Bacterial Causes and their Antimicrobial Susceptibility Testing among Urinary Tract Infection Patients in Tobruk Area, Libya

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Abstract: Urinary tract infections are very common among people of all ages, but the disease is more prevalent in women, so proper clinical and laboratory diagnosis, and the right treatment are very important to avoid complications and antimicrobial resistance. This study aimed to determine the bacterial causes and antibiotic susceptibility patterns of UTI patients. Recorded results of 6065 urine sample cultures and their antimicrobial susceptibility tests from the Department of Microbiology in Tobruk Medical Center, Tobruk City, were obtained from September 2016 to December 2018. The data was analyzed and discussed in compare with other studies. The study showed that a UTI is more common in females (78.8%) compared with male patients (21.2%). Gram-negative bacteria accounted for the majority of urinary pathogens (90%), where *E. coli* alone was (58.4%), *klebsiellae* (17.4%), *Proteus* (10.7%), while *Pseudomonas* species was the least common (3.3%) of the total. While the Gram-positive bacteria *S. aureus* constituted 9.6% of the total. The study was found statistically significant (P=0.000). The most effective antibiotics against all uropathogens were Imipenem and Amikacin, and less effective antibiotics were Ampicillin and Amoxicillin-clavulanic acid. This study concluded that the most common cause of UTI in Tobruk was *Escherichia coli* and the most effective antibiotics appear to be Imipenem and Amikacin.

Keywords: Urinary Tract Infection, Uropathogens, Susceptibility Testing, Antibiotic Resistance, Antimicrobial Agents, Disk Diffusion

INTRODUCTION

The urinary tract system, like other body systems, may become infected with various pathogens. Among uropathogens bacteria are a more common cause of Urinary tract infections (UTI) (Cunha *et al*., 2016; Ekwealor *et al*., 2016; Mohammed *et al*., 2016). Bacterial infection of the urinary system is a disease affecting people of all ages, but it is more prevalent in females compared with males (Gupta *et al*., 2019; Tambekar *et al*., 2006). Many studies showed that 150 million people reported having UTI each year worldwide (Assafi *et al*., 2015; Iregbu & Nwajiobi-Princewill, 2013; Majumder *et al*., 2017). UTI affects the lower urinary tract including the urethra, urinary bladder, prostate gland,

and ureters, or more seriously affects the upper urinary system; the kidneys. UTI may be self-limiting infections or life-threatening that necessitate prompt use of antimicrobial treatment. The infection of the urinary tract may be complicated, uncomplicated, and even asymptomatic (Gupta *et al*., 2019).

Most studies demonstrated that, UTI populations' urine cultures showed a predominance of Gram-negative bacteria belonging to the family of Enterobacteriaceae in particularly *E. coli* as the leading cause of UTI ranges from 50 to 92% of uropathogens (Assafi *et al*., 2015; Bagar *et al*., 2007; Cunha *et al*., 2016; Khan *et al*., 2014). So, *E. coli* is one of the most common urinary pathogens that need to be treated with antibiotics. Other common Gram-negative species pathogens

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include *Klebsiella*, *Proteus*, and less common *pseudomonas*. Among Gram-positive pathogens, *Staphylococcus* spp., and *Enterococcus* spp. are more common causes of UTI. Among the most commonly prescribed antibiotics used against UTI bacterial pathogens are amoxicillin-clavulanic (augmentin), ampicillin, nitrofurantoin, cephalosporins, nalidixic acid, fluoroquinolones, and trimethoprim-sulfamethoxazole (Iregbu & Nwajiobi-Princewill, 2013). Unfortunately, resistance to these antibacterial agents is increasing over time, reducing their efficiency and subsequently their clinical usage (Assafi *et al.*, 2015; Lnqer *et al.*, 2020; Salih *et al.*, 2016; Obayes AL-Khikani, 2020). UTI is mostly, treated empirically with antibiotics particularly β- lactam antibiotics without investigating causative pathogens or the exact antibiotic/s which should be used, and the introduction of invasive procedures such as a catheter. These, with time, put stress on uropathogens playing an important role in increasing rates of antimicrobial resistance. Therefore, regular monitoring of the bacterial causes of UTI and antibiotic susceptibility in a local area is an important strategy.

This study aimed to identify the pathogenic bacterial species of UTI and their antibiotic resistance for a selected antimicrobial agent commonly used in UTI treatment. The obtained data may help identify uropathogens frequency and guide the selection of antibiotics against uropathogens in clinical practice in our area.

