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Original Article

Pattern of bacterial profile and antibiotic susceptibility among neonatal sepsis cases at Cairo University Children Hospital

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الملخص

أهداف البحث: يعتبر إنتان الدم الوليدي السبب الرئيس الثالث لمرض ووفاة الأطفال في وحدات الرعاية المركزة لحديثي الولادة. تعتبر مقاومة البكتيريا للمضادات الحيوية في وحدة العناية المركزة لحديثي الولادة عبئا كبيرا. في هذه الدراسة، استهدفنا دراسة مدى انتشار البكتيريا وكذلك المضادات الحيوية المستخدمة بين المرضى الذين تم قبولهم بالإنتان، خلال العام ٢٠١٧، في وحدة العناية المركزة لحديثي الولادة في مستشفيات الأطفال بجامعة القاهرة، التي تضم ٥٠ حاضنة.

طرق البحث: تم تقييم حديثي الولادة مع اشتباه إنتان الدم عن طريق مزرعة الدم. تم تحديد التضمين في الدراسة عن طريق مزرعة الدم البكتيرية الإيجابية، في حين تم استبعاد حالات الزراعة السلبية. كما تمت زراعة العينات الإيجابية باستخدام الزراعة الفرعية على آغارات الدم، وآغار ماكونكي، وآغار الشوكولاتة. تم تحديد البكتيريا عن طريق صبغة غرام وردود الفعل الكيميائية الحيوية. وتم إجراء الحساسية للمضادات الحيوية بطريقة نشر قرص كيربي باور.

النتائج: وجدت سبعون مزرعة دم إيجابية (٣١.٧٪): ٤٥.٣٪ لكليبسيلة، و٢٢.٧٪ للمكورات العنقودية سالبة الكواجيولاز، تليها البكتيريا الراكدة، والمكورات العنقودية الذهبية المقاومة للميثيسيلين، والبكتيريا الزائفة، والمكورات العنقودية الذهبية، والأمعائية والبكتيريا العقدية. ولوحظت مقاومة عالية لجميع السيفالوسبورينات، ومجموعات البيتا-لاكتاماز، والبنسلين، والكاربابينيمات، والأمينوغليكوزيدات. أظهرت جميع بكتيريا الأمعائية سلبية الغرام أعلى حساسية لليفوفلوكساسين، في حين أن البكتيريا الزائفة والبكتيريا الراكدة كانتا شديدة الحساسية لبولي ميكسين ب. في حين استجابت البكتيريا الموجبة الجرام لمضاد القانكومايسين والليزوليد. وكانت العقدية أقل حساسية للقانكومايسين، ولكنها استجابت للغاية للماكروليدات وسيفوتاكسيم.

الاستنتاجات: توصلت الدراسة إلى أن ميكروبي الكليبسيلة والمكورات العنقودية سالبة الكواجيولاز هما أكثر الميكروبات تواجدا بمزارع الدم، وأن مقاومة الميكروبات للمضادات الحيوية قد وصلت إلى مستويات تنذر بالخطر وتشكل خطرا داهما على حياة هؤلاء الأطفال مما يستدعى التخطيط الدقيق لتنظيم استعمال المضادات الحيوية.

الكلمات المفتاحية: مقاومة المضادات الحيوية؛ الحساسية؛ انتشار البكتيريا؛ الإنتان الوليدي

Abstract

Objectives: Neonatal sepsis is the third leading contributor to mortality and morbidity. Emanating resistance to antibiotics in neonatal intensive care units (NICUs) is considered a major burden. In this study, we aimed to investigate the bacterial prevalence and antibiotic profile among patients admitted with sepsis in the NICU of Cairo University Children Hospital.

Methods: Neonates with suspected sepsis were evaluated for bacterial sepsis in their blood cultures. The neonates with positive bacterial blood culture were included in this study, whereas neonates with negative culture were excluded. Positive samples were sub-cultured on blood, MacConkey, and chocolate agar plates. Organisms were identified by Gram staining and biochemical reactions. Antibiotic susceptibility was assessed by the Kirby–Bauer disc diffusion method.

