

Prevalence and Antibiotic Resistance of Bacteria Isolated from Urinary Tract Infections of Pregnant Women in Baghdad Hospitals

Kais Kassim Ghaima*¹, Zainab Shaban Khalaf²,
Alaa Aziz Abdulhassan¹ and Noor Yahya Salman¹

¹Institute of Genetic Engineering and Biotechnology for Postgraduate Studies,
University of Baghdad, Baghdad, Iraq.

²Ministry of Health, Baghdad, Iraq.

*Corresponding author E-mail: kaiskassim@gmail.com

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The aim of this study was investigating the drug resistance patterns of bacteria isolated from pregnant women with urinary tract infections (UTIs). This study included 1140 pregnant women attending two of main hospitals in Baghdad, Iraq, between September 2016 and August 2017. The isolation and identification of uropathogenic bacteria conducted by standard microbiological methods and Vitek2 system. The disc diffusion test was used to determine the antimicrobial resistance patterns of the isolated bacteria. The present study revealed that three hundred and fifty six isolates were positive for significant bacterial growth. *Escherichia coli* were the predominant bacteria (34 %) followed by *Staphylococcus aureus* (22.2 %), *Klebsiella* spp. (14.6 %), non-coagulase *Staphylococcus* (11.5 %), *Proteus* spp. (4.5 %), *Pseudomonas* spp. (3.7 %), *Acinetobacter* spp. (2.8 %), *Citrobacter* (2.8 %), *Enterococcus* (2.5 %) and *Enterobacter* (1.4 %). High resistance to Ampicillin (85.6 %), Co-trimoxazole (72.2 %) and Tetracycline (71.3 %) was observed. Also, It was found a moderate resistance to Ceftazidime, Ciprofloxacin, Amoxicillin-clavulanic acid and Ceftriaxone. Imipenem was the most active antibiotic against isolated uropathogens. Among the 356 uropathogenic isolates, 196 (55 %) were from women in the 21 to 30 years old age group, and this rate was higher than other age groups. In conclusion, urinary tract infections are frequent among pregnant women. Early detection and continuous monitoring of antibiotic resistance pattern is very important to determine the appropriate treatment of UTIs among pregnant women without complications.

Keywords: Antibiotic resistance, Pregnant women, Urinary tract infection.

Urinary tract infection (UTI) is one of the most common bacterial infections especially in developing countries with a high rate of morbidity and financial cost.¹ UTIs in pregnant women continue to pose a clinical problem and a great challenge for physicians and there is a much higher

risk (up to 40%) of progression to pyelonephritis, and possibly increased risk of premature birth and low neonatal birth weight.² As in non-pregnant women, UTIs in pregnant women are classified as asymptomatic bacteriuria (ASB), when infection is limited by the bacterial growth in urine, or

symptomatic infections, as acute cystitis, acute pyelonephritis, when bacteria invade the urinary tract tissue, causing an inflammatory reaction.³ The main causative agents of UTIs are the bacteria (95 %), and the most prevalent microorganisms are *E. coli* which responsible for more than 80 % of infections.⁴ The species of *Enterobacteriaceae* are responsible for urinary tract infections, where *Escherichia coli* is the main bacteria which causes uncomplicated cystitis and pyelonephritis followed by *Klebsiella pneumoniae*, *Proteus mirabilis* and by Gram-positive pathogens, such as *Staphylococcus aureus*, *Enterococcus faecalis* and *Staphylococcus saprophyticus*.⁵ The emergence of antibiotic resistance is increased because of the random use of antibiotics and subsequent redundancy of antibiotics. The ineffectiveness of antibiotics for treating UTIs is associated with antibiotic resistance of uropathogens, therefore the range of antibiotics available to treat infections becomes a limit, as well as antibiotic use in pregnancy, will be harmful to the fetus.⁶ The risk of spontaneous miscarriages due to the using of antibiotics during the period of pregnancy with the infections of urinary tract.⁷ Multidrug resistant bacteria (MDR) making treatment a difficult task and therefore increase threat to both mother and fetus and will minimize chance of prescribing safe antibiotic.⁸ This study is, therefore, designed to determine the prevalence of bacterial uropathogens among pregnant women and their antibiotic resistance patterns in Baghdad hospitals, Iraq.

The main causative agents of UTIs are the bacteria (95 %), and the most prevalent microorganisms are *E. coli* which responsible for more than 80 % of infections

MATERIALS AND METHODS

Study Design and Population

This study was done at two main hospitals in Baghdad, Iraq; Al-Kindy and Al-Yarmook teaching Hospitals, to assess the prevalence of uropathogenic bacteria from urine samples among pregnant women with UTIs. All non-repetitive midstream urine (MSU) samples obtained during the study period (September 2016 to August 2017) were included in the study.

