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ORIGINAL ARTICLE

Bacterial Profile of Urinary Tract Infection and Antimicrobial Susceptibility Pattern Among Patients Attending at Bushra Medical Laboratory, Tripoli, Libya

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ABSTRACT

BACKGROUND: Urinary tract infection (UTI) is a common infection that affects the urinary tract, particularly in women, children and elderly, and it doesn't always cause classic signs and symptoms. Excessive utilization of antibiotics led to increased incidence of antibiotic resistance among uropathogens worldwide.

AIM: To identify the causative bacteria of UTI and antimicrobial susceptibility pattern among patients attended to Bushra Medical laboratory, Tripoli, Libya.

METHODS: This prospective study was conducted in Tripoli

city, on 1423 urine specimens from untreated patients with clinical symptoms of UTIs. Bacterial identification and antimicrobial susceptibility testing were done on 291 specimens which their colony count ≥ 10⁵ colony forming units (cfu)/mL, using DL-96II (Auto microbial identification and antimicrobial susceptibility testing

RESULTS: The prevalence of significant bacteriuria was 20.4% (291/1423), among them 81.4% (237/291) were females and 18.6% (54/291) were males. Just 0.7% (2/291) cases of isolated bacteria were gram positive cocci represented in Staphylococcus aureus, that exhibited hugely resistance to Penicillin (100%) and susceptible to many tested antimicrobial agents, while 99.3% (289/291) were gram negative bacilli represented in Klebsiella pneumonia 43.6% (127/291); Escherichia coli 33% (96/291); Klebsiella ozaenae 10.7% (31/291); Proteus mirabilis 9.3% (27/291); Pseudomonas mendocina 1.7% (5/291) and Pseudomonas alcaligenes 1% (3/291). Considering the results of this study, Klebsiella pneumonia isolates were the predominant pathogens in UTI. Except for Pseudomonas isolates, our data demonstrated that gram negative isolates were susceptible to Cefoperazone/Sulbactam followed by Ceftazidime then Levofloxacin (3.9%, 4.9% and 6% resistance, respectively), while Pseudomonas isolates were extremely sensitive to Amikacin, Piperacillin and Norfloxacin.

CONCLUSION: Because of highly resistant bacteria to antibiotics, physicians must prescribe appropriate antibiotics depending on antibiotics susceptibility results. Otherwise, routine monitoring of antimicrobial resistance among uropathogens must be accomplished to guide the treatment of UTIs in the country.

Key words: Bacterial profile; Urinary tract infection; Uropathogens; Antimicrobial susceptibility testing

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INTRODUCTION

Urinary tract infections (UTIs) are one of the most common bacterial infections in humans both in the community and hospital setting^[1], which can affect any part of the genitourinary tract, including urethra, bladder, ureter, renal pelvis, or renal parenchyma^[2], and occur in all populations and ages. However, various factors including race, genetic factors, age, gender, sexual activity, nocturnal enuresis and circumcision in boys^[3], make infections range from presence of bacteria in urine without symptoms (asymptomatic bacteriuria) to wide spectrum of symptoms ranging from mild excitation to bacteremia, sepsis, or even death^[4]. It is estimated that approximately 150 million cases occur globally each year^[5]. Most infections are caused by movement of bacteria through fecal from colon to urinary tract via urethra, especially in females, due to the proximity of the genital tract and urethra[6]. Anatomical structure of female urethra and vagina make them susceptible to trauma during sexual intercourse and bacterial passage through urethra up to the bladder during pregnancy and delivery^[7]. The major causative organisms responsible for the most UTI cases are bacteria^[8], mainly gram negative species which account for 80-85% and the leading causative organisms are Escherichia coli (80%) of UTI cases, followed by Klebsiella pneumonia and Proteus mirabilis[9]. Gram positive bacteria as Staphylococcus saprophyticus and Enterococcus faecalis are also an important cause of UTIs^[5]. Although UTIs are commonly curable with antibiotics, the antibiotic susceptibility patterns of UTI vary according to regional and geographical locations[10] and also change

The emergence of antibiotic resistance of UTIs is a serious public health issue, particularly in the developing countries such as Libya, because of health awareness obscurity, and poor hygienic practices. Therefore, this study was conducted to investigate microbial species isolated from patients with UTI who attended to Bushra Medical laboratory, Tripoli, Libya, and to evaluate their in vitro susceptibility patterns to commonly used antimicrobial agents in Libya.

