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16 S rRNA–based molecular identification of coagulase-negative *Staphylococcus* species in neonates with sepsis and their antibiotic resistance patterns in Ahvaz, Iran

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Abstract

Introduction Coagulase-negative staphylococci (CoNS) are among the leading causes of neonatal sepsis (NS). NS can be divided into two types: early-onset sepsis (EOS), which usually occurs less than 72 h after birth, and late-onset sepsis (LOS), which can occur 8 to 28 days after birth. According to newly published statistics, the incidence rates of EOS and LOS in neonates are 0.5–3.1% and 2–32%, respectively. This study aimed to determine the prevalence of common CoNS isolates and their antibiotic resistance patterns in NS cases in Ahvaz, Iran.

Methods This cross-sectional study (October 2022-April 2023) was conducted on all neonates (0–28 days old) with NS manifestations admitted to Imam Khomeini Hospital. Blood culture samples were collected and incubated at 37 °C for 24 h. The bacterial isolates were identified via standard biochemical tests, and the *Staphylococcus epidermidis* strains were identified via polymerase chain reaction (PCR) of the *SesC* gene. The other suspected CoNS species were identified using *16 S rRNA* sequencing.

Results In total, 1221 blood culture bottles were collected from 1330 neonates with NS manifestations. A total of 111 (9.1%) blood cultures were positive for bacterial growth. Overall, 51 staphylococcal isolates, including 39 (76.5%) CoNS species and 12 (23.5%) *S. aureus* isolates, were identified. Using *SesC* gene PCR and *16 S rRNA* sequencing, the CoNS species were as follows: 28 (71.8%) *S. epidermidis*, 5 (12.8%) *S. hominis*, 4 (10.38%) *S. haemolyticus* and 2 (5.1%) *S. warneri*. In total, the frequency of NS caused by CoNS isolates was 35.1% ($n = 39/111$). All CoNS isolates were methicillin resistant and presented the highest antibiotic resistance rates (100.0%) to cefoxitin, ampicillin, erythromycin, and linezolid.

Conclusions This study revealed a high incidence of methicillin-resistant coagulase-negative staphylococci (MRCoNS) with high antibiotic resistance rates in NS patients from Ahvaz. To prevent the spread of these isolates in healthcare systems, measures such as monitoring the optimal use of antibiotics on the basis of the results of laboratory antibiograms seem necessary.

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Keywords Antibiotic resistance, CoNS, Early onset sepsis, Late onset sepsis, Neonatal sepsis

Introduction

Neonatal sepsis (NS) is a serious life-threatening bacterial infection and the third most common cause of neonatal mortality, especially in developing countries [1]. NS can be divided into two types: early-onset sepsis (EOS), which usually occurs less than 72 h after birth, and late-onset sepsis (LOS), which can occur between 8 and 28 days after birth [1, 2]. According to newly published statistics, the incidence rates of EOS and LOS in neonates are 0.5–3.1% and 2–32%, respectively [2, 3].

NS has different signs and symptoms. Therefore, its clinical diagnosis is based on the presence of several symptoms. For this reason, the diagnosis and treatment of NS is among the most important medical challenges [4, 5]. NS is usually diagnosed on the basis of a combination of clinical findings and the use of positive septic screening parameters such as lymphocyte count, C-reactive protein (CRP), and the erythrocyte sedimentation rate (ESR) [5].

Different types of Gram-positive and Gram-negative bacteria are involved in NS. The most common bacteria causing LOS among newborns in the neonatal intensive care unit (NICU) are coagulase-negative staphylococci (CoNS) [6]. In recent decades, these bacteria have become among the most important human pathogens [7]. In low- and middle-income countries (LMICs), *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* are among the most common CoNS species causing NS [8]. According to a systematic review and meta-analysis published in recent years, CoNS are the third most common cause (14.0%) of NS in Iran [9].

CoNS strains easily acquire antibiotic resistance determinants that lead to multidrug resistance [10]. CoNS strains, in addition to their opportunistic nature in pathogenicity, are important reservoirs for the transfer of antimicrobial resistance genes, such as *mecA*, which cause resistance to methicillin [11]. Moreover, their ability to form biofilms on indwelling medical devices results in the treatment of infections caused by these species with serious problems [10]. Compared with methicillin-susceptible CoNS (MSCoNS) strains, methicillin-resistant CoNS (MRCoNS) strains are more resistant to different antibiotic classes [12].

The growing challenge in managing NS lies in the increasing prevalence of antimicrobial resistance (AMR), which is closely linked to the overuse of antibiotics, particularly those with broader spectra [13]. The latest global analysis of the epidemiology and incidence of sepsis indicates a growing relationship between antimicrobial resistance and mortality from NS [14]. Hence, considering the present circumstances and the fact that the empirical therapy for NS from CoNS bacteria may vary depending on location and over time, it is important to study their antibiotic resistance patterns. This study aimed to investigate the prevalence and antibiotic resistance profile of CoNS species in neonates with sepsis and their clinical and demographic findings in Ahvaz, Iran.

Materials and methods

Ethics

This study was approved by the Research Ethics Committee of Golestan Hospital (ethics code: IR.AJUMS.HGOLESTAN.REC.1401.119) of the Ahvaz Jundishapur University of Medical Sciences in accordance with the principles of the Declaration of Helsinki. Written consent was obtained from the parents or legal guardians of the neonates.

