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Phenotypic and Genotypic Characterization of Pathogens Causing Neonatal Sepsis in Neonatal Intensive Care Unit During the COVID-19 Pandemic

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Abstract

This study aimed to determine the distribution of bacterial and fungal pathogens in neonatal sepsis cases in a neonatal unit during the COVID-19 pandemic and to investigate antimicrobial susceptibility/resistance profiles using phenotypic and molecular methods. A total of 940 samples were analysed in this study. Pathogens were identified using conventional culture methods and the Siemens MicroScan Walkaway 96 Plus system. Antimicrobial susceptibility of the isolates was determined phenotypically using the Siemens MicroScan Walkaway 96 Plus system and by PCR. Selected resistance genes were detected using multiplex PCR. A total of 113 isolates were recovered, predominantly Gram-negative bacilli (72%). The most frequent pathogens were coagulase-negative staphylococci (n = 28), *Acinetobacter baumannii/haemolyticus* (n = 22), *Klebsiella* spp. (n = 16), *Escherichia coli* (n = 10), *Enterococcus* spp. (n = 10), *Candida albicans* (n = 8), *Stenotrophomonas maltophilia* (n = 6), *Serratia marcescens* (n = 5), *Staphylococcus aureus* (n = 5) and *Pseudomonas aeruginosa* (n = 3). High resistance rates to beta-lactam antibiotics were observed, particularly among Gram-negative isolates. ESBL production ranged from 33%-50% in *Enterobacterales*, while MDR rates varied between 20% and 95.45% across species. All *C. albicans* isolates were susceptible to amphotericin B and to caspofungin. These findings highlight the urgent need for strengthened infection control measures and tailored empirical therapy.

Keywords: Neonatal Sepsis, Antimicrobial Resistance, Pathogens, ESBL, MDR

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INTRODUCTION

Neonatal sepsis, characterized as a systemic infection within the neonatal period (0-28 days), continues to be a major contributor to infant mortality globally, posing a persistent clinical and public health concern worldwide, particularly in low- and middle-income countries.[1-3]

The emergence of resistance among bacterial pathogens has become a significant public health concern worldwide.[4] The COVID-19 pandemic has further intensified these issues in NICUs.[3,4] Increased patient loads, staffing shortages, disrupted infection control practices, and excessive antibiotic use have been associated with surges in MDR infections, including carbapenem-resistant *Enterobacterales* and non-fermenters.[3-5]

This study, which was conducted with a particular emphasis on neonatal infections, aimed to determine the distribution of bacterial and fungal agents in cases of neonatal sepsis in the neonatal unit, offering contemporary local data on pathogen resistance patterns, and implications for management during the COVID-19 pandemic.

MATERIALS AND METHODS

Study design and setting

This study was conducted at Van Yuzuncu Yil University, Turkey, between 2020 and 2022. The study included microbiological samples obtained for routine diagnostic purposes from infants aged 0-28 days who presented with a preliminary diagnosis of sepsis.

Ethical approval for this study was obtained from the Non-Interventional Clinical Research Ethics Committee of Van Yüzüncü Yıl University (Türkiye) (decision no. 2020/01-19, dated 17 January 2020). The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to their enrollment in the study.

Study material

A total of 940 clinical samples were analyzed, including 647 blood, 133 urine, 70 cerebrospinal fluid (CSF), 56 catheter, 27 total parenteral nutrition (TPN) fluid, 3 abscesses, 2 paracentesis, 1 ear swab, and 1 nasal swab. Samples yielding the growth of potential pathogens were included, and probable contaminants were excluded.

Isolation and identification of microorganisms

The samples were inoculated onto 5% sheep blood agar and Eosin Methylene Blue (EMB) agar and incubated aerobically at 37 °C for 24-48 hours. The Gram characteristics of the pure colonies were revealed using the Gram staining method. Bacterial identification was performed using the identification (ID) panel of the MicroScan WalkAway 96 Plus automated system (Siemens Healthcare Diagnostics, West Sacramento, CA, USA).

For the fungal agents, samples were inoculated onto Sabouraud Dextrose Agar (SDA) medium and incubated aerobically at 37 °C for up to 7 days. The preliminary identification was performed based on their macroscopic and microscopic morphologies.

Antimicrobial susceptibility tests

Antimicrobial susceptibility testing (AST) of bacterial isolates was performed using the appropriate AST panels on a MicroScan WalkAway 96 Plus system. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI M100, 30th ed. 2020) guidelines.[6] Different panels were employed depending on the sample origin. Screening and confirmation of extended-spectrum β-lactamase (ESBL) production, vancomycin-resistant enterococci (VRE), and methicillin-resistant *Staphylococcus aureus* (MRSA) were performed according to the algorithm of the automated system and CLSI recommendations.