Processing of Samples

According to World Health Organization

(WHO) manual.⁹ all MSU samples were cultured on routine culture media by semi-quantitative method. In brief, 1iL of urine was inoculated on MacConkey and Blood agar plate (HiMedia, India) by streaking using calibrated loop, and incubated aerobically for 24 hrs at 37 R°C. Growth of 100 colonies or more, *i.e.* 10⁵ colony forming units (CFU)/mL urine, was considered as culture positive. Isolation and identification of isolates were done following their morphology in Gram's staining, cultural characteristics and biochemical properties, as per the Manual of Clinical Microbiology.¹⁰ The identification was confirmed by using Vitek2 system (bioMérieux, France).

Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing of all isolates was performed by Kirby-Bauer's disc diffusion method and interpretation of the results was done as described in CLSI 2013.¹¹ Antibiotic discs (MAST, UK) used were Ampicillin (10 ig), Amikacin (30 ig), Cefepime (50 ig), Ceftriaxone (30 ig), Ciprofloxacin (5 ig), Nitrofurantoin (300 ig), Cotrimoxazole (25 ig), Ceftazidime (30 ig), Amoxicillin-clavulanic acid (30 ig), Tetracycline (10 ig), and Imipenem (10 ig). Control strains of *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 were used in parallel as a part of quality control.

RESULTS AND DISCUSSION

A total of 1140 urine samples were investigated, and 356 of them (31.2 %) were positive for significant bacterial growth (10⁵ CFU/mL). Uropathogenic bacterial species were isolated from pregnant women patients that were 20 to 50 years of age. A high prevalence of UTI (55 %) was identified in the 21 to 30 years old age group, and the prevalence in and this rate was more than those for the other age groups (Table 1).

Bacteriuria is common in pregnancy and the acute pyelonephritis is resulted from asymptomatic bacteruria, this state will be a serious threat to both the mother and fetus. Therefore, it is necessary to careful monitoring of the urinary tract infections when the infection occurs in the pregnant women.¹² There are many factors which encourage the survival of bacteria and makes the pregnant women more susceptible to UTI infection such as increased amino acids, vitamins, and other

nutrients in the urinary content. Also, increasing of bacterial growth may be due to the glycosuria, where during pregnancy, the physiological increase in plasma volume leads to decreases in the urine concentration and most of pregnant women develop suffered from this state.¹³ The results of our study showed that 31.2 % of the pregnant women of Baghdad city were infected with UTIs during their pregnancy. The incidence of UTI in our study was high in contrast with many studies conducted in different regions of the world. The study done by Soleymanizadeh *et al.* on 1500 pregnant women in the city of Bam, Iran, demonstrated that 12.3 of pregnant women were suffered from UTIs.¹⁴ However, lower prevalence of UTI in pregnant women compared to the present study was reported: Australia 4.9 %¹⁵, Mexico 8.4 %¹⁶ and Ghana 7.3 %¹⁷. The results of this study were in agreement with the findings of Shaheen *et al.* who reported that the frequency of UTIs during pregnancy was 32 % in Menoufia Governorate, Egypt.¹⁸

The variation in UTI percentage from one geographical region to another could be attributed to differences in UTI perception, methods of screening, and confounding risk factors. The high incidence of UTI in this study may be attributed to the low educational level among most of the women as well as lower concern with personal hygiene. According to the present results which conducted in Baghdad city, the highest rate of UTI among pregnant women in Baghdad is in the age range 21-30 years while the age range of 41 - 50 years recorded the lowest rate of infection. The similar results showed by the study of Al-Haddad in Yemen who found that the young women are more acquired for UTI than the old and the high frequency of UTI among pregnant women was in the age range of 15 - 24 years at the percentage (53.7 %).¹⁹ While the findings of Amir *et al.* revealed that the maximum prevalence of UTI was in the age group over 30 years while the minimum percentage was belong to the age group ranged from 25-30 years.²⁰

The following bacterial uropathogens were identified: *E. coli*, 121 isolates (34 %); *S. aureus*, 79 isolates (22.2 %); *Klebsiella* spp., 52 isolates (14.6 %); non-coagulase *Staphylococcus*, 41 isolates (11.5 %); *Proteus* spp., 16 isolates (4.5 %); *Pseudomonas* spp., 13 isolates (3.7 %), *Acinetobacter* spp., 10 isolates (2.8 %);

Citrobacter, 10 isolates (2.8 %), *Enterococcus*, 9 isolates (2.5 %) and *Enterobacter*, 5 isolates (1.4 %). (Table 2). *E. coli* was the predominant isolate among pregnant women in this study.