Results: Seventy blood cultures (31.7%) were bacteria-positive: 45.3% for *Klebsiella*, 22.7% for coagulase-

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negative staphylococci (CoNS), and for *Acinetobacter* (10.7%), methicillin-resistant *Staphylococcus aureus* (MRSA) (9.3%), *Pseudomonas* (5.3%), *Enterobacter* (4%), and streptococci (2.7%). High resistance to all cephalosporins, B-lactamase combinations, penicillin, carbapenems, and aminoglycosides was observed. All Gram-negative *Enterobacteria* showed the highest sensitivity to levofloxacin, whereas *Pseudomonas* and *Acinetobacter* were highly sensitive to polymyxin B. Gram-positive samples were sensitive to vancomycin and linezolid. *Streptococci* were slightly sensitive to vancomycin and highly sensitive to macrolides and cefotaxime.

Conclusions: In our study, *Klebsiella* and CoNS were the most common isolates in neonatal sepsis. The levels of multidrug-resistant strains were alarmingly high. This finding negatively affected the outcomes, prompting the need for a strict guideline for antibiotics use.

Keywords: Antibiotic resistance; Antibiotic sensitivity; Bacterial prevalence; Neonatal sepsis

Introduction

The outcome of neonatal sepsis is a crucial factor affecting neonatal mortality and morbidity rates. In developing countries, 30−50% of neonatal mortality is attributed to sepsis. Despite recent advances in health care, delayed identification and inappropriate treatment remain as key factors causing high neonatal mortality[1,2]; however, these problems can be averted through judicious antimicrobial selection and advanced adjuvant care.[3] Contributing factors in developing countries include the lack of people awareness of alarming signs of sepsis, lack of training of medical personnel, and limited availability of reliable laboratories, particularly blood culture, in areas far from hospitals; these factors lead to compromised care, outdated antibiotic guidelines, and emergence of resistance.[4]

Neonatal sepsis is a syndrome of clinical manifestations of inflammatory responses due to a spectrum of systemic infections,[2] such as septicaemia, pneumonia, bone-related infections, and meningitis.[3] Clinical manifestations of sepsis are non-specific and resemble many non-infection-related disorders. However, despite this dilemma, consensus definition and global guidelines are still lacking[5,6] owing to variability among different studies and clinical practices, as well as epidemiologic differences.[5] Although blood culture is considered the gold standard in the diagnosis of neonatal sepsis, low rates of positivity constitute a real management challenge.[6,7]

Neonatal sepsis is classified according to the onset of its presentation: early-onset sepsis (EOS) occurs at <72 h of age and late-onset sepsis (LOS) occurs at >72 h of age, with implications on possible risk factors, probable organism, and proposed empirical treatment.[3] Empirical antibiotics are usually initiated once sepsis is suspected. However, increasing emergence of multidrug-resistant organisms reduces antibiotics options and defers adequate treatment implementation[8]; hence, there is a need for institutional guidelines based on local microbial prevalence and their antibiotic susceptibility patterns.[9]

Our study was conducted in Cairo University Children Hospitals, which contains 623 beds, 125 paediatric ICU beds, 50 NICU incubators, and 10 postoperative NICU incubators. We aimed to analyse bacterial spectra in blood cultures collected from cases suspected of sepsis in the NICU over a year. Antibiotic sensitivity and resistance patterns, which are associated comorbidities, as well as outcomes were evaluated to help develop management guidelines for suspected neonatal sepsis in light of continuously evolving challenges of antimicrobial resistance.

Materials and Methods

This is a cross-sectional retrospective study conducted in the NICU of Cairo University Children Hospitals, which is a tertiary care referral unit. Blood cultures from neonates admitted with suspected sepsis between January 2017 and December 2017 were evaluated. Inclusion criteria: neonatal sepsis cases with positive bacterial blood culture. Exclusion criteria: suspected sepsis with negative blood cultures.

Clinical data were collected, including name, age, gender, gestational age, associated diagnoses, place of referral, and birth weight. Neonates were screened for complete blood counts, particularly total leucocyte count (immature/total ratio and absolute neutrophilic count were calculated), and C-reactive protein level.