Study design

This cross-sectional study (October 2022-April 2023) was conducted on neonates (0–28 days old) with NS manifestations such as bradycardia, poor feeding, fever, apnea, and lethargy admitted to the NICU of Imam Khomeini Hospital [15, 16]. NS was confirmed and diagnosed by an attending pediatrician at the hospital. In addition to clinical symptoms, laboratory tests, such as C-reactive protein (CRP) and white blood cell count (WBC), are also used in the diagnosis of NS [15]. The demographic and clinical characteristics of neonates with NS were recorded on the basis of the statements of mothers or other companions [17].

Inclusion and exclusion criteria

All infants who had two or more symptoms of NS were included in this study [18]. All neonates with a history of surgery, antibiotic consumption, or congenital anomalies were excluded from the study [19].

Sample collection and processing

The blood culture bottles (Baharafshan, Iran) containing trypticase soy broth (TSB) were filled with approximately 1 to 2 mL of neonatal blood after the area was disinfected with povidone-iodine and 70% alcohol [20, 21]. Immediately after collection, the samples were incubated at 37 °C for 24 h. Subcultures were performed on sheep blood agar and McConkey agar (Condalab, Spain) after 24 h. The plates were incubated at 37 °C for 24 h. If bacterial growth was negative, the process was repeated on sheep blood agar and McConkey agar for 7 days. The plates were evaluated for bacterial growth. Blood culture bottles were deemed negative if no bacterial growth was observed after seven days of incubation [20, 21]. In total,

three blood samples were taken from patients with suspected bloodstream infections over a three-day period. If an organism grows only in one set of blood cultures or when different organisms grow on the same culture set, it is considered a contaminant. When the same bacterium was grown in multiple blood culture sets, it was considered true bacteremia [22]. Additionally, several clinical and laboratory indices, including hyperthermia and leucocytosis, have been evaluated to determine true infection [22].

Primary identification of CoNS species

Primary identification of CoNS species was performed via standard microbiological methods, such as colony morphology, Gram staining, catalase, mannitol salt agar (MSA), deoxyribonuclease (DNase), and coagulase tests [23].

Confirmation and final determination of the identity of CoNS species

In this step, genomic DNA was extracted from all CoNS species via the boiling method as previously described [24]. The process involved suspending 2–3 overnight culture colonies in microtubes containing 500 µl of sterile deionized water. These microtubes were incubated in dry blocks (Denville Scientific, USA) (100 °C) for 5 min. Next, the microtubes were centrifuged at 14,000 rpm for 10 min at 4 °C. The resulting supernatants were used as genomic DNA templates for molecular analysis [24]. *S. epidermidis* strains were identified and confirmed via polymerase chain reaction (PCR) of the *SesC* gene using previously described specific primers (Table 1) [25]. The other suspected CoNS species were identified using *16 S rRNA* sequencing with the previously mentioned primers (Table 1) [26]. PCRs were performed in a total volume of 25 µL as follows: 12.5 µL of Master Mix 1.5X (Sinaclon, Tehran, Iran), 0.5 µL of forward and reverse primers (10 pM), 5 µL of genomic DNA (50 ng/µL), and 6.5 µL of sterile distilled water. The PCR protocols are described in Table 1. The PCR products were analyzed via electrophoresis in a 1.5% agarose gel containing 2 µl of safe stain (Sinaclon, Tehran, Iran) [27]. The resulting PCR products were sent to Pishgam Company (Tehran, Iran) for sequencing. Separate confirmation of the *16 S rRNA* gene sequences for each isolate was achieved through BLAST analysis. The sequences were aligned with the relevant sequences belonging to CoNS species retrieved from the GenBank database via MEGA 6.0 software [28]. After the sequences of the *16 S rRNA* gene of CoNS species were checked via Chromas software version 2.01 and trimmed, the sequences were compared with the data in the GenBank database (https://www.ncbi.nlm.nih.gov/genebank/), and their percentage similarity was checked. The sequences of the *16 S rRNA* gene of CoNS species were subsequently deposited in the GenBank database. The positive quality control for the amplification of the *SesC* and *16 S rRNA* genes included the use of *S. epidermidis* ATCC 49,134 [12]. Additionally, sterile distilled water was used as a negative control for each PCR run.

Detection of MRCoNS

MRCoNS isolates were detected via a phenotypic test (cefoxitin, 30 µg) and PCR amplification of the *mecA* gene as previously described (Table 1) [29]. The PCR protocol is described in Table 1. The positive and negative controls for *mecA* gene detection were *S. aureus* ATCC® 33,591™ and ATCC® 25,923™, respectively.

Antibiotic susceptibility testing (AST)

The AST of CoNS species was determined via Kirby–Bauer disk diffusion according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [30]. This test involved the use of 14 antibiotic disks (MAST, United Kingdom) as follows: cefoxitin (30 µg), ampicillin (10 µg), erythromycin (15 µg), clindamycin (2 µg), ciprofloxacin (CIP, 5 µg), amikacin (30 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), gentamicin (10 µg), meropenem (10 µg), imipenem (10 µg), rifampicin (5 µg), chloramphenicol (30 µg), linezolid (30 µg), and doxycycline (30 µg). Quality control of AST was performed using *S. aureus* ATCC® 25,923™ [30].

Data analysis

The obtained data were analyzed and interpreted through the use of SPSS version 22 software provided by IBM in Armonk, New York, USA.