MATERIALS AND METHODS

A retrospective study was performed in the microbiology section of Tobruk Medical Center, Tobruk, Libya, from September 2016 to December 2018. A total of 6065 urine culture results from all UTI suspected patients (3796 females and 2269 males) were included. Urine samples were collected from patients as instructed by microbiology staff, where the first morning mid-stream urine was collected in a sterile urine container and delivered as soon as possible for culturing. Suprapubic collection was indicated for babies.

Urine samples were inoculated aerobically onto plates of MacConkey agar and blood agar. Then, the plates were incubated at 37°C for 24 hours. On the following day, the plates were examined for bacterial growth and evaluated. Each urine culture containing 100 000 (10^5) or more bacterial cells/ml was considered as significant growth. Significant cultures were proceeds for identification processes and were sub-cultured on solid media such as nutrient agar and mannitol salt agar. Microscopic examination of stained smear, in particular Gram stain, biochemical tests such catalase, coagulase, urease, and oxidase were performed when needed (Cheesbrough, 2006).

An antimicrobial susceptibility test was done on all pathogenic bacteria using the disc diffusion technique of the Kirby-Bauer method on Müller-Hinton agar (Jenkins and Schuetz, 2012). All plates were incubated aerobically at 37°C for 24 hours. Then, plates were checked for zones diameter of inhibition, and the results were expressed in millimeters (mm) as sensitive and resistant and interpreted according to standard guidelines (CLSI, 2013). Susceptibility testing discs used were obtained from Oxoid UK or Becton and Dickinson Company-BD.

Data Analysis: The statistical analysis was performed using SPSS software version 23 (SPSS Inc., Chicago III, USA), and Microsoft Excel 2010. All data were expressed as frequencies and percentages. Pearson Chi-Square test was used for statistical comparisons between antibiotics groups for Gram-negative and Gram-positive pathogens that were statistically significant (P=0.001).

RESULTS

A total of 6065 urine samples collected from

inpatients and outpatients primarily diagnosed as having UTIs were cultured. Urine samples from female patients were 3796 (62.6%), and 2296 (37.4%) were from male patients. Out of 6065 urine cultures, 1517 (25%) showed growth of pathogenic bacteria belonging to five species, while 4548 (75%) were negative or showed non-significant growth. The prevalence of positive urine cultures was higher 1195/1517 (78.8%) among females compared with males 322/1517 (21.2%). Different Gram pattern bacteria were isolated. Gram-negative pathogens were 1372/1517 (90.4%), while Gram-positive was 145/1517 (9.6%) (Table.1).

Table (1). Prevalence of Gram-negative and Gram-positive uropathogens

	Gram-negative pathogens	Gram-positive pathogens	Total
Number of isolated bacteria	1372	145	1517
Percentage %	90.4	9.6	100

Among Gram-negative isolates, *E. coli* was the most common pathogen, followed by *klebsiellae* spp., *Proteus* spp., while *Pseudomonas* spp. was less common. *S. aureus* was the main isolated Gram-positive pathogen as shown in (Table 2).

Table (2). Species and frequency of pathogenic bacteria

Isolated bacteria	Frequency	Percentage (%)
Esherichia coli	886	58.4
Klebsiella species	264	17.4
Proteus species	162	10.7
Pseudomonas sp.	60	3.9
S. aureus	145	9.6
Total	1517	100

The susceptibility testing results of 12 selected antibiotics for isolated uropathogens were recorded. Overall antibiotics susceptibility testing for Gram-negative pathogens showed 94.51% were sensitive to Imipenem and 70.73% to

Amikacin, while their sensitivity to Ciprofloxacin was 65.14% (Table 3).

Table (3). The sensitivity and resistance of Gram-negative pathogens for individual antibiotics in percentages *(T=total, S=sensitivity, R=resistance)*

Antibiotic (symbol)	Total	Sensitivity (%)	Resistance (%)
Ceftazidime (CAZ)	405	45.68	54.32
Cefoxitin (FOX)	725	62.34	37.66
Ciprofloxacin (CIP)	568	65.14	34.86
Nalidixic acid (NA)	747	27.31	48.59
Nitrofurantoin (F)	892	50.45	49.55
Sulfamethoxazole-Trimethoprim (SXT)	492	38.62	61.38
Amoxicillin Clavulanic acid (AMC)	803	7.97	92.03
Ampicillin (AMP)	312	4.81	95.19
Amikacin (AK)	492	70.73	29.27
Cefotaxime (CTX)	248	36.69	63.31
Gentamicin (CN)	426	38.49	61.5
Imipenem (IMP)	91	94.51	5.49

