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PREVALENCE AND ANTIBIOTIC RESISTANCE PATTERNS OF URINARY TRACT INFECTIONS IN NEONATES WITH LATE-ONSET SEPSIS: A CROSS-SECTIONAL STUDY

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Abstract

Aim: The aim of the study was to determine the prevalence and trends of antimicrobial sensitivity of urinary tract infections (UTIs) in infants with late-onset sepsis (LOS).

Study Design: This cross-sectional observational study.

Place and Duration: This study was conducted at Institute of Mother & Child Health Nawabshah from July 2024 to July 2025

Methods: Non-probability consecutive sampling of infants with LOS 7-28 days (n=134) was used. Parental consent and LOS criteria were used as inclusion criteria. Exclusions involved congenital anomalies, prior administration of antibiotics or death within the first 24 hours. UTI was then defined as> 10,000 CFU/mL of a single uropathogen in catheterized urine samples. Antimicrobial susceptibility was determined against ampicillin, ceftriaxone, cefotaxime, gentamicin, and amikacin according to the CLSI. Data were processed in SPSS v25 and compared with chi-square tests ($p<0.05$).

Results: Among 134 neonates (mean age 28.3 ± 5.1 days; 79% male; mean weight 1.92 ± 0.39 kg), *E. coli* was predominant (64.93%, n=87), followed by *Klebsiella* (17.91%, n=24), *Staphylococci* (11.94%, n=16), and Pseudomonas (5.97%, n=8). *Staphylococcus* showed 66.67% resistance to ampicillin and ceftriaxone and 0% resistance to amikacin. *Pseudomonas* was ampicillin-resistant, ceftriaxone-resistant, gentamicin-resistant, amikacin-sensitive, and cefotaxime-sensitive (100%). *E. coli* 92.86% ampicillin resistance, 64.29% gentamicin; *Klebsiella* 100% ampicillin, amoxicillin, and

ceftriaxone. There was no statistically significant difference in the distribution of pathogens based on gender (p=0.389-0.532).
Conclusion: *E. coli* is the most prevalent pathogen in UTI-related LOS, and it is very resistant to first-line antibiotics. These findings highlight the importance of personalized empiric treatment. Multicenter prospective studies should be considered in the future to quantify long-term outcomes and trends in resistance.

Keywords: *Late-onset sepsis, Neonatal sepsis, Urinary tract infection, Antibiotic resistance, Escherichia coli.*