Antimicrobial susceptibility analyses of the fungal isolates were performed using the gradient test (E-test) method.[7] E-test strips (BM Bioanalyse, Turkey) of fluconazole (FLU, 0.016-256 µg/ml), voriconazole (VO, 0.002-32 µg/ml), amphotericin B (AMB, 0.002-32 µg/ml), and caspofungin (CAS, 0.002-32 µg/ml) were used.

Multidrug-resistance (MDR) was defined as non-susceptibility to at least one agent in three or more antimicrobial categories.[5]

Molecular analysis

DNA extraction

DNA extraction from bacteria and yeast

Table 1. Primer pairs used for the PCR process

Gene	Target	Primers	Annealing (°C)	Product (bp)	Ref.
TEM	ESBL	F-5'-ATGAGTATTCAACATTTCCGTG-3' R-5'-TTACCAATGCTTAATCAGTGAG-3'	55	861	[11]
CTX-M	ESBL	F-5'-TTTGCGATGTGCAGTACCAGTAA-3' R-5'-CGATATCGTTGGTGGTGCCATA-3'	57	544	[12]
CMY-2	AmpC β-lactamase	F-5'-GACAGCCTCTTTCTCCACA-3' R-5'-TGGAACGAAGGCTACGTA-3'	50	1000	[13]
VIM	Carbapenemase	F-5'-GATGGTGTTTGGTCGCATA-3' R-5'-CGAATGCGCAGCACCAG-3'	54	390	
NDM	Carbapenemase	F-5'-GGTTTGGCGATCTGGTTTTC-3' R-5'-GAATGGCTCATCACGATC-3'	54	621	[14]
KPC	Carbapenemase	F-5'-ATGTCACTGTATCGCCGTCT-3' R-5'-TTTTCAGAGCCTTACTGCCC-3'	54	893	
SUL-2	Sulfonamide	F-5'-CCAATACCGCCAGCCCGTCG-3' R-5'-TGCCTTGTCGCGTGGTGTGG-3'	56	489	[15]
MecA	Methicillin	F-5'-CCTAGTAAAGCTCCGGAA-3' R-5'-CTAGTCCATTCGGTCCA-3'	60	314	[16]
vanA	Glycopeptide	F-5'-GGGAAAACGACAATTGC-3' R-5'-GTACAATGCGGCCGTTA-3'	54	732	
vanB	Glycopeptide	F-5'-ATGGGAAGCCGATAGTC-3' R-5'-GATTTCGTTCCTCGACC-3'	54	647	[17]
vanC	Glycopeptide	F-5'-GAAAGACAACAGGAAGACCGC-3' R-5'-ATCGCATCACAAGCACCAATC-3'	54	796	
AaDB	Aminoglycoside	F-5'-TTACGCAGCAGGGCAGTCGC-3' R-5'-GCGGCACGCAAGACCTCAAC-3'	56	551	[15]
AaDA25	Aminoglycoside	F-5'-GCAGTGGATGGCGGCCTGAA-3' R-5'-TCGGCGCGATTTTGCCGGTT-3'	56	503	
ERG11	Fluconazole	F-5'-CATAACTCAAATATGGCTATT-3' R-5'-CTTTTGACGACATGATTCGA-3'	50	245	[18]
		F-5'-TTGAAACTGTCATTGATGGC-3' R-5'-GGTTGTTGACCATATGAAGC-3'		193	
FUR1	Flucytosine	F-5'-CGCAACCTGATTTTGTCCATA-3' R-5'-ATCGGAAGAATATCATGAAAATCC-3'	50	340	[19]
FKS1	Caspofungin	F-5'-GAAATCGGCATATGCTGTGTC-3' R-5'-AATGAACGACCAATGGAGAAG-3'	50	450	[20]

ESBL, Extended-spectrum β-Lactamases

isolates was performed according to the phenol-chloroform-isoamyl alcohol method reported by Saran et al.[8]

Candida albicans specific PCR

Candida albicans-specific PCR was performed using forward (CA,5' -TCAACTTGTCACACCAGATTATT-3') and reverse (ITS4, 5'-TCCTCCGCTTATTGATATGC-3') primers designed by Li et al.[9] and White et al.[10] respectively. The products of 402 bp in size were identified as *C. albicans* based on 1.5% agarose gel electrophoresis.

