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Antimicrobial resistance pattern of bacteria isolated from patients with urinary tract infection in Tripoli city, Libya

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ABSTRACT

Urinary tract infections (UTIs) remain one of the most common bacterial infections worldwide diagnosed in outpatients as well as hospitalized patients. In last decades, the widespread uses of antibiotics has resulted in the appearance of pathogens with increasing resistance to antimicrobial agents among urinary tract pathogens all over the world.The aim of this study was to investigate the prevalence and antibiotic resistance patterns of bacterial pathogens isolated from patients with urinary tract infections (UTIs) at the red-crescent private clinic, Tripoli, Libya.This retrospective study was conducted at a private clinic in Tripoli city during February to May, 2016. Urine was collected from suspected cases of UTI patients. Identification of bacterial isolates was performed by conventional methods. Antimicrobial susceptibility testing of culture positive bacterial isolateswas done by disk diffusion method according to Clinical Laboratory Standard Institute guidelines (CLSI).A total number of 224 urine samples were collected of which 62 (27.7%) showed significant bacterial growth. The commonest bacterium isolated from the culture positive urine sample was Staphylococci (64.5%) followed by Escherichia coli (29.0%) and Klebsiella pneumonia (6.5%). K. pneumonia and E. coli were highly resistant (38.11%, 34.13%, respectively) than staphylococci spp (26.29%), and exhibit resistance to wide range of tested antimicrobials. Ciprofloxacin was the most (12.3% resistance) powerful antibiotic agent followed by Gentamycin (17.6% resistance) and Erythromycin (19.0% resistance) which affected 87.7%, 82.4% and 81.0% of tested UTI causative pathogens, respectively; on the other hand, Coamoxclav and Amoxicillin were the least (63.0% & 50.3% resistance, respectively) affected only 37.0% and 49.7% of tested isolates, respectively. The obtained findings emphasized the need for ongoing investigations to show the pattern of antibiotic resistance which can help clinicians in antibiotic prescription in their clinics.

INTRODUCTION

Urinary tract infections (UTIs) are one of the most common human bacterial infections encountered by clinicians in outpatients as well as in hospitalized patients, and can lead to high risk of mortality [1]. It is the second most common infections after respiratory tract infections. Globally, about 150 million people are diagnosed with UTI annually, resulting in at least 6 billion dollars in health care expenses [2,3].Studies have shown that UTIs in women are very common due to anatomical relationship of the urinary tract. In some cases, clinicians prefer to start prophylactic therapy before culture and sensitivity results are present [4,5].The incidence in women aged 20- 40 years ranges from 25 to 30% while in older women above 60 years of age, it ranges from 4 to 43% [6,7]. Pregnancy also makes the women more susceptible to the infection, with 20% of pregnant women reported to be affected with UTI and therefore admitted to obstetrical wards [8]. However, UTI can also affects men, especially if men were

having prostate enlargement and neurogenic bladder with advancing age [9]. It has been observed that 95% of UTIs are caused by a single bacterial species. Staphylococci, Klebsiella, E. coli, Pseudomonas, Proteus, and Enterococci species are more preponderance isolated bacteria from UTIs patients [10]. Escherichia coli is found to be the most frequently isolated bacteria from urine samples in both inpatients and outpatients of both males and females [11].

Although UTIs are commonly treated with empirical antibiotics, the irrational use of antibiotics as well as prescribing of antibiotics without proper antibiotic susceptibility testing has leda new form of infectious diseases caused by drugresistant bacteria [12,13]. This has made antibiotic choice for empirical and rational treatment very complicated [14]. Therefore, to ensure convenient therapy, current awareness of the organisms that cause UTIs and their antibiotic susceptibility is obligatory [15]. The aim of this study is to determine the types of bacteria isolated from the urinary tract infections and their resistance patterns in patients referred to red-crescent clinic, Tripoli, Libya.

METHODOLOGY

Specimen Collection and Bacterial Isolates

This retrospective study was carried out in the department of Medical laboratory at red-crescent private clinic in Tripoli city during the period of Feb 2016 to May 2016, and was approved by the ethical committee of department of pharmacy, university of Tripoli Alahlia, Libya. All the adult male and female patients with an age of more than 18 years attending outpatient and inpatient clinic with the clinical features of UTI were included for this study. A total of 204 urine samples were collected during this study period.The collected samples were firstly labelled with patient information including age, gender, and clinical symptoms, subsequently sent to the microbiological laboratory for isolation and identification of presence of any potential bacterial pathogen, in which samples were processed immediately within 30 min.

