Emergence of Drug-Resistant Pathogens in a Neonatal Intensive Care Unit

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J Pediatr Infect Dis 2023;18:326–330.

Abstract

Objective Neonatal bloodstream infections (BSIs) due to drug-resistant pathogens are a major cause of neonatal morbidity and mortality. Unfortunately, data regarding the pathogens and their resistance profile are limited in developing countries. The aim of this study was to determine the bacteriological profile and antimicrobial susceptibility patterns in neonatal BSI at a university hospital in Türkiye.

Methods Medical records of neonates with suspected sepsis were retrospectively reviewed during the study period (between January 1, 2018, and December 31, 2020) for demographic data, blood culture, and antimicrobial susceptibility test results.

Results During the study period, 117 BSI episodes were encountered in 106 neonates. The most common pathogen isolated was *Staphylococcus epidermidis* ($n = 86$, 73.5%), followed by *Klebsiella pneumoniae* ($n = 11$, 9.4%). Methicillin resistance among staphylococci (77/93, 82.8%) and extended-spectrum beta-lactamase (ESBL) production among Enterobacterales (14/17, 82.4%) were common. Gentamicin resistance was detected in 70.1% (54/77) of methicillin-resistant staphylococci and 78.6% (11/14) of ESBL (+) Enterobacterales. Vancomycin and colistin resistance were not detected.

Conclusion The high rate of resistant pathogens encountered in neonatal BSIs underline the importance of constant surveillance of the local pathogens and their antimicrobial susceptibility patterns, which is crucial for implementing appropriate therapy that could save lives and lower the burden of antimicrobial resistance.

Keywords
- bloodstream infection
- neonatal sepsis
- blood culture
- antibiotic resistance
- neonatal ICU

Introduction

The global neonatal mortality rate has declined approximately 50% in the past two decades; nevertheless, in 2020, 2.4 million newborns lost their lives worldwide, amounting to 6,500 newborn deaths per day.[1] According to the latest global burden of disease data in 2019, 227,000 neonates died due to sepsis, accounting for 4.5% (4.1–4.9) of deaths under 5 years.[2]

Neonatal sepsis occurs due to an infection involving the bloodstream, and it remains a leading cause of mortality and morbidity among neonates especially in middle- and low-income countries.[3] Neonates with sepsis may present with nonspecific signs and symptoms when infected, but identifying the pathogen via blood culture or other methods is the key in diagnosing that it is truly sepsis.[3] Importantly, the sensitivity of blood cultures is significantly improved with appropriate blood volumes.[4] However, antibiotic treatment decisions cannot await the results of blood cultures. Instead, antimicrobial therapy is usually implemented empirically on clinical suspicion of sepsis. To prevent the emergence of antimicrobial-resistant organisms, therapy should be instituted according to the local pathogen profiles and their

received
July 17, 2023
accepted after revision
September 11, 2023

DOI https://doi.org/10.1055/s-0043-1775838.
ISSN 1305-7707.

antimicrobial susceptibility patterns. Unfortunately, data on this topic are limited in low- and middle-income countries.[5,6]

Türkiye is a middle-income country in the World Health Organization (WHO) European region and has the highest ratio of child population to the general population, when compared with countries in the European Union. As with the rest of the world, in Türkiye, the infant mortality rate has declined gradually over the last decade, from 13.9 in 2009 to 9.2 in 2021.[7] However, approximately 1 in 10 newborn deaths are due to sepsis and other infections,[8] most of which could be prevented with prompt diagnosis and appropriate and timely treatment.

In this study, we aimed to investigate the burden of bloodstream infections (BSIs), determine the spectrum of pathogens and their antimicrobial susceptibility patterns in a neonatal intensive care unit (NICU), and report the preliminary results of a new university hospital in Istanbul, Türkiye.

Materials and Methods

In this retrospective cross-sectional study, medical records of all neonates suspected to have sepsis admitted to NICU between January 1, 2018, and December 31, 2020, were analyzed. Patients' laboratory records were reviewed for demographic data, blood culture, and antimicrobial susceptibility test results.