PCR analysis of antimicrobial resistance genes

Antimicrobial resistance gene analysis was performed in parallel with MicroScan WalkAway 96 Plus AST and gradient assay results. Common genes of clinical importance that are responsible for resistance were selected. PCRs was performed using the primer pairs listed in Table 1.[11-20]

The PCR products were evaluated for the relevant resistance genes by electrophoresis on a 1.5% agarose gel (Figure 1). Positive controls (*S. aureus, K. pneumoniae, E. coli, E. faecalis, C. albicans*) belonging to the culture collection of the Department of Microbiology, Faculty of Veterinary Medicine, were used in the study.

Statistical analysis

The data were analyzed using the IBM SPSS Version 25.0 software. Chi-square and Fisher's exact tests were used for the data analysis of variables to determine whether there was a relationship between categorical variables. $P < 0.05$ was taken statistically significant. Due to the limited number of isolates for some species and antibiotics, confidence intervals and advanced statistical tests were not routinely performed.

RESULTS

Identification findings

Analysis results of the 940 clinical samples were found to be positive for bacterial agents in 105 (11.17%) and fungal agents in 8 (0.85%) (Table 2). The culture positivity was calculated at rates that varied significantly by sample type (Pearson's chi-square test). The culture positivity was calculated as 100%, 100%, 50%, 33.33%, 30.36%, 13.53%, 10.66%, 5.71%, and 3.7% for ear, nose, paracentesis, abscess, catheter, urine, blood, CSF, and TPN samples, respectively. The most common sources of isolated microorganisms were blood 69 (7.34%), urine 18 (1.91%), and catheter 17 (1.80%).

The distribution of the isolates is shown in Table 2. The most frequent pathogens were coagulase-negative staphylococci (28 isolates, 24.8%), *A. baumannii/haemolyticus* (22, 19.5%), *Klebsiella* spp. (16, 14.2%), *E. coli* and *Enterococcus* spp. (10 each, 8.8%), *C. albicans* (8, 7.1%), *S. maltophilia* (6, 5.3%), *S. marcescens* (5, 4.4%), *S. aureus* (5, 4.4%), and *P. aeruginosa* (3, 2.7%). All *Candida* isolates were confirmed to be *C. albicans* using species-specific PCR.

Phenotypic antimicrobial susceptibility findings

The antimicrobial susceptibility results are summarized in Table 3. Gram-positive isolates exhibited high resistance to penicillin, oxacillin, and aminoglycosides but universal susceptibility to glycopeptides. Vancomycin-resistance was not observed in enterococci. Methicillin-resistance was prevalent in CoNS and *S. aureus.* Gram-negative isolates showed extensive resistance to beta-lactam antibiotics, including high rates of resistance to carbapenems in *A. baumannii/*

