Curr Pediatr Res 2021; 25 (8): 781-786 *ISSN 0971-9032*
www.currentpediatrics.com

Microbiological study and antibiotic susceptibility pattern among lethargic neonates in Najaf city, Iraq.

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Abstract

The antibiotic susceptibility pattern differs from regions and countries, and physicians must be aware of their specific regions' pattern. Therefore, the recent study aimed to evaluate the microbiological features and the antibiotic susceptibility pattern among neonates admitted into Al-Zahraa teaching hospital, Najaf, Iraq.

Materials and Methods: This cross-sectional descriptive study was conducted in the pediatric emergency department and the Neonatal Intensive Care Units (NICUs) of Al-Zahraa teaching hospital in Najaf city, Iraq, for ten months from February 1 to November 1, 2016. Sampling was performed on all lethargic neonates brought to the emergency department and NICUs before they were given any empirical antibiotic therapy. Urine samples were collected using urine bags or suprapubic aspiration in aseptic condition. Positive urine cultures were defined as more than 100,000 CFU/mL bacterial colonies by bag specimen collection and the presence of any colonies regarding suprapubic aspiration. Antimicrobial sensitivity was assessed using the disk diffusion (Kirby-Bauer) method.

Results: The most common isolated pathogens were *E. coli* (45%), followed by Klebsiella spp (25%), Proteus spp (15%). The only gram-positive uropathogen was *S. aureus* affecting four (10%) neonates. Our study showed the highest overall drug sensitivity to imipenem (97.2%), amikacin (88.89%), and gentamicin (88.89%) and the highest resistance to amoxicillin (91.67%), ampicillin (77.78%), nalidixic acid (77.78%), and nitrofurantoin (77.78%) among gram-negative pathogens. Only one gram-positive pathogen (*S. aureus*) was detected, affecting four neonates. They were completely sensitive to vancomycin (100%) and linezolid (100%) but resistant to penicillin G (100%). Most *E. coli* pathogens were resistant to tetracycline (94.44%) and amoxicillin (94.44%), followed by ampicillin and cotrimoxazole (both 88.89%). All the *E. coli* were sensitive to colistin, imipenem, and meropenem.

Discussion: In our study, the most frequent causative organism was *E. coli* isolates (45%), followed by Klebsiella spp (25%) and Proteus spp (15%). This is in agreement with almost all previous studies with different age groups worldwide. However, in another study conducted by Aljanaby et al. in Najaf governorate, the most frequent causative organism in outpatient adult cases was *Klebsiella pneumonia* (41.53%). Taheri et al. in Iran showed that most isolates were resistant to ampicillin (95.9%) and gentamicin (52.6%). Although 77.78% of our isolates were resistant to ampicillin, gentamicin accounted for one of the most potent antimicrobial agents (88.89%) against gram-negative organisms, reflecting regional differences. Our finding was consistent with another study conducted in Duhok city, Iraqi Kurdistan, which showed high susceptibility of gram-negative bacteria to aminoglycoside agents and carbapenem.

Keywords: UTI, Neonatal septicemia, Neonates, Antibiotics, Resistance, *E. coli*.

Accepted on August 15th, 2021

Introduction

Unlike the overall prevalence, Urinary Tract Infections (UTIs) are more common in boys than the girls during the first year of life. UTI's overt clinical features may be absent during this period, and children may develop with other non-specific symptoms such as lethargy and poor feeding. UTI can damage kidneys, progress into urosepsis, and cause morbidity and mortality in the absence of proper treatment [1,2].

Gram-negative bacteria are the most common causes of UTI in all age groups and between both genders. The *Escherichia coli* (*E. coli*) bacteria affect the urinary tract more frequently than the other gram-negatives such as klebsiella spps, *Proteus mirabilis*.

Fungi are not common but frequently occurred in immune suppressed patients, long-term antibiotics, and patients with Foley catheters [3,4]. An increase in the rate of antibiotic resistance has been reported in several studies. This leads to

more hospitalization, more complication, and more recurrent infections.