It was found that *E. coli* as the most common isolates (34 %) followed by *Staphylococcus* spp. In concurrent to our study, several studies showed that *E. coli* was the dominant uropathogen causing urinary tract infection during pregnancy.^{8,9,20} The rectal and vaginal area contain high levels of *E. coli* as the most common bacteria, also the anatomical and functional changes in females and difficulty of maintaining personal hygiene during pregnancy may increase the risk of UTI by *E. coli*.²¹ Mobbasheri *et al.* in Iran showed that the major causes of UTIs among pregnant women *E. coli* (33.3 %), coagulase negative *Staphylococcus* (30.3%) and *klebsiella* (15.2 %).²² The present findings indicated to *Staphylococcus aureus* as a second most common causative agent. One of the studies showed the high prevalence of *Staphylococcus* and was the main cause of UTI in Nigeria, where, the most prevalent isolates were *S. aureus* and *S. saprophyticus*.²³ some of studies had previously linked the increasing cause of UTIs by *Staphylococcus* to increased use of instrumentation such as bladder catheterization.^{13,24} Our findings revealed that *Klebsiella* (14.6 %) was the third most frequent pathogen and this result is consistent with the study conducted in Kenya.²⁵ The differences in the percentages of uropathogens could be due to differences in the traditions of the community, the environment factors, the methods of personnel hygiene and differences in health facilities. The other uropathogenic bacteria detected in this study were *Proteus*, *Pseudomonas*, *Acinetobacter* and *Citrobacter* and this result is consistent with those of recent studies which indicated to the isolation of these bacteria but in low percentages in comparison with *E. coli*, *Staphylococcus* and *Klebsiella*.^{20,26} Additionally, these bacteria regard as opportunistic pathogen of humans and are etiologic agents of nosocomial infections. One of the main causes of infections during pregnancy is the contamination, where in females the urethra is closer to the anus; also, the abdomen distension makes anal cleaning more difficult.²⁷ Antibiotic resistance pattern of uropathogenic isolates is shown in Table 3.

The results of antimicrobial resistance of isolated bacteria showed that the percentage

of resistance as the following; high resistance to Ampicillin (85.6 %), Co-trimoxazole (72.2 %) and Tetracycline (71.3 %) was observed. Also, It was found a moderate resistance to Ceftazidime (52.2 %), Ciprofloxacin (49.4 %), Amoxicillin-clavulanic acid (47.2 %) and Ceftriaxone (42.7 %). Imipenem was the most active antibiotic against isolated uropathogens with the lower resistance prevalence (3.1 %). The predominant uropathogens in this study exhibited high resistance to cephalosporins and penicillins and this may be due to the production of Extended-spectrum beta-lactamases (ESBLs) especially with Ampicillin and Ceftazime. The main risk factors ESBL-producing

pathogens isolated from pregnant women with UTI are recurrent urinary tract infections and antibiotic exposure.²⁸ Also, obvious resistance to most antibiotics, except Imipenem, was recorded for the isolated bacteria *Pseudomonas* and *Acinetobacter* while the genus *Enterobacter* revealed high sensitivity to most used antibiotics except Ampicillin. The present findings showed that the Gram-positive isolates were most sensitive to nitrofurantoin in contrast with fluoroquinolones.

Table 1. Distribution of uropathogenic isolates according to the age groups

| Age groups (years) distribution | No. (%) of bacteria |
|---------------------------------|---------------------|
| <20 | 42 (11.8 %) |
| 21–30 | 196 (55.0 %) |
| 31–40 | 91 (25.6 %) |
| 41–50 | 27 (7.6 %) |
| Total No. (%) | 356 (100 %) |

Table 2. Distribution of uropathogenic bacteria isolated from positive urine samples

| Type of bacteria isolates | Frequency | Percent |
|-------------------------------------|-----------|---------|
| <i>Escherichia coli</i> | 121 | 34.0 % |
| <i>Staphylococcus aureus</i> | 79 | 22.2 % |
| <i>Non-coagulase staphylococcus</i> | 41 | 11.5 % |
| <i>Klebsiella spp</i> | 52 | 14.6 % |
| <i>Protues spp</i> | 16 | 4.5 % |
| <i>Pseudomonas spp</i> | 13 | 3.7 % |
| <i>Acinetobacter spp</i> | 10 | 2.8 % |
| <i>Citrobacter</i> | 10 | 2.8 % |
| <i>Enterococcus</i> | 9 | 2.5 % |
| <i>Enterobacter</i> | 5 | 1.4 % |
| Total | 356 | 100.0 % |