Two sets of blood samples (1 mL) were obtained from each neonate for culture. Each set consisted of one aerobic and one anaerobic bottle; if adequate volume of blood sample could not be obtained for two sets, the entire blood sample was inoculated into the aerobic bottle. Blood samples were incubated for 5 days in the automated hemoculture system BacT/Alert (bioMerieux); isolates were identified by VITEK 2 compact (bioMerieux). Blood samples were incubated for 5 days in the automated hemoculture system BacT/Alert (bioMerieux); isolates were identified by VITEK 2 compact (bioMerieux). Blood cultures with no growth after 5 days incubation was considered as negative. When growth was detected, Gram staining was performed, and the broth was subcultured onto blood, chocolate, and Sabouraud's dextrose agars. Isolates of microorganisms that are common skin commensals (e.g., coagulase-negative staphylococci [CoNS], *Corynebacterium* spp., *Propionibacterium acnes*), from a single blood culture bottle, were considered as contaminants. True bacteremia with these organisms required isolation of the same species with similar antibiograms from at least two blood culture sets.[9,10]

Data were collected from culture-proven BSI cases in NICU between January 2018 and December 2020. Descriptive data analysis was performed, and the numbers and percentages of isolates and their antimicrobial susceptibility tests were summarized.

As this study was retrospective and laboratory based, additional clinical information regarding the probable source of BSIs, risk factors for BSIs, and comorbidities were not available.

Primary outcome measures were the pathogen distribution and rates of methicillin, vancomycin, gentamicin, carbapenem resistance and extended-spectrum beta-lactamase (ESBL) production among isolated strains.

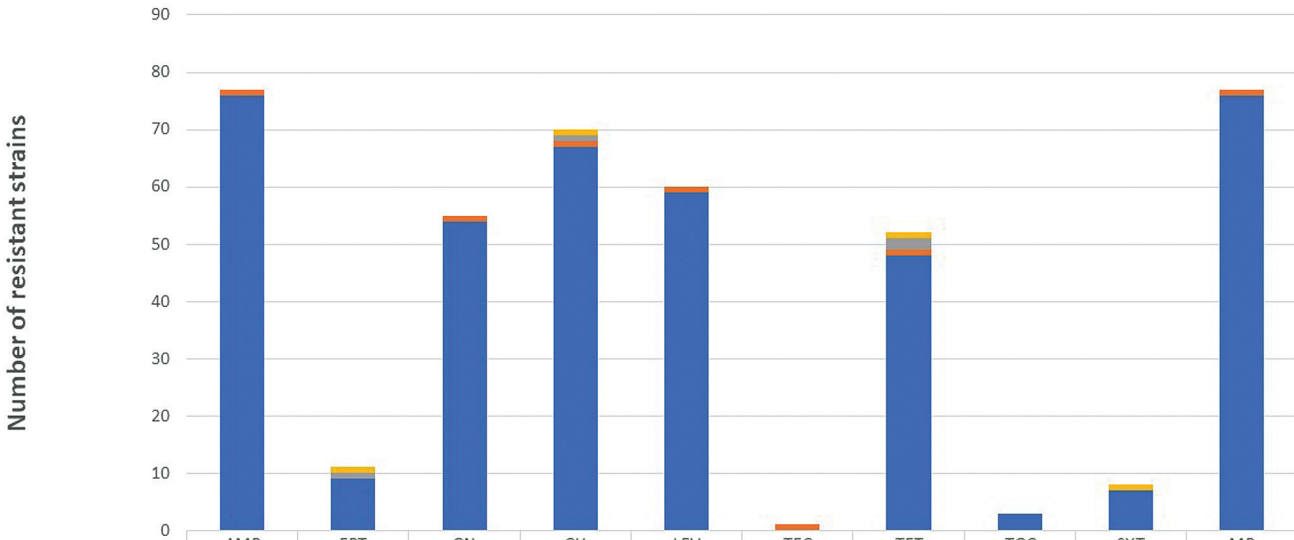
In this study, laboratory data were collected retrospectively and used in compliance with procedures and principles laid down in Personal Data Protection Law (No: 6698, date: March 24, 2016). Patient identifying information was removed from the data set by the laboratory personnel in charge of data control before analysis, and then sent to authors.