The antibiotic susceptibility pattern differs from regions and countries, and physicians must be aware of their specific regions' pattern. Therefore, the recent study aimed to evaluate the microbiological features and the antibiotic susceptibility pattern among neonates admitted into Al-Zahraa teaching hospital, Najaf, Iraq.

Materials and Methods

This cross-sectional descriptive study was conducted in the pediatric emergency department and the Neonatal Intensive Care Units (NICUs) of Al-Zahraa teaching hospital in Najaf city, Iraq, for ten months from February 1 to November 1, 2016. Sampling was performed on all lethargic neonates brought to the emergency department and NICUs before they were given any empirical antibiotic therapy.

Exclusion criteria were negative urine culture in the presence of other etiology such as diarrhea. Other laboratory and chestx-ray and Lumbar Puncture (LP) were also performed, although their results were not shown in detail.

We immediately started empirical antibiotic therapy with Intravenous (IV) vancomycin 20 mg/kg loading dose (maintenance dosage was adjusted regarding gestational age and serum creatinine level) and IV cefotaxime (in neonates less than one week: 50 mg/Kg every twelve-hour and every eight-hour in neonates older than one week).

Urine samples were collected using urine bags or suprapubic aspiration in aseptic condition, and about five milliliters were poured into sterile tubes. The samples were brought to the laboratory within one hour, followed byinoculation using 0.001 ml caliber loop; they were cultured in MacConkeys media and 5% sheep blood agar.

All sample plates were incubated for 24 to 48 hours at 37°C in 5%-10% carbon dioxide for anaerobic growth. Positive urine cultures were defined as more than 100,000 CFU/mL bacterial colonies by bag specimen collection and the presence of any colonies regarding suprapubic aspiration.

Antimicrobial sensitivity was assessed using the disk diffusion (Kirby-Bauer) method [5]. Only one consultant microbiologist in a constant laboratory assessed all the plates. If the growth revealed more than one type of organism, the culture was repeated. Colony counts of less than 10,000 CFU/mL were excluded; meanwhile, we repeated samples between 10,000 CFU/mL to 100,000 CFU/mL.

Demographic data, including age, gender, maternal history, were collected. The guardians were exclusively asked whether the mother experienced any UTI or asymptomatic bacteria, and they fulfilled a written informed consent. Study protocol approved by the scientific department in the Al-Kufa medical college.

Collected data were analyzed using the Statistical Package for Social Sciences, version 20.0 (SPSS Inc., Chicago, IL, USA). Quantitative variables were presented as mean ± Standard Deviation (SD), although categorical variables were shown as frequencies (numbers and percentages).

We used the chi-square test or fisher's exact test to compare categorical variables and independent t-test or Mann-Whitney U test for quantitative variables. The value of $p \leq 0.05$ was considered statistically significant.

Results

Totally seventy lethargic neonates were brought to our units during the ten months; 45 of them (64.3%) were boys. Forty out of 70 neonates had a positive urine culture with the mean age (SD) of 15.57 (8.12) days old, ranging from 2-30 days old.

Thirty neonates had negative urine culture; therefore, they were excluded from the study; fourteen had positive blood culture, eight neonates had diarrhea and were dehydrated, four had hyperbilirubinemia, one had a history of falling down, and three neonates had positive LP.

Table 1 summarized the demographic information of the neonates with positive U/C. The majority of the neonates were terms (72.5%) and boys (67.5%). Furthermore, thirty-one neonates (77.5%) were older than seven days. Twenty-six mothers (65%) had a positive history of UTI during pregnancy.

We had to admit fifteen neonates (37.5%) in the NICU due to their unstable conditions. The most common isolated pathogens were *E. coli* (45%), followed by Klebsiella spp (25%), Proteus spp (15%). The only gram-positive uropathogen was *S. aureus* affecting four (10%) neonates.