Blood cultures were collected from all cases on admission prior to the initiation of antimicrobial therapy. Next, 1−2 ml of blood was drawn from a unilateral venipuncture under aseptic conditions, and then injected to a blood culture bottle for analysis with an automated BacT/ALERT 3D 60 microbial system (bioMerieoux, France). This is an automated microbial detection system that monitors microbial growth through a chemical sensor that detects increased production of carbon dioxide resulting from microbial growth. Blood cultures without any microbial growth after 5 days of incubation were considered negative. Positive samples were subcultured on blood, MacConkey, and chocolate agar plates. The plates were examined for growth after 24−48 h of incubation at 37 °C. Organisms were identified by the colony morphology, Gram staining, and examination of biochemical characteristics according to the Clinical Microbiology Procedures Handbook.[10] Biochemical assays for Gram-positive isolates included catalase, DNase agar, mannitol salt agar, and haemolysis assays. Biochemical assays for Gram-negative isolates included triple sugar iron agar (TSI); lysine iron agar (LIA), motility, indole, ornithine (MIO); citrate; urease; and oxidase assays.[11] The quality control of all media and biochemical reactions was guaranteed according to the standards of the American Type Culture Collection.

Antibiotic susceptibility tests were carried out by the Kirby−Bauer disc diffusion method, as stated by the

standards of Clinical and Laboratory Standards Institute (CLSI). Three to four colonies of the isolated organism were mixed with sterile saline until the turbidity of the mixture was equivalent to 0.5 MacFarland, and then the suspension was swabbed over Muller-Hinton agar (MHA) plates. Antibiotic discs were then added within 15 min and incubated overnight at 37 °C (18 Hours). The different groups of antibiotics were represented. The Gram-negative bacilli antibiotics used included beta lactam drugs, such as ampicillin (10 μg), amoxicillin/clavulanic acid (20/10 μg), piperacillin/tazobactam (100/10 μg), cefoperazone/sulbactam (75/30 μg), cefoxitin (30 μg), cefotaxime (30 μg), ceftriaxone (30 μg), ceftazidime (30 μg), cefepime (30 μg), meropenem (10 μg), and imipenem (10 μg); aminoglycosides, such as amikacin (30 μg) and gentamicin (10 μg); fluoroquinolones, such as ciprofloxacin (5 μg) and levofloxacin (5 μg); and sulfamethoxazole/trimethoprim (SXT) (923.75/1.25 μg). Polymyxin B (300 units) was tested for identifying non-fermenter Gram-negative organisms (*Pseudomonas* and *Acinetobacter*). The Gram-positive bacteria antibiotics used included ampicillin (10 μg), cefoxitin (30 μg), vancomycin (30 μg), clindamycin (2 μg), erythromycin (15 μg), doxycycline (30 μg), chloramphenicol (30 μg), linezolid (30 μg), rifampicin (10 μg), gentamicin (10 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), and SXT (923.75/1.25 μg). All discs were obtained from Oxoid, England. Inhibition zones of different antibiotics were interpreted according to the CLSI recommendations.[12] Multidrug-resistant (MDR) bacteria were defined as bacteria that showed resistance to at least one agent from three or more of the antimicrobial classes tested.[13]

Statistical methods: Data were transferred to Microsoft Excel. Categorical data were analysed with descriptive statistics, and the results were expressed as percentages and frequencies. The Chi square test was used for correlation analysis of more than one variable, and P values less than 0.05 were considered significant.

Results

A total of 911 neonates were admitted to the NICU of Cairo University Children Hospitals during the studied period. There were 221 blood cultures collected upon admission: 99 cases (44.8%) were clinically suspected of sepsis, 45 (20.4%) had respiratory distress, 15 (6.8%) had pneumonia, 62 (28.1%) had other diagnoses. Seventy patients (31.7%) had positive blood culture and 151 (68.3%) had negative blood culture. In this study, only sepsis cases with positive blood culture were included, analysed, and categorized according to the onset of symptoms into two groups: EOS and LOS. Characteristics of neonates with sepsis-positive blood culture are presented below (Table 1).

Among the neonates, 39 (55.7%) were males and 31 (44.3%) were females. A total of 34 neonates were preterm (48.6%) and 36 were full term (51.4%). There were insignificant differences in gender, gestational age, birth weight, mode of delivery, history of premature rupture of membranes, presence of intervention such as central venous line, use of umbilical catheter or mechanical ventilation, and outcome according to onset of sepsis. Comorbidities such as congenital heart disease, congenital anomalies, and respiratory distress syndrome were present in 33 patients, of which 14 (42.4%) showed EOS and 19 (57.6%) showed LOS.

Table 1: Characteristics of patients with sepsis-positive blood culture.

