Identification and Antimicrobial Resistance Profile of Bacterial Species Isolated from Urine Samples Obtained from Patients who Attended some Clinics in Misrata City

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Abstract

Urinary tract infection (UTI) is a common healthcare concern worldwide. Many risk factors predispose to UTIs such as sex, age, and underlying diseases, and identifying the causative agent and the effective antimicrobial will improve the outcome of medical treatment.

The urine samples of 56 patients who attended the Algheran clinic, Alzaweia clinic, and Misrata Medical Centre were collected for bacterial isolation and to determine their antimicrobial susceptibility. Bacterial identification and antimicrobial susceptibility were performed according to the Clinical and Laboratory Standards Institute guidelines (CLSI).

Out of 56 included patients, 50 showed positive urine cultures. The incidence of UTIs among women, in included patients, was 94.6% higher than men's 79%. The isolated bacterial species' prevalence was *Staphylococcus. aureus* 32%, *Escherichia. coli* 26%, *Klebsiella. pneumonia* 24%, *Pseudomonas. aeruginosa* 5%, and *Proteus. mirabilis* 8%. Of the fifty identified bacterial species, resistance to ciprofloxacin and kanamycin was 44% and 66%, respectively, where high resistance to cefotaxime at 90% was reported.

The prevalence of positive urine bacterial culture of patients who attended the clinics was high, and resistance to commonly used antimicrobials is a major medical concern. Therefore, antimicrobial use strategies and policies need to be enforced and followed for better control and consequences of UTIs.

Keywords: Identification, Antimicrobials, Urinary tract infection, Misrata City.

Introduction

Infection of the urinary tract is considered one of the most common and costly health concerns worldwide (Schmiemann et al. 2010). Urine is generally sterile and free of pathogens; viruses and fungi are rarely possible causes of UTI, however, bacteria are the most commonly identified uropathogen.

Both men's and women's urinary tracts may be infected, yet UTIs among women are more frequent due to the anatomy structure and physiology (Vasudevan 2014). Pregnancy was considered a risk factor preloading to asymptomatic and symptomatic UTIs (Haider et al. 2010).

The association between UTIs and age group is not clearly identified; some studies found UTIs more frequent in adolescent females, in contrast, the incidence of UTIs among adolescent males was less than among men older than 50 years (Horowitz and Cohen 2007).

The frequently isolated bacteria of UTI are *Escherichia coli* (*E. coli*), which is responsible for 80-85% of recorded cases, followed by *Staphylococcus aureus* (*S. aureus*), which causes 5-10% of identified bacterial species, and other bacterial species associated with UTIs are Klebsiella, proteus *species*, *pseudomonas aeruginosa* and *enterococcus faecalis* (Vasudevan 2014).

This study aimed to investigate the prevalence of bacterial species in urine samples of patients who attended the Algheran clinic, Azaweia clinic, and Misrata Medical Centre, and evaluate their resistance to commonly used antimicrobials.

Methods

Study site and demographic data samples of urine were collected from 56 patients who showed clinical symptoms of UTIs from the Algheran clinic (n = 18), Alzaweia clinic (n = 18), and Misrata Medical Centre (n = 20). Of included patients, 37 were females, and 19 were males. Samples were collected from 28/03/2021 to 06/06 2021.

Samples Processing and Bacterial Identification

Samples were collected in the early morning by clean-catch midstream urine after well urethral area cleaning. The urine sample was collected in a wide-mouthed sterile container closed tightly and was processed within two hours of collection. Standard capacity loop used to inoculate 0.01 mL of urine sample into the blood and MacConkey agar. All inoculated plates were incubated aerobically at 37°C for 18-24 hours.

Colony counts yielded bacterial growth of $\geq 10^5$/ml of urine is considered a positive case of UTIs. Preliminarily identification of pathogenic bacterial

species was performed using colony morphology on plates and Gram-stain; Gram-positive bacterial isolates were furtherly identified by catalase and coagulase tests, while Gram-negative bacterial isolates were additionally identified by oxidase and multi-Biochemical test analytical profile index 20E (API 20E) (bioMérieux's). Identified bacterial species stored in 25% glycerol at -20°C.