Age (days.); Mean ± SD	**15.57 ± 8.12**
Age more than 7 day-old; N (%)	31 (77.5%)
Gender; N (%)	
Boy	27 (67.5%)
Girl	13 (32.5%)
MOD; N (%)	
NVD	26 (65%)
C/S	14 (35%)

NICU admission; N (%)	15 (37.5%)
UTI in mother during pregnancy; N (%)	26 (65%)
Living place; N (%)	
Rural	27 (67.5%)
Urban	13 (32.5%)
Term Delivery a; N (%)	
Term	29 (72.5%)
Preterm	7 (17.5%)
Post-term	4 (10%)
U/C; N (%)	
E. coli	18 (45%)
Klebsiella	10 (25%)
Proteus	6 (15%)
S. aureus	4 (10%)
Enterobacter	1 (2.5%)
Pseudomonas	1 (2.5%)

Table 1. Characteristics of the neonates with UTI (n=40). A preterm birth was defined as any birth before 37 weeks completed weeks of gestation, Post Term: 42 weeks of gestation and beyond. SD: Standard Deviation; N: Number; MOD: Mode of Delivery; NVD: Normal Vaginal Delivery; C/S: Caesarian Section; NICU: Neonatal Intensive Care Unit; UTI: Urinary Tract Infection; U/C: Urine Culture.

Our study showed the highest overall drug sensitivity to imipenem (97.2%), amikacin (88.89%), and gentamicin (88.89%) and the highest resistance to amoxicillin (91.67%), ampicillin (77.78%), nalidixic acid (77.78%), and nitrofurantoin (77.78%) among gram-negative pathogens. Only one gram-positive pathogen (S. aureus) was detected, affecting four neonates.

They were completely sensitive to vancomycin (100%) and linezolid (100%) but resistant to penicillin G (100%). The detailed pattern of antimicrobial resistance was reported in Table 2. Most *E. coli* pathogens were resistant to tetracycline (94.44%) and amoxicillin (94.44%), followed by ampicillin and cotrimoxazole (both 88.89%). All the *E. coli* was sensitive to colistin, imipenem, and meropenem.

	Uropathogenic organisms n=40					
Antibiotics	*E. coli* **18(45%)**	**Klebsiella 10(25%)**	**Proteus 6(15%)**	*S. aureus* **4 (10%)**	**Enterobacter 1 (2.5%)**	**Pseudomonas 1(2.5%)**
Colistin	0	0	100	-	0	0
Imipenem	0	0	16.67	-	0	0
Meropenem	0	20	33.33	-	100	0
Piperacillin-tazobactam	16.67	20	0	-	0	0
Amikacin	5.56	20	0	-	0	100
Gentamicin	11.11	10	0	50	0	100
Chloramphenicol	22.22	30	100	75	0	-
Ciprofloxacin	33.33	20	-	25	0	100
Cefepime	55.56	40	50	75	0	0
Ceftazidime	44.44	40	0	-	0	100
Cefotaxime	61.11	50	50	-	0	-

Ceftriaxone	61.11	50	0	75	0	-
Cefixime	72.22	50	0	75	0	-
Nitrofurantoin	66.67	90	100	25	100	-
Cefuroxime	66.67	60	100	-	0	-
Nalidixic acid	77.78	60	100	50	100	100
Cephalexin	66.67	70	-	-	0	-
Piperacillin	83.33	70	0	-	0	-
Ampicillin	88.89	90	33.33	75	100	-
Cotrimoxazole	88.89	60	0	50	0	-
Amoxicillin	94.44	90	100	-	100	-
Tetracycline	94.44	40	100	75	0	-
Penicillin G	-	-	-	100	-	-
Doxycycline	-	-	-	75	-	-
Azithromycin	-	-	-	75	-	-
Erythromycin	-	-	-	75	-	-
Linezolid	-	-	-	0	-	-
Vancomycin	-	-	-	0	-	-
Rifampin	-	-	-	25	-	-

Table 2. The antibiotic susceptibility pattern of the urine specimens.

