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Original Article

Microbial Etiology and Antibiotic Susceptibility of Samples from Neonatal Intensive Care Units Patients in a Tertiary Care Center in Iran

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ABSTRACT

Background: Bacterial infection among neonatal patients admitted to the neonatal intensive care unit (NICU) is a global crisis and is among the first causes of neonatal mortality globally. Hence, this study aimed to evaluate the prevalence of bacterial pathogens from collected samples of neonatal infection in the NICU of a tertiary care center in Tehran, Iran.

Methods: In this study, we used data from blood, ulcer/fluids, and urine samples of NICU neonates at Imam Khomeini Hospital from 2019 to 2022.

Results: Overall, 712 urine cultures (144 positives), 4578 blood cultures (376 positives), and 1771 ulcer/fluid cultures (411 positives) were gathered. Among positive blood cultures, the most prevalent bacterial species was *Klebsiella pneumonia* (120, 32.88%). Among urine cultures, the most prevalent bacterial species was *Klebsiella pneumonia* (71, 49.31%). Among positive ulcer/fluid cultures, the most prevalent bacterial species were *Coagulase Negative Staphylococci* (150, 36.49%). The highest sensitivity of *Klebsiella pneumonia* was against *Ciprofloxacin* (15.83%) in blood samples and resistant to *Gentamycin* (81.67%). Among urine samples, the highest sensitivity of *Klebsiella pneumonia* was against *Ciprofloxacin* (29.58%%) and the highest resistance against *Gentamycin* (84.51%). Among the ulcer/fluid samples, the highest sensitivity of *Coagulase Negative Staphylococci* was against *Ciprofloxacin* (13.48%) and the highest resistance against *Cotrimoxazole* (14.81%).

Conclusion: The most prevalent bacterial species found were *Klebsiella pneumonia* and *Coagulase Negative Staphylococci.* These species showed high resistance against multi drugs such as *Cotrimoxazole, Ampicillin-sulbactam,* and *Piperacillin-tazobactam.* National health policymakers should focus on surveillance programs to control and monitor such trends in antibiotic resistance.

Keywords: Antibiogram, Antibiotic resistance, Antibiotic susceptibility, Iran, Neonatal intensive care unit

Introduction

A neonatal bacterial infection causes a great burden and mortality on healthcare systems annually (1). Based on a global estimate, of the 6.3 million deaths before the age of 5 among children, more than 50% have died from a bacterial infection (2). Iran is a low-middle-income country with high rates of neonatal mortality due to early infection (3-5). Treating such infections is complicated by the

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rising trend of antibiotic resistance and multi-drug resistant species in neonatal intensive care units (NICUs). Based on a recent national systematic review and meta-analysis study, the national prevalence of neonatal sepsis was around 16% (5). These numbers are significantly lower in the United States, with an estimated incidence of 1 to 2 cases per 1000 live births (6, 7). Better surveillance programs and lower use of unnecessary antibiotic prescriptions can be a certain factor regarding this difference (8).

Studies that report the prevalence of certain common pathogens and their susceptibility and resistance against a great spectrum of antibiotics can guide healthcare policymakers in guiding institutional protocols regarding infection control and prevention. Also, with the increased human travel and globalization, the rising prevalence of one pathogen in one country can be rapidly reported in the neighboring countries and increase the chance of an epidemic or a pandemic, such has been the case with the SARS-CoV-2 or COVID-19 recently (9-17). Hence, this study aimed to evaluate the prevalence of bacterial pathogens from collected samples of neonatal infection in the NICU of a tertiary care center in Tehran, Iran.

Methods

We used data from blood, ulcer/fluids, and urine samples of neonatal intensive care unit (NICU) neonates of Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran. This hospital is affiliated with the top-rank medical school in Iran and is a referral center. Hence, numerous neonates are referred to this hospital. Thus, the data can be seen as a viable depiction of antibiotic susceptibility and resistance in the general population of Iran. The primary objective of our study was to isolate and identify bacterial organisms in NICU neonates suffering from infections amenable to antibiotic treatment. We also evaluated the antimicrobial susceptibility patterns of the identified organisms. All neonates admitted to the NICU for various reasons and for whom a caregiver or family member signed a consent form were included in the study. Exclusion criteria were neonates with a NICU stay of less than 2 days or incomplete data in hospital records. The laboratory reports from 2019 to 2022 were gathered and examined in our study. The study was retrospective, and data were collected by reviewing and analyzing hospital records. Also, informed consent was obtained from all the parents of neonates, whose data was used in our study.

The blood, ulcer/fluids, and urine cultures were taken by NICU nurses according to the currently available standard protocols (18-22). After taking the sample, all types of microorganisms and susceptibility testing were recorded. These bacteria were isolated using the streak plate method on blood agar and MacConkey agar cultures standard by loops (34/1 mm id). But only for blood cultures, firstly, blood culture bottles were placed in the Bact-Alert instrument and if this device had alarms, this specimen was transferred to the mentioned protocol. After that, Culture plates were incubated at 37°C for 24 hours. A culture consisting of more than 10^5 colonies of a particular bacterium was considered a positive culture. Incubation of negative 24-hour cultures was extended for an additional 24 hours. Species were identified by biochemical tests (indole, citrate, oxidase and H2S production, lysine decarboxylase, lactose fermentation, urea hydrolysis, gas production, catalase, coagulase, mannitol fermentation, and susceptibility testing for novobiocin).

After identification, the antibiotic susceptibility and resistance were tested using the disk diffusion method on the Mueller-Hinton medium (Merck, Germany). After inoculating the bacteria onto Mueller-Hinton agar and inserting antibiotic discs, the plates were incubated in an incubator for 24 hours. Results were then classified according to growth inhibition zone size and international number and reported into three groups: (1) susceptible and sensitive, (2) intermediate susceptibility or sensitivity, and (3) resistance. We used STATA V.17.0 to analyze and report the results.