Table 1 List of primers and protocols used for polymerase chain reaction (PCR) in this study

Gene	Primers (5′–3′)	Product size (bp)	PCR protocol
SesC	F: GTTGATAACCGTCAACAAGG R: CATGTTGATCTTTTGAATCCC	388	First denaturation: 94 °C for 5 min 30 cycles: 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s Terminal extension: 72 °C for 5 min
16 S rRNA	27 F: AGAGTTTGATCMTGGCTCAG 1492R: TACGGYTACCTTGTTACGACTT	1500	First denaturation: 94 °C for 5 min 30 cycles: 95 °C for 30 s, 63 °C for 30 s, 72 °C for 60 s Terminal extension: 72 °C for 5 min
mecA	F: GTAGAAATGACTGAACGTCCGATAA R: AGCCAAGCCTTGACGAACTAAAGC	310	First denaturation: 94 °C for 5 min 30 cycles: 95 °C for 30 s, 57 °C for 30 s, 72 °C for 30 s Terminal extension: 72 °C for 5 min

Results

Identification of CoNS species

In total, 1221 blood culture samples were collected from 1330 hospitalized neonates referred to Imam Khomeini Hospital. In total, from 1221 blood culture samples, 111 (9.1%) were positive in terms of bacterial growth, and 90.9% were negative. The number of positive cultures included 75 (67.6%) Gram-positive and 36 (32.4%) Gram-negative bacteria. *Acinetobacter* species made up the majority of Gram-negative isolates ($n=21$, 51.4%), followed by *Klebsiella pneumoniae* ($n=8$, 82.2%), *Escherichia coli* ($n=3$, 8.3%), *Pseudomonas aeruginosa* ($n=3$, 8.3%), and *Enterobacter cloacae* ($n=1$, 2.8%). The isolated bacteria included 74 (98.7%) *Staphylococcus* species and one (1.3%) *Enterococcus faecalis* strain. Among the 74 *Staphylococcus* isolates, only 51 with clinical symptoms of sepsis were included in the study. Overall, 51 staphylococcal strains comprised 39 (76.5%) CoNS species and 12 (23.5%) *S. aureus* isolates. Using *SesC* gene PCR and *16 S rRNA* sequencing, the CoNS species were as follows: 28 (71.8%) *S. epidermidis*, 5 (12.8%) *S. hominis*, 4 (10.38%) *S. haemolyticus*, and 2 (5.1%) *S. warneri*. The data for the *16 S rRNA* genes of CoNS species are shown in Table 2. In total, the frequency of NS caused by CoNS isolates was 35.1% ($n=39/111$) in neonates. One of the neonates died due to this NS.

Clinical and demographic characteristics of neonates with sepsis caused by CoNS species

Tables 3 and 4 present the main clinical and demographic characteristics of neonates with sepsis caused by 39 CoNS species. The frequencies of CoNS isolates were 56.4% ($n=22$) and 43.6% ($n=17$) in males and females, respectively. The gestational age of the neonates ranged from 26 w to 39 w, with a mean of 34 weeks. In total, 37 cases (94.9%) were born via the cesarean section method. Only 3 patients (7.7%) experienced spontaneous rupture of membranes (ROMs) for ≥18 h. The average birth weight was 2.118 ± 0.69 kg, with a minimum of 950 g and a maximum of 4000 g. The incidences of EOS and LOS caused by CoNS were 32 (82.1%) and 7 (17.9%), respectively. For EOS, the most prevalent CoNS isolates were *S. epidermidis* (22, 56.5%), *S. hominis* (5, 12.8%), *S. haemolyticus* (3, 7.7%), and *S. warneri* (2, 5.1%). The leading CoNS isolates in LOS patients were *S. epidermidis* (6, 15.4%) and *S. haemolyticus* (1, 2.5%). In neonates who had peripherally inserted central catheters (PICCs) and urine catheters, the *S. epidermis* was predominant. In neonates intubated for sepsis, 100% of the CoNS isolates were *S. epidermis*. Sepsis caused by CoNS isolates was more common in women who delivered via cesarean Sect. (94.9%) than in women who delivered vaginally (5.1%).

Prevalence of MRCoNS

The results of the cefoxitin phenotypic test revealed that all 39 CoNS isolates presented resistance patterns. Additionally, all of them harbored the *mecA* gene and were confirmed as MRCoNS. In addition, 10 methicillin-resistant *S. aureus* (MRSA) strains were detected.

AST patterns of CoNS isolates

The antibiotic susceptibility rates of the CoNS isolates are shown in Table 5. Accordingly, CoNS isolates presented the highest susceptibility rates to chloramphenicol (76.9%) and rifampin (74.4%). However, the CoNS isolates presented the highest antibiotic resistance rates (100.0%) to cefoxitin, ampicillin, erythromycin, and linezolid (Table 5). Besides the aforementioned antibiotics, *S. epidermis* isolates showed the highest resistance rates (96.4%) against amikacin and clindamycin; *S. hominis* isolates showed the highest resistance rates (100.0%) against amikacin, rifampin, doxycycline, trimethoprim/sulfamethoxazole, meropenem, and clindamycin; *S.*

Table 2 Accession numbers of the *16 S rRNA* sequences of the *Staphylococcus* species in this study