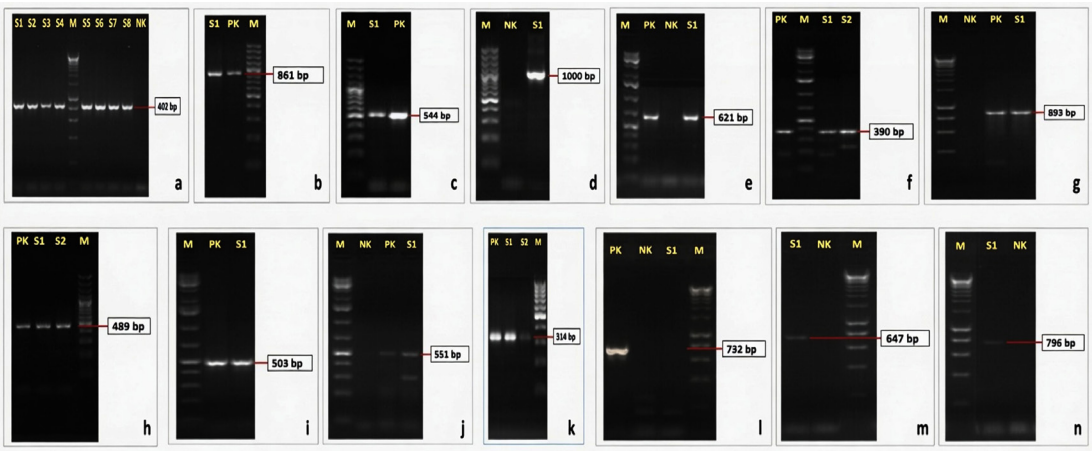

Figure 1. Electrophoresis of PCR products. *C. albicans* species-specific PCR (a); *TEM* (b), *CTX-M* (c), *CMY-2* (d), *NDM* (e), *VIM* (f), *KPC* (g), *SUL-2* (h), *AaDA25* (i), *AaDB* (j), *mecA* (k), *vanA* (l), *vanB* (m), and *vanC* (n) resistance gene-specific PCR. M: DNA ladder (GeneRuler 1 kb Plus DNA ladder, SM1331, Thermo Fisher Sci. (a, e, f, i, j), GeneRuler 100 bp plus DNA ladder, SM0321, Thermo Fisher Sci. (b, c, d, h), HyperLadder 1 kb DNA ladder, BIO-33053, Bioline (g, l, m, n), GeneRuler 100 bp DNA ladder, SM0241, Thermo Fisher Sci.) (k). S1, S2, …, Sn: Field strains, PK: Positive control, NK: Negative control

