

## **Antimicrobial sensitivity pattern of pathogenic bacteria isolated from older women with asymptomatic bacteriuria.**

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### **Abstract**

**Objectives:** Asymptomatic bacteriuria is one of the most important silent diseases frequently infect women in worldwide.

**Aims:** To determine the antimicrobial susceptibility pattern of pathogenic bacteria isolated from older women with asymptomatic bacteriuria.

**Materials and methods:** A total of 263 culture positive bacterial isolates from 862 urine samples were collected from older women (60-70 years old) admitted to Al-Najaf hospital in Al-Najaf Governorate, Iraq during January 2016 to December 2017. All bacterial isolates were identified according to standard microbiological tests. Antimicrobial susceptibility test was done according to method by Kirby-Bauer.

**Results:** Out of 263 culture positive urine samples, *Escherichia coli* was the most dominant bacterial isolates 109 (41.44%) followed by *Klebsiella pneumoniae* 85 (32.32%), *Acinetobacter baumannii* 32 (12.16%), *Pseudomonas aeruginosa* 18 (6.84%), *Serratia marcescens* 11 (4.18%) and *Staphylococcus saprophyticus* 8 (3.06%). Among all antimicrobials, Imipenem 10 µg showed the highest activity (100%) against *E. coli*, *S. marcescens* and *S. saprophyticus* isolates While, Amikacin 30 µg showed the highest activity (100%) against *S. marcescens* only. Most bacterial isolates 240 (91.3%) were multi-drug resistance (MDR), 16 isolates (6%) and 7 isolates (2.7%) were extensive drug-resistance (XDR) and pan-drug-resistance (PDR), respectively.

**Conclusions:** There were many older women infected with asymptomatic bacteriuria caused by bacteria with high resistant to different antimicrobials; therefore, the antimicrobial sensitivity test must be done periodically.

**Keywords:** Asymptomatic bacteriuria, Older women, Antimicrobials, Pathogenic bacteria.

*Accepted on May 15, 2018*

### **Introduction**

Urinary tract infection (UTI) is one of the most important common infections in worldwide [1]. Older women and children are the most infected with this infection [2,3]. Urinary tract infection is broadly define as an infection of the urinary system and may involve the lower urinary tract or the upper urinary tract or both [4,5]. Multi-drug resistance pathogenic bacteria such as *E. coli* and *K. pneumoniae* are the main pathogens cause urinary tract infection [6,7]. The presence of pathogenic bacteria in the urine of an individual without symptoms of urinary tract infections is defined as a symptomatic bacteriuria [8]. Asymptomatic bacteriuria is generally not recommended in all times only during in some cases such as in pregnancy and at preoperative of men before urological procedure [9,10]. Overuse of different antimicrobials in asymptomatic bacteriuria treatment in older women infected with recurrent urinary tract infections is common, in whom antibiotics are routinely administered, therefore, most pathogenic bacteria cause asymptomatic

bacteriuria characterized by highly resistant against many antimicrobials [11,12]. In Iraq, there are no data focuses on the prevalence of asymptomatic bacteriuria, which is among the facilitative factors urinary tract infections and no data on the relationship between asymptomatic bacteriuria treatment and the risk of higher antibiotic resistance. Therefore, the aim of this study was to investigations of the prevalence of asymptomatic bacteriuria in older women and the antimicrobials resistance pattern of pathogenic bacteria isolated from this infection during two years in Al-Najaf Governorate, Iraq.

### **Materials and Methods**

#### ***Study design and women selection***

This is a cross-sectional study carried out in Al-Najaf hospital in laboratory of microbiology in Al-Najaf Governorate, Iraq during January 2016 to December 2017. A total of 862 older women (60-70 years old) were included in this study. All

women were not suffering from any signs and symptoms of urinary tract infections, not received any antibiotic treatments for any reason within the last one week and accepted being a part in the study.

### **Samples collection, bacterial identification and antimicrobials susceptibility test**

Eight hundred and sixty two midstream urine samples were collected from women (two samples from each women for two days) in sterile disposable containers (Hi-media, India), immediately the urine samples were streaked by loop (Hi-media, India) onto blood agar (Oxoid, UK) surface and MacConkey agar (Oxoid,UK) surface and incubated under aerobic conditions at 37°C for 24 h. If the single bacterial isolate were in titer  $\leq 10^5$  colony forming units were considered as a positive growth. All bacterial isolates were identified according to standard microbiological methods [13,14]. Antimicrobials susceptibility testing was determined by a disc diffusion test method (Kirby-Bauer) [15]. Antimicrobial susceptibility and resistance was determined according to CLSI guidelines (2017) [16] according to strain growth zone diameter.

### **Statistical analysis**

Statistical analysis was performing according to (SPSS) version 12.0 for windows software to compare (in percentages) between the prevalence of pathogenic bacteria isolated from women and between sensitive and resistance of pathogenic bacteria to antimicrobials.

