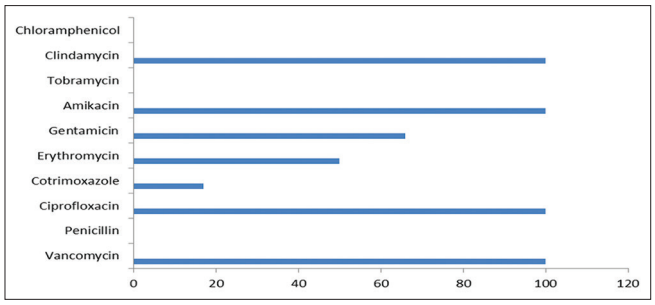

Figure 1: Antimicrobial drugs' efficacy (%) against Gram positive bacteria

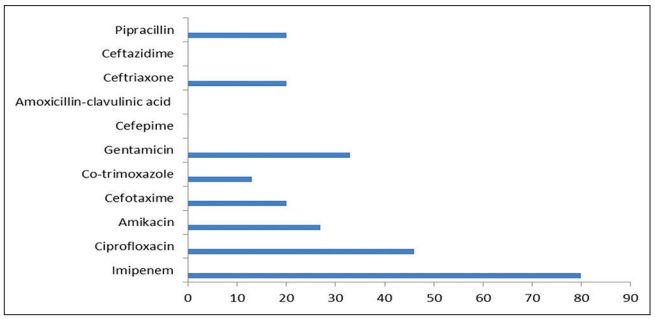

Figure 2: Antimicrobial drugs' efficacy (%) against Gram negative bacteria

Table 3: Gram-negative bacteria sensitivity and resistance towards the used antimicrobials

Antimicrobials	Number of antimicrobial uses	Sensitivity, *n* (%)	Resistance, *n* (%)
Imipenem	15	12 (80)	3 (20)
Ciprofloxacin	15	7 (46)	8 (54)
Amikacin	15	4 (27)	11 (73)
Cefotaxime	15	3 (20)	12 (80)
Co-trimoxazole	15	2 (13.3)	13 (86.7)
Gentamicin	15	5 (33.3)	10 (66.7)
Cefepime	15	0	15 (100)
Amoxicillin-clavulinic acid	15	0	15 (100)
Ceftriaxone	15	3 (20)	12 (80)
Ceftazidime	15	0	15 (100)
Pipracillin	15	3 (20)	12 (80)

Table 4: Dominant microbial species sensitivity percentage towards antimicrobials

Antimicrobial drugs	Bacterial sensitivity percentage		
	Klebsiella	*E. coli*	*S. aureus*
Vancomycin	NT	NT	100
Imipenem	90	100	NT
Ciprofloxacin	60	0	100
Gentamicin	33	50	66
Amikacin	0	50	100
Pipracillin	0	0	NT
Penicillin	NT	NT	0

NT means the antimicrobial did not tested. *S. aureus: Staphyllococcus aureus, E. coli: Escherichia coli*

followed by *S. aureus* and *E. coli*. Near to these results, a study conducted in India has shown that for the period between 1998 and 2004 the most predominant bacterial pathogen causing NS was Pseudomonas spp.(33.2%) followed by *Klebsiella* (31.4%) and acinobacter (14%). Majority of the isolates (90.8%) were Gram-negative microbes.[10] In fact there is quite different etiological pattern of developed from developing countries, in developing countries Group B Streptococci were the predominant etiological agent for early onset NS.[11] A recent Iraqi study has shown that staphylococcal Spp. were the most predominant pathogen and Gram-positive bacteria were more frequent than Gram negative in both early and late onset NS.[4] The diversity of bacterial pathogens in NS varies from one geographical region to another. Furthermore, it changes over time even in the same region.[12] Another recent Iraqi study conducted in Duhok revealed that Gram-negative bacteria were the cause of most causes of NS (68.1%) while Gram positive bacteria were (31.9%). The most frequent isolated pathogens were *Klebsiella* pneumoniae (33%), Coagulase negative staphylococcus (26.4%), and *E. coli* (20.9%), respectively.[13] These results are close to results obtained by this study. *Klebsiella* was the most common isolated bacteria causing NS in several recent studies.[14-16]

