Prevalence and antibiotic susceptibility patterns of bacteria causing urinary tract infections in Youssef Hospital Center: first report from Akkar governorate, North Lebanon

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Abstract

Background. Urinary tract infection (UTI) is common infection feature worldwide. Infected patients are usually treated empirically without culture or antibiotics susceptibility testing, and which may lead to increase antibiotic resistance level. This study aims to determine the prevalence and antibiotic susceptibility patterns of common uropathogenic bacteria isolated at Youssef Hospital Center, Akkar governorate, North of Lebanon.

Methods. Spot midstream of urine samples from 9662 patients with UTI symptoms who came for medical investigation to Youssef Hospital Center located in Akkar governorate. Urine specimens were collected in sterile plastic bottles. Culture, identification and antibiotic susceptibility testing were performed using conventional tools according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing.

Results. A total of 1009 bacterial uropathogens were isolated. *Escherichia coli* was most presented (72.5%) of all isolates, followed by *Klebsiella pneumoniae* (8.2%), *Enterococcus* spp. (5.5%), *Pseudomonas aeruginosa* (4.5%), *Proteus* spp. (3%), *Enterobacter* spp. (2%), *Staphylococcus aureus* (2%), *Streptococcus agalactiae* (1.6%), *Staphylococcus saprophyticus* (0.4%), *Acinetobacter baumannii* (0.2%) and *Providencia rettgeri* (0.1%). Moreover, the mean antibiotic resistance rates of isolates was relatively high, but comparable to previously published data in Lebanon.

Conclusion. To the best of our knowledge, this is the first investigation reporting epidemiological data regarding the prevalence and antibiotic susceptibility patterns of bacterial uropathogens isolated from patients in the Akkar governorate. Our data indicated the urgent need of a strategic plan to tackle antibiotic resistance, particularly in deprived regions with poor healthcare centers.

Keywords: Uropathogens, Epidemiology, Antibiotic susceptibility, Risk factors, Akkar governorate, North Lebanon.

Introduction

Urinary tract infections (UTIs) are the most common infections in humans that can affect any part of the urinary system including kidneys, ureters, bladder, or urethra. It is estimated that approximately 150 million cases occur yearly globally each year [1]. Clinically, UTI is categorized as uncomplicated or complicated cases. Uncomplicated UTIs occur in healthy individuals and usually can be cured within 3 days of treatment. These infections are more common causing cystitis (lower UTI) and pyelonephritis (upper UTI) [2,3]. Numerous risk factors can increase the likelihood of developing a UTI, including female gender, sexual intercourse, poor personal hygiene, diabetes, obesity, vaginal infections and heavy use of antibiotics [4]. In contrast, complicated UTIs are found in an individual with factors that compromise the urinary tract or immune system, such as immunosuppression, renal failure, renal transplantation, pregnancy, problems of emptying the bladder completely, kidney stones and the presence of foreign bodies particularly indwelling catheters [5]. Additionally, this type of UTI requires longer periods of antibiotic treatment [6].

The leading pathogens of UTIs are caused mainly by Gram-negative bacteria including uropathogenic

Escherichia coli (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Proteus mirabilis* and *Pseudomonas aeruginosa* (*P. aeruginosa*). Gram-positive bacteria like *S. aureus*, *Staphylococcus saprophyticus* (*S. saprophyticus*), *Enterococcus faecalis* (*E. faecalis*) and *Streptococcus agalactiae* (*S. agalactiae*), and yeasts like *Candida* spp. are also an important cause of UTIs [1].

Recently, The Center for Disease Control and Prevention (CDC) declared that the human race is now in the post-antibiotic era [7]. The World Health Organization (WHO) also warned that the antibiotic resistance crisis is becoming serious all around the world [7]. In the past decade in Lebanon, several recent studies documented the occurrence of high rates of antibiotic resistant bacteria [8-21]. However, no data was reported from the Akkar governorate located in North of Lebanon. Akkar covers 788 km² of territory and is one of the most deprived regions in Lebanon. It shows all the typical features of a poor and relatively isolated rural community, with bad infrastructure and low quality of education and health services.