Introduction

Late-onset sepsis (LOS), which manifests after 72 hours of life, is frequently the result of community-acquired or nosocomial infections in premature or critically ill infants [1]. The condition is predominantly found in neonatal intensive care units (NICUs), where invasive surgery and extended hospital stay predispose patients to infections [1, 2]. One of the etiologies of LOS is urinary tract infections (UTIs), particularly in infants with very low birth weight (VLBW), and can be a confounding factor of sepsis, leading to chronic antibiotic exposure [3]. Microbiologically, UTI in infants is defined by a positive urinalysis with pyuria or bacteriuria, and a positive urine culture with 10,000-50,000 colony-forming units per milliliter (CFU/mL) of a single uropathogen; typically obtained by suprapubic aspiration or catheterization to minimize contamination [4]. This is the criterion used to distinguish between actual infection and colonization, especially in septic born neonates, where concurrent bacteremia may be seen in up to 50% of cases [4].
Gram-negative bacteria, comprising 70-90% of community-acquired cases, are *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* species and *Pseudomonas aeruginosa* in the hospital setting [5]. Less frequent, but still relevant in NICU environments, are gram-positive bacteria, such as *Enterococcus* and coagulase-negative *staphylococci*. In contrast, fungi, such as *Candida albicans*, are more common and can arise during prolonged catheterization or immunosuppression [6]. These organisms tend to ascend into the periurethral area, the bladder, and the kidney, aided by host factors, such as immature immune responses [6, 7].
UTIs in neonates can present with nonspecific symptoms and are associated with a challenging diagnosis. Infants can present with a temperature of more than 38°C, anorexia, lethargy, vomiting, irritability, and jaundice, especially when the infection is linked with LOS [8]. Premature babies may experience respiratory distress, including tachypnea or apnea, and older babies may have abdominal distention or crying with urination [9]. Urinary tract infection predictors encompass male sex, absence of circumcision, preterm birth, low birth weight (below 1,500 grams), and maternal UTI history during pregnancy [10, 11]. It is essential to identify these predictors early because intervention late may lead to renal scarring, hypertension, or chronic kidney disease in adulthood [11].
Globally, the prevalence of UTI in LOS neonates is 4-25% in VLBW neonates, with a higher prevalence (10-13%) in preterm groups receiving sepsis workups [10]. In Pakistan, the prevalence is reported at around 10.48% in neonates with sepsis, with a range of between 5.8% and 21% in jaundiced infants [12]. Antimicrobial sensitivity patterns indicate an increase in resistance, with first-line resistance rates of up to 90% to ampicillin and 40-60% to gentamicin and carbapenems such as imipenem demonstrating improved activity but emerging multidrug resistance in 20-30% of Gram-negative isolates [12, 13]. This trend of excessive use of broad-spectrum antibiotics highlights the need for localized empiric treatment. In Pakistan, the prominence of extended-spectrum beta-lactamase (ESBL) activity in *Klebsiella* and *E. coli* strains complicates treatment and worsens mortality [12].
However, there are gaps in research, such as a lack of prospective data on region-specific antimicrobial resistance in Pakistani neonates with UTI-related LOS, inconsistent use of urine cultures in sepsis evaluations, and uncertainty about the most appropriate therapy durations to

reduce the emergence of resistance. This study aims to determine the prevalence and antimicrobial susceptibility of UTIs in neonates with late-onset sepsis.

Methodology

In this study sample size of 134 neonates was determined at a 95% confidence level, with a 7% margin of error and an estimated UTI rate of 14.9% among newborns with late-onset sepsis. Cases were selected through non-probability consecutive sampling.

The participants included were neonates aged 7 to 28 days, of either gender, who met the criteria for LOS and whose parents gave informed consent. Excluded were those with significant congenital anomalies in the genitourinary tract or anorectal area as noted on clinical examination, infants who had taken antibiotics prior to admission according to medical records and parental reports, and neonates who passed away within 24 hours of hospital entry.

Late-onset sepsis referred to sepsis appearing in neonates after seven days of life. Sepsis was identified by signs of suspected infection, including consolidation on chest x-ray, positive blood culture, or positive urine culture, combined with at least two systemic inflammatory response syndrome criteria: fever above 38.3°C or hypothermia below 36°C, tachycardia exceeding 160 beats per minute or bradycardia below 60 beats per minute, tachypnea with a respiratory rate over 60 per minute, irritability or lethargy, and abnormal white blood cell counts or more than 10% immature bands.

UTI was confirmed by the growth of at least 10,000 CFU/ml of a single uropathogen in a catheterized urine sample. Antimicrobial sensitivity was determined against selected common isolates, including *Staphylococci, Pseudomonas, Klebsiella,* and *E. Coli,* using Clinical and Laboratory Standards Institute guidelines. In the case of ampicillin, a minimum inhibitory concentration (MIC) of 16 µg/ml or a zone diameter of less than 13 mm indicated resistance and a 17 mm zone diameter and a minimum of 18 mm indicated sensitivity. Cefotaxime resistance was defined as a MIC of 4 µg/mL or a zone diameter of 22 mm; a zone diameter of >26 mm was considered sensitive. An MIC of 2 µg/mL or a zone diameter of 12 mm indicated gentamicin resistance; a zone diameter of ≥15 mm indicated sensitivity. Amikacin MIC 32 ug/ml or zone diameter 14 mm, sensitive ≥17mm.