## **Results**

Of the 862 urine samples were collected from women, 263 were positive for bacterial growth (30.5% of women were

infected with asymptomatic bacteriuria). The most frequently isolated bacteria included *E. coli* (41.44%), *K. pneumoniae* (32.32%), *A. baumannii* (12.16%), *P. aeruginosa* (6.84%), *S. marcescens* (4.18%) and *S. saprophyticus* (3.06%) (Table 1). Among the antimicrobials used for susceptibility testing, Imipenem 10 µg showed the full activity (100%) against *S. marcescens* and *S. saprophyticus*, While, Amikacin 30 µg showed the highest activity (100%) against *S. saprophyticus* only. Most isolates were resistance to Amoxiclav 30µg, Cefotaxime 30 µg, Ceftriaxone 30 µg and Ceftazidime 30 µg with highly percentages (<50%). The antimicrobials susceptibility pattern of all isolates is shown in Table 2 and Figure 1. Among all 263 isolates, 240 (91.3%) were MDR, 16 (6%) XDR and 7 (2.7%) PDR, The resistance type of all isolates is shown in Table 3.

**Table 1.** Numbers and percentages of pathogenic bacteria isolated from older women with asymptomatic bacteriuria.

<i>E. coli</i>	109 (41.44)
<i>K. pneumoniae</i>	85 (32.32)
<i>A. baumannii</i>	32 (12.16)
<i>P. aeruginosa</i>	18 (6.84)
<i>S. marcescens</i>	11 (4.18)
<i>S. saprophyticus</i>	8 (3.06)
Total	263 (100)

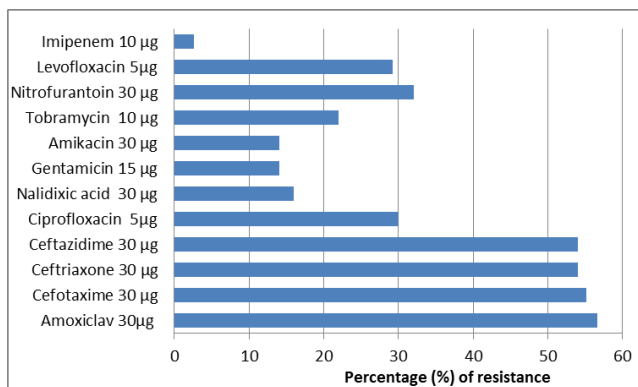
Data presented as numbers and percentage of bacterial isolates - no. (%)

**Table 2.** Numbers and percentages of pathogenic bacteria isolated from older women with asymptomatic bacteriuria that were sensitive to antimicrobials. N=263.

Antimicrobials	<i>E. coli</i> (N=109)	<i>K. pneumoniae</i> (N=85)	<i>A. baumannii</i> (N=32)	<i>P. aeruginosa</i> (N=18)	<i>S. marcescens</i> (N=11)	<i>S. saprophyticus</i> (N=8)
Amoxiclav 30 µg	45 (41.2)	40 (47)	14 (43.7)	8 (44.4)	6 (45.5)	1 (12.5)
Cefotaxime 30 µg	40 (36.6)	42 (49.4)	20 (62.5)	7 (38.8)	7 (63.6)	2 (25)
Ceftriaxone 30 µg	43 (39.4)	45 (52.9)	17 (53.1)	7 (38.8)	8 (72.7)	2 (25)
Ceftazidime 30 µg	43 (39.4)	48 (56.4)	15 (46.8)	6 (33.3)	7 (63.6)	2 (25)
Ciprofloxacin 5 µg	88 (80.7)	55 (64.7)	20 (62.5)	10 (55.5)	8 (72.7)	3 (37.5)
Nalidixic acid 30 µg	97 (88.9)	74 (87)	28 (87.5)	8 (44.4)	8 (72.7)	6 (75)
Gentamicin 15 µg	90 (82.5)	77 (90.5)	28 (87.5)	16 (88.8)	9 (81.8)	6 (75)
Amikacin 30 µg	91 (83.4)	74 (87)	28 (87.5)	15 (83.3)	11 (100)	7 (87.5)
Tobramycin 10 µg	89 (81.6)	70 (82.3)	20 (62.5)	11 (61.1)	8 (72.7)	7 (87.5)
Nitrofurantoin 30 µg	78 (71.5)	65 (76.4)	18 (56.2)	9 (50)	6 (45.5)	3 (37.5)
Levofloxacin 5 µg	85 (77.9)	65 (76.4)	19 (59.3)	8 (44.4)	5 (45.4)	4 (50)

Imipenem 10 µg	109 (100)	80 (94.1)	31 (96.8)	17 (94.4)	11 (100)	8 (100)
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Data presented as numbers and percentage of bacterial isolates-no (%).



**Figure 1.** Overall of resistance to 12 antimicrobials of 263 bacterial isolates from older women with asymptomatic bacteriuria.

**Table 3.** Prevalence of MDR, XDR and PDR pathogenic bacteria isolated from older women with asymptomatic bacteriuria, N=263.