(Pearson Chi-Square = 1087.086; DF = 11; P-Value = 0.000)

Nitrofurantoin sensitivity among Gram-negative pathogens was 50.45%, and only 27.31% were sensitive to Nalidixic acid. Cephalosporins (β-lactam antibiotics) susceptibility showed 62.34% and 36.69% of Gram-negative isolates were sensitive to Cefoxitin and Cefotaxime respectively, while 54% were resistant to Ceftazidime. In respective to penicillins, 95.19% and 92.03% of Gram-negative pathogens were resistant to each of Ampicillin and Amoxicillin-clavulanic acid (Augmentin) respectively. Finally, the resistance rate of (61.38%) was observed among Gram-negatives to Sulfamethoxazole-trimethoprim, and 61.5% was to Gentamicin.

The sensitivity of *Staphylococcus aureus* to Imipenem was 84.6%, amikacin 70.1% Nitrofurantoin 62.61%, and to ciprofloxacin it was 58.3%. While it was highly resistant to Ampicillin 98.4%, Nalidixic acid 90%, Amoxicillin-Clavulanic acid 78.95%, Ceftazidime 87.62%, Sulfamethoxazole-Trimethoprim 69.23%, last-

ly to Cefotaxime was 65.31% and 57.835% to Cefoxitin as shown in (Table 4).

Table (4). Sensitivity and resistance rate of S. aureus for individual antibiotic in percentages. *(T=total, S=sensitivity, R=resistance)*

Antibiotic (symbol)	Total	Sensitivity (%)	Resistance (%)
Ceftazidime (CAZ)	105	12.38	87.62
Cefoxitin (FOX)	145	42.18	57.83
Ciprofloxacin (CIP)	84	58.33	41.67
Nalidixicacid (NA)	100	10.00	90.00
Nitrofurantoin (F)	115	62.61	37.39
Sulfamethoxazole-Trimethoprim (SXT)	52	30.77	69.23
Amoxicillin Clavu-lanic acid (AMC)	114	21.05	78.95
Ampicillin (AMP)	63	01.59	98.41
Amikacin (AK)	97	70.10	29.9
Cefotaxime (CTX)	49	34.69	65.31
Gentamicin (CN)	87	31.03	68.97
Imipenem (IMP)	13	84.62	15.38

(Pearson Chi-Square = 218.159; DF = 11; P-Value = 0.000)

DISCUSSION

This study provides information relating to bacterial uropathogens and also a pattern of local susceptibility to main antibiotics regularly used in UTI treatment. UTI occurs in all people of all ages, but the rate of positive urine cultures in this study is much higher in females (78.8%) because of the high prevalence of UTI among them. The same finding is well documented in other studies (Mahdi *et al*., 2020; Mohamed Hayir *et al*., 2019).

This is referring to differences in female anatomy, short urethra and its proximity to the anus, sexual activity, moisture environment of the urogenital area let bacteria multiply and become more ready to reach the urinary system (Gupta *et al*., 2019; Jatileni *et al*., 2015; Mohammed *et al*., 2016). However, personal hygiene and education play an important role in UTI prevention in both females and males. Gram-negative bacteria comprised the majority 90.4% of isolated uropathogens in this study. This finding is compared to a study from India

done by Chander and Singla (2008), who estimated that 83.68% of UTI were referred to Gram-negative isolates.

Gram-positive pathogens in the present study were 9.6%. However, predominance isolation of Gram-positive bacteria as uropathogenic was reported in a study from Nigeria (Ekwealor *et al*., 2016). Among Gram-negatives, *E. coli* was predominant and accounted for (58.4%). A similar result 58% showed in a study performed in India (Tambekar *et al*., 2006) and 57.3% in study performed in Sudan (Badri & Mohamed, 2017). *E. coli* isolation rate in this study also was in line with other studies rates, 60% from Brazil (Cunha *et al*., 2016), from India 61% (Akram *et al*., 2007), from Libya 55.6 (Mohammed *et al*., 2016). Much higher rates 92% (Bagar *et al*., 2007) and 73% (Assafi *et al*., 2015) were reported in other studies performed in Libya and Iraq respectively. Whereas a much lower rate of 36% was also reported (Lnqer *et al*., 2020).