Ethical Approval

This study was approved by Research Ethics Board at Tehran University of Medical Sciences: IR.TUMS.IKHC.REC.1402.074.

Results

Overall, 712 urine cultures, 4578 blood cultures, and 1771 ulcer and fluid cultures were gathered. Among the urine and stool cultures, 144 were positive for growth, and 568 were negative. Of the blood cultures, 376 were positive for growth, and 4202 were negative. Among the ulcer and fluid cultures, 411 samples were positive for growth and 1360 negative samples.

Among the positive blood cultures, the most prevalent bacterial species were *Klebsiella*

pneumonia (120, 32.88%) and *Coagulase Negative Staphylococci* (110, 29.25%), respectively. Among the positive urine cultures, the most prevalent bacterial species were *Klebsiella pneumonia* (71, 49.31%), *Escherichia coli* (11, 7.64%), and *Klebsiella oxytoca* (8, 5.56%), respectively. Among the positive ulcer and fluid cultures, the most prevalent bacterial species were *Coagulase Negative Staphylococci* (150, 36.49%) and *Klebsiella pneumonia* (89, 21.87%), respectively. More details regarding the prevalence of other species are summarized in Table 1.

Among the blood samples: the highest sensitivity of *Klebsiella pneumonia* was against *Ciprofloxacin* (15.83%), *Imipenem* (15%), and *Gentamycin* (10.83%), respectively. The highest resistance of *Klebsiella pneumonia* was against *Gentamycin* (81.67%) and *Piperacillin-tazobactam* (79.17%). The highest sensitivity of *Staphylococcus epidermidis* (the most common species of *Coagulative Negative Staphylococcus*) was against *Vancomycin* (66.67%) and *Linezolid* (57.89%), respectively. The highest resistance of *Staphylococcus epidermidis* was against *Clindamycin* (87.72%) and *Erythromycin* (63.16%). Further details regarding the susceptibility and resistance of bacterial species from blood-positive blood cultures are summarized in Tables 2 and 3.

Table 1. Prevalence of different species among the samples

Name	Blood		Urine		Ulcer/Fluid	
	N	%	N	%	N	%
Achromobacter xylosoxidans	1	0.27	-	-	-	-
Acinetobacter baumannii	15	4.11	1	0.69	28	6.88
Burkholderia cepacian	-	-	3	2.08	-	-
Acinetobacter lwoffii	-	-	-	-	1	0.25
Citrobacter diversus	-	-	3	2.08	-	-
Citrobacter freundii	1	0.27	-	-	-	-
Citrobacter sedlakii	-	-	1	0.69	-	-
Coagulase Negative Staphylococci	53	14.52	2	1.39	81	19.90
Diphtheroid	-	-	-	-	2	0.49
Escherichia coli	8	2.19	11	7.64	31	7.62
Enterococcus faecalis	-	-	3	2.08	4	0.98
Enterococcus faecium	13	3.56	5	3.47	9	2.21
Enterococcus cloacae	7	1.92	4	2.78	3	0.74
Enterococcus subspecies	3	0.82	-	-	5	1.23
Enterobacter aerogenes	1	0.27	-	-	-	-
Klebsiella oxytoca	4	1.10	8	5.56	8	1.97
Klebsiella ozaenae	-	-	-	-	1	0.25
Klebsiella pneumoniae	120	32.88	71	49.31	89	21.87
Klebsiella subspecies	8	2.19	3	2.08	22	5.41
Kocuria kristinae	4	1.10	-	-	-	-
Micrococcus subspecies	1	0.27	-	-	1	0.25
Pseudomonas aeruginosa	4	1.10	5	3.47	15	3.69
Pseudomonas subspecies	4	1.10	-	-	-	-
Ralstonia pickettii	2	0.55	-	-	-	-
Sphingomonas paucimobilis	1	0.27	-	-	-	-
Serratia marcescens	-	-	1	0.69	-	-
Staphylococcus aureus	-	-	-	-	11	2.70
Staphylococcus hominis	9	2.47	-	-	-	-
Staphylococcus epidermidis	57	15.62	1	0.69	69	16.95
Staphylococcus haemolyticus	10	2.74	-	-	-	-
Staphylococcus saprophyticus	-	-	-	-	1	0.25
Stenotrophomonas maltophilia	7	1.92	-	-	-	-
Staphylococcus gallinarum	1	0.27	-	-	-	-
Streptococcus Group D	-	-	-	-	2	0.49
Streptococcus salivarius	-	-	-	-	2	0.49
Streptococcus viridans	1	0.27	-	-	6	1.47
Streptococcus mitis	2	0.55	-	-	-	-
Streptococcus pyogen	1	0.27	-	-	-	-
Streptococcus pneumonia	1	0.27	-	-	1	0.25
Streptococcus subspecies	-	-	-	-	3	0.74
Stenotrophomonas maltophilia	-	-	-	-	2	0.49
Yeast (Candida albicans)	7	1.92	2	1.39	2	0.49
Yeast subspecies (non-albicans)	21	5.75	10	6.94	3	0.74
Bacillus subspecies	-	-	-	-	2	0.49