Results

During the study period, there were 117 BSI episodes in 106 neonates; 11 neonates had 2 episodes of BSI caused by different species. Among the 106 neonates, 75 (70.8%) were male and 31 (29.2%) were female. Mean age was 13.2 (±8.1) days. The most common pathogen was *Staphylococcus epidermidis* ($n = 86$, 73.5%), followed by *Klebsiella pneumoniae* ($n = 11$, 9.4%) (►**Table 1**). Among 86 *S. epidermidis* isolates, 76 (88.4%) were resistant to methicillin, whereas only 1 (14.3%) *Staphylococcus aureus* isolate was methicillin-resistant. Overall, methicillin resistance among staphylococci was 82.8% (77/93) (►**Fig. 1**). Vancomycin resistance was not detected. All *K. pneumoniae* and *E. coli* isolates were resistant to ampicillin and were ESBL producing. The overall rate of ESBL production among Enterobacterales was 82.4% (14/17) (►**Fig. 2**).

Table 1 Number of isolated pathogens

Group (*N*, %)	Microorganism	Number of isolates (%)
Gram-positive (96, 82.1%)	*Staphylococcus epidermidis*	86 (73.5%)
	Staphylococcus aureus	7 (5.9%)
	Streptococcus agalactiae	2 (1.7%)
	Streptococcus pyogenes	1 (0.9%)
Gram-negative (21, 17.9%)	*Klebsiella pneumoniae*	11 (9.4%)
	Enterobacter cloacae	3 (2.6%)
	Acinetobacter baumannii	2 (1.7%)
	Escherichia coli	2 (1.7%)
	Pseudomonas aeruginosa	1 (0.9%)
	Serratia marcescens	1 (0.9%)
	Stenotrophomonas maltophilia	1 (0.9%)



	AMP	ERT	GN	CLI	LEV	TEC	TET	TGC	SXT	MR
S.pyogenes (n=1)	0	1	0	1	0	0	1	0	1	0
S.agalactiae (n=2)	0	1	0	1	0	0	2	0	0	0
S.aureus (n=7)	1	0	1	1	1	1	1	0	0	1
S.epidermidis (n=86)	76	9	54	67	59	0	48	3	7	76