Characteristics	Early-onset sepsis (n = 29)	Late-onset sepsis (n = 41)	Chi square	P value
Gender				
Male	17(58.6%)	22(53.7%)	0.17	0.681
Female	12(41.4%)	19(46.3%)		
Gestational age				
Preterm	15(51.7%)	19(46.3%)	0.197	0.657
Full term	14(48.3%)	22(53.7%)		
Birth weight				
<2500 g	15(51.7%)	20(48.8%)	0.059	0.808
>2500 g	14(48.3%)	21(51.2%)		
Mode of delivery				
Vaginal	9(31%)	11(26.8%)	0.147	0.701
Caesarean section	20(69%)	30(73.2%)		
Premature rupture of membranes	2(6.9%)	1(2.4%)	0.823	0.364
Intervention	11(37.9%)	18(43.9%)	0.25	0.617
Mortality	11(37.9%)	13(31.7%)	0.292	0.589

Data are presented as numbers (percentages).

As shown in Tables 2 and 3, 75 bacteria were isolated from our patients, with two bacteria isolated from five cases. Of the 75 organisms found, 49 (65.3%) were Gram-negative and 26 (34.7%) were Gram-positive. The predominantly isolated strains were *Klebsiella* species (34/75, 45.3%), whereas the second-most prevalent organism was CoNS (17/75, 22.7%), followed by *Acinetobacter* (8/75, 10.7%), MRSA (7/75, 9.3%), *Pseudomonas* (4/75, 5.3%), *Enterobacter* (3/75, 4%), and streptococci (2/75, 2.7%). Gram-positive organisms were significantly more prevalent in LOS than in EOS (19/26, 73.1%), specifically CoNS (13/17, 76.5%).

Gram-positive isolates were significantly more abundant in previously non-hospitalized cases than in those referred from other hospitals (Table 4), but there was no significant difference in Gram-negative isolates between the two types of cases. Two isolates were found in two previously hospitalized cases and four isolates were found in previously non-hospitalized cases, but this difference was not significant.

As shown in Table 5, among neonates with two isolates, two (33.3%) died, with one of them showing comorbidity, whereas the other four (66.7%) were discharged. In comparison, 22 (34.4%) of neonates with a single isolated organism died, with the majority of them (16/22) showing comorbidities, whereas the other 42 cases (65.6%) were discharged. No significant difference was observed.

Antibiotic resistance/susceptibility profiles (Table 6):

- Gram-negative bacteria:
  - *Klebsiella* showed the highest susceptibility to levofloxacin (53%) as well as low susceptibility to ciprofloxacin, SXT, and amikacin (15%, 15%, and 18%, respectively). *Klebsiella* was highly resistant to all beta

Table 2: Distribution of isolated bacteria according to sepsis onset and gestational age.

Isolated organism (total = 75)	N (%)	Early onset N (%)	Late onset N (%)	Test proportion	P value	Preterm N (%)	Full term N (%)	Test proportion	P value
Gram-negative									
Klebsiella species	34(45.3)	17(50)	17(50)	0.5	1	15(44.1)	19(55.9)	0.5	0.6
Enterobacter	3(4)	2(66.7)	1(33.3)	0.5	1	2(66.7)	1(33.3)	0.5	1
Pseudomonas	4(5.3)	0(0)	4(100)	0.5	0.12	2(50)	2(50)	0.5	1
Acinetobacter	8(10.7)	5(62.5)	3(37.5)	0.5	o.72	3(37.5)	5(62.5)	0.5	0.72
Total	49(65.3)	24(49)	25(51)	0.5	1	22(44.9)	27(55.1)	0.5	0.56
Gram-positive									
CoNS	17(22.7)	4(23.5)	13(76.5)	0.5	**0.04***	9(52.9)	8(47.1)	0.5	1
MRSA	7(9.3)	1(14.3)	6(85.7)	0.5	0.125	4(57.1)	3(42.9)	0.5	1
Strept.	2(2.7)	2(100)	0(0)	0.5	0.12	0(0)	2(100)	0.5	0.12
Total	26(34.7)	7(26.9)	19(73.1)	0.5	***0.02****	13(50)	13(50)	0.5	1

*P < 0.05 is significant.
CoNS: coagulase-negative *staphylococci*; MRSA: methicillin-resistant *Staphylococcus aureus*; Strept.: streptococci.
Data are presented as numbers (percentages).