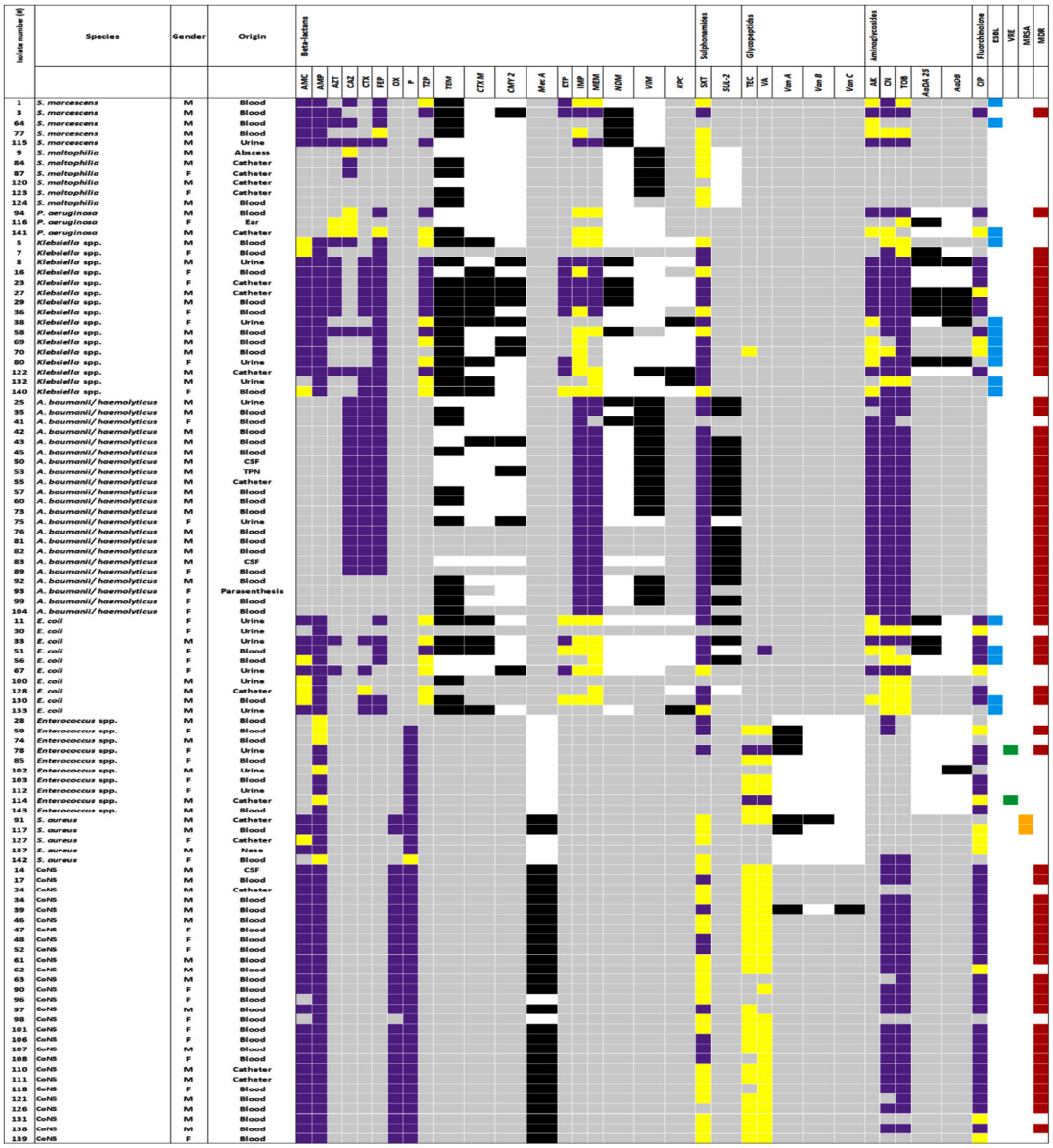

Figure 2. Antibiotic resistance and related gene positivity of bacterial isolates. ■ resistant; ■ sensitive; ■ not determined; ■ positive; ■ ESBL= extended-spectrum β-lactamases; ■ VRE= vancomycin-resistant enterococci; ■ MRSA= methicillin-resistant *Staphylococcus aureus*; ■ MDR= multidrug-resistance;
CoNS= coagulase-negative staphylococci; CSF= cerebrospinal Fuid; TPN= total parenteral nutrition, AMC= amoxicillin-clavulanate; AMP= ampicillin; AZT= aztreonam, CAZ= ceftazidime; CTX= cefotaxime; FEP= cefepime, OX= oxacillin; P= penicillin; TZP= piperacillin/tazobactam, ETP= ertapenem; IMP= imipenem; MEM= meropenem; SXT= trimethoprim/sulfamethoxazole; TEC= teicoplanin; VA= vancomycin; AK= amikacin; CN= gentamicin; TOB= tobramycin; CIP= ciprofloxacin

haemolyticus and *Klebsiella* spp. Multidrug -resistance (MDR) was frequent, reaching 95.45% in *A. baumannii/haemolyticus* and 81.25% in *Klebsiella* spp. The ESBL phenotype was detected in 33%-50% of *Enterobacterales*. All *C. albicans* isolates were susceptible to amphotericin B and caspofungin, with 50% resistance to fluconazole and voriconazole.

Antimicrobial resistance gene analysis findings

At least one antimicrobial resistance gene was detected in 91 (86.67%) of the 105 bacterial isolates. The detailed gene distribution is shown in Figure 2. Among Gram-negative isolates, bla_{TEM} was the most prevalent ESBL gene, ranging from 33.33% (*P. aeruginosa*) to 93.33% (*Klebsiella* spp.). bla_{CTX-M} was detected in 66.67% of *E. coli* and *Klebsiella* spp., but was rare (6.25%) in *A. baumannii/haemolyticus* and absent in *S. marcescens, S. maltophilia,* and *P. aeruginosa*. bla_{CMY-2} (AmpC-type) was found in 46.67% of *Klebsiella* spp., 20% of *S. marcescens*, 17.65% of *A. baumannii/haemolyticus*, and 16.67% of *E. coli*. Carbapenemase genes showed distinct patterns: bla_{NDM} was common in *S. marcescens* (80%) and *Klebsiella* spp. (33.33%), but low in *A. baumannii/haemolyticus* (11.76%) and absent in other species. bla_{VIM} predominated in *A. baumannii/haemolyticus* (88.24%) and *S. maltophilia* (83.33%), with a low prevalence in *Klebsiella* (7.14%). bla_{KPC} was detected only in *Klebsiella* spp. (23.08%) and *E. coli* (16.67%). The sulfonamide resistance gene, *sul2*, was highly prevalent in *A. baumannii/haemolyticus* (94.44%) and *E. coli* (60%). Aminoglycoside-modifying enzyme genes were frequently found *in Klebsiella* spp. (*aadA25* 60%, *aadB* 60%) and *E. coli* (*aadA25* 50%), with lower rates in other species. Among Gram-positive isolates, *mecA* was detected in 26 CoNS and two *S. aureus* isolates. Glycopeptide resistance genes were infrequent: *vanA* in 40% of *S. aureus*, 30% of *Enterococcus* spp., and one CoNS; *vanB* in 20% of *S. aureus*; and *vanC* in one CoNS.

No significant difference was observed in overall resistance rates between Gram-negative and Gram-positive isolates (chi-square test, P = 0.527), though Gram-negatives showed broader resistance patterns. Carbapenemase gene distribution showed species-specific patterns: bla_{VIM} was significantly more prevalent in non-fermenters (*A. baumannii/haemolyticus* 88.24%, *S. maltophilia* 83.33%) than in *Enterobacterales* (Fisher's exact test, P = 0.048). The *mecA* gene was detected in 92.9% of CoNS and 40% of *S. aureus* isolates, with a significantly higher prevalence in CoNS than in other Gram-positive species (Fisher's