This variation may refer to the difference in people, bacterial strains, and geographic area. The second more frequently isolated pathogens were *Klebsiella spp*., comprising (17.4%) and *Proteus species* (10.7%). A less common pathogen was *Pseudomonas species* accounting only for (3.9%), while isolation and reporting of these bacteria as uropathogens is verified by others. This research showed *Staphylococcus aureus* as a Gram-positive pathogen, comprising 9.6% of uropathogen frequency. A comparable finding (8%) was demonstrated by Ghenghesh *et al*. (2003). While higher rates of 28% and 31% were reported by (Ekwealor et al., 2016) and (Odoki *et al*., 2019) respectively. Gram-positive bacteria as uropathogens are reported particularly among individuals who are elderly, pregnant, or have other UTI risk factors (Kline & Lewis, 2016). Moreover, one can recall that the urogenital area and distal urethra are normally colonized with various species of Gram-positive bacteria, which, under certain conditions, can cause infection to the urinary system.

Many studies reported the isolation of other pathogenic bacterial species other than those isolated in this study (Jatileni *et al.*, 2015; Mohammed *et al.*, 2016; Natesan & Banu, 2017). Additionally, this study showed that nearly all urine cultures had grown a single pathogen. This finding was previously mentioned, where 95% and 96% of urine cultures had grown a single pathogen (Aboderin *et al.*, 2009; Naqid *et al.*, 2020). Because the majority of uropathogens are Gram-negative enteric bacteria inhabiting the gut as commensals, this may be the major source of urinary system infections (Magruder *et al.*, 2019).

This study showed that the highest drugs activity against all isolated uropathogens were Imipenem and Amikacin. For Gram-negative isolates, 94.51% were sensitive to Imipenem and 70.73% to Amikacin. A closer result was reported by (Elabidi *et al.*, 2018), who found 96.3% were sensitive to Imipenem, but the sensitivity to Amikacin was higher and estimated at (96.2%) among Gram-negative isolates. The present study also showed that 84.62 % of *S. aureus* isolates were sensitive to Imipenem. A sensitivity rate of 85.7% for this antibiotic has been observed by (Muhammad *et al.*, 2020) and 100% in a study by (Prakash & Saxena, 2013). A70.1% of *S. aureus* showed sensitivity to Amikacin. High activity of Amikacin was in agreement with another study that reported 71.6% of *S. aureus* were sensitive (Sukumaran & Kumar, 2017) but a higher sensitivity rate (80%) has been reported earlier (Iregbu & Nwajiobi-Princewill, 2013).

Sensitivity to Ciprofloxacin was 65.14% among Gram-negative pathogens. A rate of 63.19% was reported by (Gupta *et al.*, 2019) and 62% by (Janifer *et al.*, 2009). However, higher sensitivity (80%) was reported (Elabidi *et al.*, 2018). Nitrofurantoin sensitivity was 50.45%, other studies showed higher sensitivity 75.5% (Jatileni *et al.*, 2015). This study showed high resistance of uropathogens (61.38%) to Sulfamethoxazole-trimethoprim, higher resistance rate (91%) to this antibiotic was reported (Tambekar *et al.*, 2006).

With regard to Cephalosporins (β-lactam antibiotics) susceptibility, 62% of Gram-negative pathogens were sensitive to Cefoxitin. But one study found a higher rate (69.2%) among Gram-negative uropathogens in Benghazi, Libya (Elabidi *et al.*, 2018). Also, this study showed a high resistant rate (63%) observed to Cefotaxime and 54% to Ceftazidime, but higher rates (84% and 86.8%) to both antibiotics respectively, had been reported (Ejaz *et al.*, 2019).

Additionally, Gram-negative pathogens in this study demonstrated the highest rate of resistance for Ampicillin 95.19%. High resistance rates of 93.8% were reported by Ajayi *et al.* (2019) and 100% reported by (Salih *et al.*, 2016). Furthermore, Gram-negative isolates in the present study showed very high resistance for Amoxicillin-clavulanic acid (Augmentin), estimated at 92.3%. A closer result (87.2%) was reported by (Mohamed Hayir et al., 2019), and also a high resistance level of (79.16%) shown by (Gupta *et al.*, 2019). A lower resistance rate (43%) has also been reported in a study from Iraq (Lnqer *et al.*, 2020). Such high Augmentin resistance in the current study did not agree with a study from Brazil that reported that 82.2% of *E .coli*, 81.1% of *Klebsiella* spp., and 95.7% *Proteus* spp. were sensitive to this antibiotic (Cunha *et al.*, 2016).