Determination of Antimicrobial Susceptibility

Isolated species were tested for their susceptibility to antimicrobial agents using the Kirby-Bauer method and performed according to the Clinical and Laboratory Standards Institute guidelines (CLSI) (Humphries et al. 2018). The following antimicrobial discs are been used: Ampicillin (AMP, 10µg), Amoxicillin + clavulanic acid (AMC, 10µg), Kanamycin (K, 30µg), Imipenem (IPM, 10µg), Meropenem (MEM, 10µg), Ciprofloxacin (CIP, 5µg), and Cefotaxime (CTX, 30µg), and all discs were made by Bioanalyse, Turkey.

Stored isolates inoculated in blood or MacConkey agar plate, and colonies of the pure culture inoculated on Muller-Hinton agar and antimicrobial discs were added. Plates were incubated at 37°C for 24h, then the inhibition zone was measured to evaluate antimicrobial susceptibility according to the CLSI reference table.

Data Analysis

The results of the study were analysed by using SPSS 22 (SPSS Inc., Chicago, USA). Obtained data presented in numbers and percentages and analysed by using Anova one-way test. Significance was established at P value ≤0.05.

Results and Discussion

Bacterial growth was identified in 50 (89%) urine samples of 56 patients included in this study (Fig 1). This study finding is higher than the results reported in a study performed by Salim and others, which showed only 25.2% of tested urine samples were positive for bacterial growth (Salim, Murad, and Elbareg 2017). In another study, only 13.9% of tested urine samples showed uropathogens growth (Mohammed et al. 2016).

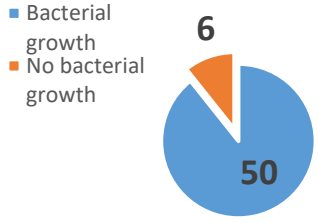


Figure 1: positve cultures of urine samples

The high prevalence of positive urine culture in this study may be due to the low economic status of the tested population, and most of the included patients have been diagnosed with UTIs by urology specialists before the urine sample collection.

Fifty urine cultures showed bacterial growth; 35 of which were obtained

from females, and 15 from males. The prevalence of positive cultures among females, 94.6% (35 of 37), was higher than among males, 79% (15 of 19) (Fig 2). The incidence of UTI among females is higher than among males was reported in previous studies as a result of different anatomical structure, and the length of urethra is shorter in women's body (Salim et al. 2017, Foxman 2002).

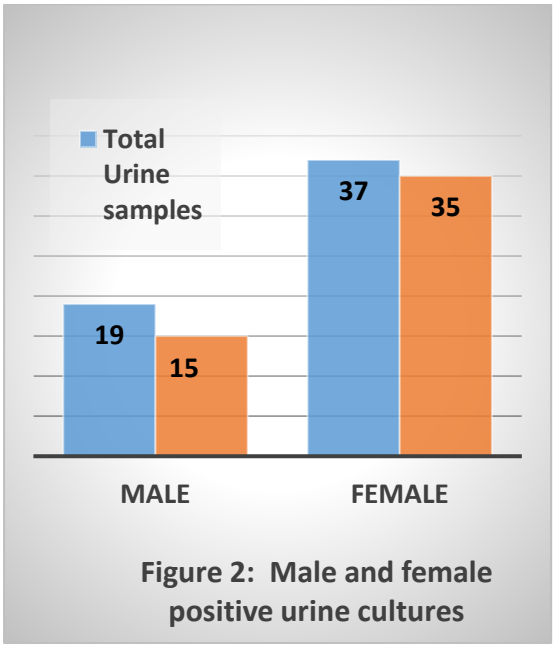
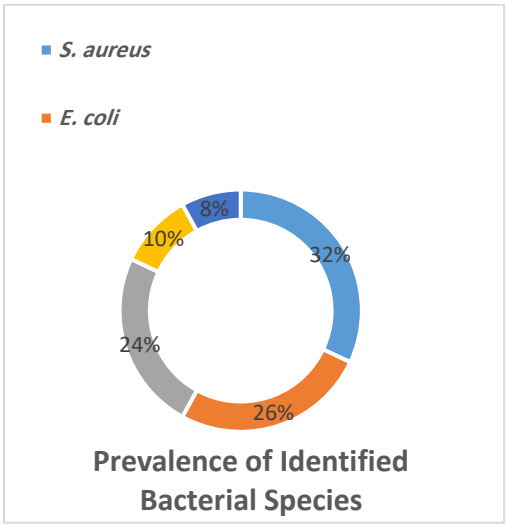