Table 2. NICU Blood Cultures – Sensitive

	Amikacin	Ampicillin	Ampicillin sulbactam	Cefepime	Cefotaxime	Cefoxitin	Ciprofloxacin	Ceftazidime	Ceftriaxone	Clindamycin	Colistin	Cotrimoxazole	Doxycycline	Ertapenem
Acinetobacter baumannii	0	0	6.66% 1/15	0	6.66% 1/15	0	6.66% 1/15	0	0	0	0	0	0	0
Coagulase Negative Staphylococci	0	0	0	0	0	28.30% 15/53	15.09% 8/53	0	0	15.09% 8/53	0	11.32% 6/53	0	0
Escherichia coli	25% 2/8	0	0	0	0	0	62.50% 5/8	0	0	0	0	50% 4/8	0	0
Enterococcus faecium	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enterobacter cloacae	57.14% 4/7	0	14.29% 1/7	14.29% 1/7	0	0	71.43% 5/7	57.14% 4/7	0	0	0	71.43% 5/7	0	14.29% 1/7
Enterobacter subspecies	0	0	0	0	0	0	33.33% 1/3	0	0	0	0	100% 3/3	0	0
Klebsiella oxytoca	25% 1/4	0	0	0	0	0	100% 4/4	0	0	0	0	25% 1/4	0	25% 1/4
Klebsiella pneumoniae	3.33% 4/120	0	1.67% 2/120	0	0.83% 1/120	0	15.83% 19/120	0	0	0	4.17% 5/120	15% 18/120	0	0
Klebsiella subspecies	25% 2/8	0	12.50% 1/8	0	0	0	100% 8/8	0	12.50% 1/8	0	0	50% 4/4	0	0
Kocuria kristinae	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pseudomonas aeruginosa	25% 1/4	0	0	25% 1/4	0	0	50% 2/4	50% 2/4	0	0	0	0	0	0
Staphylococcus haemolyticus	0	0	0	0	0	10% 1/10	0	0	0	0	0	0	0	0
Staphylococcus hominis	0	0	0	0	0	11.11% 1/9	11.11% 1/9	0	0	0	0	11.11% 1/9	11.11% 1/9	0
Staphylococcus epidermidis	0	0	0	0	10.53% 6/57	15.79% 9/57	1.75% 1/57	0	0	8.77% 5/57	0	22.81% 13/57	1.75% 1/57	0
Stenotrophomonas maltophilia	14.29% 1/7	0	14.29% 1/7	0	0	0	57.14% 4/7	14.29% 1/7	0	0	0	57.14% 4/7	0	0
Other species	6.25% 1/16	0	0	0	0	6.25% 1/16	43.75% 7/16	31.25% 5/16	0	0	0	50% 8/16	0	0

Table 2. Continued

	Erythromycin	Gentamycin	Imipenem	Levofloxacin	Linezolid	Meropenem	Oxacillin	Piperacillin T.	Rifampicin	Temocillin	Tetracycline	Tigecycline	Tobramycin	Vancomycin
Acinetobacter baumannii	0	6.66% 1/15	6.66% 1/15	0	0	0	0	6.66% 1/15	0	0	0	0	0	0
Coagulase Negative Staphylococci	3.77% 2/53	16.98% 9/53	0	0	28.30% 15/53	0	0	0	75.47% 40/53	0	0	1.89% 1/53	0	83.02% 44/53
Escherichia coli	0	37.50% 3/8	37.50% 3/8	50% 4/4	0	25% 2/8	0	37.50% 3/8	0	0	0	0	0	0
Enterococcus faecium	0	0	0	0	76.92% 10/13	0	0	0	0	0	0	23.08% 3/13	0	0
Enterobacter cloacae	0	0	85.71% 6/7	14.29% 1/7	0	0	0	71.43% 5/7	0	0	0	0	0	0
Enterobacter subspecies	0	33.33% 1/3	100% 3/3	66.67% 2/3	0	0	0	33.33% 1/3	0	0	0	0	0	0
Klebsiella oxytoca	0	25% 1/4	100% 4/4	0	0	0	0	100% 4/4	0	0	0	0	0	0
Klebsiella pneumoniae	0	10.83% 13/120	15% 18/120	3.33% 4/120	0	5% 6/120	0	8.33 10/120	0	0	0	7.50% 9/120	0	0
Klebsiella subspecies	0	62.50% 5/8	100% 8/8	50% 4/8	0	0	0	87.50% 7/8	0	0	0	0	0	0
Kocuria kristinae	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pseudomonas aeruginosa	0	25% 1/4	25% 1/4	0	0	0	0	25% 1/4	0	0	0	0	0	0
Staphylococcus haemolyticus	0	0	0	0	70% 7/10	0	0	0	0	0	0	0	0	80% 8/10
Staphylococcus hominis	0	11.11% 1/9	0	0	66.67% 6/9	0	0	0	77.78% 7/9	0	0	44.44% 4/9	0	77.78% 7/9
Staphylococcus epidermidis	5.26% 3/57	1.75% 1/57	0	1.75% 1/57	57.89% 33/57	0	0	0	26.32% 15/57	0	7.02% 4/57	7.02% 4/57	0	66.67% 38/57
Stenotrophomonas maltophilia	0	57.14% 4/7	0	42.86% 3/7	0	0	0	28.57% 2/7	0	0	0	0	0	0
Other species	6.25% 1/16	18.75% 3/16	43.75% 7/16	0	31.25% 5/16	0	0	18.75% 3/16	6.25% 1/16	0	0	6.25% 1/16	0	31.25% 5/16