Table 3: Types of isolates.

Isolates	Type	Number of patients	Percentage (%)
A single isolated organism	Gram-negative	42	60
	Gram-positive	22	31.4
Two isolated organisms	Gram-negative/-positive	4	5.7
	Gram-negative/-negative	1	1.4
	Gram-negative/candida	1	1.4

Data are presented as numbers and percentages.

Table 4: Differences in isolates between referred cases from another hospital and previously non-hospitalized cases.

Isolated organism	Referred cases from another hospital n = 28	Previously non-hospitalized cases n = 42	Chi square	P value
Gram-negative (49)	22	27	2.3	***0.00****
Gram-positive (26)	7	19		
Two isolates (6)	2	4	0.12	0.72
Single isolate (64)	26	38		

*P < 0.05 is significant.
Data are presented as numbers.

Table 5: Comparison of outcomes between single-isolate and two-isolate cases and their relation to comorbidities.

Patients (n = 70)		Single isolate (n = 64, 91.4%)	Two isolates (n = 6, 8.6%)	Chi square	P value
Mortality (n = 24)	Comorbidity	16(25%)	1(16.7%)	3.6	0.36
	No comorbidity	6 (9.4%)	1(16.7%)		
Discharged (n = 46)	Comorbidity	13(20.3%)	3(50%)		
	No comorbidity	29(45.3%)	1(16.7%)		

Data are presented as numbers (percentages).

lactam antibiotics, even imipenem and meropenem (91% and 94%, respectively). Most of the isolated *Klebsiella* were MDR (69.7%).

- *Acinetobacter* was highly susceptible to polymyxin (100%), but showed limited susceptibility to gentamycin (25%), amikacin (25%), ciprofloxacin (25%), levofloxacin (25%), and SXT (12%). All *Acinetobacter* isolates were MDR.
- *Pseudomonas* had 100% susceptibility to polymyxin and 50% susceptibility to amikacin, as well as 100% resistance to gentamicin, fluoroquinolones, and all beta lactam antibiotics.
- *Enterobacter* showed 100% sensitivity to levofloxacin and 67% sensitivity to amikacin and ciprofloxacin, as well as 100% resistance to SXT, gentamicin, and all beta lactam antibiotics.

- Gram-positive bacteria:
  - CoNS were 100% sensitive to vancomycin and linezolid, as well as 100% resistant to imipenem, ciprofloxacin, B-lactamase combinations, and cephalosporins. They also showed high resistance to gentamicin, SXT, and levofloxacin (93%), erythromycin (88%), chloramphenicol (76%), and clindamycin (71%).
  - MRSA showed 100% sensitivity to vancomycin and linezolid and 71% to chloramphenicol and clindamycin, as well as 100% resistance to gentamycin, ciprofloxacin, levofloxacin, SXT, ampicillin-sulbactam, and amoxiclav. It also showed high resistance to rifampicin and doxycycline (83%).
  - Streptococci were highly sensitive to macrolides and cefotaxime (100%), slightly sensitive (50%) to vancomycin, and highly resistant to amikacin, quinolones, linezolid, ampicillin-sulbactam, and amoxiclav.

Table 6: Relative resistance of isolated organisms to relevant antibiotics.