Isolate number (#)	Species	Gender	Origin	Azoles		Echinocandins	Polyene macrolide				MDR
				FLU	VO	CAS	AMB	*FUR1*	*ERG11*	*FKS1*	
1C	*C. albicans*	M	CSF					■			
2C	*C. albicans*	M	Blood	■	■				■	■	
3C	*C. albicans*	M	Blood	■	■			■	■		
5C	*C. albicans*	M	Urine								
6C	*C. albicans*	M	Catheter					■			
7C	*C. albicans*	F	Blood					■	■	■	
8C	*C. albicans*	M	Urine	■	■			■	■	■	
9C	*C. albicans*	F	Blood	■	■			■	■	■	

Figure 3. Antibiotic resistance and related gene positivity of bacterial isolates. ■ = resistant, ■ = sensitive, ■ = positive; CSF = cerebrospinal fluid, FLU = fluconazole, VO = voriconazole, CAS = caspofungin, AMB = amphotericin B, MDR = multidrug-resistance

Table 2. Distribution of the isolates according to species and sample origin

Microorganism	No. of isolates	Distribution of isolates by sample origin								
		Blood n = 647	Urine n = 133	Catheter n = 56	CSF n = 70	Abscess n = 3	Paracentesis n = 2	Ear svap n = 1	Nasal svap n = 1	TPN n = 27
CoNS	28 (24.78%)	24 (21.24%)	0 (0%)	3 (2.65%)	1 (0.88%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
A. baumanii/ haemolyticus	22 (19.47%)	15 (13.27%)	2 (1.77%)	1 (0.88%)	2 (1.77%)	0 (0%)	1 (0.88%)	0 (0%)	0 (0%)	1 (0.88%)
Klebsiella spp.	16 (14.16%)	9 (7.96%)	4 (3.54%)	3 (2.65%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
E. coli	10 (8.85%)	3 (2.65%)	6 (5.31%)	1 (0.88%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Enterococcus spp.	10 (8.85%)	6 (5.31%)	3 (2.65%)	1 (0.88%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
S. maltophilia	6 (5.31%)	1 (0.88%)	0 (0%)	4 (3.54%)	0 (0%)	1 (0.88%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
S. marcescens	5 (4.42%)	4 (3.54%)	1 (0.88%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
S. aureus	5 (4.42%)	2 (1.77%)	0 (0%)	2 (1.77%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.88%)	0 (0%)
P. aeruginosa	3 (2.65%)	1 (0.88%)	0 (0%)	1 (0.88%)	0 (0%)	0 (0%)	0 (0%)	1 (0.88%)	0 (0%)	0 (0%)
Subtotal	105 (11.17%)	65 (10.05%)	16 (12.03%)	16 (28.57%)	3 (4.29%)	1 (33.33%)	1 (50%)	1 (100%)	1 (100%)	1 (3.7%)
C. albicans	8 (0.85%)	4 (0.62%)	2 (1.5%)	1 (1.79%)	1 (1.43%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total	113 (12.02%)	69 (10.66%)	18 (13.53%)	17 (30.36%)	4 (5.71%)	1 (33.33%)	1 (50%)	1 (100%)	1 (100%)	1 (3.7%)

CoNS, Coagulase-negative staphylococci; CSF, Cerebrospinal Fluid; TPN, Total Parenteral Nutrition

Table 3. Resistance percentages of microorganisms obtained from cultural analysis to antimicrobial agents

Bacterial isolates	Antimicrobial Resistance Rates of Isolates (%)																		
	Beta-lactams									Carbapenems			Sulfo-namides	Glyco-peptides		Amino-glycosides			Fluoro-quinolones
	AMC	AMP	AZT	CAZ	CTX	FEP	OX	P	TZP	ETP	IMP	MEM	SXT	TEC	VA	AK	CN	TOB	CIP
CoNS	92.86	100	-	-	-	-	96.43	100	-	-	-	-	29.63	0	0	-	100	100	88.89
S. aureus	75	80	-	-	-	-	100	80	-	-	-	-	0	0	0	-	100	100	0
Enterococcus spp.	-	50	-	-	-	-	-	100	-	-	-	-	100	28.57	28.57	-	100	100	71.43
A. baumannii/ haemolyticus	-	-	-	81.82	81.82	81.82	-	-	-	-	100	95.45	95.45	-	-	95.45	100	95.45	-
Klebsiella spp.	80	100	100	100	100	100	-	-	57.14	88.89	30.77	54.55	68.75	0	-	58.33	80	81.25	70
E. coli	55.56	100	100	-	80	100	-	-	14.29	40	0	0	75	-	100	33.33	30	33.33	75
S. marcescens	100	100	100	100	100	80	-	-	66.67	100	50	66.67	25	-	-	40	75	50	100
P. aeruginosa	-	-	0	0	-	50	-	-	50	-	0	0	-	-	-	50	50	50	50
S. maltophilia	-	-	-	66.67	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-
Fungal isolates	Polyene macrolides			Echinocandins										Azoles					
	AMB			CAS										FLU				VO	
C. albicans	0			0										50				50	