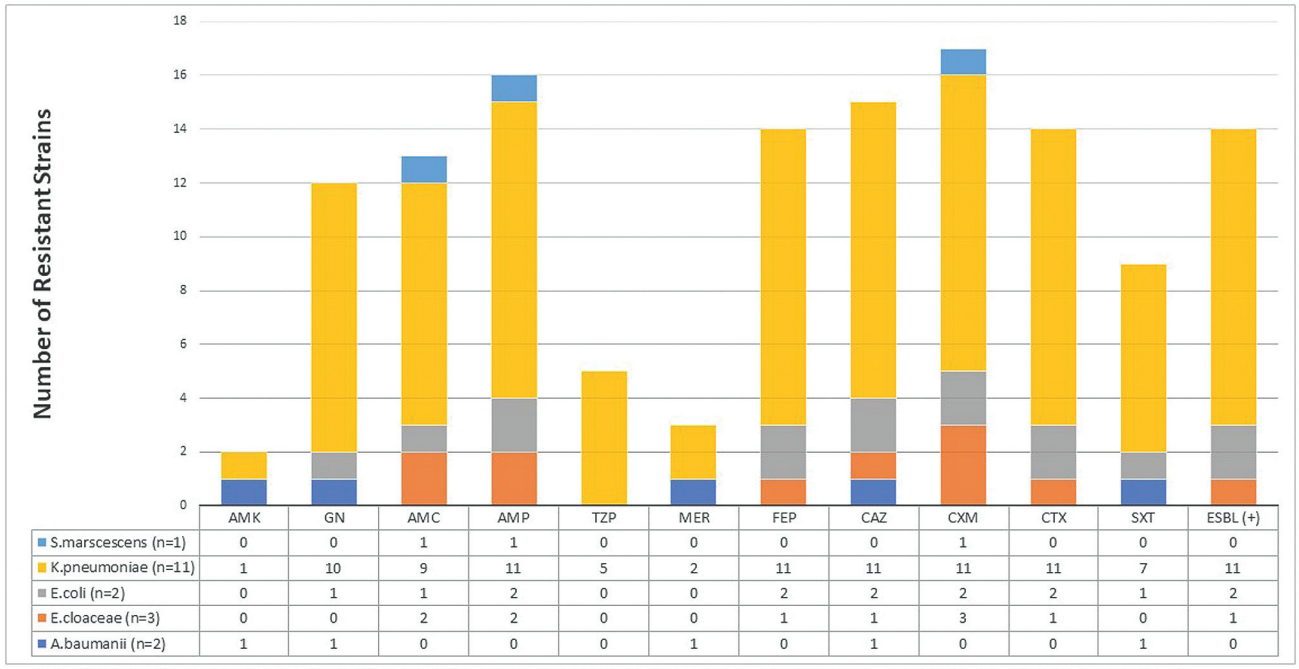
AMP: Ampicillin;ERT: Erythromycin, GN: Gentamicin, CLI: Clindamycin, LEV: Levofloxacin, TEC: Teicoplanin, TET: Tetracyclin, TGC: Tigecyclin, SXT: Co-trimoxazole, MR: Methicillin resistant

Fig. 1 Antimicrobial resistance in gram-positive bacteria isolated from neonatal blood cultures.

Gentamicin resistance was common among methicillin-resistant staphylococci and ESBL-producing gram-negative bacilli (►**Fig. 3**). Among 77 methicillin-resistant staphylococci, 54 strains (70.1%), 1 of which was methicillin-resistant S. aureus and 53 were methicillin-resistant S. epidermidis, were resistant to gentamicin. Gentamicin resistance was detected in 11 (78.6%) of the 14 ESBL-producing Enterobacterales (1 *E. coli* and 10 *K. pneumoniae*) (►**Fig. 3**). Two *K. pneumoniae* (2/11, 18.2%) and one *Acinetobacter baumannii* (1/2, 50%) were resistant to meropenem (►**Fig. 2**).

Discussion

Our study underlines the importance of local surveillance of pathogen distribution and antimicrobial resistance patterns. Rates of methicillin resistance (82.8%) among staphylococci



	AMK	GN	AMC	AMP	TZP	MER	FEP	CAZ	CXM	CTX	SXT	ESBL (+)
S.marscescens (n=1)	0	0	1	1	0	0	0	0	1	0	0	0
K.pneumoniae (n=11)	1	10	9	11	5	2	11	11	11	11	7	11
E.coli (n=2)	0	1	1	2	0	0	2	2	2	2	1	2
E.cloaceae (n=3)	0	0	2	2	0	0	1	1	3	1	0	1
A.baumanii (n=2)	1	1	0	0	0	1	0	1	0	0	1	0

AMK: Amikacin, GN: Gentamicin, AMC: Amoxycilin/clavulanic acid, AMP: Ampicillin,TZP: Piperacillin/tazobactam, MER: Meropenem, FEP: Cefepim, CAZ: ceftazidime, CXM: Cefuroxim, CTX: Cefotaxim, SXT: Co-trimoxazole, ESBL: Extended spectrum beta lactamase

Fig. 2 Antimicrobial resistance in gram-negative bacteria isolated from neonatal blood cultures.