Antibiotics tested	Gram-negative				Gram-positive		
	Klebsiella	*Pseudomonas*	*Enterobacter*	*Acinetobacter*	CoNS	MRSA	Strept.
	R/T (%)	R/T (%)	R/T (%)	R/T (%)	R/T (%)	R/T (%)	R/T (%)
Polymyxin		0/4(0)		0/8(0)			
Gentamycin	32/34(94)	4/4(100)	3/3(100)	6/8(75)	13/14(93)	7/7(100)	1/1(100)
Amikacin	28/34(82)	1/2 (50)	1/3(33)	6/8(75)			
Meropenem	32/34(94)	1/1(100)	3/3(100)	1/1(100)			
Imipenem	31/34(91)	4/4(100)	3/3(100)	8/8(100)			
Ciprofloxacin	28/34(82)	4/4(100)	1/3(33)	6/8(75)	13/13(100)	7/7(100)	2/2(100)
Levofloxacin	16/34(47)	4/4(100)	0/3(0)	6/8(75)	13/14(93)	7/7(100)	2/2(100)
SXT	29/34(85)	IR	3/3(100)	7/8(88)	13/14(93)	7/7(100)	
Ampicillin-sulbactam	34/34(100)	IR	3/3(100)	8/8(100)	17/17(100)	7/7(100)	2/2(100)
Amoxiclav	34/34(100)	IR	3/3(100)	8/8(100)	15/15(100)	7/7(100)	1/1(100)
Pip-tazo	34/34(100)	1/2(50)	3/3(100)	8/8(100)			
Sulperazone	34/34(100)	1/1(100)	3/3(100)	8/8(100)			
Ceftazidime	34/34(100)		3/3(100)	8/8(100)			
Cefotaxime	34/34(100)	IR	3/3(100)	8/8(100)	14/15(93)	7/7(100)	0/1(0)
Ceftriaxone	34/34(100)	IR	3/3(100)	8/8(100)	14/14(100)	7/7(100)	
Cefuroxime	34/34(100)	IR	3/3(100)	IR	14/14(100)		
Cefoperazone	34/34(100)	IR	3/3(100)	8/8(100)	14/14(100)	7/7(100)	
Cefepime	34/34(100)	3/3(100)	3/3(100)	8/8(100)	14/14(100)		
Cefoxitin	34/34(100)	1/2(50)	3/3(100)	8/8(100)	14/14(100)	7/7(100)	
Rifampicin					13/17(76)	5/6(83)	
Vancomycin					0/17(0)	0/7(0)	1/2(50)
Chloramphenicol					13/17(76)	2/7(29)	
Linezolid					0/6(0)	0/3(0)	2/2(100)
Clindamycin					12/17(71)	2/7(29)	0/1(0)
Erythromycin					15/17(88)	7/7(100)	0/1(0)

R/T: Number of resistant organisms/total; IR: intrinsic resistance; CoNS: coagulase-negative staphylococci; MRSA: methicillin-resistant *Staphylococcus aureus*; strept: streptococci; amociclav: amoxicillin-clavulanic acid; pip-tazo: piperacillin tazobactam; SXT: sulfamethoxazole trimethoprim.
Data are presented as numbers (percentages).

Discussion

The incidence of neonatal sepsis, a major causal factor of mortality in the NICU, is increasing owing to the surge in antibiotic resistance.[14] In our study, the proven sepsis cases were mostly EOS and showed mortality rate of 34.3%, similar to the 30.8% previously reported by Turhan et al.[15] Immediate microbial identification and implementation of appropriate antibiotics are crucial.[16] Blood culture remains the gold standard for microbial identification despite the long time needed, low sensitivity, and potential contamination. Moreover, blood culture positivity varies considerably among studies owing to different techniques or study designs. In our cases, positive results were shown by 31.7% of blood cultures, similar to the 33% reported by Kabwe et al.,[17] but different from other reports of 20.7%,[18] 15%,[19] 38.9% from a single site, and 46.5% from two cultures simultaneously collected from different sites.[20] The relatively low percentage of sepsis-positive blood cultures in this study can be explained by the fact that 51 (23.1%) negative cultures out of the 221 blood cultures tested were neonates referred from another hospital, who might have previously received antibiotics.[21]

The majority of sepsis-positive cases were LOS (58.6%); a comparable result (55.8%)[22] and a higher result (71.2%)[21] have also been reported. This might reflect the higher incidence of community-acquired infections among neonates. The result of our study was contrast to that of another study[18] where EOS predominated at 78.3%. The male-to-female ratio among our cases (39:31) was similar to that in a study by Kabwe et al.[17]

Owing to the causative agents that vary from region to region[23] and the emergence of antibiotic resistance,[24] knowledge of prevailing organisms in the local environment of an NICU and their antibiotic sensitivity pattern according to periodic surveys are essential for realizing effective treatment and favourable outcomes. In the present study, Gram-negative bacteria were the most notable (65.3%), consistent with the results of previous studies.[18,23] In contrast, Gram-positive organisms predominated in other studies.[22,25]

Klebsiella predominated our isolates (45.3%) equally among both EOS and LOS cases, the same as in former studies.[18,26] However, *Klebsiella* predominated among EOS cases in another study,[26] probably because their patients included more EOS neonates. *Klebsiella* also predominated among LOS cases in another study.[22] Other major isolates found in different studies included CoNS,[27,28] staphylococci,[25,29] and *Acinetobacter*.[23] In our study, however, CoNS was the second-most (22.7%) prevalent isolate, followed by *Acinetobacter* (10.7%), MRSA (9.3%), *Pseudomonas* (5.3%), *Enterobacter* (4%), and streptococci (2.7%).