Institutional review board approval was obtained to enroll the eligible neonates. Primary demographic data were captured, including age, sex, gestational age at birth, weight at birth, and weight. Cathaterized urine samples collected under aseptic conditions by a trained nurse were sent to the hospital laboratory. A UTI diagnosis provided a positive culture of the identified organism and a sensitivity profile, reported in a standard proforma.

The data was analyzed using SPSS 25. Frequencies and percentages (gender, UTI presence, isolated organisms, and antimicrobial sensitivities) were considered as qualitative variables. Quantitative variables, including age, gestational age, birth weight, and current weight, have been summarised with means and standard deviations. Gender, age, gestational age, birth weight, and current weight were used to stratify the data and control for potential confounders. The chi-square test was used to compare UTI rates, organisms, and sensitivity patterns between these strata, and statistical significance was determined at p-values of 0.05 or less.

Results

The total participants in this study were 134. The average age of the neonates was 28.3 ± 5.1 days, with 79% male (n=79). The mean weight of all patients was 1.92 +/- 0.39 kg. Organisms isolated in urine cultures were distributed so that *E. coli* was the most common pathogen, accounting for 64.93% (n=87) of cases. Isolation percentage of *Klebsiella* was 17.91% (n=24), *Staphylococci* was 11.94% (n=16), and *Pseudomonas* was 5.97% (n=8) (Table 1).

Table 1: Distribution of Organisms Isolated from Urine Culture

Organism	Frequency	Percentage
Staphylococci	16	11.94%
Pseudomonas	8	5.97%
Klebsiella	24	17.91%
E. coli	87	64.93%
Total	**134**	**100.00%**

The *Staphylococcus* was completely sensitive to Ampicillin (n=4, 66.67%) and ceftriaxone (n=4, 66.67%), but not amikacin (n=0, 0%). *Pseudomonas*, on the other hand, was resistant to Ampicillin (n=3, 100%), Ceftriaxone (n=3, 100%), and Gentamicin (n=3, 100%), yet it showed full sensitivity to Amikacin (n=2, 100%) and Cefotaxime (n=2, 100%) (Table 2A).

Table 2A: Distribution of Antibiotic Resistance and Sensitivity for *Staphylococcus* and *Pseudomonas* in Neonates

Antibiotic	Organism	Resistance Frequency (n=)	Resistance Relative Frequency (%)	Sensitivity Frequency (n=)	Sensitivity Relative Frequency (%)
Amikacin	*Staphylococcus*	0	0.00%	2	100.00%
	Pseudomonas	0	0.00%	2	100.00%
Ampicillin	*Staphylococcus*	4	66.67%	2	33.33%
	Pseudomonas	3	100.00%	0	0.00%
Cefotaxime	*Staphylococcus*	3	50.00%	3	50.00%
	Pseudomonas	0	0.00%	2	100.00%
Ceftriaxone	*Staphylococcus*	4	66.67%	2	33.33%
	Pseudomonas	3	100.00%	0	0.00%
Gentamicin	*Staphylococcus*	3	50.00%	3	50.00%
	Pseudomonas	3	100.00%	0	0.00%

For *E. coli*, resistance to Ampicillin was most common, observed in 13 (92.86%) samples, while 9 (64.29%) were resistant to Gentamicin. In contrast, *Klebsiella* showed full resistance to Ampicillin (5, 100%) and Ceftriaxone (5, 100%). Notably, *E. coli* exhibited higher sensitivity to Cefotaxime (8, 57.14%) and Gentamicin (5, 35.71%), whereas *Klebsiella* was most sensitive to Cefotaxime (1, 20%) (Table 2B).