Table 3. Antibiotic resistance of the uropathogenic bacteria isolated from pregnant women

| Uropathogenic isolate | AMP% (No) | CPM% (No) | CTR% (No) | CAZ% (No) | IPM% (No) | AK% (No) | COT% (No) | NIT% (No) | T% (No) | AMC% (No) | CIP% (No) |
|--|------------|------------|------------|------------|-----------|------------|------------|------------|------------|------------|------------|
| <i>Escherichia coli</i> (N = 121) | 86.8 (105) | 33.9 (41) | 50.4 (61) | 54.5 (66) | 4.1 (5) | 51.2 (62) | 62.8 (76) | 33.9 (41) | 70 (81) | 4.1 (55) | 52.9 (64) |
| <i>Staphylococcus aureus</i> (N = 79) | 86.1 (68) | 7.6 (6) | 34.2 (27) | 53.2 (42) | 00 (00) | 17.7 (14) | 91.1 (72) | 8.9 (7) | 80.9 (64) | 43 (34) | 41.8 (33) |
| <i>non coagulase staphylococcus</i> (N = 41) | 87.8 (36) | 24.4 (10) | 51.2 (21) | 43.9 (18) | 00 (00) | 26.8 (11) | 95.1 (39) | 4.8 (2) | 82.9 (36) | 43.9 (18) | 58.5 (24) |
| <i>Klebsiella spp</i> (N = 52) | 96.2 (50) | 53.8 (28) | 55.8 (29) | 75 (39) | 7.7 (4) | 17.3 (9) | 67.3 (35) | 42.3 (22) | 82.7 (43) | 57.7 (30) | 59.6 (31) |
| <i>Protues spp</i> (N = 16) | 50 (8) | 00 (00) | 12.5 (2) | 6.3 (1) | 00 (00) | 12.5 (2) | 37.5 (6) | 56.3 (9) | 25 (4) | 56.3 (9) | 31.3 (5) |
| <i>Pseudomonas spp</i> (N = 13) | 100 (13) | 46.1 (6) | 53.8 (7) | 69.2 (9) | 00 (00) | 38.5 (5) | 69.2 (9) | 76.9 (10) | 76.9 (10) | 76.9 (10) | 76.9 (10) |
| <i>Acinetobacter spp</i> (N = 10) | 90 (9) | 50 (5) | 50 (5) | 70 (7) | 20 (2) | 40 (4) | 80 (8) | 70 (7) | 70 (7) | 60 (6) | 70 (7) |
| <i>Citrobacter</i> (N = 10) | 60 (6) | 10 (1) | 00 (00) | 20 (2) | 00 (00) | 00 (00) | 40 (4) | 50 (5) | 40 (4) | 20 (2) | 00 (00) |
| <i>Enterococcus</i> (N = 9) | 55.5 (5) | 33.3 (3) | 00 (00) | 22.2 (2) | 00 (00) | 11.1 (1) | 55.5 (5) | 22.2 (2) | 44.4 (4) | 44.4 (4) | 00 (00) |
| <i>Enterobacter</i> (N = 5) | 100 (5) | 20 (1) | 00 (00) | 00 (00) | 00 (00) | 00 (00) | 60 (3) | 20 (1) | 20 (1) | 00 (00) | 00 (00) |
| Total (N = 356) | 85.6 (305) | 28.4 (101) | 42.7 (152) | 52.2 (186) | 3.1 (11) | 30.3 (108) | 72.2 (257) | 29.8 (106) | 71.3 (254) | 47.2 (168) | 49.4 (174) |

AK: Amikacin, AMP: Ampicillin, AMC: Amoxicillin-clavulanic acid, CPM: CTR: Ceftriaxone, CAZ: Ceftazidime, Cefepime, CIP: Ciprofloxacin, NIT: Nitrofurantoin, COT: Co-trimoxazole, T: Tetracycline, IPM: Imipenem

Our finding in agreement with the study done in Addis Ababa in which most Gram negative isolates from patients with UTIs showed high susceptibility to aminoglycosides and nitrofurantoin.²⁹ Because of the lack of surveys for antimicrobial resistance and the absence of control prescribing policies, the antibiotic resistance among pathogenic bacteria is a major health problem in developing countries and affects the whole world. The developing multidrug resistant bacteria (MDR) making treatment a difficult task and therefore increase threat to both mother and fetus and will minimize chance of prescribing safe antibiotic, infection difficult to eliminate and treatment successes hard to achieve.^{30,31} In pregnant women, UTIs are complicated problems because of there is no suitable antibiotics for treatment and due to the greater risk of pyelonephritis. The high prevalence of isolates which resistant to cephalosporins and amoxicillin in our local strains limits the use of these antibiotics in pregnant women, therefore, safer alternatives were used such as amoxicillin/clavulanate and nitrofurantoin for cystitis, while pyelonephritis should be treated with fourth-generation cephalosporins.^{32,33}

CONCLUSION

In conclusion, high frequency of UTI was recorded among pregnant women in our hospitals and it was observed the high level of multidrug resistance strains of uropathogens. There was a need for control of the use of non-prescribed antibiotics. Also, continuous monitoring of antibiotic resistance profiles of uropathogenic isolates in the pregnant women. The antibiotic susceptibility testing before antibiotic prescription is very necessary in order to ensure adequate treatment of urinary tract infection.

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