Gram-positive organisms, particularly CoNS, were significantly more prevalent among LOS cases (13/17, 76.5%), consistent with the results of previous studies,[22,25] but different from the findings of Pokhrel et al.[18] and Shretha et al.[26] Two published studies involving 5 NICUs in the Gulf Cooperation Council area reported that, in contrast to our findings, Group B Streptococci was the most prevalent organism in EOS cases (60%), followed by CoNS (6%) and *Klebsiella* (4%).[30] CoNS was the most prevalent (34%) isolates among their LOS cases,[31] which is similar to our result (31%); however, the prevalence of *Klebsiella* was different between our and their results (41.5% vs 22.8%). Another study in KSA over 5 years showed Group B Streptococci prevalence of 33.3% among EOS cases, as well as Staphylococci and *Klebsiella* prevalence of 47.2% and 17.9%, respectively, among LOS cases.[32]

Among our cases, 28 were referred from other hospitals. Gram-positive bacteria were significantly more prevalent among previously non-hospitalized, whereas the prevalence of Gram-negative isolates was not significantly different between the two types of cases. Previous use of antibiotics leads to difficulties in interpreting blood cultures.[7] Furthermore, prior use of antibacterial drugs, particularly cephalosporins, ampicillin, and gentamycin, along with prolonged exposure, are associated with high prevalence of MDR bacteria.[33] MDR bacteria are not limited to hospitals; they are also widely spread in the community environment, especially in the Middle East area owing to the excessive use and over-the-counter availability of antibiotics.[34]

Two isolates were found in 8.6% of our cases; however, the presence of two isolates was not significantly associated with mortality or comorbidities. Pokhrel et al.[18] found a close percentage of 7.2%. Lower results were reported by Tsai et al.[35] over 8 years (4.4%), with similar insignificant difference in coexisting chronic illnesses or sepsis-related mortality between two-isolate and single-isolate cases. However, in contrast, a 16-year study reported an average of 14% polymicrobial episodes in the NICU, with >3-fold increase in mortality.[36]

The Gram-negative organisms isolated in our study were resistant to most of the commonly used antibiotics, such as ampicillin, ampicillin-sulbactam, amoxiclav, cephalosporins, gentamicin, and SXT, similar to the findings of other studies.[18,27,37,38] Isolates resistant to carbapenems were also found in our cases, on the contrary to previous reports,[18,26,38] but similar to others.[23] High sensitivity to polymyxin (100%) was shown by *Pseudomonas* and *Acinetobacter* in the present and other studies.[18,39]

In our findings, *Klebsiella* showed 53% susceptibility to levofloxacin and low susceptibility to ciprofloxacin, amikacin, and gentamicin (12%, 12%, and 6%, respectively). Sharma et al.[29] also found that *Klebsiella* is susceptible to amikacin and gentamicin (20% and 0%, respectively), but less susceptible (30%) to levofloxacin. More than half of *Klebsiella* isolates and 43% of all Gram-negative isolates from LOS neonates in the GCC study[31] were resistant to third-generation cephalosporins, in contrast to our results (100% resistance). Higher susceptibilities of *Klebsiella* isolates to amikacin (89%), gentamycin (83%), and cephalosporins (83–85%) were reported by Almatary et al.,[32] which may reflect relatively more controlled use of antibiotics.

The *Enterobacter* in our study showed 100% susceptibility to levofloxacin, 67% susceptibility to amikacin and ciprofloxacin, and 100% resistance to imipenem. Sharma et al.[29] reported that *Enterobacter* showed 100% susceptibility to levofloxacin, gentamicin, and imipenem, as well as 25% susceptibility to amikacin and ciprofloxacin. Almatary et al.[32] reported that *Enterobacter* was 100% sensitive to amikacin and 83% sensitive to gentamycin and cefepime. These variabilities among studies can be explained by differences in preference of the antibiotics used among different communities, which might lead to different patterns of development of antibiotics resistance.