Discussion

About 5%-14% of hospital visits in the pediatric emergency department are annually due to UTIs [6]. UTI's classic signs and symptoms, including dysuria, frequency, and urgency, are common among older children, although these findings are usually absent in the neonates.

These patients may develop with poor feeding and lethargy in the presence of any infections such as UTI. The importance of urinary tract infection is its ability to damage the kidney leading to renal scarring, end-stage renal disease, and hypertension, specifically in recurrent patients. Therefore, early diagnosis and proper treatment are mandatory in neonates to decrease mortality and further morbidity.

In the recent survey, the overall prevalence of proven UTI among neonates during the ten months was 57.14%. Preterm neonates and Low Birth Weight (LBW) are more prone to develop UTI [7].

However, our study showed a higher incidence in neonates (72.5% *vs.* 15.5%). This is consistent with a study conducted by Taheri et al. who claim that this high incidence might result from a bias in population selection of the study [8]. In our study, this finding may be due to the overall lower frequency of preterm neonates during the study period.

In our study, the most frequent causative organism was *E. coli* isolates (45%), followed by Klebsiella spp (25%) and Proteus spp (15%). This is in agreement with almost all previous studies with different age groups worldwide [9-13]. However, in another study conducted by Aljanaby et al. in Najaf governorate, the most frequent causative organism in outpatient adult cases was *klebsiella pneumonia* (41.53%) [14].

E. coli is the most common normal flora in the gastrointestinal tract affecting the urinary tract through the ascending pathway from the anus to the urethra [15,16]. This is the main reason for the higher UTI frequency in the females than the males. However, factors including a higher incidence of urethral anomalies, congenital obstruction in the urinary tract, and phimosis lead to a higher frequency in boys than the girls during the neonatal period [17,18]. In our study, in agreement with the mentioned studies, the prevalence of UTI in boys was higher than the girls during the neonatal period.

The recent survey showed the highest antimicrobial resistance among *E. coli* to tetracycline and amoxicillin, followed by ampicillin and cotrimoxazole, while all of the isolates were sensitive to colistin and carbapenems. Previous studies in Iraq showed the same results; Mansoor et al. in Erbil City, north of Iraq, showed that almost all isolated *E. coli* were resistant to penicillin G and ampicillin antibiotics (100% and 95.8%, respectively) [19].

In Basra province, south of Iraq, Hadi et al. concluded that there was a high sensitivity rate among *E. coli* isolates to imipenem (100%) and amikacin (97%) antibiotics [20]. All *E. coli* were resistant to ampicillin and sensitive to imipenem in Baghdad, Iraq's capital, as reported by Hussein et al. [21].

Taheri et al. in Iran showed that most isolates were resistant to ampicillin (95.9%) and gentamicin (52.6%). Although 77.78% of our isolates were resistant to ampicillin, gentamicin accounted for one of the most potent antimicrobial agents (88.89%) against gram-negative organisms, reflecting regional differences. Our finding was consistent with another study conducted in Duhok city, Iraqi Kurdistan, which showed high susceptibility of gram-negative bacteria to aminoglycoside agents and carbapenem [22].

Klebsiella spps were the second most frequent pathogen in our study. They showed a high rate of resistance against amoxicillin, ampicillin, nitrofurantoin (90% against all of them); meanwhile, they exhibited complete susceptibility against colistin and imipenem (both 100%) followed by gentamicin (90%) and amikacin (80%).

This is in agreement with the overall susceptibility pattern in the recent study [21-24]. Similarly, multiple studies accounted Klebsiella spps as the second most common pathogen. Pouladfar et al. showed the highest sensitivity to gentamicin, colistin, and imipenem, although they were resistant to amoxicillin [17].

Conclusion

According to our findings, physicians must be aware of antimicrobial agents' susceptibility patterns in their specific region. This awareness, along with; the routine urine culture, leads to early diagnosis and proper treatment and subsequently prevents further complications.

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