Table 3. NICU Blood Cultures – Resistant

	Amikacin	Ampicillin	Ampicillin sulbactam	Cefepime	Cefotaxime	Cefoxitin	Ciprofloxacin	Ceftazidime	Ceftriaxone	Clindamycin	Colistin	Cotrimoxazole	Doxycycline	Ertapenem
Acinetobacter baumannii	0	0	86.67% 13/15	0	73.33% 11/15	0	86.67% 13/15	0	0	0	0	93.33% 14/57	0	0
Coagulase Negative Staphylococci	0	0	0	0	3.77% 2/53	67.92% 36/53	52.83% 28/53	0	1.89% 1/53	83.02% 44/53	0	62.26% 33/53	0	0
Escherichia coli	12.50% 1/8	0	0	0	37.5% 3/8	0	37.5% 3/8	37.5% 3/8	0	0	12.50% 1/8	50% 4/4	0	0
Enterococcus faecium	0	84.62% 11/13	0	0	15.38% 2/13	30.77% 4/13	38.46% 5/13	0	0	53.85% 7/13	0	23.08% 3/13	0	0
Enterobacter cloacae	14.29% 1/7	14.29% 1/7	28.57% 2/7	0	0	0	0	14.29% 1/7	0	0	0	14.29% 1/7	0	0
Enterobacter subspecies	33.33% 1/3	0	100% 3/3	0	0	0	0	33.33% 1/3	0	0	0	0	0	0
Klebsiella oxytoca	25% 1/4	0	75% 3/4	0	0	0	0	75% 3/4	0	0	0	75% 3/4	0	0
Klebsiella pneumoniae	46.67% 56/120	2.50% 3/120	70.83% 85/120	7.50% 9/120	20% 30/120	0.83% 1/120	71.67% 86/120	45.83% 55/120	0	0	19.17% 23/120	73.33% 88/120	0	0
Klebsiella subspecies	25% 2/8	0	62.50% 5/8	0	0	0	0	37.50% 3/8	0	0	0	25% 2/8	0	0
Kocuria kristinae	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pseudomonas aeruginosa	50% 2/4	0	0	0	50% 2/4	0	50% 2/4	0	0	0	0	50% 2/4	0	0
Staphylococcus haemolyticus	0	0	0	0	50% 5/10	40% 4/10	10% 1/10	0	0	90% 9/10	0	50% 5/10	0	0
Staphylococcus hominis	0	0	0	0	11.11% 1/9	77.78% 7/9	44.44% 4/9	0	0	100% 9/9	0	88.89% 8/9	0	0
Staphylococcus epidermidis	0	0	0	0	29.82% 17/57	57.89% 33/57	17.54% 10/57	0	0	87.72% 50/57	0	33.33% 19/57	8.77% 5/57	0
Stenotrophomonas maltophilia	0	0	42.86% 3/7	14.29% 1/7	28.57% 2/7	0	14.29% 1/7	14.29% 1/7	0	0	0	14.29% 1/7	0	0
Other species	12.50% 2/16	18.75% 3/16%	18.75% 3/16%	0	0	0	0	6.25% 1/16	12.50% 2/16	25% 4/16	6.25% 1/16	31.25% 5/16	0	0

Table 3. Continued

	Erythromycin	Gentamycin	Imipenem	Levofloxacin	Linezolid	Meropenem	Oxacillin	Piperacillin T.	Rifampicin	Temocillin	Tetracycline	Tigecycline	Tobramycin	Vancomycin
Acinetobacter baumannii	0	86.67% 13/15	86.67% 13/15	0	0	0	0	86.67% 13/15	0	0	0	0	0	0
Coagulase Negative Staphylococci	84.91% 45/53	28.30% 15/53	0	0	0	0	0	0	13.21% 7/53	0	0	0	0	0
Escherichia coli	0	37.5% 3/8	50% 4/8	0	0	12.50% 1/8	0	37.5% 3/8	0	0	0	0	0	0
Enterococcus faecium	69.23% 9/13	53.85% 7/13	38.46% 5/13	0	15.38% 2/13	0	0	0	15.38% 2/13	0	0	0	0	92.31% 12/13
Enterobacter cloacae	0	0	0	0	0	0	0	14.29% 1/7	0	0	0	0	0	0
Enterobacter subspecies	0	0	0	0	0	0	0	66.67% 2/3	0	0	0	0	0	0
Klebsiella oxytoca	0	75% 3/4	0	0	0	0	0	0	0	0	0	0	0	0
Klebsiella pneumoniae	0	81.67% 98/120	79.17% 95/120	11.67% 14/120	0	29.17% 35/120	0	79.17% 95/120	0	0	0	3.33% 4/120	0.83% 1/120	0
Klebsiella subspeciesp	0	37.50% 3/8	0	0	0	0	0	12.50% 1/8	0	0	0	0	0	0
Kocuria kristinae	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pseudomonas aeruginosa	0	75% 3/4	75% 3/4	0	0	0	0	50% 2/4	0	0	0	0	0	0
Staphylococcus haemolyticus	50% 5/10	10% 1/10	0	0	0	0	10% 1/10	0	10% 1/10	0	0	0	0	10% 1/10
Staphylococcus hominis	100% 9/9	33.33% 3/9	0	11.11% 1/9	0	0	11.11% 1/9	0	11.11% 1/9	0	0	0	0	0
Staphylococcus epidermidis	63.16% 36/57	14.04% 8/57	1.75% 1/57	0	3.51% 2/57	0	15.79% 9/57	0	17.54% 10/57	0	7.02% 4/57	0	0	3.51% 2/57
Stenotrophomonas maltophilia	0	14.29% 1/7	14.29% 1/7	0	0	14.29% 1/7	0	14.29% 1/7	0	0	0	0	14.29% 1/7	0
Other species	25% 4/16	31.25% 5/16	12.50% 2/16	0	0	0	0	25% 4/16	0	0	0	0	0	0

Among the ulcer and fluid samples, the highest sensitivity of *Klebsiella pneumonia* was against *Ciprofloxacin* (15.83%), *Imipenem* (15%), and *Gentamycin* (10.83%), respectively. The highest resistance of *Klebsiella pneumonia* was against *Gentamycin* (81.67%) and *Piperacillin-tazobactam* (79.17%). The highest sensitivity of *Coagulase Negative Staphylococci* was against *Ciprofloxacin* (13.48%) and *Cotrimoxazole* (10.11%), *Piperacillin-tazobactam* (10.11%) respectively. The highest resistance of *Coagulase Negative Staphylococci* was against *Cotrimoxazole* (14.81%), *Ciprofloxacin* (13.58%), and *Clindamycin* (13.58%). Further details regarding the susceptibility and resistance of bacterial species from blood-positive blood cultures are summarized in Tables 4 and 5.