MATERIALS AND METHODS

Specimens collection and bacterial isolates

This study was conducted from January 2017 to August 2017 on 1423 untreated patients with different clinical symptoms of UTIs, 414 males and 1009 females, aged 2-83 years were attended to Bushra Medical laboratory, Tripoli, Libya. In case of adults, clean catch midstream urine samples (MSU) were collected in sterile disposable containers (20 mL) with tight-fitting lid to prevent leakage, while in case of infants, adhesive urine collection bags were used for specimen collection. The collected urine specimens were subjected to general urine examinations using direct microscopy for white blood cells (WBCs) counting, then cultured on 5% Blood agar, MacConkey agar, and Eosin-Methylene blue (EMB) agar (Liofilchem srl, Zona Ind. Ie. Roseto d. Abruzzi "TE"- ITALY) using calibrated loops for semi-quantitative method[12], and incubated aerobically for 24 hours at 37°C. Cultures without any colony at the end of 24 hours incubation were further incubated for 48 hours. Samples with colony count $\geq 10^5$ cfu/mL were considered positive isolates.

Identification of uropathogens and antibiotic susceptibility testing

Positive isolates of 291 specimens (54 males and 237 females) were identified and confirmed using The DL-96II Auto microbial identification and antimicrobial susceptibility testing system (Zhuhai DL Biotech Co., Ltd., China), according to the manufacturer's

recommendations. The DL-96II was designed for both identification of clinical pathogens and semi-quantitative analysis of antimicrobial drugs (MIC), using test cards (DL-96E and DL-96STAPH) which composed of biochemical reaction wells and wells of antimicrobial MIC determination tests. The diluted solution bottle was, individually, inoculated with bacterial colonies from a pure culture to make 0.5 McFarland standard bacterial suspensions using DL-96 turbidumeter. Using a CrystalSpec nephelometer (DL-96II), 100 μl of bacterial suspension was injected into all biochemical reaction wells, and 50 µl of bacterial suspension was also injected into broth bottle and mixed well before injecting 100 µl of the mixture into each well of the all antimicrobial determination test card wells. Two drops of sterile paraffin oil was added into wells A1 to A7 of DL-96E test card and into wells A1, A2 and A3 of DL-96STAPH test card, for anaerobic conditions before incubating the test cards for 18-24 hrs at 35-37°C. After incubation period, one drop of VP reagent A and another of VP reagent B were added into wells A12 (VP) of both DL-96E and DL-96STAPH test cards; for DL-96E test card, one drop of indole reagent was added into well A11 (IND) after 20 minutes and one drop of phenylalanine reagent was added into well A10 (PHE) few minutes later, while for DL-96STAPH test card a drop of PYR chromogenic reagent was added into well A10 after 30 minutes, and another drop of NIT reagent A&B was added into well A11.

Immediately, the test cards were inserted into DL-96 II and analysis was started automatically to determine the bacterial species and their antimicrobial susceptibility results.

Statistical analysis

Statistical analyses were done by using Microsoft Office Excel 2007 and SPSS version 12.0.1. Discrete variables were expressed as percentages and proportions were compared using the Chi-square test. Value of $p \le 0.05$ was considered statistically significant.

RESULTS

Of the 1423 urine specimens tested, 291 (20.4%) specimens were positive for the presence of bacterial pathogens. The distribution of infection among patients' gender was (237/291, 81.4%) in female patients, while it was (54/291, 18.6%) in male patients. The order of prevalence of uropathogens isolated from female patients was *Klebsiella ozaenae* (87.1%); followed by *Escherichia coli* (83.3%); *Klebsiella pneumonia* (81.9%); *Proteus mirabilis* (77.8%); *Pseudomonas mendocina* (60.0%); *Staphylococcus aureus* (50.0%) and *Pseudomonas alcaligenes* (33.3%); otherwise this order in male patients was *Pseudomonas alcaligenes* (66.7%); *Staphylococcus aureus* (50.0%); *Pseudomonas mendocina* (40.0%); *Proteus mirabilis* (22.2%); *Klebsiella pneumonia* (18.1%); *Escherichia coli* (16.7%) and *Klebsiella ozaenae* (12.9%). (Table 1). No significant difference was found between isolated bacteria and gender of the patient (*p* = 0.460)

Patients' age ranged from 2 to 83 years with an average age of 41.5 years. As shown in table 2, the highest distribution of infection among patients' age groups was (135/291, 46.4%) in adults (25-64 years); followed by elderly (\geq 65 years); then children (2-14 years); and the lowest incidence was (36/291, 12.4%) in youth (15-24 years). Significant difference was found between isolated bacteria and age of the patient (p = 0.009). The most common microorganism isolated in all age groups and from both sexes was *Klebsiella pneumonia* (127/291, 43.6%); followed by *Escherichia coli* (96/291, 33.0%); then *Klebsiella ozaenae* (31/291, 10.7%); *Proteus mirabilis* (27/291, 9.3%); *Pseudomonas mendocina* (5/291, 1.7%); *Pseudomonas*

alcaligenes (3/291, 1.0%) and the fewest microorganism isolated was *Staphylococcus aureus* (2/291, 0.7%).