Number	Isolate code	Accession numbers	Bacteria
1	E42	PQ008312	*Staphylococcus epidermidis* strain Ajums EA-AM.E42
2	E38	PQ008311	*Staphylococcus hominis* strain Ajums EA-AM.E38
3	E35	PQ008310	*Staphylococcus warneri* strain Ajums EA-AM.E35
4	E31	PQ001723	*Staphylococcus haemolyticus* strain Ajums EA-AM.E31
5	E25	PQ001664	*Staphylococcus hominis* strain Ajums EA-AM.E25
6	E22	PQ001585	*Staphylococcus haemolyticus* strain Ajums EA-AM.E22
7	E15	PP979720	*Staphylococcus hominis* strain EA-AM.E15
8	E8	PP977009	*Enterococcus faecalis* strain AjumsEa-AM.E8
9	E7	PP976979	*Staphylococcus hominis* strain AjumsEa-AM.E7
10	E1	PQ097764	*Staphylococcus epidermidis* strain Ajums EA-AM.E1
11	E18	PQ164247	*Staphylococcus haemolyticus* strain EA-AM.E18
12	E30	PQ198417	*Staphylococcus haemolyticus* strain Ajums EA-AM.E30
13	E9	PQ203544	*Staphylococcus hominis* strain AjumsEa-AM.E9
14	E39	PQ210209	*Staphylococcus warneri* strain Ajums EA-AM.E39

Table 3 Sociodemographic characteristics of the study neonates with sepsis caused by coagulase-negative staphylococci (CoNS)

Variables	Frequency (*N* = 39)	Percentage (%)
Gender	22	56.4
Male	17	43.6
Female		
Gestational age (weeks)	1	2.5
< 28 w	17	43.5
28–33 w	14	35.9
34–36 w	7	17.9
≥ 37		
Mode of delivery	2	5.1
Vaginal	37	94.9
Caesarean section		
Duration of ROM (hour)	31	79.5
No ROM	5	12.8
< 18 h	3	7.7
≥ 18 h		
Onset of sepsis (hour)	32	82.1
Early (≤ 72)	7	17.9
Late (> 72)		
Birth Weight (kg)	27	69.2
< 2.5	12	30.7
≥ 2.5	2.118 ± 0.69	
Mean ± SD		
APGAR 1st min	13	33.3
< 7	26	66.7
≥ 7		
APGAR 5th min	4	10.2
< 7	35	89.8
≥ 7		
Symptom	21	53.8
Tachypnea	10	25.6
Cyanosis	2	5.1
Hypotonia	2	5.1
Poor feeding	1	2.6
Hypothermia	1	2.6
Mottling	1	2.6
Abdominal distention	1	2.6
Knee arthritis		
Maternal disorders	36	92.2
No disease	1	2.6
GHTN	2	5.2
GDM		
Maternal antibiotic therapy	2	5.1
Yes	37	94.9
No		
TPN therapy	11	28.2
Yes	28	71.8
No		
Surfactant therapy	13	33.3
Yes	26	66.7
No		

CPAP, continuous positive airway pressure; NEC, necrotizing enterocolitis (Bell stage IIA or greater); PDA, patent ductus arteriosus; ROM, rupture of membranes; SGA, small for gestational age

haemolyticus isolates showed the highest resistance rates (100.0%) against amikacin, doxycycline, gentamicin, ciprofloxacin, trimethoprim/sulfamethoxazole, meropenem, and clindamycin; and *S. warneri* isolates showed the highest resistance rates (100.0%) against amikacin, meropenem, clindamycin, trimethoprim/sulfamethoxazole, imipenem, and doxycycline. The resistance rates to other antibiotics, including clindamycin, ciprofloxacin, amikacin, trimethoprim/sulfamethoxazole, meropenem, imipenem, and doxycycline, were greater than 70.0%.

Table 4 Blood culture isolates by gestational age, place of birth and type of sepsis

Isolates	Gender		Gestational age				Type of sepsis		PICC insertion	Mode of delivery		Intubation	Urine catheter	ROM	Outcomes	
	Male	Female	< 28	28–33	34–36	≥ 37	EOS	LOS		VD	C/S				Discharge	expire
Staphylococcus epidermis	17 (43.6%)	11 (28.2%)	1 (2.5%)	12 (30.7%)	10 (25.6%)	5 (12.8%)	22 (56.5%)	6 (15.4%)	4 (10.2%)	2 (5.1%)	26 (66.6%)	5 (12.8%)	4 (10.2%)	6 (15.4%)	27	1
Staphylococcus hominis	3 (7.7%)	2 (5.1%)	0 (0.0%)	3 (7.7%)	2 (5.1%)	0 (0.0%)	5 (12.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (12.8%)	0 (0.0%)	1 (2.5%)	1 (2.5%)	5	0
Staphylococcus haemolyticus	2 (5.1%)	2 (5.1%)	0 (0.0%)	1 (2.5%)	2 (5.1%)	2 (5.1%)	3 (7.7%)	1 (2.5%)	1 (2.5%)	0 (0.0%)	4 (10.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4	0
Staphylococcus warneri	0 (0.0%)	2 (5.1%)	0 (0.0%)	2 (5.1%)	0 (0.0%)	0 (0.0%)	2 (5.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (5.1%)	0 (0.0%)	0 (0.0%)	1 (2.5%)	2	0

EOS, early-onset sepsis; LOS, late-onset sepsis; C/S, caesarean section delivery; VD, vaginal delivery; ROM, rupture of membranes; PICC, peripherally inserted central catheter

Discussion

CoNS play a significant role in the development of neonatal sepsis [31]. Hence, to determine suitable approaches for treatment and effective infection control, it is necessary to identify CoNS species and assess their antibiotic resistance mechanisms in hospital settings [31]. This study revealed a frequency of 35.1% for CoNS isolates in NS patients. Additionally, compared with other Gram-positive and Gram-negative bacteria, CoNS isolates were the most common bacteria in both the EOS and LOS patients. In line with our results, Majigo et al. [4] from Tanzania (35.4%) and Hosseini et al. [33] from Iran (35.2%) reported similar prevalence rates for CoNS strains in NS patients. However, compared with this study, in a previous report from India, the percentage of NS caused by CoNS isolates was lower (13.83%) [7]. Inadequate infection control practices in the studied region may have led to frequent CoNS isolate occurrence in this study [7]. Inconsistent with these findings, Boskabadi et al. [34] from Iran reported *Klebsiella pneumoniae* isolates as the predominant bacteria in both LOS and EOS patients. However, in line with this study, Hosseini et al. [33] from Tabriz, Iran reported CoNS strains as the main cause of both EOS and LOS.