	MDR	XDR	PDR
<i>E. coli</i> (N=109)	106 (97.2)	3 (2.7)	0 (0.0)
<i>K. pneumoniae</i> (N=85)	71 (83.5)	9 (10.5)	5 (5.8)
<i>A. baumannii</i> (N=32)	28 (87.5)	3 (9.3)	1 (3.1)
<i>P. aeruginosa</i> (N=18)	16 (88.9)	1 (5.5)	1 (5.5)
<i>S. marcescens</i> (N=11)	11 (100)	0 (0.0)	0 (0.0)
<i>S. saprophyticus</i> (N=8)	8 (100)	0 (0.0)	0 (0.0)
Total 263 (100)	240 (91.3)	16 (6)	7 (2.7)

Data presented as numbers and percentage of bacterial isolates-no. (%). MDR: Multi-Drug Resistance; XDR: Extensive Drug Resistance; PDR: Pan-Drug Resistances.

**Discussion**

This is the first study in Iraq (to the best of our knowledge) aimed to investigate the prevalence of older women infected with asymptomatic bacteriuria caused by pathogenic bacteria and antimicrobial susceptibility pattern of these pathogens during two years. According to our data, of the 862 older women, 263 (30.5%) were infected with asymptomatic bacteriuria, this result is similar to the previous reports [17-20]. Mostly, asymptomatic bacteriuria is common in the older women over than 60 years old; many factors may be lead to asymptomatic bacteriuria such as; age related in urologic function, multiple comorbid chronic disease and neurogenic bladder caused by various pathologies [21]. In this study as shown in table 1, of the 263 positive culture, *E. coli* 109 (41.44%) and *K. pneumoniae* 85 (32.32%) were the most predominant bacteria were responsible of asymptomatic bacteriuria, while *S. saprophyticus* was the lowest predominant bacteria 8 (30.06%). These results are in agreement with

previous studies [22,23]. Many members of enterobacteriaceae such as *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *A. baumannii* are able to cause recurrent urinary tract infections lead to asymptomatic bacteriuria [24,25]. Mainly, recurrent urinary tract infections are caused by gram-negative bacteria as well as by gram-positive bacteria such as uropathogenic *E. coli*, *K. pneumoniae*, *S. saprophyticus* and *P. aeruginosa* [26-28]. These uropathogens have the ability to bind to the bladder epithelium and form biofilms-like intracellular bacterial communities that are responsible for colonization and persistence and protect their members from antibiotics and neutrophils in urinary tract and lead to cause infection [29,30]. In this study, most bacterial isolates were sensitive to Imipenem 10 µg, Amikacin 30 µg, Gentamicin 15 µg and Nalidixic acid 30 µg, respectively as shown in Table 3 and Figure 1. *Klebsiella pneumoniae*, *A. baumannii* and *P. aeruginosa* were the most pathogens resistant to most antimicrobials. Amoxiclav 30 µg, Cefotaxime 30 µg, Ceftriaxone 30 µg and Ceftazidime 30 µg were with low activity against all isolates. These results are in agreement with many previous studies [31-34]. The recurrent urinary tract infection is one of the most serious problems particularly in older individuals who are living in developing countries [35]. The overuse of the same antimicrobials such as 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins against same bacteria without using of antimicrobial sensitivity test lead to cause asymptomatic bacteriuria and emerging of new bacterial strains with highly resistance to different antimicrobials [36-38]. Our finding in the current study showed that among 263 bacterial isolates, 240 (91.3%) were MDR (bacteria resist three different classes of antimicrobials), *E. coli* 3 (2.7%), *K. pneumoniae* 9 (10.5%), *A. baumannii* 3 (9.3%) and *P. aeruginosa* 1 (5.5%) were sensitive to only one or two types of antimicrobials (XDR isolates), while *K. pneumoniae* 5 (5.8%), *A. baumannii* 1 (3.1%) and *P. aeruginosa* 1 (5.5%) were resistance to all types of antimicrobial classes (PDR isolates) (Table 3). These results are similar with other studies in different countries [39-41]. Multi-drug resistance gram negative and gram positive bacteria became the most problem numerous pathogens cause serious infections worldwide such as burns and urinary tract infections [42,43]. Recently, extensive and pan- drug resistance bacteria such as *K. pneumoniae*, *A. baumannii* and *P. aeruginosa* were considered are the most problem pathogens resist about 98% of antimicrobials [44]. Many reasons lead to increase the prevalence of MDR, XDR and PDR uropathogenic bacteria include, the incorrect administration of antimicrobials and increase in the production of beta-lactamases enzymes by pathogenic bacteria and lack of good controlling mechanisms in hospitals [45].

## Conclusion

In Iraq, asymptomatic bacteriuria is a common neglected disease among older women over than 60 years old. *E. coli* is the most commonly bacterium were isolated. *Klebsiella pneumoniae*, *A. baumannii* and *P. aeruginosa* are a highly dangerous drug resistant pathogens cause this silent infection. In spite of imipenem 10 µg is expensive, but is still one of the best antimicrobials against different pathogenic bacteria cause asymptomatic bacteriuria.

## Limitations

The definition of asymptomatic bacteriuria relies on a single urine sample. This is not entirely in accordance with the Infectious Diseases Society of America guidelines for the diagnosis of asymptomatic bacteriuria in adults that requires two consecutive urine specimens with isolation of the same bacterial strain in women.

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