Gram-positive bacteria have shown different patterns of susceptibility toward the used antimicrobials. Vancomycin, ciprofloxacin, amikacin, clindamycin, and chloramphenicol caused the optimal bacterial susceptibility (100%). Recent Iraqi antimicrobial susceptibility assessment studies have shown that vancomycin and amikacin had an excellent antimicrobial effect for different pathogenic species of both Gram negative and positive bacteria.[17,18]

Gram-positive isolated bacteria have shown complete resistance toward penicillin and tobramycin. Staphylococcal spp. were completely resistant to tobramycin in a study conducted in Nepal.[14] Another study has shown low susceptibility of *S. aureus* (6.25%) toward penicillin in NS,[19] other Indian study have shown complete resistance.[20]

The majority of isolates were Gram negative similar to many other studies.[12,14,16] Gram-negative bacteria have shown less susceptibility toward the used antimicrobials than Gram positive microbes. The highest susceptibility was toward imipenem (80%). An Indian study has shown 100% susceptibility of Gram-negative bacteria toward imipenem,[20] while the other antimicrobials in this study showed far less effect since ciprofloxacin has shown 46% susceptibility which is lower than the same Indian study (72%) than aminoglycosides gentamycin (33.3%) and amikacin (27%) these results indicate reduced susceptibility of aminoglycosides toward Gram-negative microbes as recorded in other studies,[10,20] but another recent study demonstrated a higher resistance rate of Gram-negative bacteria towards both amikacin and gentamycin (50% and 60% resistance rate, respectively).[14] Cephalosporins have shown a complete resistance (cefipem and Ceftazidime) or weak susceptibility (ceftriaxone), these results indicate an increased

cephalosporin bacterial resistance in comparison with previous local and foreign studies[17,18,20] which is mostly due to the increased uncontrolled antimicrobial prescription.

Regarding the susceptibility of the most common isolated bacteria, *Klebsiella* was susceptible to imipenem (90%), ciprofloxacin (60%), and gentamycin (33%) and completely resistant to amikacin and Pipracillin.

Carbapenems, a beta–lactam group of dugs including imipenem, carbapenem and meropenem has shown a high antimicrobial effect against Gram-negative pathogens, different studies agree with this study results.[14,19-22] This group is reserved for multidrug resistant bacteria.

Aminoglycosides (gentamycin and amikacin) susceptibility toward *Klebsiella* was lower than expected in this study in comparison with other previous studies[20-22] indicating that therapeutic protocols including empirical aminoglycosides may need to be reviewed.

Klebsiella ciprofloxacin susceptibility remained within the intermediate range in comparison with other recent studies.[10,20,22]

The susceptibility of *E. coli* toward imipenem was complete (100%), but for ciprofloxacin was totally resistant. For aminoglycosides both of the susceptibility and resistance were equal (50%). *E. coli* bacteria showed variable susceptibility patterns in the previous studies, ciprofloxacin susceptibility ranged from 20% (10) to 75% in more recent studies.[22]

Vancomycin has shown an excellent efficacy (100%) against *Staphylococcus* spp. this is because its high bactericidal effect through inhibition of bacterial cell wall synthesis. In addition, it is effective against Gram-negative microbes as stated in different studies including Iraq[14,17-22] but it was not tested in this study against Gram-negative isolates.

Conclusion

Klebsiella is the most predominant bacterial pathogen isolated from the blood cultures of neonates suspected to have NS followed by *E. coli* and *S. aureus*. Gram-negative bacteria were more resistant toward the used antimicrobials than Gram-positive microbe. Vancomycin, clindamycin and amikacin were totally effective with 100% Gram-positive bacterial susceptibility. Imipenem caused the greatest Gram-negative bacterial susceptibility (80%) followed by ciprofloxacin (46%). *Klebsiella* pneumoniae bacteria showed its minimum antimicrobial resistance (10%) toward imipenem. According to these results, NS management regimes should take in consideration increasing microbial resistance and susceptibility toward the commonly used antimicrobials.

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Conflicts of interest

There are no conflicts of interest.

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