Klebsiella isolates, the predominant causative of UTIs, showed resistance to Ampicillin, Minocycline and Amikacin, whilst Cefoperazone/Sulbactam, Levofloxacin and Ceftazidime had stronger effect against Klebsiella ozaenae than Klebsiella pneumonia.

E. coli, the important uropathogen causative, displayed a similar resistance pattern as for Klebsiella, were showed resistance to Ampicillin and Minocycline and high susceptible to Cefoperazone/Sulbactam, Ceftazidime and Levofloxacin.

Proteus mirabilis also comparable to Klebsiella, showed high resistance to Ampicillin, Minocycline and Cefepime, in return showed high sensitivity to Cefoperazone/ Sulbactam, Piperacillin/Tazobactam, Ticracillin/Clavulanin, Moropenem, Levofloxacin, Ciprofloxacin, Ceftazidime, and Nitrofurantion that were significantly similar to Klebsiella ozaenae.

Pseudomonas isolates, showed high resistance to Trimethoprim/ Sulfamethoxazole, Ceftazidime, Topramycin and Polymyxin B, and high susceptible to Amikacin, Piperacillin and Norfloxacin. However, that Pseudomonas alcaligenes showed increased resistance to Cefepime, Imipenem and Levofloxacin, while Pseudomonas mendocina showed increased resistance to Moropenem, Piperacillin/ Tazobactam and Gentamicin.

Staphylococcus aureus, showed high susceptibility to most tested antibiotics, were appeared high resistance to Penicillin, while showed resistance to Thrimethoprim/Sulfamethoxazole, Cefoxitin, Clindamycin, Erythromycin, Oxacillin, Clarithromycin and Azithromycin in a half of isolates (Table 3).

DISCUSSION

Because the management of UTI is essential to isolate and identify the bacterial causative agent, this study was undertaken to determine the distribution of microbial species isolated from patients with UTI at Bushra Medical Laboratory in Tripoli, Libya, and their susceptibility patterns to antimicrobial agents were tested. The overall prevalence of UTI in examined urine specimens was 20.4%. This result nearly similar to that previously documented one (20.7%) in a study conducted at Zawiya city, Libya^[13]. However; the rate of prevalence in the current study was higher than in two studies previously documented by Mohammed et al at Messalata city, Libya and Toumi et al at Gafsa city, Tunisia (13.9% and 16.5% respectively)[14,15], but it is lower compared to the result in an investigation previously conducted in Misurata city, Libya (25.2%) and much lower compared to another study conducted in Zliten city, Libya (37.3%)^[16,17]. The low growth rate of UTI in this study could result from illogical use of antibiotics in this country which are given without beforehand culture and antibiotics susceptibility tests and other clinical conditions. The deficient dose of antibiotics may be is another factor as well, could be responsible for insignificant bacteriuria or no growth[18].

Approximately 55% of women will suffer from UTI during their lifetime^[19]. According to patients' gender in this study, total uropathogens were significantly isolated from females 237/291 (81.4%) compared to males 54/291 (18.6%). This result was comparable with the previous studies conducted in other regions of Libya^[14,16,17]. Sexual activity has been reported to influence higher prevalence of UTI in females^[16]. Alternations in vaginal microflora also play a critical role in encouraging vaginal colonization with coliforms and this can lead to urinary tract infection^[20]. Furthermore, management mistakes made by women include cleaning perineum

Table 1 Frequency distribution of isolated uropathogens among tested patients according to gender.

Isolated bacteria	No. (%)	Female, No, (%)	Male, No (%)	
Klebsiella pneumonia	127 (43.6)	104 (81.9)	23 (18.1)	
Klebsiella ozaenae	31 (10.7)	27 (87.1)	4 (12.9)	
Proteus mirabilis	27 (9.3)	21 (77.8)	6 (22.2)	
Pseudomonas alcaligenes	3 (1.0)	1 (33.3)	2 (66.7)	
Pseudomonas mendocina	5 (1.7)	3 (60.0)	2 (40.0)	
Escherichia coli	96 (33.0)	80 (83.3)	16 (16.7)	
Staphylococcus aureus	2 (0.7)	1 (50.0)	1 (50.0)	
Total	291 (100)	237 (81.4)	54 (18.6)	

Table 2 Frequency distribution of isolated uropathogens among tested patients according to age.