Table 4. NICU Ulcer Cultures – Resistant

	Amikacin	Ampicillin	Ampicillin sulbactam	Cefepime	Cefotaxime	Cefoxitin	Ciprofloxacin	Ceftazidime	Ceftriaxone	Clindamycin	Colistin	Cotrimoxazole	Doxycycline	Ertapenem
Acinetobacter baumannii	7.14% 2/28	0	89.29% 25/28	0	67.86% 19/28	0	92.86% 26/28	0	0	0	0	92.86% 26/28	0	0
Coagulase Negative Staphylococci	0	0	0	0	0	12.35% 10/81	13.58% 11/81	0	0	13.58% 11/81	0	14.81% 12/81	0	0
Escherichia coli	0	0	32.26% 10/31	0	6.45% 2/31	0	3.23% 1/31	3.23% 1/31	0	0	0	16.13% 5/31	0	0
Enterococcus faecium	0	55.56% 5/9	0	0	22.22% 2/9	0	11.11% 1/9	0	0	44.44% 4/9	0	11.11% 1/9	0	0
Enterobacter cloacae	33.33% 1/3	0	66.67% 2/3	0	0	0	0	0	0	0	0	33.33% 1/3	0	0
Enterobacter subspecies	0	0	60% 3/5	0	0	0	40% 2/5	40% 2/5	0	0	0	0	0	0
Enterococcus faecalis	0	0	0	0	0	50% 2/4	0	0	0	50% 2/4	0	0	0	0
Klebsiella oxytoca	0	0	87.50% 7/8	0	50% 4/8	0	0	0	0	0	0	50% 4/8	0	0
Klebsiella pneumoniae	6.74% 6/89	0	82.02% 73/89	1.12% 1/89	46.07% 41/89	0	69.66% 62/89	5.62% 5/89	0	0	1.12% 1/89	75.28% 67/89	0	0
Klebsiella subspecies	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pseudomonas aeruginosa	20% 3/15	0	60% 9/15	0	13.32% 2/15	0	26.67% 4/15	13.32% 2/15	0	0	13.32% 2/15	20% 3/15	0	0
Staphylococcus aureus	0	0	0	0	9.09% 1/11	27.27% 3/11	18.18% 2/11	0	0	36.36% 4/11	0	27.27% 3/11	0	0
Staphylococcus epidermidis	0	0	0	0	65.22% 45/69	33.33% 23/69	2.90% 2/69	0	0	86.96% 60/69	0	13.04% 9/69	0	0
Streptococcus viridans	0	50% 3/6	0	0	50% 3/6	16.67% 1/6	0	0	0	83.33% 5/6	0	16.67% 1/6	0	0
Streptococcus subspecies	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Other species	0	16.67% 3/18	5.56% 1/18	0	0	5.56% 1/18	22.22% 4/18	0	0	27.78% 5/18	0	0	0	0

Table 4. Continued

	Erythromycin	Gentamycin	Imipenem	Levofloxacin	Linezolid	Meropenem	Oxacillin	Piperacillin T.	Rifampicin	Temocillin	Tetracycline	Tigecycline	Tobramycin	Vancomycin
Acinetobacter baumannii	0	85.71% 24/28	89.29% 25/28	0	0	0	0	92.86% 26/28	0	0	0	0	0	0
Coagulase Negative Staphylococci	12.35% 10/81	8.64% 7/81	0	0	0	0	0	0	1.23% 1/81	0	0	0	0	0
Escherichia coli	0	3.23% 1/31	3.23% 1/31	0	0	0	0	6.45% 2/31	0	0	0	0	0	0
Enterococcus faecium	44.44% 4/9	44.44% 4/9	33.33% 3/9	0	0	0	0	0	0	0	0	0	0	55.56% 5/9
Enterobacter cloacae	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enterobacter subspecies	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enterococcus faecalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Klebsiella oxytoca	0	50% 4/8	0	0	0	0	0	12.50% 1/8	0	0	0	0	0	0
Klebsiella pneumoniae	0	73.03% 65/89	71.94% 64/89	1.12% 1/89	0	4.49% 4/89	0	73.03% 65/89	0	0	0	1.12% 1/89	0	0
Klebsiella subspecies	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pseudomonas aeruginosa	0	6.67% 1/15	40% 6/15	6.67% 1/15	0	6.67% 1/15	0	26.67% 4/15	0	0	0	0	0	0
Staphylococcus aureus	27.27% 3/11	9.09% 1/11	0	0	0	0	0	0	9.09% 1/11	0	0	0	0	0
Staphylococcus epidermidis	14.49% 10/69	2.90% 2/69	0	0	0	0	0	0	5.80% 4/69	0	0	0	0	0
Streptococcus viridans	16.67% 1/6	66.67% 4/6	0	0	0	0	0	0	33.33% 2/6	0	0	0	0	0
Streptococcus subspecies	0	5.56% 1/18	5.56% 1/18	0	0	0	0	0	0	0	11.11% 2/18	0	0	0