The activity of ampicillin and amoxicillin-clavulanic acid is significantly reduced over time, they could be used extensively and empirically in clinical practice by physicians or people. The drug quality, changing of uropathogens susceptibility results due to variance in bacterial strains, and differences in study area, right drug dosage, and therapy duration, all these factors collectively or individually can largely affect the right management of UTI and lead to the emergence of such high resistant rates among uropathogens to these drugs which resulted in difficulty in the treatment of UTIs. Another important reason is that drugs are readily obtained in developing countries, including Libya, either from private or govern-

mental pharmacies and stores which can give rise to such problems. A 62.6% of *S. aureus* in this study were susceptible to Nitrofurantoin (Table 4), but much higher (98.2% and 81%) rates were reported earlier by (Cunha *et al*., 2016) and (Iregbu & Nwajiobi-Princewill, 2013) respectively. A 58% of S. *aureus* were sensitive to Ciprofloxacin. Other studies showed a higher rate of 73.9% of resistance to this antibiotic (Sukumaran & Kumar, 2017). *S. aureus* isolates in this study were highly (98.41%) resistant to Ampicillin and (78.75%) to Amoxicillin-clavulanic acid. The same finding was reported in other studies (Ekwealor *et al*., 2016; Mezal *et al*., 2011). *S. aureus* showed a high rate of resistance estimated at (65.31%) for Cefotaxime and 57.83 for Cefoxitin but very high resistance (87.62%) was reported for Ceftazidime. This study showed high resistance of *S. aureus* for cephalosporins antibiotics and the same observed by others (Sukumaran & Kumar, 2017). *S. aureus* isolates in this study showed high resistance for Sulfamethoxazole-trimethoprim (69.23%), higher resistance rate (79.5%) was reported by (Sukumaran & Kumar, 2017).

CONCLUSIONS

This study confirmed that *E. coli, Klebsiella species, Proteus species, Pseudomonas* species, and *Staphylococcus aureus* are the major uropathogens usually associated with UTI. Results also demonstrated that females are more susceptible to UTI than males. Additionally, Imipenem and Amikacin showed the highest activity against Gram-negative and Grampositive pathogenic bacteria, and they could be the drugs of choice for the treatment of UTI. On the contrary, Ampicillin and Amoxicillinclavulanic acid should be excluded for UTI treatment in our area due to a very high resistance rate.

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المسببات البكتيرية وحساسيتها لمضادات الميكروبات بين مرضى التهاب المسالك البولية في منطقة طبرق، ليبيا

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المستخلص: تعد التهابات المسالك البولية شائعة جدًا، وتؤثر على الأشخاص من جميع الأعمار، ولكن المرض أكثر انتشارًا عند النساء ، لذا فإن التشخيص السريري، والمختبري، والعلاج الصحيح مهمان للغاية لتجنب المضاعفات، ومقاومة المضادات الميكروبية. هدفت هذه الدراسة إلى تحديد الأسباب البكتيرية، ونمط حساسية المضادات الحيوية لمرضى المسالك البولية. تم الحصول على النتائج المسجلة لـ 6065 عينة من عينات البول، واختبار الحساسية لمضادات الميكروبات من قسم الأحياء الدقيقة في مركز طبرق الطبي، مدينة طبرق، من سبتمبر 2016 إلى ديسمبر 2018 حيث تم تحليل البيانات، ومناقشتها مع دراسات أخرى. أظهرت الدراسة أن عدوى المسالك البولية أكثر شيوعًا عند الإناث بنسبة (78.8%)، مقارنة بالذكور (21.2%). شكلت البكتيريا سالبة الجرام غالبية مسببات أمراض المسالك البولية (90 %)، حيث كانت *E. coli* وحدها (58.4%)، *Klebsiellae* (17.4%)، *Proteus* (10.7%)، بينما كان نوع *Pseudomonas* الأقل شيوعا (3.3%) من المجموع. بينما شكلت البكتيريا الموجبة الجرام نوع *S. aureus* نسبة (9.6%) من المجموع. كانت نتائج هذه الدراسة ذات دلالة احصائية (P=0.000). المضادات الحيوية الأكثر فعالية ضد جميع الممرضات البولية هي: Imipenem وAmikacin، والمضادات الحيوية الأقل فعالية كانت Ampicillin، و Amoxicillin-clavulanic acid. خلصت هذه الدراسة إلى أن السبب الأكثر شيوعًا للإصابة بالتهاب المسالك البولية في منطقة طبرق هو *E. coli*، ويبدو أن المضادات الحيوية الأكثر فعالية هي Imipenem، وAmikacin.

الكلمات المفتاحية: التهابات المسالك البولية، الممرضات البولية، اختبار الحساسية للمضادات الحيوية، مقاومة المضادات الحيوية، العوامل المضادة للجراثيم، انتشار القرص.

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