Acinetobacter was highly susceptible to only polymyxin and highly sensitive to minocycline, consistent with other reports.[18,23] In our study, *Acinetobacter* showed limited susceptibility (25%) to levofloxacin and ciprofloxacin, consistent with the report by Shrestha et al.,[26] as well as to gentamycin and amikacin, consistent with the result of Ahmed et al.[23] Higher sensitivity of *Acinetobacter* to gentamicin (66.7%) was reported by Sharma et al.[40]

MDR isolates accounted for 69.7% of *Klebsiella* isolates and 100% of *Acinetobacter* isolates. High prevalence of MDR isolates were also reported by others.[22,41] The MDR isolates in EOS and LOS cases were consistent with the findings of a previous study in India.[42]

The Gram-positive bacteria isolated in our study showed the highest sensitivity to vancomycin and linezolid, consistent with other findings.[18] However, streptococci was 50% resistant to vancomycin, inconsistent with other studies,[32,40] and highly resistant to linezolid, also contrary to another report.[23] In addition, streptococci in our cases were highly sensitive (100%) to cefotaxime and macrolides, consistent with the report by Sharma et al.[40] The low susceptibility of streptococci to rifampicin in our cases was inconsistent with the finding of Ahmad et al.,[23] in which CoNS and streptococci were 83% and 75% susceptible to rifampicin, respectively. MRSA showed 100% sensitivity to linezolid and vancomycin in our

study, showing higher sensitivity than that observed by Shehab El Din et al.[22]

CoNS showed the highest susceptibility (100%) to linezolid, consistent with previous reports,[18,23] and vancomycin comparable to other reports.[18,23,32,39,43] The high resistance of CoNS to penicillin, gentamicin, quinolones, and cephalosporins has been previously reported.[18,23,40] Other studies also found that CoNS was sensitive to gentamycin and amikacin21. In our cases, CoNS showed 76% resistance to chloramphenicol, in contrast to the 100% sensitivity reported in another study.[23]

Consistent with the aim of our study, we identified Gram-negative organisms, mainly *Klebsiella* isolates, as the most prevalent organisms in our unit. We also confirmed high levels of antibiotics resistance (69.7% of the isolates were MDR), leaving extremely limited antibiotic choices (the highest sensitivity was 53% to levofloxacin). The second-most prevalent organism was CoNS, which showed 100% sensitivity to vancomycin and linezolid and resistant to most other antibiotics. *Acinetobacter* (the second-most common Gram-negative isolate) showed only 25% sensitivity to levofloxacin and 100% to polymyxin.

Taking these findings together, short- and long-term strategies can be planned. Our understanding of this pattern would help us avoid using highly ineffective empirical choices of antibiotics, such as ampicillin, gentamycin, and cephalosporins, which are currently being used. Changes should then be made based on further identification of isolated organisms and results of specific sensitivity assay. In the long term, such data can complement similar and consecutive studies in meta-analyses to further develop local, national, and international guidelines.

Limitations

The limitations of this study included the relatively small number of sepsis-positive blood cultures and the high number of patients who received prior antibiotics treatment, in addition to being a single centre study of over one year.

Conclusions

Bacterial prevalence and antibiotic resistance differed considerably among studies. Our cases showed higher prevalence of Gram-negative bacteria in both EOS and LOS cases, whereas Gram-positive bacteria were common in LOS cases. Resistance to antibiotics, notably to the commonly used ones, was at distressing levels, and appropriate implementation of likely susceptible antibiotics would have a considerable impact on outcomes.

Recommendations

Longer periods of study are recommended to include more patients and to monitor changing patterns. Although each NICU needs to develop local protocols based on their specific microbial pattern, multicentre collection of data and analysis of antibiotic resistance emergence are suggested to help develop a national protocol for better outcomes. Our study also suggested that raising awareness on judicious use of antibiotics was essential to curb the escalation of resistance levels.

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Conflict of interest

There is no conflict of interest.

Ethical approval

This study was approved and confirmed by the relevant provisions of the Ethics Committee of Cairo University Children Hospital.

Authors contributions

MA conceived and designed the study, collected the data and references, reviewed the results, and wrote the manuscript. EE participated in the study designing, reviewed the results, analysed the data, and carried out the statistical analysis of the results. NMR performed the laboratory work, interpreted the results, and participated in manuscript writing and reference collection. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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