Figure 2: Male and female positive urine cultures

In this study, bacterial identification revealed *S. aureus* as the most prevalent species at 32% (16/50), followed by *E. coli* at 26% (13/50), *Klebsiella pneumonia* (*K. pneumonia*) at 24% (12/50), *Pseudomonas aeruginosa* (*P. aeruginosa*) at 10% (5/50), and *Proteus mirabilis* (*Pr. mirabilis*) at 8% (4/50) (Fig 3).

In agreement to our result, studies enrolled in Tripoli found the



Prevalence of Identified Bacterial Species

predominant uropathogens were *S. aureus* 64%, followed by *E. coli* 29%. In contrast, other studies demonstrated *E. coli* as the dominant agent causing UTIs among included patients (Kibar, Yaman and Dündar 2004, Nerurkar et al. 2012, Odoki et al. 2019).

Another study investigated the prevalence of UTIs conducted in Messalata Central Hospital, Libya, and found that all identified bacterial species, Gram-negative and *E. coli,* were the most dominant pathogen (Mohammed et al. 2016, Ghenghesh et al. 2003).

In agreement with other studies, *E. coli* was the dominant Gram-negative uropathogen identified in tested urine samples (Ghenghesh et al. 2003, Salim et al. 2017). This study's findings showed the dominant reported uropathogen; *S. aureus* found as normal flora on the skin including the area around urethral orifice, and *E. coli* known microflora of intestinal tract which is considered a predisposing

factor of reaching the urogenital tract and lead to UTIs.

Table 1: Antimicrobial resistance of identified pathogenic bacterial species.

Bacterial species	Antimicrobial agents						
	AMP N (%)	**AMC N (%)**	**K N (%)**	**IPM N (%)**	**MEM N (%)**	**CIP N (%)**	**CTX N (%)**
***S. aureus* (n = 16)**	13 (81)	13 (81)	11 (68.7)	15 (93.7)	15 (93.7)	10 (62.5)	15 (93.7)
***E. coli* (n =13)**	10 (77)	10 (77)	9 (69.2)	9 (69.2)	9 (69.2)	6 (46)	11 (84.6)
***K. pneumonia* (n = 12)**	12 (100)	10 (83)	8 (66.6)	8 (66.6)	7 (58.3)	5 (41.6)	11 (91.6)
***P. aeruginosa* (n = 5)**	5 (100)	3 (60)	1 (20)	3 (60)	3 (60)	0	5 (100)
***Pr. mirabilis* (n = 4)**	3 (75)	4 (100)	4 (100)	2 (50)	3 (75)	1 (25)	3 (75)
Total = 50	43 (86)	40 (80)	33 (66)	37 (74)	37 (74)	22 (44)	45 (90)

The highest resistance among the tested antibiotics was reported against cefotaxime (90%), ampicillin (86%), and amoxicillin-clavulanic acid (80%), while the most effective antimicrobial agents were ciprofloxacin (56%), K (67%), and imipenem and meropenem (63%).

Uropathogens collected from patients in Sobrata and Algmel in Libya were highly susceptible to ciprofloxacin (Khalifa, Harb and Alkout 2014). High resistance of *S. aureus* to all tested antimicrobial agents was established except ciprofloxacin and kanamycin. The susceptibility of *S. aureus* to ciprofloxacin was significantly higher than other tested antimicrobial agents (P = 0.015).

In agreement with this study finding, ciprofloxacin was the most effective antimicrobial agents against Gram-positive Uropathogens (Nermeen et al. 2011). *E. coli* isolates resistant to cefotaxime, ampicillin, and amoxicillin-clavulanic acid was 84.6%, 77%, and 77% respectively. It was more susceptible to kanamycin, imipenem, and meropenem, while ciprofloxacin showed the highest effect against *E. coli* (46%). Significance between *E. coli* resistance to tested antimicrobials not recorded.