Table 5. NICU Ulcer Cultures – Sensitivity

	Amikacin	Ampicillin	Ampicillin sulbactam	Cefepime	Cefotaxime	Cefoxitin	Ciprofloxacin	Ceftazidime	Ceftriaxone	Clindamycin	Colistin	Cotrimoxazole	Doxycycline	Ertapenem
Acinetobacter baumannii	0	0	7.14% 2/28	0	3.57% 1/28	0	3.57% 1/28	3.57% 1/28	0	0	0	7.14% 2/28	0	0
Coagulase Negative Staphylococci	0	0	0	0	0	4.94% 4/81	2.47% 2/81	0	0	3.70% 3/81	0	2.47% 2/81	0	0
Escherichia coli	22.58% 7/31	0	9.68% 3/31	0	25.81% 8/31	0	38.71% 12/31	3.23% 1/31	0	0	0	25.81% 8/31	0	0
Enterococcus faecium	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enterobacter cloacae	0	0	33.33% 1/3	0	0	0	100% 3/3	0	0	0	0	66.67% 2/3	0	0
Enterobacter subspecies	0	0	0	0	0	0	20% 1/5	20% 1/5	0	0	0	60% 3/5	0	0
Enterococcus faecalis	0	50% 2/4	0	0	0	0	0	0	0	0	0	50% 2/4	0	0
Enterococcus faecium	0	11.11% 1/9	0	0	0	0	0	0	0	0	0	11.11% 1/9	0	0
Klebsiella oxytoca	0	0	0	0	25% 2/8	0	87.50% 7/8	0	0	0	0	37.5% 3/8	0	0
Klebsiella pneumoniae	2.25% 2/89%	0	1.12% 1/89	0	4.49% 4/89	0	13.48% 12/89	0	0	0	0	10.11% 9/89	0	0
Klebsiella subspecies	0	0	18.18% 4/22	0	13.64% 3/22	0	18.18% 4/22	0	0	0	0	13.64% 3/22	0	0
Pseudomonas aeruginosa	0	0	0	13.33% 2/15	13.33% 2/15	0	73.33% 11/15	26.67% 4/15	0	0	0	0	0	0
Staphylococcus aureus	0	0	0	0	0	18.18% 2/11	0	0	0	9.09% 1/11	0	9.09% 1/11	0	0
Staphylococcus epidermidis	0	0	0	0	11.59% 8/69	59.42% 41/69	2.90% 2/69	0	0	8.70% 6/69	0	5.80% 4/69	0	0
Streptococcus viridans	0	16.67% 1/6	0	0	0	0	0	0	0	16.67% 1/6	0	33.33% 2/6	0	0
Streptococcus subspecies	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Other species	0	5.56% 1/18	5.56% 1/18	0	0	0	11.11% 2/18	0	0	5.56% 1/18	0	22.22% 4/18	0	0

Table 5. Continued

	Erythromycin	Gentamycin	Imipenem	Levofloxacin	Linezolid	Meropenem	Oxacillin	Piperacillin T.	Rifampicin	Temocillin	Tetracycline	Tigecycline	Tobramycin	Vancomycin
Acinetobacter baumannii	0	3.57% 1/28	3.57% 1/28	3.57% 1/28	0	0	0	7.14% 2/28	0	0	0	0	0	0
Coagulase Negative Staphylococci	1.23% 1/81	1.23% 1/81	0	0	0	0	0	0	12.35% 10/81	0	0	0	0	17.28% 14/81
Escherichia coli	0	12.90% 4/31	6.45% 2/31	3.23% 1/31	0	0	0	32.26% 10/31	0	0	0	0	0	0
Enterococcus faecium	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enterobacter cloacae	0	66.67% 2/3	66.67% 2/3	0	0	0	0	100% 3/3	0	0	0	0	0	0
Enterobacter subspecies	0	60% 3/5	60% 3/5	0	0	0	0	0	0	0	0	0	0	0
Enterococcus faecalis	50% 2/4	0	0	0	50% 2/4	0	0	0	0	0	0	0	0	50% 2/4
Enterococcus faecium	0	0	11.11% 1/9	0	66.67% 6/9	0	0	0	0	0	0	0	0	22.22% 2/9
Klebsiella oxytoca	0	12.50% 1/8	25% 2/8	0	0	0	0	75% 6/8	0	0	0	0	0	0
Klebsiella pneumoniae	0	5.62% 5/89	7.87% 7/89	1.12% 1/89	0	0	0	10.11% 9/89	0	0	0	0	0	0
Klebsiella subspecies	0	9.09% 2/22	4.55% 1/22	0	0	0	0	18.18% 4/22	0	0	0	0	0	0
Pseudomonas aeruginosa	0	93.33% 14/15	46.67% 7/15	0	0	0	0	60% 9/15	0	0	0	0	13.33% 2/15	0
Staphylococcus aureus	0	0	0	0	9.09% 1/11	0	0	0	18.18% 2/11	0	0	0	0	36.36% 4/11
Staphylococcus epidermidis	0	2.90% 2/69	0	0	81.16% 56/69	0	0	0	10.14% 7/69	0	0	1.45% 1/69	0	89.96% 60/69
Streptococcus viridans	16.67% 1/6	16.67% 1/6	0	0	100% 6/6	0	0	0	16.67% 1/6	0	0	0	0	100% 6/6
Streptococcus subspecies	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Other species	5.56% 1/18	5.56% 1/18	0	16.67% 3/18	33.33% 6/18	0	0	5.56% 1/18	0	0	0	11.11% 2/18	0	22.22% 4/18

Among the urine samples: the highest sensitivity of *Klebsiella pneumonia* was against *Ciprofloxacin* (29.58%%), *Imipenem* (18.31%), and *Piperacillin-tazobactam* (16.90%), respectively. The highest resistance of *Klebsiella pneumonia* was against *Gentamycin* (84.51%) and *Ampicillin-sulbactam* (83.10%). The highest sensitivity of *Escherichia coli* was against *Imipenem* (63.64%), *Gentamycin* (54.55%), *Piperacillin-tazobactam* (54.55%), and *Nitrofurantoin* (54.55%), respectively. The highest resistance of *Escherichia coli* was against *Cotrimoxazole* (72.73%) and *Ciprofloxacin* (63.64%), respectively. Further details regarding the susceptibility and resistance of bacterial species from blood-positive blood cultures are summarized in Tables 6 and 7.