The lack of detection of group B *Streptococcus* (GBS) isolates in the current study was consistent with previous reports from Iran, which indicated a very low rate of GBS-related NS [33, 34]. Hence, it seems that in the study area, GBS is not a common bacterium in the genital tract of pregnant women. Another possibility is the ineffectiveness of current traditional microbial detection methods in identifying GBS strains [33]. These variations could be explained by differences in geographical features, climates, sample sizes, and races across various regions [7, 32].

In accordance with previous reports from India [4], Ghana [19], and Peru [35], *S. epidermidis* (56.5%) was the most prevalent CoNS species in NS cases in the studied area. Since *S. epidermidis* is part of the skin flora, it is often considered a contaminant in positive blood cultures and creates challenges in differentiating between true infection and contamination [35]. Clinical and laboratory evidence indicative of infection, along with a positive blood culture result, are the only ways in which CoNS isolates are considered true pathogens in our medical centers [35].

The AST results revealed a high frequency rate (100.0%) for MRCoNS isolates, which was in line with previous studies from India (70%) [7], Kuwait (92.4%) [36], and Iran (54.4%) [37]. The presence of the *mecA* gene may be the reason for this observation. This gene is responsible for encoding penicillin-binding protein 2a (PBP2a), leading to a reduced affinity for β-lactam antibiotics [12]. It is located in a mobile genetic element called

Table 5 Antibiotic susceptibility testing of coagulase-negative staphylococci (CoNS)

Antibiotic disc		Frequency (%)		
Linezolid	Resistant	39 (100.0%)		
	Susceptible	0 (0.0%)		
Cefoxitin	Resistant	39 (100.0%)		
	Susceptible	0 (0.0%)		
Erytheromycin	Resistant	39 (100.0%)		
	Susceptible	0 (0.0%)		
Ampicillin	Resistant	39 (100.0%)		
	Susceptible	0 (0.0%)		
Gentamicin	Resistant	26 (66.7%)	*S. epidermis*	20 (71.4%)
			S. hominis	1 (20.0%)
			S. haemolyticus	4 (100.0%)
			S. warneri	1 (50.0%)
	Susceptible	13 (33.3%)	*S. epidermis*	8 (28.6%)
			S. hominis	4 (80%)
			S. haemolyticus	0 (0.0%)
			S. warneri	1 (50.0%)
Amikacin	Resistant	38 (97.4%)	*S. epidermis*	27 (96.4%)
			S. hominis	5 (100.0%)
			S. haemolyticus	4 (100.0%)
			S. warneri	2 (100.0%)
	Susceptible	1 (2.6%)	*S. epidermis*	1 (3.6%)
			S. hominis	0 (0.0%)
			S. haemolyticus	0 (0.0%)
			S. warneri	0 (0.0%)
Meropenem	Resistant	37 (94.9%)	*S. epidermis*	26 (92.9%)
			S. hominis	5 (100.0%)
			S. haemolyticus	4 (100.0%)
			S. warneri	2 (100.0%)
	Susceptible	2 (5.1%)	*S. epidermis*	2 (7.1%)
			S. hominis	0 (0.0%)
			S. haemolyticus	0 (0.0%)
			S. warneri	0 (0.0%)
Clindamycin	Resistant	38 (97.4%)	*S. epidermis*	27 (96.4%)
			S. hominis	5 (100.0%)
			S. haemolyticus	4 (100.0%)
			S. warneri	2 (100.0%)
	Susceptible	1 (2.6%)	*S. epidermis*	1 (3.6%)
			S. hominis	0 (0.0%)
			S. haemolyticus	0 (0.0%)
			S. warneri	0 (0.0%)
Trimethoprim/sulfamethoxazole	Resistant	31 (79.5%)	*S. epidermis*	20 (71.4%)
			S. hominis	5 (100.0%)
			S. haemolyticus	4 (100.0%)
			S. warneri	2 (100.0%)
	Susceptible	8 (20.5%)	*S. epidermis*	8 (28.6%)
			S. hominis	0 (0.0%)
			S. haemolyticus	0 (0.0%)
			S. warneri	0 (0.0%)

Table 5 (continued)