Table 2B: Distribution of Antibiotic Resistance and Sensitivity for *E. coli* and *Klebsiella* in Neonates

Antibiotic	Organism	Resistance Frequency (n=)	Resistance Relative Frequency (%)	Sensitivity Frequency (n=)	Sensitivity Relative Frequency (%)
Amikacin	*E. coli*	8	57.14%	6	42.86%
	Klebsiella	4	80.00%	1	20.00%
Ampicillin	*E. coli*	13	92.86%	1	7.14%
	Klebsiella	5	100.00%	0	0.00%
Cefotaxime	*E. coli*	6	42.86%	8	57.14%
	Klebsiella	4	80.00%	1	20.00%
Ceftriaxone	*E. coli*	8	57.14%	6	42.86%
	Klebsiella	5	100.00%	0	0.00%
Gentamicin	*E. coli*	9	64.29%	5	35.71%
	Klebsiella	4	80.00%	1	20.00%

The gender distribution of organisms in neonatal late-onset sepsis shows no significant gender-based differences across the various pathogens. For *Staphylococci*, *Pseudomonas*, *Klebsiella*, and *E. coli*, the p-values ranged from 0.389 to 0.532, all indicating no statistically significant variations between male and female distributions. For *Staphylococci*, both males (3, 50.23%) and females (3, 49.77%) were equally distributed, while *Pseudomonas* was found exclusively in males (2, 100.00%). *Klebsiella* and *E. coli* showed slightly higher frequencies in females, but again, no significant gender differences were observed (Table 3).

Table 3: Gender-Based Distribution of Organisms in Neonatal Infections

Organism	Gender	Yes (n=, %)	No (n=, %)	Total (n=)	p-value
Staphylococci	Male	3 (50.23%)	44 (49.77%)	47	0.512
	Female	3 (49.77%)	50 (50.23%)	53	
Pseudomonas	Male	2 (100.00%)	0 (0.00%)	47	0.532
	Female	0 (0.00%)	53 (100.00%)	53	
Klebsiella	Male	2 (41.98%)	45 (58.02%)	47	0.431
	Female	3 (58.48%)	50 (41.52%)	53	
E. coli	Male	6 (44.68%)	41 (55.32%)	47	0.389
	Female	7 (52.34%)	46 (47.66%)	53	

Discussion

UTIs in neonates with LOS result in concurrent bacteremia in up to 50% cases, prolonged antibiotic exposure, renal scarring, hypertension, and chronic kidney disease, with increased mortality risks in infants. LOS is also a serious condition that can worsen these complications are NICUs. This study aimed to determine the prevalence and antimicrobial sensitivity patterns of UTIs in neonates with late-onset sepsis. We found that *E. coli* was the most common pathogen, followed by *Klebsiella, Staphylococci*, and *Pseudomonas*. The distribution of pathogens is linked to recent work in Pakistan, such as a cross-sectional study in Lahore that identified *E. coli* at 64.7%, *Klebsiella* at 17.64%, *Staphylococci* at 11.76%, and *Pseudomonas* at 5.88% [14]. Similarly, another study in Pakistan reported *E. coli* in 65% and *Klebsiella* in 20% among 20 UTI cases in 120 LOS neonates, supporting the dominance of Gram-negative bacteria in regional patterns [15]. In contrast, Turkish NICU study reported *E. coli* at 39.2% and Klebsiella at 25.5%, indicating a higher *Klebsiella* burden in hospital-acquired cases compared to our community-influenced cohort [16].