Table 6. NICU Urine Cultures – Sensitive

	Amikacin	Ampicillin	Ampicillin sulbactam	Cefepime	Cefotaxime	Cefoxitin	Ciprofloxacin	Ceftazidime	Ceftriaxone	Clindamycin	Colistin	Cotrimoxazole	Doxycycline	Ertapenem
Burkholderia cepacian	0	0	0	0	100% 3/3	0	100% 3/3	0	0	0	0	100% 3/3	0	0
Citrobacter diversus	33.33% 1/3	0	100% 3/3	0	0	0	100% 3/3	0	100% 3/3	0	0	66.67% 2/3	0	0
Enterobacter Cloacae	0	0	50% 2/4	0	0	0	100% 4/4	0	50% 2/4	0	0	50% 2/4	0	0
Enterococcus faecalis	0	66.67% 2/3	0	0	33.33% 1/3	0	0	0	0	0	0	0	0	0
Enterococcus faecium	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Escherichia coli	27.27% 3/11	0	18.18% 2/11	0	9.09% 1/11	0	36.36% 4/11	0	0	0	0	27.27% 3/11	0	0
Klebsiella oxytoca	12.50% 1/8	0	0	0	12.50% 1/8	0	37.50% 3/8	0	12.50% 1/8	0	0	25% 2/8	0	0
Klebsiella pneumoniae	1.41% 1/71	0	5.63% 4/71	0	1.41% 1/71	0	29.58% 21/71	0	4.23% 3/71	0	0	12.68% 9/71	0	0
Klebsiella subspecies	0	0	100% 3/3	0	100% 3/3	0	100% 3/3	0	0	0	0	100% 3/3	0	0
Pseudomonas aeruginosa	0	0	0	0	0	0	20% 1/5	0	0	0	0	0	0	0
Other species	16.67% 1/6	0	0	0	0	0	33.33% 2/6	0	0	0	0	16.67% 1/6	0	0

Table 6. Continued

	Erythromycin	Gentamycin	Imipenem	Levofloxacin	Linezolid	Meropenem	Oxacillin	Piperacillin T.	Rifampicin	Temocillin	Tetracycline	Tigecycline	Tobramycin	Vancomycin	Nitrofurantoin
Burkholderia cepacian	0	100% 3/3	100% 3/3	0	0	0	0	0	0	0	0	0	0	0	0
Citrobacter diversus	0	100% 3/3	100% 3/3	0	0	0	0	100% 3/3	0	0	0	0	0	0	100% 3/3
Enterobacter Cloacae	0	50% 2/4	75% 3/4	0	0	0	0	75% 3/4	0	75% 3/4	0	0	0	0	50% 2/2
Enterococcus faecalis	0	0	0	0	100% 3/3	0	0	0	0	0	0	0	0	66.67% 2/3	100% 3/3
Enterococcus faecium	0	0	0	0	100% 5/5	0	0	0	0	0	0	0	0	0	40% 2/5
Escherichia coli	0	54.55% 6/11	63.64% 7/11	9.09% 1/11	0	9.09% 1/11	0	54.55% 6/11	0	27.27% 3/11	0	0	0	0	54.55% 6/11
Klebsiella oxytoca	0	37.50% 3/8	62.50% 5/8	0	0	0	0	37.50% 3/8	0	25% 2/8	0	0	0	0	12.5% 1/8
Klebsiella pneumoniae	0	9.86% 7/71	18.31% 13/71	0	0	1.41% 1/71	0	16.90% 12/71	0	9.86% 7/71	0	0	0	0	14.08% 10/71
Klebsiella subspecies	0	100% 3/3	100% 3/3	0	0	0	0	100% 3/3	0	100% 3/3	0	0	0	0	100% 3/3
Pseudomonas aeruginosa	0	60% 3/5	20% 1/5	0	0	0	0	0	0	0	0	0	0	0	0
Other species	0	50% 3/6	16.67% 1/6	16.67% 1/6	16.67% 1/6	16.67% 1/6	0	16.67% 1/6	0	0	0	0	0	33.33% 2/6	16.67% 1/6

Table 7. NICU Urine Cultures – Resistant

	Amikacin	Ampicillin	Ampicillin sulbactam	Cefepime	Cefotaxime	Cefoxitin	Ciprofloxacin	Ceftazidime	Ceftriaxone	Clindamycin	Colistin	Cotrimoxazole	Doxycycline	Ertapenem
Burkholderia cepacia	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Citrobacter diversus	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enterobacter Cloacae	25% 1/4	0	50% 2/4	0	25% 1/4	0	0	0	25% 1/4	0	0	25% 1/4	0	0
Enterococcus faecalis	0	33.33% 1/3	0	0	33.33% 1/3	33.33% 1/3	33.33% 1/3	0	33.33% 1/3	100% 3/3	0	33.33% 1/3	0	0
Enterococcus faecium	0	60% 3/5	0	0	20% 1/5	20% 1/5	0	0	60% 3/5	80% 4/5	0	80% 4/5	0	0
Escherichia coli	9.09% 1/11	0	54.55% 6/11	0	27.27% 3/11	0	63.64% 7/11	0	27.27% 3/11	0	0	72.73% 8/11	0	0
Klebsiella oxytoca	0	0	100% 8/8	0	12.50% 1/8	0	62.50% 5/8	0	75% 6/8	0	0	75% 6/8	0	0
Klebsiella pneumoniae	23.94% 17/71	0	83.10% 59/71	5.63% 4/71	39.44% 28/71	0	66.20% 47/71	1.41% 1/71	28.17% 20/71	0	7.04% 5/71	74.65% 53/71	0	0
Klebsiella subspecies	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pseudomonas aeruginosa	20% 1/5	0	80% 4/5	0	60% 3/5	0	80% 4/5	0	20% 1/5	0	0	20% 1/5	0	0
Other species	0	0	50% 3/6	0	0	50% 3/6	33.33% 2/6	0	16.67% 1/6	50% 3/6	0	50% 3/6	0	0