AMC: Amoxicillin-Clavulanate; AMP: Ampicillin; AZT: Aztreonam; CAZ: Ceftazidime; CTX: Cefotaxime; FEP: Cefepime; OX: Oxacillin; P: Penicillin; TZP: Piperacillin/Tazobactam; ETP: ertapenem; IMP: Imipenem; MEM: Meropenem; SXT: Trimethoprim/Sulfamethoxazole; TEC: Teicoplanin; VA: Vancomycin; AK: Amikacin; CN: Gentamicin; TOB: Tobramycin; CIP: Ciprofloxacin; AMB: Amphotericin B; CAS: Caspofungin; FLU: Fluconazole; VO: Voriconazole; CoNS: Coagulase-negative staphylococci

exact test, P < 0.0001). Glycopeptide resistance genes (*vanA, vanB,* and *vanC*) were infrequent, predominantly in *S. aureus* and *Enterococcus* spp.

At least one antifungal resistance gene was detected in seven (87.5%) *C. albicans* isolates. Among the isolates, *FUR1* positivity was 75%, *ERG11* positivity was 62.5%, and *FKS1* positivity was 50%, respectively (Figure 3).

Correlation between phenotypic resistance, resistance genes, and MDR patterns

Strong correlations were observed between phenotypic resistance and gene detection for beta-lactams and aminoglycosides in *Klebsiella* spp., *Acinetobacter baumannii/haemolyticus, E. coli,* and other species, contributing to high rates of MDR. Notable findings included multiple gene positivity in MDR isolates of *S. marcescens, S. maltophilia,* and *P. aeruginosa*. However, discrepancies were evident, such as the presence of resistance genes in phenotypically susceptible isolates or the absence of tested genes in resistant isolates.

In *C. albicans,* azole resistance was associated with higher gene diversity, although genes were also present in the susceptible isolates.

DISCUSSION

Neonatal sepsis remains a major cause of morbidity and mortality in newborns, particularly in neonatal intensive care units (NICUs), where immature immune systems and invasive procedures increase susceptibility to infection.[1,3,5] Advances in neonatal care have improved the survival rates of preterm and low-birth-weight infants; however, prolonged hospitalization and device use have also increased the risk of healthcare-associated infections. The widespread use of broad-spectrum antibiotics has driven the emergence of multidrug-resistant (MDR) pathogens, complicating empirical therapy and worsening patient outcomes.[1,5]

The bacterial profile of neonatal infections varies depending on the neonatal period.[1,2] *Streptococcus agalactiae, E. coli, S. aureus, Enterococcus* spp., and *Streptococcus pneumoniae* are the most common bacteria reported in the early-period of neonatal infection cases, whereas, Gram-negative bacteria, CoNS, *K. pneumoniae* and *A. baumannii* are mostly reported in the late-period cases. *Candida* spp. are the most common fungal species detected in these cases.[1,2] In this study, conducted during the COVID-19 pandemic, Gram-negative bacilli accounted for 77.1% of bacterial isolates, with CoNS (24.8%), *A. baumannii/haemolyticus* (19.5%), and *Klebsiella* spp. (14.2%) was the most common. In addition, *C. albicans* (n = 8, 7.1%) was also isolated in this study. This Gram-negative predominance is potentially exacerbated by pandemic-related factors, such as increased antibiotic pressure and infection control challenges.[3,4] The data in this study indicate that critical changes occurred in pathogen profiles and resistance mechanisms, particularly during the COVID-19 pandemic, and these changes are consistent with studies reported in the current literature.[3,4] This situation aligns with findings reported during the pandemic, where researchers determined that empirical antibiotic pressure and disruptions in infection control measures during the pandemic altered the epidemiology of neonatal sepsis in favor of Gram-negative pathogens.[3] Gram-negative dominance is a major threat to neonatal survival, especially in low- and middle-income regions.[4,21]

The presence of ESBL, MRSA, VRE, and MDR in hospitals has become a significant problem in treatment and infection control.[4,22,23] In the current study, phenotypic susceptibility testing showed high glycopeptide susceptibility among Gram-positive isolates, whereas marked resistance to penicillins, oxacillin, and aminoglycosides was observed. In contrast, Gram-negative isolates exhibited extensive resistance to beta-lactams, with particularly high carbapenem resistance in *A. baumannii/haemolyticus* and *Klebsiella* spp. MDR rates reached 95.5% in *A. baumannii/haemolyticus* and 81.3% in *Klebsiella.* The ESBL phenotype was detected in 33%-50% of *Enterobacterales* isolates. This study showed that ESBL positivity and MDR rates can vary significantly among different bacterial species. It is striking that special resistances, such as MRSA and VRE, were determined at similar rates in certain bacterial species.