Isolated bacteria	No. (%)	2-14 (%)	15-24 (%)	25-64 (%)	> 65 (%)
Klebsiella pneumonia	127 (43.6)	15 (11.8)	15 (11.8)	68 (53.5)	29 (22.8)
Klebsiella ozaenae	31 (10.7)	6 (19.3)	2 (6.4)	13 (42.0)	10 (32.3)
Proteus mirabilis	27 (9.3)	8 (29.6)	4 (14.8)	9 (33.3)	6 (22.2)
Pseudomonas alcaligenes	3 (1.0)	1 (33.3)	0 (00.0)	1 (33.3)	1 (33.3)
Pseudomonas mendocina	5 (1.7)	2 (40.0)	1 (20.0)	2 (40.0)	0 (00.0)
Escherichia coli	96 (33.0)	24 (25.0)	13 (13.5)	42 (43.8)	17 (17.7)
Staphylococcus aureus	2 (0.7)	0 (00.0)	1 (50.0)	0 (00.0)	1 (50.0)
Total	291 (100)	56 (19.2)	36 (12.4)	135 (46.4)	64 (22.0)

forward from the anus to the vulva that can cause urinary tract infection^[21]. Males are less prone to UTIs possibly because of their longer urethra and the presence of antimicrobial substances in prostatic fluid^[8].

The distribution of infection among patients' age groups was higher (46.4%) in adults (25-64 years) compared to other groups. This might be due to adults in this age group being more sexually active which might predispose them to UTI, followed by elderly (22%) then children under 14 years (19.2%). This was nearly agreement with previous studies^[14,17].

Regarding ethological agents, gram-negative bacteria were the most common isolates in this study, which is identical with other reports from different areas^[14,16,17,22]. The present study detected the dominance of *Klebsiella pneumonia* (43.6%) and *Escherichia coli* (33%) (Table 1), which was with different rates when compared with other studies in Libya and other countries. In Northwest Libya, Abujnah *et al* have found predominance of *Escherichia coli* (55.8%) and *Klebsiella pneumonia* (18.5%)^[13]. In another study in Messalata, Libya, Mahammed *et al* have reported the predominance of *Escherichia coli* (55.6%) and *Klebsiella pneumonia* (16.3%)^[14]. In Southern Tunisia, The authors have found *Escherichia coli* (67.1%) and *Klebsiella pneumonia* (12.8%) as predominance uropathogens among patients of UTIs^[15]. A study in Poland have reported uropathogens with a predominance of *Escherichia coli* (73%) and *Proteus spp.* (8.9%)^[23].

Psedumonas isolates in the current study showed extreme sensitivity to Amikacin, Piperacillin and Norfloxacin, a compatible result with that in a study that has been conducted in Messalata, Libya^[14]. On the other hand, other gram negative uropathogens exhibited high sensitivity to Cefoperazone/sulbactam, followed by Ceftazidime and Levofloxacin, then Trimethoprim/sulfamethoxazole, which was inconsistent with the result in other studies in Libya^[13,14,16], however, gram positive isolates (*S. aureus*) were extremely resistant to Penicillin.

In conclusion, we evidenced that Klebsiella pneumonia and

Table 3 Antimicrobial Resistance of Uropathogens Isolated in this Study.