For this reason, we conducted this investigation in order to determine for the first time the prevalence of bacterial pathogens causing UTIs and their antibiotic susceptibility profiles of isolates from pa-

tients in Youssef hospital center in the Akkar governorate, North of Lebanon.

Material and Methods

This study was conducted in the clinical microbiology laboratory of Youssef hospital center in the Akkar governorate during the period from September 2015 to January 2017. This hospital created in 1995, contains 120 beds available to deliver high-quality patient care in one center. Spot midstream of urine samples were collected from 9662 patients presenting with UTI symptoms. The sterile urine specimens transported immediately to the microbiology department. The count of white bloodcells (WBC) was carried out on centrifuged urine specimens using routine microscopy. Culture of uncentrifuged specimens was performed using a calibrated loop (10 µl) on UriSelect™ 4 agar (Bio-Rad®, France) after an incubation for 18 to 24 hours at 35°C. The bacterial identification was performed according to the manufacturer's recommended procedures and through the use of API-20E and API-20NE (bioMérieux® - France), Pastorex™ Staph Plus kit (Bio-Rad®, France), and Pastorex™ Strep kits (Bio-Rad®, France).

The antibiotic susceptibility testing was performed by the disk diffusion method on Mueller Hinton agar for *Enterobacteriaceae* and non-*Enterobacteriaceae* Gram negative rods, *Staphylococcus* spp., *Enterococcus* spp. (Bio-Rad®, France), and on Mueller Hinton agar supplemented with 5% blood and 20 mg/l β-NAD (MHF) for *S. agalactiae* according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

In addition, statistical analyses were performed with GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA) using the Fisher's exact test to explore the association between gender and infection, and between the sex ratio (male/female) and the age of infected patients (<70 years and ≥70 years). The general significance level was set at a P-value below 0.05.

Results

A total of 9662 consecutive urine samples were included in our study. Of these, 8653 (89.6%) were negative for the growth of bacteria or showed insignificant growth, and 1009 (10.4%) were positive for significant bacterial growth. Among positive samples, 244 belonged to males and 765 belonged to females, ranging in age from 1 month to 97 years, with a mean age of 47.5 years. All the patients came from the Akkar governorate. Moreover, the positive patients had more than 200 white blood cells/mm³ in their urine specimens as determined by routine microscopy. *E. coli* caused the highest infection rate (72.5%), followed by *K. pneumoniae* (8.2%), *Enterococcus* spp. (5.5%), *P. aeruginosa* (4.5%), *Proteus* spp. (3%), *Enterobacter* spp. (2%), *S. aureus* (2%), *S. agalactiae* (1.6%), *S. saprophyticus* (0.4%), *Acinetobacter baumannii* (*A. baumannii*) (0.2%) and *Providencia rettgeri* (*P. rettgeri*) (0.1%).

Tables 1 and 2 express distribution of the major bacterial isolates based age and sex of patients, respectively. The mean sex ratio (male/female) of infection was 0.32. The sex ratio (male/female) of urine infections showed the lower rates among the children and younger populations and the higher rates among older populations, particularly in patients aged more than 70 years (**Figure 1**). In addition, according to EUCAST guidelines, the mean percentages of susceptibility of Gram-negative and Gram-positive isolates are showed in **Table 3, 4 and 5**, respectively.