In contrast, resistance of *E.coli* uropathogen was 0% and 55.5% to imipenem and cefotaxime respectively in a study conducted in Iraq (Alshewelee 2012), and similar results were established in resistance of *E.coli* to cefotaxime and ampicillin (Salim et al. 2017, Jabbar 2013).Powerful resistance was reported by the included *K. pneumonia* isolates to ampicillin (100%) and cefotaxime (91.6%).

On the other hand, ciprofloxacin was significantly more effective than other

tested antimicrobials (*P*. value = 0.018) and showed variable resistance to other tested antimicrobials.

This result correspondences with that reported by Azizi and others; where the identified *K. pneumonia* isolates showed 3.51% and 5.26% resistance to ciprofloxacin and kanamycin, respectively (Azizi et al. 2014). All identified *P. aeruginosa* were sensitive to ciprofloxacin, and 80% to kanamycin, and presented 100% resistance to ampicillin and cefotaxime.

Our study results showed a high significance between the effectiveness of used antimicrobials against *P. aeruginosa* (*P*. value = 0.003). A similar finding was reported by Mohammed and others, in 2016, where they reported the resistance of tested *P. aeruginosa* isolates against ciprofloxacin at 11.1%, and absolute susceptibility to kanamycin (Mohammed et al. 2016). Resistance of *Pr. mirabilis* tested isolates to amoxicillin-clavulanic acid and kanamycin were 100%, and more susceptible to ciprofloxacin 25%. Isolates of *Pr. mirabilis* obtained from patients who attended Messalata Central Hospital, Libya, reported high sensitivity to kanamycin and moderated susceptibility to ciprofloxacin (Mohammed et al. 2016).

Amongst tested population, the prevalence of uropathogens in urine samples was high compared to other studies, and *S. aureus* was the predominant identified bacterial species. The highest effectiveness of tested antimicrobials was ciprofloxacin and the third generation of included cephalosporin (cefotaxime). Surveillance and studies of uropathogens and their resistance to antimicrobials should be performed in each geographical area; as there were high differences in the prevalent and predominant identified bacterial species, and the effectiveness of used antimicrobials.

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تعريف ومقاومة مضادات الميكروبات للأنواع البكتيرية المعزولة من عينات البول المأخوذة من المرضى ببعض العيادات في مدينة مصراته

الملخص

تعد عدوى المسالك البولية (UTI) مصدر قلق شائع للرعاية الصحية في جميع أنحاء العالم. هناك العديد من عوامل الخطر لعدوى المسالك البولية مثل الجنس والعمر والأمراض الكامنة، تحديد العامل المسبب والمضاد الفعال للميكروبات سيحسن نتائج العلاج الطبي.
جمعت عينات بول 56 مريضاً ممن حضروا لعيادة الغيران وعيادة الزاوية ومركز مصراته الطبي لعزل البكتيريا وتحديد مدى قابليتهم لمضادات الميكروبات. تم إجراء تحديد نوع البكتيريا وقابلية مضادات الميكروبات وفقًا لإرشادات معهد المعايير السريرية والمخبرية (CLSI).
من بين 56 مريضاً، أظهر 50 مريضاً نتيجة ايجابية في زراعة البول. كان معدل الإصابة بعدوى المسالك البولية بين النساء، أعلى بنسبة 94.6 ٪ من الرجال بنسبة 79 ٪. كان انتشار الأنواع البكتيرية المعزولة هو *Staphylococcus. aureus* 32٪ *Escherichia. coli* 26٪ ، *Klebsiella. pneumonia* 24٪ ، *Pseudomonas. aeruginosa* 5٪ *Proteus. mirabilis* 8٪. من بين الخمسين نوعًا من البكتيريا التي تم تحديدها، كانت المقاومة Ciprofloxacin وkanamycin 44٪ و 66٪ على التوالي ، حيث لوحظ مقاومة عالية Cefotaxime عند 90٪.
كان معدل انتشار البكتيريا في البول للمرضى الذين حضروا العيادات مرتفعًا ، تعد مقاومة مضادات الميكروبات شائعة الاستخدام مصدر قلق طبي كبير. لذلك ، يجب تنفيذ استراتيجيات معينه لاستخدام مضادات الميكروبات واتباعها بطرق صحيحة من أجل مراقبة عواقب عدوى المسالك البولية بشكل أفضل.