Antibiotic disc		Frequency (%)		
Ciprofloxacin	Resistant	32 (82.1%)	*S. epidermis*	23 (82.1%)
			S. hominis	4 (80.0%)
			S. haemolyticus	4 (100.0%)
			S. warneri	1 (50.0%)
	Susceptible	7 (17.9%)	*S. epidermis*	5 (17.9%)
			S. hominis	1 (20.0%)
			S. haemolyticus	0 (0.0%)
			S. warneri	1 (50.0%)
Imipenem	Resistant	32 (82.1%)	*S. epidermis*	24 (85.7%)
			S. hominis	3 (60.0%)
			S. haemolyticus	3 (75.0%)
			S. warneri	2 (100.0%)
	Susceptible	7 (17.9%)	*S. epidermis*	4 (14.3%)
			S. hominis	2 (40.0%)
			S. haemolyticus	1 (25.0%)
			S. warneri	0 (0.0%)
Rifampin	Resistant	10 (25.6%)	*S. epidermis*	9 (32.1%)
			S. hominis	0 (0.0%)
			S. haemolyticus	0 (0.0%)
			S. warneri	0 (0.0%)
	Susceptible	29 (74.4%)	*S. epidermis*	19 (67.9%)
			S. hominis	5 (100.0%)
			S. haemolyticus	4 (100.0%)
			S. warneri	1 (50.0%)
Chloramphenicol	Resistant	9 (23.1%)	*S. epidermis*	7 (25.5%)
			S. hominis	1 (20.0%)
			S. haemolyticus	0 (0.0%)
			S. warneri	1 (50.0%)
	Susceptible	30 (76.9%)	*S. epidermis*	21 (75.0%)
			S. hominis	4 (80.0%)
			S. haemolyticus	4 (100.0%)
			S. warneri	1 (50.0%)
Doxycycline	Resistant	35 (89.7%)	*S. epidermis*	24 (85.7%)
			S. hominis	5 (100.0%)
			S. haemolyticus	4 (100.0%)
			S. warneri	2 (100.0%)
	Susceptible	4 (10.3%)	*S. epidermis*	4 (14.3%)
			S. hominis	0 (0.0%)
			S. haemolyticus	0 (0.0%)
			S. warneri	0 (0.0%)

the staphylococcal cassette chromosome *mec* (SCC*mec*) [12]. In this study, all MRCoNS isolates harbored the *mecA* gene. These findings emphasize the importance of MRCoNS strains as potential stores of antibiotic resistance genes.

In this study, the cefoxitin phenotypic test and PCR of the *mecA* gene were used to detect methicillin resistance within CoNS species. Both methods yielded similar results. However, in a previous study from Kuwait [36], the results of the phenotypic test did not match those of the molecular method. The *mecA* gene being placed outside the region covered by the primers employed in those investigations may be the cause of the inconsistent findings [36]. These findings indicate the importance of using multiple methods to test the antibiotic susceptibility of CoNS isolates in hospital settings [36].

In this study, chloramphenicol (76.9%) and rifampin (74.4%) were the most effective antibiotics against CoNS isolates. However, cefoxitin, ampicillin, erythromycin, and linezolid, with 100.0% resistance rates, were less effective. Moreover, CoNS isolates showed high resistance rates (more than 70.0%) to the majority of the tested antibiotics. Consistent with the results of this study, Majigo et al. [4] from Tanzania and Al-Haqan et

al. [36] from Kuwait reported high antibiotic resistance rates against ampicillin, erythromycin, clindamycin, and trimethoprim/sulfamethoxazole. The overuse and inappropriate prescription of these antibiotics in our healthcare systems may have contributed to the high resistance shown by the CoNS isolates in the current study [4]. The elevated levels of resistance to most antibiotics observed in this research emphasize the need to reevaluate existing treatment protocols and enforce antibiotic stewardship in the studied region.

One unexpected observation was the high resistance rate to linezolid because this antibiotic is not among the common treatment regimens in our region. However, in a previous study, a high rate of resistance to linezolid was observed among CoNS isolates from the same geographical area [38]. This observation necessitates further investigation of the molecular mechanisms involved in this resistance. The main factor contributing to resistance against linezolid is predominantly attributed to mutations in the chloramphenicol florfenicol resistance (*cfr*) gene or domain V of the *23 S rRNA* gene [39]. Additionally, linezolid resistance has been reported to be caused by biofilm formation and efflux pump expression [40].

Limitations

In this study due to lack of enough financial supports, we had several limitations. The small sample size and single-center design were among the limitations of this study. Also, the lack of evaluation of the clonal relatedness of CoNS isolates via accurate methods such as pulse field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) due to financial constraints was another limitation.

Conclusion

The present research highlights the high occurrence of MRCoNS with a high antibiotic resistance rate in NS patients from Imam Khomeini Hospital, Ahvaz, Iran. To prevent the spread of these isolates in healthcare systems, measures such as monitoring the optimal use of antibiotics on the basis of the results of laboratory antibiograms seem necessary. Additionally, the use of automatic methods for identifying bacteria is another appropriate measure to increase the accuracy and speed of response. Additionally, infection control requires the reinforcement of practices such as environmental cleaning and disinfection and hand hygiene.

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Author contributions

A.M., S.D., and E.A.M. wrote the main manuscript text. E.A.M., M.R.A., M.D. and S.M.H.A. contributed to the study conception and design. S.D. contributed to laboratory experiments and data analysis. All authors reviewed the manuscript.

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Data availability

The sequencing data presented in the study are deposited in the (https://www.ncbi.nlm.nih.gov/) with following accession numbers: PQ008312, PQ008311, PQ008310, PQ001723, PQ001664, PQ001585, PP979720, PP977009, PP976979, PQ097764, PQ164247, PQ198417, PQ203544, and PQ210209.