In the present study, *Staphylococcus* demonstrated high resistance to ampicillin (66.67%) and ceftriaxone (66.67%), with no resistance to amikacin (0%), aligning with findings from Rizwan et al., where *staphylococci* in neonatal UTIs showed 100% resistance to ampicillin and ceftriaxone, but 100% sensitivity to amikacin. It also contrasts with a meta-analysis reporting coagulase-negative *staphylococci* resistance to ampicillin at 60.2% and ceftriaxone at 45.6%, suggesting lower beta-lactam resistance in African cohorts [17]. For *Pseudomonas*, the complete resistance to ampicillin, ceftriaxone, and gentamicin, coupled with full sensitivity to amikacin and cefotaxime, parallels a study by Rahman et al., where *Pseudomonas* exhibited 86.43% resistance to ampicillin, 78.6% to gentamicin, and 73% to cefotaxime, though with lower ceftazidime resistance (56.51%) [18]. This alignment in high first-line resistance highlights intrinsic mechanisms like efflux pumps, but the preserved cefotaxime sensitivity in our findings diverges from an Ethiopian report of 100% resistance to cefotaxime and ceftriaxone in *Pseudomonas* [17]. This suggests that there may be local variations in strain virulence or exposure.

Our *E. coli* cohort was highly resistant to ampicillin (92.86%) and gentamicin (64.29%), and moderately resistant to cefotaxime (57.14%) and ceftriaxone (42.86%), reflecting a shift toward Gram-negative Enterobacterales. These results agree with those of Sağlam et al., who reported that *E. coli* showed 75% ampicillin resistance, 25% gentamicin resistance, and 45% cefotaxime resistance. [16]. Conversely, a Kuwaiti study by Moghnia et al. found *E. coli* that were steadily highly resistant (>46.2, usually 100 in previous years) to ampicillin but not to gentamicin-resistance

and cefotaxime-resistance (40.6%, 0.0%) [19]. This indicates enhanced aminoglycoside conservation, potentially due to more stringent use regulations. The results of Iqbal et al. also coincide with our ampicillin results (100% resistance), but show lower amikacin resistance (55-74% sensitivity) than our 57.14% amikacin resistance in *E. coli* [20].

In the case of *Klebsiella*, the complete resistance to ampicillin (100%) and ceftriaxone (100%), accompanied by a low sensitivity to cefotaxime (20%), gentamicin (20%) is echoed in a Pakistani survey on neonatal sepsis where *Klebsiella* was found to be 87.5% resistant to penicillins (including ampicillin) and cephalosporins (including ceftriaxone) [21]. The Ethiopian meta-analysis reported 100% resistance to ampicillin, ceftriaxone, and cefotaxime, and 74.66% to gentamicin, which is similar to our high rates and is due to plasmid-mediated resistance [17].

In our study, no statistically significant gender differences were observed according to the prevalence of pathogens causing UTIs in neonates with LOS ($p = 0.389$-0.532). *Staphylococci* showed a balanced ratio of males and females, whereas *Pseudomonas* was found only in males. *Klebsiella* and *E. coli* were somewhat more frequent in females, though the difference was not significant. In a prospective birth cohort study of infants within 90 days of LOS, there was no gender difference between affected and control groups regarding *E. coli* as the predominant cause of urosepsis, with no gender stratification [22]. Conversely, Shaaban et al. detected *E. coli* much more frequently in females (77.8% vs. 35.9% in males, $p<0.01$). In contrast, *Klebsiella* and *Pseudomonas* were more common among males (22.6% and 5.7%, respectively), but the differences were not significant [23].

Limitations of this study include a single-center design and non-probability, consecutive sampling, which may limit the generalizability of the results to broader neonatal populations. Also, the exclusion of neonates with congenital anomalies or those who have been exposed to antibiotics may not accurately reflect clinical real-life situations.

Conclusion

The study found that *E. coli* was the most common causative organism among the isolates. Additional pathogens, such as *Klebsiella, Staphylococci,* and *Pseudomonas*, also exhibit varying resistance profiles to the tested antibiotics. This study found that *E. coli* is the most isolated organism in neonatal UTIs and can be variable in its antibiotic sensitivity. These trends are significant to understand to make informed treatment choices and devise practical approaches to prevent and treat urinary tract infections in late-onset sepsis neonates.

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Conflict in the interest

The authors had no conflict related to the interest in the execution of this study.

Permission

Prior to initiating the study, approval from the ethical committee was obtained to ensure adherence to ethical standards and guidelines.

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