The samples were midstream urine specimen, catheterized urine samples, collected in sterile disposable container (approximately 15 ml) collected with standard procedure [16] and transported immediately to the laboratory. The urine samples were centrifuged at 3000 rpm for 15 minutes. Thereafter, the supernatant was discarded and one drop of the sediment was put onto the glass slide to examine the presence of significant pus cell in our samples. Samples lack of significant pus cell containing urine was discarded.The samples were cultured for isolation of the microbial agents of UTI on blood agar and MacConky agar media using calibrated sterile wire loop, and then incubated aerobically for 24 hours at 37°C.Cultures without any colony at the end of 24h incubation were further incubated for 48h.The isolates were then observed for bacterial growth using standard microbiological methods including Gram staining,colony morphology, growth on selective media, and conventional biochemical tests [17-18].

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed onMueller- Hinton agar using disk diffusion (Kirby Bauer's) technique according to the clinical and laboratory standards institute (CLSI) guidelinesto determine susceptibility ofUTIs agents using the following antimicrobial agents; ceftriaxone (30µg), cephalothin (30µg), Clindamycin (10µg), Amoxicillin (30 µg), Amoxiclav (30 µg), Ciprofloxacin (5 µg), Nitrofurantoin (300 µg), gentamicin (10µg), trimethoprim-sulfamethoxazole (25µg), Tetracycline (30µg), Erythromycin (15µg),cefotaxime (10µg) [19]. Data were entered and analyzed using SPSS version 22.0(SPSS Inc, Chicago, IL, USA). Discrete variables were expressed as percentagesand proportions were compared using the Chi-square test.

RESULTS

In this study, out the 224 urine samples tested,62 (27.7%) samples yielded bacterial growth while 162 (72.3%) were negative. The isolate yielding bacterial growth 50 (80.6%) urine cultures belonged to female samples and 12(19.4%) belonged to male patients (Table 1).Out of the positive urine cultures 7 (11.3%) were from indoor patients and rest 55 (88.7%) from outdoor patients. Gram-positive staphylococci constituted the most common bacteria isolated in both genders which accounts for 64.5% of the total positive isolates. The percentage of isolated bacteria were Staphylococci (64.5%), Escherichia coli (29.0%), and Klebsiella pneumonia (6.5%).

All isolated bacteria, in this study, showed increased resistance to several used antimicrobials. The isolated bacterial exhibited wide differences in their susceptibility to the tested antibiotics. Our results established that isolated Klebsiella

Table 1 : Frequency of bacterial agents isolated from urine specimens and their relation to sex in this study

Isolated bacteria	No (%)	Female (%)	Male (%)
Gram-negative bacilli			
Escherichia coli	18(29.0%)	16(25.8)	2(3.2%)
Klebsiella	4(6.5%)	4(6.5%)	-
Gram-positive cocci			
Staphylococci	40(64.5%)	30(48.3)	10(16.2%)
Total	62(100)	50 (80.6%)	12(19.4%)

Table 2 : Prevalence and Antimicrobial Resistance of bacterial agents isolated from urine specimens

Frequency and distribution of bacterial isolates		Percentage (%) of resistance to antimicrobial agent											
Isolates	**NO**	**CRO**	**CE**	**DA**	**AML**	**AMC**	**CIP**	**F**	**CN**	**SXT**	**TE**	**E**	**CXM**
Escherichia coli	18	11.1	22.2	33.3	60	85.7	20	33.3	25	44.4	25	0	50
Klebsiella	4	28	14.1	41.2	63.6	79.0	17	25	14.5	63.6	54.5	11.1	45.8
Staphylococci	40	40	36.8	28.5	18.7	25	0	35.7	13.3	25	21.4	46.1	25

Abbreviations: CRO, Ceftriaxone; CE, Cephalothin; DA, Clindamycin; AML, Amoxicillin; AMC,Coamoxclav; CIP, Ciprofloxacin; F, Nitrofurantoin; CN, Gentamycin; SXT, Sulfamethoxazole; TE, Tetracycline; E, Erythromycin; CXM; cefotaxime.

pneumonia subspecies, E. coli, and Staphylococci exhibit resistance (38.1%, 34.1% and 26.3%, respectively) to almost all tested antimicrobials. E. coli and K. pneumonia were sensitive to Ciprofloxacin and Erythromycin consecutively.The antimicrobial resistance pattern of Klebsiella pneumonia, E. coli, and Gram positive Staphylococci spp is shown in Table 2.

DISCUSSION

This study describes the distribution and antibiotic susceptibility pattern of microbial species isolated from patients with UTI at a diagnostic laboratory center in red-crescent clinic in Tripoli city. Moreover, we described the relationship between gender and isolated bacterial agents of UTI.The results of this study revealed that bacterial pathogens were isolated from 27.7% of the requested urine culture. This finding almost similar to previously published data [9, 20] however, this rate of prevalence is higher than that previously (13.9%) documented in a recent study conducted at Messalatacity, Libya [21], but it is lower than that previously reported [22].