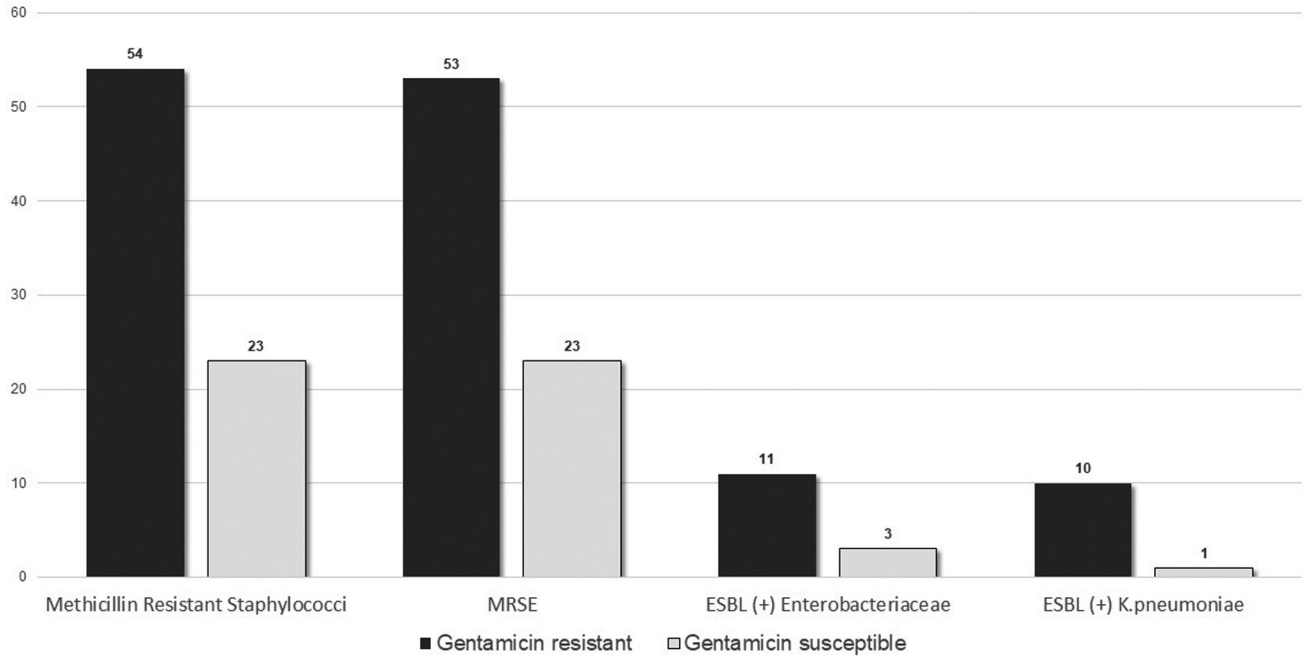


Fig. 3 Number of gentamicin-resistant isolates among methicillin-resistant staphylococci and extended-spectrum beta-lactamase-producing Enterobacteriaceae.

and of ESBL production (82.4%) among Enterobacterales in our setting are alarming. Empirical treatment of neonatal sepsis recommended by WHO consists of ampicillin/penicillin (or cloxacillin if staphylococci are suspected) with gentamicin.[11] In accordance with WHO's recommendations, empirical treatment in our setting consists of ampicillin with gentamicin. Vancomycin or meropenem usage is limited with the neonates not responding to the initial treatment. Therefore, we have considered gentamicin resistance rates among methicillin-resistant staphylococci and ESBL-producing Enterobacterales. Unfortunately, gentamicin resistance rates were high at 70.1% in methicillin-resistant staphylococci and 78.6% in ESBL-producing Enterobacterales. Vancomycin and colistin resistance were not detected.