Table 7. Continued

	Erythromycin	Gentamycin	Imipenem	Levofloxacin	Linezolid	Meropenem	Oxacillin	Piperacillin T.	Rifampicin	Temocillin	Tetracycline	Tigecycline	Tobramycin	Vancomycin	Nitrofurantoin
Burkholderia cepacia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100% 3/3
Citrobacter diversus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enterobacter Cloacae	0	50% 2/4	25% 1/4	0	0	0	0	25% 1/4	0	25% 1/4	0	0	0	0	50% 2/4
Enterococcus faecalis	66.67% 2/3	66.67% 2/3	33.33% 1/3	33.33% 1/3	0	0	0	0	0	0	0	0	0	33.33% 1/3	0
Enterococcus faecium	80% 4/5	100% 5/5	80% 4/5	0	0	0	0	0	20% 1/5	0	0	0	0	100% 5/5	40% 2/5
Escherichia coli	0	45.45% 5/11	36.36% 4/11	0	0	0	0	18.18% 2/11	0	0	0	0	0	0	9.09% 1/11
Klebsiella oxytoca	0	62.5% 5/8	37.50% 3/8	0	0	0	0	50% 4/8	0	25% 2/8	0	0	0	0	87.50% 7/8
Klebsiella pneumoniae	0	84.51% 60/71	78.87% 56/71	0	0	4.23% 3/71	0	78.87% 56/71	0	23.94% 17/71	0	0	1.41% 1/71	0	50.70% 36/71
Klebsiella subspecies	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pseudomonas aeruginosa	0	40% 2/5	80% 4/5	0	0	0	0	80% 4/5	0	40% 2/5	0	0	0	0	80% 4/5
Other species	33.33% 2/6	16.67% 1/6	16.67% 1/6	0	0	0	0	16.67% 1/6	33.33% 2/6	0	0	0	0	16.67% 1/6	16.67% 1/6

Discussion

Several studies have previously reported the etiology and antibiotic susceptibility of bacterial strains in neonates admitted to the NICU. These studies can guide healthcare decision makers in initiating and implementing surveillance programs to reduce the potential for antibiotic resistance. (23-26).

In our study, the most common bacterial species found among our samples were *Klebsiella pneumonia, Coagulase Negative Staphylococci, Escherichia coli,* and *Staphylococcus epidermidis.* These microorganisms have shown reduced susceptibility to numerous antibiotics and high resistance against various antibiotics such as *Gentamycin, Imipenem, Ampicillin-sulbactam, Vancomycin,* and *Piperacillin-tazobactam.* Recent national reports also support such claims. Based on a recent study by Bahmani on neonatal sepsis

cases in the west of Iran, the most prevalent bacterial species was *Coagulase-negative Staphylococci* and *Acinetobacter* subspecies. They were both resistant to *Cephalosporins* and were similarly susceptible to *Clindamycin, Vancomycin,* and *Ciprofloxacin* (27). Another study regarding the antibiotic susceptibility of bacterial species from NICU admissions reported that the most prevalent sample was the *Staphylococcus* subspecies. These samples were resistant to *Penicillin, Ampicillin,* and *Cotrimoxazole,* similar to the findings of our study. Also, low susceptibility was found against *Vancomycin* and *Ciprofloxacin*.

A recent systematic review and meta-analysis study was performed regarding neonatal sepsis cases in Iran and the prevalence of causative pathogens. According to the results of this study and similar to the findings of our research, the most prevalent pathogens were *Enterobacter subspecies, Klebsiella pneumonia, Coagulase-negative Staphylococci, Escherichia coli,* and *Pseudomonas aeruginosa* (5). Such findings highlight the importance of monitoring and preventive assessment to lower the burden of neonatal sepsis. Another study in the east of Iran has shown that *Coagulase-negative Staphylococci, Escherichia coli,* and *Klebsiella* subspecies. The highest resistance was against *Penicillin, Ampicillin, Imipenem,* and *Gentamycin* (28). Also, based on another report from Hamedan province in Iran, among the urine, spinal fluid, stool, and other secretive fluid cultures, the most prevalent species were *Escherichia coli* and *Klebsiella* subspecies. Drug resistance was reported to be high among the gathered cultures (29).

Similar results have also been reported in other countries. Based on a recent study, *Klebsiella pneumoniae* and *gram-negative* bacterial species were the most prevalent pathogens in India. *Klebsiella pneumonia* was also resistant to *Colistin, Cephalosporin,* and *Carbapenem* (30). Based on another study in the United Kingdom, the majority of the pathogens found among the NICU samples were susceptible to *Benzylpenicillin* and *Gentamycin* and showed high resistance against *Flucloxacillin* (31). Another study in Ethiopia reported the high prevalence of *Klebsiella pneumonia* and *Escherichia coli* among neonates admitted to the NICU. These species were resistant to *Ampicillin, Cefotaxime, Gentamycin, Piperacillin-tazobactam, Amikacin,* and *Imipenem* (32). Such findings pinpoint the global hazard of multi-drug resistant species of bacterial infection among neonates (1, 10, 33). Overall, it appears that we are still dealing with high rates of antibiotic resistance in the NICUs in Iran, and this issue needs to be actively addressed. Antibiotic resistance can be reduced through better neonate care, including shorter hospital stays and appropriate antibiotic use. Limitations of our study include the fact that it included a relatively small population and was conducted at a single center. Therefore, we recommend additional multicenter studies using larger sample sizes to obtain more accurate predictions of antibiotic resistance rates in NICUs.

Conclusion

Based on the findings of our study, from samples gathered from NICU neonates in a tertiary care center in Tehran, Iran, the most prevalent bacterial species found were *Klebsiella pneumonia, Coagulase Negative Staphylococci, Escherichia coli,* and *Staphylococcus epidermidis.* These species were susceptible to *Ciprofloxacin, Imipenem,* and *Gentamycin*. However, the susceptibility has been reduced. Also, these species showed high resistance against multi drugs such as *Cotrimoxazole, Ampicillin-sulbactam,* and *Piperacillin-tazobactam*. A national imitative should be implemented for surveillance of prevalent pathogens in NICU-related infections and monitoring the antibiotic susceptibility and resistance.

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Conflicts of interest

There was no conflict of interest.

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