Discussion

To our best knowledge, the present study represents the first investigation from a hospital in the Akkar governorate, North of Lebanon. In addition, no data is available in the literature regarding to the distribution of bacterial uropathogens and their antibiotic resistance patterns in this region. Hence, this investigation provides important labo-

Table 1. Distribution of the isolated bacterial strains according to the age of patients

Age class	Sex ratio (male/ female)	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>Proteus</i> spp.	<i>Enterobacter</i> spp.	<i>P. rettgeri</i>	<i>P. aeruginosa</i>	<i>A. baumannii</i>	<i>Enterococcus</i> spp.	<i>S. aureus</i>	<i>S. saprophyticus</i>	<i>S. agalactiae</i>
[0-10]	0.21	139	7	6	3	0	6	1	13	1	1	0
[10-20]	0.12	21	1	2	1	0	0	0	1	2	0	0
[20-30]	0.11	73	15	4	3	0	9	0	6	8	0	3
[30-40]	0.09	59	7	4	0	0	4	0	7	1	1	2
[40-50]	0.40	51	3	1	2	0	10	0	0	1	0	2
[50-60]	0.35	74	8	1	2	0	4	0	6	2	0	3
[60-70]	0.35	99	14	2	2	0	2	0	6	1	0	2
[70-80]	0.55	120	15	4	7	0	4	0	10	1	1	2
[80-90]	0.69	79	11	4	0	1	3	1	5	3	1	2
[90-100]	0.65	17	2	2	0	0	3	0	2	0	0	0
Total	0.32	732	83	30	20	1	45	2	56	20	4	16

Table 2. Distribution of the isolated bacterial strains according to the sex of patients and inpatient/ outpatient status

Bacteria	Male	Female	Sex ratio (male/ female)	Inpatient status	Outpatient status	Total (%)
<i>E. coli</i>	154	578	0.27	475	257	732 (72.5%)
<i>K. pneumoniae</i>	17	66	0.26	62	21	83 (8.2%)
<i>Proteus</i> spp.	10	20	0.5	13	17	30 (3%)
<i>Enterobacter</i> spp.	5	15	0.33	15	5	20 (2%)
<i>P. aeruginosa</i>	25	20	1.25	33	12	45 (4.5%)
<i>Enterococcus</i> spp.	21	35	0.6	30	26	56 (5.5%)
<i>S. aureus</i>	5	15	0.33	9	11	20 (2%)
<i>S. agalactiae</i>	4	12	0.33	9	7	16 (1.6%)
<i>Others</i>	3	4	0.75	4	3	7 (0.7%)
Total	244	765	0.32	650	359	1009

Figure 1. The variation of the sex ratio (male/female) according to the age of infected patients.

ratory data that could support national surveillance of antibiotic resistance and will improve treatment recommendations in this geographical region of our country. Overall, a total of 1009 isolates were collected from Akkari patients. The majority of urine infection cases were found in female (765/1009). A significant gender difference in the prevalence of the infection was found (OR: 3.5 CI: 3.0–4.1, $P < 0.0001$) as reported in other study [1]. Women, particularly sexually active and aged between 16 and 64 years have significantly more risk to experience UTIs than men because they have a shorter urethra [22]. However, UTIs often occur in both genders and across all age groups, including elderly population [23]. In the same context, the sex ratio (male/female) has significantly increased with age, particularly in patients older than 70 years (OR: 2.6 CI: 1.9–3.5, $P < 0.0001$). Older adults are associated with high burden of UTI and need improvement in diagnosis and treatment of their infections. According to our laboratory records, *E. coli* was the most common pathogen causing UTIs, followed by other well-known uropathogens; *K. pneumoniae*, *Proteus*

spp, *Enterobacter* spp. and *P. aeruginosa* (Table 3) and these results are comparable to other studies from various countries [22,24]. No significant difference was found concerning the distribution of uropathogens according to age of patients. All types of bacterial pathogens were found in both females and males. However, out of all *P. aeruginosa* isolates, 13 (28.9%) were from hospitalized patients. This organism is becoming an important cause of UTI associated with hospital-acquired UTIs [1].

Furthermore, this investigation showed a relatively high antibiotic resistance rates among the majority of uropathogens isolated in our hospital (Tables 3-5). *E. coli* isolates showed high level of resistance to commonly used empirical antibiotics such as beta-lactams, quinolones and trimethoprim/sulfamethoxazole. In the present study, 35.7% of *E. coli* isolates were ESBL-producers. Our findings showed similar percentages of resistance to antibiotics described in previous studies conducted in Lebanon. Numerous recent investigations found a high prevalence of ESBL-producing *E. coli* [8,15,25]. Moreover, we found that resistance to ertapenem, imipenem and

Table 3. Percentage of antibiotic susceptibility of major Gram-negative bacilli isolates.