Bacterial isolates (n)	Klebsiella pneumonia 127	Klebsiella ozaenae 31	Proteus merabilis 27	Escherichia coli 96	Pseudomonas	Pseudomonas mendocina 5	Staphylococcus
Tested antimicrobials Cefazolin	91 (71.6%)	23 (74.2%)	18 (66.6%)	68 (70.3%)	alcaligenes 3 ND	ND	aureus 2 ND
Gentamicin	70 (55%)	8 (25.8%)	9 (33.3%)	43 (44.8%)	0 (00.0%)	4 (80%)	0 (00.0%)
Ampicillin	127 (100%)	31 (100%)	27 (100%)	93 (96.9%)	ND	ND	ND
•	, ,	, ,	, ,		ND	ND	ND
Cefuroxime Sodium	91 (71.6%)	15 (48.4%)	18 (66.6%)	68 (70.3%)			
Cefepime	77 (60.6%)	23 (74.2%)	23 (85.2%)	62 (64.6%)	3 (100%)	2 (40%)	ND
Cefoxitin	67 (52.7%)	8 (25.8%)	4 (14.8%)	40 (41.7%)	ND	ND	1 (50%)
Ceftriaxone	64 (50.4%)	15 (48.4%)	9 (33.3%)	59 (61.5%)	ND	ND	ND
Cefoperazone/ Sulbactam	11 (8.6%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	1 (33.3%)	3 (60%)	ND
Piperacillin/ Tazobactam	22 (17.3%)	0 (00.0%)	0 (00.0%)	6 (6.3%)	0 (00.0%)	5 (100%)	ND
Ticracillin/ Clavulanin	39 (30.7%)	8 (25.8%)	0 (00.0%)	22 (23%)	0 (00.0%)	3 (60%)	ND
Ampicillin/ Sulbactam	77 (60.6%)	0 (00.0%)	13 (18.1%)	46 (48%)	ND	ND	ND
Moropenem	25 (19.7%)	0 (00.0%)	0 (00.0%)	16 (16.7%)	2 (66.6%)	5 (100%)	ND
Imipenem	95 (74.8%)	15 (48.4%)	18 (66.6%)	50 (52%)	3 (100%)	1 (20%)	ND
Amikacin	91 (71.6%)	31 (100%)	9 (33.3%)	56 (58.3%)	0 (00.0%)	0 (00.0%)	ND
Trimethoprim/ Sulfamethoxazole	18 (14.2%)	0 (00.0%)	4 (14.8%)	19 (19.8%)	3 (100%)	5 (100%)	1 (50%)
Levofloxacin	14 (11%)	0 (00.0%)	0 (00.0%)	3 (3.1%)	3 (100%)	1 (20%)	0 (00.0%)
Ciprofloxacin	32 (25.2%)	0 (00.0%)	0 (00.0%)	16 (16.7%)	2 (66.6%)	3 (60%)	ND
Ceftazidime	14 (11%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	3 (100%)	5 (100%)	ND
Minocycline	116 (91.3%)	31 (100%)	27 (100%)	71 (74%)	1 (33.3%)	2 (40%)	ND
Nitrofurantion	46 (36.2%)	0 (00.0%)	0 (00.0%)	6 (6.3%)	ND	ND	ND
Piperacillin	ND	ND	ND	ND	0 (00.0%)	0 (00.0%)	ND
Topramycin	ND	ND	ND	ND	3 (100%)	5 (100%)	ND
Aztreonam	ND	ND	ND	ND	1 (33.3%)	2 (40%)	ND
Polymyxin B	ND	ND	ND	ND	3 (100%)	5 (100%)	ND
Norfloxacin	ND	ND	ND	ND	0 (00.0%)	0 (00.0%)	ND
Clindamycin	ND	ND	ND	ND	ND	ND	1 (50%)
Erythromycin	ND	ND	ND	ND	ND	ND	1 (50%)
Oxacillin	ND	ND	ND	ND	ND	ND	1 (50%)
Penicillin	ND	ND	ND	ND	ND	ND	2 (100%)
Clarithromycin	ND	ND	ND	ND	ND	ND	1 (50%)
Azithromycin	ND	ND	ND	ND	ND	ND	1 (50%)
Rifampin	ND	ND	ND	ND	ND	ND	0 (00.0%)
Vancomycin	ND	ND	ND	ND	ND	ND	0 (00.0%)
Tetracycline	ND	ND	ND	ND	ND	ND	0 (00.0%)
Tigecycline	ND	ND	ND	ND	ND	ND	0 (00.0%)
Linezolid	ND	ND	ND	ND	ND	ND	0 (00.0%)
Teicoplanin	ND	ND	ND	ND	ND	ND	0 (00.0%)
Moxifloxacin	ND	ND	ND	ND	ND	ND	0 (00.0%)
Chloramphenicol	ND	ND	ND	ND	ND	ND	0 (00.0%)
(%) Total	1186 (46.7%)	208 (33.5%)	179 (33.1%)	744 (38.8%)	28 (51.9%)	51 (56.7%)	6 (16.7%)

Escherichia coli were the most causative agents of UTIs in patients frequenting Bushra Medical Laboratory, especially female patients, with nearly similar rates compared with other Libyan regions. Of the antimicrobial tested, we recommend Cefoperazone/Sulbactam, Ceftazidime, Amikacin and Norfloxacin as drug of choice to treatment of gram negative uropathogens, however, Vancomycin and Gentamicin showed excellent activity against Staphylococcus aureus isolates.

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