Declarations

Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of Golestan Hospital (ethics code: IR.AJUMS.HGOLESTAN.REC.1401.119) of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, in line with the principles of the Declaration of Helsinki. All samples were collected after written consent was obtained from the parents or legal guardians of the neonates.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Milas GP, Issaris V, Niotis G. Pentraxin-3 and neonatal sepsis: a systematic review and meta-analysis. J Matern Fetal Neonatal Med. 2023;36(1):2205986.
2. Camargo JF, Caldas JPS, Marba STM. Early neonatal sepsis: prevalence, complications and outcomes in newborns with 35 weeks of gestational age or more. Rev Paul Pediatr. 2021;40:e2020388.
3. De Rose DU, Ronchetti MP, Martini L, Rechichi J, Iannetta M, Dotta A, et al. Diagnosis and management of neonatal bacterial sepsis: current challenges and future perspectives. Trop Med Infect Dis. 2024;9(9):199.
4. Majigo M, Makupa J, Mwazyunga Z, Luoga A, Kisinga J, Mwamkoa B, et al. Bacterial aetiology of neonatal sepsis and antimicrobial resistance pattern at the regional referral hospital, Dar Es Salam, Tanzania; a call to strengthening antibiotic stewardship program. Antibiotics. 2023;12(4):767.
5. Arun Babu T, Shankaralingappa A, Vijayadevagaran V, Sharmila V, Kalidoss VK. Comparative efficacy of microerythrocyte sedimentation rate (m-ESR) and C-reactive protein (CRP) as a neonatal septic screening marker—a single-center, retrospective observational study. Egypt Paediatr Assoc Gaz. 2023;71(1):32.
6. Nazir A. Neonatal sepsis due to coagulase negative staphylococci: a study from Kashmir valley, India. Int J Contemp Pediatr. 2019;6(2):650-655.7
7. Ansari F, Banerjee T, Kumar A, Anupurba S. Coagulase-negative staphylococci in neonatal blood: How concerning? J Lab Physicians. 2023;15(1):126–130.
8. Afeke I, Amegan-Aho KH, Adu-Amankwaah J, Orish VN, Mensah GL, Mbroh HK, et al. Antimicrobial profile of coagulase-negative staphylococcus isolates from categories of individuals at a neonatal intensive care unit of a tertiary hospital, Ghana. Pan Afr Med J. 2023;44:92.