There are several other studies in the literature investigating the species distribution of pathogens isolated from neonates with suspected sepsis. In a recent meta-analysis, investigating the global incidence of neonatal sepsis found that in approximately one-third of culture-proven sepsis, the causative agents were *S. aureus* and *Klebsiella* spp.[12] Another meta-analysis focusing on developing countries determined *Klebsiella* spp. (26.36%), *S. aureus* (23.22%), CoNS (23.22%), and *E. coli* (15.30%) as the most common causes of neonatal sepsis.[13] A systematic review and meta-analysis investigating the etiologies and antimicrobial resistances of invasive bacterial infections in neonates in sub-Saharan Africa reported that *S. aureus*, *Klebsiella* spp., and *E. coli* accounted for 25, 21, and 10% of bacteremia or sepsis cases, respectively.[14] A recent literature review[15] of retrospective studies regarding culture-proven neonatal sepsis conducted in China between 2016 and 2018 showed a predominance of gram-positive pathogens (59.2%) and among gram-positive bacteria, CoNS (40.2%) ranked first, followed by *Streptococcus* spp. (6.81%), *Enterococcus* spp. (6.10%), and *S. aureus* (5.15%). Enterobacterales were the most common gram-negative pathogens; *Klebsiella* spp. were isolated in 14.5%, *E. coli* in 12.1%, and *Enterobacter cloacae* in 1.9%. *Pseudomonas aeruginosa* was isolated only in 1.4% of cultures. Similarly, in our study, the most common pathogens were *Staphylococcus* and *Klebsiella* species; however, CoNS predominated to a far greater extent than that in other studies.

Antimicrobial susceptibility patterns vary between settings and countries. In our study, methicillin resistance among *S. epidermidis* and ESBL production among *K. pneumoniae* are striking. In an Italian study, methicillin resistance was detected in 30% of *S. aureus* and 94.7% of CoNS isolated from neonates with sepsis.[16] A study from Germany reported high rate of oxacillin resistance (88.2%) among *S. epidermidis* and ampicillin resistance (73.9%) among *E. coli* isolates.[17] Methicillin resistance among staphylococci was reported to be high in an Ethiopian study, in which 69% of *S. aureus* and 100% of CoNS strains were resistant to methicillin; 56.6 and 91% of these strains were also resistant to gentamicin. Antimicrobial resistance was also common among gram-negative bacteria, with ampicillin and gentamicin resistance detected in 66.7 and 55.6% of *E. coli* strains and 91 and 82% of *Klebsiella* strains, respectively.[18] In the systematic review and meta-analysis of studies conducted in sub-Saharan Africa, resistance to beta-lactam and aminoglycoside drugs recommended by WHO was found to be 68 and 27%, respectively.[14] In the study conducted by the Burden of Antibiotic Resistance in Neonates from Developing Societies (BARNARDS) group assessing the burden of antimicrobial

resistance in neonatal sepsis cases in low- and middle-income countries, 60% of gram-negative bacteria were found to be resistant to the first-line empirical treatment for neonatal sepsis (both ampicillin and gentamicin).[19] Not all studies reported high levels of resistance. In a study conducted in Türkiye, cases of early-onset neonatal sepsis were investigated and 27.6% (8 out of 29) of *E. coli* strains were found to be ESBL positive; all of these ESBL producers were susceptible to gentamicin.[20] A study investigating the epidemiology of culture-proven neonatal sepsis from Saudi Arabia reported that all isolated gram-negative and gram-positive bacteria were susceptible to aminoglycoside antibiotics (amikacin and/or gentamicin) tested.[21]

This retrospective, laboratory data-based study had some limitations. Due to the lack of baseline characteristics and comorbidities of the patients and data regarding the treatment applied to patients and its outcome, we could not assess risk factors related with sepsis due to drug-resistant bacteria or treatment failure. Nevertheless, our study provided significant data regarding the etiology of neonatal sepsis in our setting and their susceptibility patterns which may be used to employ better medical decisions.

In conclusion, our study represents the preliminary results of the NICU in a new university hospital with high levels of resistance which emphasizes the importance of constant surveillance of local pathogen distribution and their antimicrobial resistance profiles to keep treatment protocols and guidelines up-to-date. Multicentered, prospective studies investigating the etiology and antimicrobial susceptibility patterns in neonatal sepsis are required to establish feasible management strategies.

Conflict of Interest
None declared.

Acknowledgment
We would like to express our sincere gratitude to the laboratory personnel, Kazime Ayan for her support in data collection and control.

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