Molecular analysis revealed species-specific patterns of resistance genes. bla_{TEM} was the most prevalent ESBL gene among Gram-negatives (33%-93%), while bla_{VIM} predominated in non-fermenters (*A. baumannii/haemolyticus*

88.2%; *S. maltophilia* 83.3%) compared with *Enterobacterales* (P = 0.048, Fisher's exact test). bla_{NDM} was frequently detected in *S. marcescens* (80%) and *Klebsiella* spp. (33.3%). The *mecA* gene was commonly found in CoNS isolates (92.9%) and showed a significant association with the species (P < 0.0001). In this study, the detection of resistance genes in phenotypically susceptible isolates was a significant discrepancy; this is likely due to unexpressed genes or silent genes. Furthermore, genetically unproven but phenotypically expressed resistance is due to the presence of alternative operational genes. PCR-based data highlight the importance of genomic studies and the value of combining phenotypic and genotypic surveillance methods.[23,24]

The microorganisms that cause neonatal infections and their responses to antimicrobials vary depending on the time and geography.[25] This could be due to factors such as different laboratory methods, sample origins, and infection control measures in different geographical regions. One of the most striking findings of our study was the 95.5% rate of multidrug-resistance (MDR) detected in *A. baumannii/haemolyticus* isolates. MDR has been reported to increase the risk of septic shock in Gram-negative sepsis.[5] The predominance of bla_{NDM}-producing *Klebsiella* spp. and bla_{VIM}-producing *A. baumannii/haemolyticus* suggests possible nosocomial transmission of resistant clones within the unit, warranting enhanced infection control measures and molecular epidemiological typing in future investigations. Furthermore, the high prevalence of bla_{VIM} and bla_{NDM} at the molecular level was consistent with the global increase in carbapenem-resistant strains in the post-pandemic era.[4]

In this study, we found 100% susceptibility to amphotericin B and 50% resistance to azoles. These results contradict those of a study reporting 39.1% resistance to amphotericin B and complete susceptibility to azoles.[26] This discrepancy is molecularly supported by the presence of genes in our unit. This is critical for demonstrating the decisive influence of antifungal surveillance on local empirical treatment decisions and the geographic/unit-based evolution of resistance. Among the *C. albicans* isolates, at least one antifungal resistance gene was identified in 87.5% of the isolates. *FUR1* (75%) and *ERG11* (62.5%) were the most common mutations associated with azole resistance. The presence of resistance genes in phenotypically susceptible isolates may indicate an emerging resistance potential under antifungal pressure.[27,28]

Molecular screening did not include common *OXA-48* genes, potentially underestimating the rate of carbapenemase-producing *Enterobacterales*. Epidemiological typing was not performed to confirm the clonal relatedness of MDR isolates. The limited number of isolates for some species reduced the statistical power. The clinical outcomes were not correlated with the resistance profiles. Despite these limitations, the data clearly demonstrate that empirical treatment in the management of neonatal sepsis after the pandemic needs to be restructured based on local resistance genetics.

CONCLUSION

Antimicrobial resistance has become an urgent and significant problem among the identified microorganisms. This underscores the necessity of genomic surveillance for the early detection and management of outbreaks in neonatal intensive care units. The high rates of multidrug-resistance (MDR) and the presence of silent resistance genes have once again highlighted the importance of infection control and rational antibiotic use in neonatal units. PCR-based studies for detecting neonatal pathogens are insufficient. In particular, the detection of genetic resistance burden despite phenotypic susceptibility underscores the need to strengthen surveillance protocols using molecular methods.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

EK, FB and YB conceptualized the study and designed the experiments. EK, FB and YE screened the samples. EK, FB and YE performed the experiments and designed the figures. EK, FB, YB and YE wrote the manuscript. FB and YB

edited the manuscript. FB critically analyzed and approved the final manuscript for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Non-Interventional Clinical Research Ethics Committee of Van Yüzüncü Yıl University (Türkiye), approval number 2020/01-19, dated 17/01/2020.

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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