Bacterial infection of the UTI is one of the most widespread infectious diseases worldwide [23]. Effective treatment of patients with UTIs commonly relies on the identification of the bacterial isolate and the proper selection of a suitable antibiotic [24].Urinary tract infections are remarkably common in women, and previous study reported that 40% to 50% of women will suffer from UTI during their lifetime, owing to anatomic and physical factors [25].The sex distribution of patients in our study is consistent with those in other previously reported studies, showing a statistically predominance of females (80.6% of the positive cultures) [26-28].

Regarding the most common UTI causative pathogens from patients enrolled in this study, gram-positive staphylococci was the most frequent causative agent of UTIs in 40 cases (64.5%) followed by E. coli in 18 cases (29%) and Klebsiella pneumonia in 4 cases (6.5%).In agreement to our findings, asimilar studies conducted at several poly clinics in Benghazi, Libya in 2017 and at antenatal clinics in Nigeria 2004 revealed that staphylococci was the most frequent bacteria accounting for 31.2% and 21.3% of the total culture positive isolates [29,30].However, many other studies around the world have been reported that the Gram-negative bacteria of E. coli and Klebsiella spp. being the most frequent organisms causing UTIs [21,22,31-33].

The resistance of microbes to antibiotic is a major clinical issue in treating infections worldwide. Resistance rates vary from country to country [34].In this study, all isolated bacteria showed increased resistance to wide range of used antibiotics. Our results established that isolated K. pneumonia and E. coli, were highly resistant (38.11%, 34.13%, respectively) than staphylococci spp (26.29%), and exhibit resistance to wide range of tested antimicrobials. E. coli and K. pneumoniae showed the highest percentage of resistance to Amoxicillin, Coamoxclav, Trimethoprim/Sulfamethoxazole, Tetracycline, and Cefotaxime. On the other hand, very low resistance was detected to antibiotics such as Ceftriaxone, Cephalothin, Clindamycin, Ciprofloxacin, Nitrofurantoin, Gentamycin, and Erythromycin. On contrary, the gram positive staphylococci shows high resistance rate to only Ceftriaxone and Erythromycin.

Considering our results, Ciprofloxacin was the most (12.3% resistance) powerful antibiotic agent followed by Gentamycin (17.6% resistance) and Erythromycin (19.0% resistance) which affected 87.7%, 82.4% and 81.0% of tested UTI causative pathogens, respectively; on the other hand, Coamoxclav and Amoxicillin were the least (63.0% &50.3% resistance, respectively) affected only 37.0% and 49.7% of tested isolates, respectively. The percentage of resistance effects of remaining antimicrobials were ordered as follow; Cephalothin (24.0%), Ceftriaxone (26.3%), Nitrofurantoin (31.0%), Tetracycline (33.3%), Clindamycin (34.0%), cefotaxime (40.0%), and Trimethoprim/Sulfamethoxazole (44.6%).This study is comparable with the results reported by Al-Mijalli et al. [26] who

revealed high level of resistance of uropathogens to Ampicillin, Amoxicillin, and many cephalosporin generations, while very low resistance was detected to antibiotics such as Ciprofloxacin and Trimethoprim/Sulfamethoxazole for all these isolates. Concerning the remarkable susceptibility of bacteria to Ciprofloxacin in our study, this findingwas similar to many other studies which declared high susceptibility of bacteria to Ciprofloxacin, nitrofurantionand other antibiotics [35-37].

In our study, the most effective antimicrobial agent reported was Ciprofloxacin and the least effective one was Coamoxclav, whileCephalothin, Ceftriaxone, Nitrofurantoin, Tetracycline, Clindamycin, cefotaxime, and Trimethoprim/Sulfamethoxazole were effective at different levels. In a study conducted by Tamalli et al [38] among pregnant patients at Al-khums, Libya revealed thatseveral bacterial isolated were highly sensitive to Ciprofloxacin, Norfloxacin,Nitrofurantoin, Ofloxacin, Cefotaxime, and Amikacin; however, higher degree of resistance was observed againstAmpicillin and Cotrimoxazole. Therefore, in developing countries like Libya, physicians advised to stop prescribing these agents as an empiric treatment for UTIs. Besides, they are also taken up directly from the pharmacies without doctors' prescription as self-medication is a common practice. Thus restrictions should be put on antibioticprescribing.

CONCLUSION

It is concluded that, females have a higher prevalence of UTI than males. Gram-positive staphylococci were the most common bacteria responsible for urinary tract infections followed by E. coli. It is quite alarming the increased resistance of almost all of the isolates included in this study to most of commonly used antimicrobials including Penicillins and several Cephalosporins. Antibiotics resistance among UTI causative pathogens is a remarkable health problem. Education of physician, pharmacists, and other healthcare workers on the health risks associated with this issue and on the usefulness of restrictive use of antibiotics will help in tackling such a problem. Furthermore, other surveillance studies on antimicrobial resistance will help to guide the clinical treatment of UTIs in Libya in the future.

CONFLICT OF INTEREST

Authors declared that there is no conflict of interest concerning this manuscript

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