9. Akbarian-Rad Z, Riahi SM, Abdollahi A, Sabbagh P, Ebrahimpour S, Javanian M, et al. Neonatal sepsis in Iran: a systematic review and meta-analysis on national prevalence and causative pathogens. PLoS ONE. 2020;15(1):e0227570.
10. Marincola G, Liong O, Schoen C, Abouelfetouh A, Hamdy A, Wencker FDR, et al. Antimicrobial resistance profiles of coagulase-negative staphylococci in community-based healthy individuals in Germany. Front Public Health. 2021;9:684456.
11. Røken M, lakhno S, Haaland AH, Bjelland AM, Wasteson Y. The home environment is a reservoir for methicillin-resistant coagulase-negative staphylococci and mammaliicocci. Antibiotics. 2024;13(3):279.
12. Abbasi Montazeri E, Seyed-Mohammadi S, Asarehzadegan Dezfuli A, Khosravi AD, Dastoorpoor M, Roointan M, et al. Investigation of SCC *Mec* types I–IV in clinical isolates of methicillin-resistant coagulase-negative staphylococci in Ahvaz, Southwest Iran. Biosci Rep. 2020;40(5):BSR20200847.
13. Shah MH, McAleese S, Kadam S, Parikh T, Vaidya U, Sanghavi S, et al. Emerging antibiotic resistance patterns in a neonatal intensive care unit in Pune, India: a 2-year retrospective study. Front Pediatr. 2022;10:864115.
14. Rallis D, Giapros V, Serbis A, Kosmeri C, Baltogianni M. Fighting antimicrobial resistance in neonatal intensive care units: rational use of antibiotics in neonatal sepsis. Antibiotics. 2023;12(3):508.
15. Celik IH, Hanna M, Canpolat FE, Mohan P. Diagnosis of neonatal sepsis: the past, present and future. Pediatr Res. 2022;91(2):337–50.
16. Oumer M, Abebaw D, Tazebew A. Time to recovery of neonatal sepsis and determinant factors among neonates admitted in Public hospitals of Central Gondar Zone, Northwest Ethiopia, 2021. PLoS ONE. 2022;17(7):e0271997.
17. Tumuhamye J, Sommerfelt H, Bwanga F, Ndeezi G, Mukunya D, Napyo A, et al. Neonatal sepsis at Mulago national referral hospital in Uganda: etiology, antimicrobial resistance, associated factors and case fatality risk. PLoS ONE. 2020;15(8):e0237085.
18. Nordberg V, Iversen A, Tidell A, Ininbergs K, Giske CG, Navér L. A decade of neonatal sepsis caused by gram-negative bacilli—a retrospective matched cohort study. Eur J Clin Microbiol Infect Dis. 2021;40:1803–13.
19. Wuni FK, Kukeba MW, Dzotsi KS, Abu O, Atobrah P, Ofosu-Poku R. Incidence of blood culture-related sepsis in neonates and antibiotics sensitivity of implicated organisms in a secondary healthcare facility in Ghana. Ghana Med J. 2023;57(2):134–40.
20. Mansouri S, Savari M, Malakian A, Abbasi Montazeri E. High prevalence of multidrug-resistant Enterobacterales carrying extended-spectrum beta-lactamase and AmpC genes isolated from neonatal sepsis in Ahvaz, Iran. BMC Microbiol. 2024;24(1):136.
21. El-Amir MI, El-Feky MA, Abo Elwafa DA, Abd-Elmawgood EA. Rapid diagnosis of neonatal sepsis by PCR for detection of 16S rRNA gene, while blood culture and PCR results were similar in E. Coli-predominant EOS cases. Infect Drug Resist. 2019;12:2703–10.
22. Murni IK, Duke T, Daley AJ, Kinney S, Soenarto Y. True pathogen or contamination: validation of blood cultures for the diagnosis of nosocomial infections in a developing country. J Trop Pediatr. 2018;64(5):389–94.
23. Nocera FP, Ferrara G, Scandura E, Ambrosio M, Fiorito F, De Martino L. A preliminary study on antimicrobial susceptibility of *Staphylococcus* spp. and *Enterococcus* spp. grown on mannitol salt agar in European wild boar (*Sus scrofa*) hunted in campania region—Italy. Animals. 2021;12(1):85.
24. Sarani S, Rashki A, Salari S, Najimi M. Molecular detection of enterotoxin-producing *Staphylococcus aureus* isolates from sheep in the Sistan region southeast of Iran. J Zoonotic Dis. 2024;8(3):524–33.
25. Behshood P, Tajbakhsh E, Momtaz H. Recognition of (Sesc) for easy identification of Staphylococcus epidermidis and molecular and phenotypic study of b-lactam resistance in Staphylococcus epidermidis isolates in Isfahan. Rep Biochem Mol Biol. 2020;9(3):309–14.
26. Tourlousse DM, Kurisu F, Tobino T, Furumai H. Sensitive and substrate-specific detection of metabolically active microorganisms in natural microbial consortia using community isotope arrays. FEMS Microbiol Lett. 2013;342(1):70–5.
27. Saki M, Amin M, Savari M, Hashemzadeh M, Seyedian SS. Beta-lactamase determinants and molecular typing of carbapenem-resistant classic and hypervirulent Klebsiella pneumoniae clinical isolates from southwest of Iran. Front Microbiol. 2022;13:1029686.
28. Hambal M, Admi M, Safika S, Sari WE, Ferasyi TR, Dasrul D, et al. Identification of Staphylococcus species isolated from preputium of Aceh cattle based on 16S rRNA gene sequences analysis. Vet World. 2019;12(10):1540–5.
29. Abbasi Montazeri E, Khosravi AD, Khazaei S, Sabbagh A. Prevalence of methicillin resistance and superantigenic toxins in Staphylococcus aureus strains isolated from patients with cancer. BMC Microbiol. 2021;21(1):262.
30. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. CLSI document M100. 32th ed. CLSI 2022.
31. França A. The role of coagulase-negative staphylococci biofilms on late-onset sepsis: current challenges and emerging diagnostics and therapies. Antibiot (Basel). 2023;12(3):554.
32. Moftian N, Rezaei-Hachesu P, Arab-Zozani M, Samad-Soltani T, Esfandiari A, Tabib MS, et al. Prevalence of gram-negative bacteria and their antibiotic resistance in neonatal sepsis in Iran: a systematic review and meta-analysis. BMC Infect Dis. 2023;23(1):534.
33. Hosseini MB, Abdoli Oskouei S, Heidari F, Sadat Sharif A, Salimi Z, Sharif SAA. Determination of the frequency of microbial agents and drug susceptibility pattern of the neonatal sepsis in the neonatal intensive care unit at Alzahra Hospital, Tabriz, Iran. Iran J Neonatol. 2019;10(4):33–40.
34. Boskabadi H, Zakerihamidi M, Bagheri F, Taghipour A. Bacterial microorganisms in definitive neonatal sepsis. J Guil Uni Med Sci. 2023;32(2):96–105.
35. Herbozo C, Julca I, Flores F, Hernandez R, Zegarra J. Incidence and microbiological characteristics of neonatal late onset sepsis in a neonatal intensive care unit in Peru. Int J Infect Dis. 2021;108:171–5.
36. Al-Haqan A, Boswihi SS, Pathan S, Udo EE. Antimicrobial resistance and virulence determinants in coagulase-negative staphylococci isolated mainly from preterm neonates. PLoS ONE. 2020;15(8):e0236713.
37. Khosravi AD, Roointan M, Abbasi Montazeri E, Aslani S, Hashemzadeh M, Taheri Soodejani M. Application of *tuf* gene sequence analysis for the identification of species of coagulase-negative staphylococci in clinical samples and evaluation of their antimicrobial resistance pattern. Infect Drug Resist. 2018;11:1275–82.
38. Akrami S, Montazeri EA, Saki M, Neisi N, Khedri R, Dini SA, et al. Bacterial profiles and their antibiotic resistance background in superinfections caused by multidrug-resistant bacteria among COVID-19 ICU patients from southwest Iran. J Med Virol. 2023;95(1):e28403.
39. Mittal G, Bhandari V, Gaind R, Rani V, Chopra S, Dawar R, et al. Linezolid resistant coagulase negative staphylococci (LRCoNS) with novel mutations causing blood stream infections (BSI) in India. BMC Infect Dis. 2019;19(1):717.
40. AbdAlhafiz AI, Elleboudy NS, Aboshanab KM, Aboulwafa MM, Hassouna NA. Phenotypic and genotypic characterization of linezolid resistance and the effect of antibiotic combinations on methicillin-resistant *Staphylococcus aureus* clinical isolates. Ann Clin Microbiol Antimicrob. 2023;22(1):23.

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