	<i>E.coli</i> N=732 (%)	<i>K. pneumoniae</i> N=83 (%)	<i>Proteus spp.</i> N=30 (%)	<i>Enterobacter spp.</i> N=20 (%)	<i>P. aeruginosa</i> N=45 (%)
AMP	26.2	0	30	0	-
AMC	79.6	89.2	83.3	0	-
TIC	29.2	0	40	85	64.4
TCC	82.0	86.7	93.3	90	64.4
PIP	29.2	0	40	85	64.4
PPT	90.7	90.4	93.3	95	64.4
CFX	50.5	68.7	70	0	-
CXM	53.3	68.7	70	85	-
FOX	84.2	95.2	83.3	0	-
CFM	57.9	71.1	80	90	-
CTX	60.1	71.1	83.3	90	-
CAZ	63.3	73.5	83.3	90	71.1
FEP	68.6	75.9	90	95	71.1
ATM	62.7	72.3	90	90	-
ERT	98.9	98.8	100	95	-
IMP	99.6	100	100	100	60
MEM	99.6	100	100	100	60
GMN	80.1	77.1	66.7	100	93.3
TMN	73.4	74.7	63.3	95	93.3
NET	99.6	98.8	76.7	100	95.6
AKN	99.3	100	90	100	93.3
NA	45.5	71.1	46.7	90	-
NXM	60.5	74.7	53.3	95	-
CIP	61.7	74.7	56.7	95	53.3
LVX	61.7	79.5	56.7	95	53.3
TGC	99.9	-	-	-	-
CS	100	100	0	100	100
FSF	98.9	92.8	66.7	75	88.9
FUR	99.3	89.2	0	85	-
SXT	55.7	55.4	43.3	80	-

AMP, ampicillin (10 µg); AMC, amoxicillin/clavulanate (20/10 µg); TIC, ticarcillin (75 µg); TCC, ticarcillin/clavulanate (75/10 µg); PIP, piperacillin (30 µg); PPT, piperacillin/tazobactam (30/4 µg); ATM, aztreonam (30 µg); IMP, imipenem (10 µg); MEM, meropenem (10 µg); ERT, ertapenem (10 µg); CFX, cefalexin (30 µg); CXM, cefuroxime (30 µg); FOX, cefoxitin (30 µg); CFM, cefixim (5 µg); CTX, cefotaxime (5 µg); FEP, cefepime (30 µg); CAZ, ceftazidime (30 µg); GMN, gentamicin (10 µg); TMN, tobramycin (10 µg); AKN, amikacin (30 µg); NET, netilmicin (10 µg); NA, nalidixic acid (30µg); NXM, norfloxacin (10µg); CIP, ciprofloxacin (5 µg); LVX, levofloxacin (5µg); TGC, tigecycline (15 µg); CS, colistin (50 µg); FSF, fosfomycin (200 µg); FUR, nitrofurantoin (100 µg); SXT, trimethoprim/sulfamethoxazole (1,25/23,75 µg).

Table 4. Percentage of antibiotic susceptibility of ESBL-producing isolates.

	<i>E. coli</i> N=261 (%)	<i>K. pneumoniae</i> N=23 (%)	<i>Proteus spp.</i> N=4 (%)
AMP	0	0	0
AMC	82.4	95.7	100
TIC	0	0	0
TCC	75.1	82.6	100
PIP	0	0	0
PPT	88.9	95.7	100
CFX	0	0	0
CXM	0	0	0
FOX	85.4	95.7	100
CFM	2.7	4.3	25
CTX	1.9	0	0
CAZ	13	13	25
FEP	16.9	17.4	25
ATM	8	8.7	25
ERT	100	100	100
IMP	100	100	100
MEM	100	100	100
GMN	58.6	34.8	25
TMN	43.3	21.7	25
NET	99.6	100	25
AKN	99.6	100	25
NA	20.3	30.4	25
NXM	34.5	34.7	25
CIP	35.2	34.7	25
LVX	35.2	47.8	25
TGC	100	-	-
CS	100	100	0
FSF	98.1	87	50
FUR	100	91.3	0
SXT	36	8.7	0

meropenem was about 1.1%, 0.4% and 0.4%, respectively. Several recent studies reported the emergence carbapenem-resistance strains in North of Lebanon, mainly by OXA-48 production [25-30]. Gentamicin and ciprofloxacin resistance were about 19.9% and 38.3%, respectively, which are in the range of previous community based studies in Lebanon [8,15,18,25]. Regarding to the other isolated

Enterobacteriaceae, the resistance rate was lower than that observed in *E. coli* isolated isolates, except with *Proteus* spp. which had the highest resistance rate between isolates of *Enterobacteriaceae* to fluoroquinolones, with 46.7% to norfloxacin, 43.3% to ciprofloxacin and 43.3% to levofloxacin. Furthermore, this study showed a trend of increasing resistance in *P. aeruginosa* isolates to all commonly used antibiotic agents. *P. aeruginosa* resistance to carbapenem and fluoroquinolones was 40% and 46.7%, higher than the previous rates of (8.7% to 28.5% and 8.7 to 20%, respectively) which was reported in other studies [8,13]. The rise in carbapenem resistance could be the result of increasing use of carbapenem and which is associated with spread of ESBL-producing strains in the last few years in Lebanon [8]. In the same context, the increase of fluoroquinolone resistance could be explained by the misuse and overuse of this class of antibiotics in the Middle East region.

Among Gram-positive cocci isolates, *Enterococcus* spp. was the predominant, followed by *S. aureus*, *S. agalactiae*, and *S. saprophyticus* (Table 5). The susceptibility of *Enterococcus* spp. to ampicillin and imipenem was equal to 73.2%. High level of resistance was recorded to trimethoprim/sulfamethoxazole, tetracycline, ampicillin, imipenem, erythromycin and fluoroquinolones (Table 5). No vancomycin-resistant enterococci was observed in our study. During the last decade, limited studies were done dealing with the identification of *Enterococcus* spp. circulating in Lebanon and their antibiotic resistance patterns. A recent study in Lebanon has showed that among 53 *Enterococcus* spp. isolates, *E. faecalis* was predominant (72.5%), followed by *Enterococcus faecium* (22.9%), *Enterococcus avium* (3.3%) and *Enterococcus gallinarum* (1.3%) [31].

With regard to *S. aureus*, the clinical significance of a positive urine culture for this pathogen is still unclear. Recent studies have reported that *S. aureus* can cause approximately 0.5 to 6% of UTIs [32]. The prevalence of methicillin-resistant *S. aureus*

Table 5. Percentage of antibiotic susceptibility of major Gram-positive cocci isolates.

	<i>S. aureus</i> N=20 (%)	<i>Enterococcus</i> spp. N=56 (%)	<i>S. agalactiae</i> N=16 (%)
PG	10	-	100
AMP	-	73.2	-
FOX	70	-	-
IMP	-	73.2	-
VAN	100	100	100
TEI	100	100	100
FSF	90	89.3	-
E	60	35.7	68.8
CD	95	-	75
PTN	100	-	100
TET	75	15.4	25
TGC	100	100	100
GMN	85	73.2	87.5
LZD	100	100	100
FUS	100	-	-
NXM	85	64.3	87.5
LVX	85	64.3	87.5
RIF	100	60.7	93.8
SXT	95	10.7	43.8
FUR	100	96.4	100

PG, penicillin G (1 unit); AMP, ampicillin (2 µg); FOX, cefoxitin (30 µg); IMP, imipenem (10 µg); GMN, gentamicin (10 µg for *S. aureus*; 30 µg for *Enterococcus* spp. and 500 µg for *S. agalactiae*); TET, tetracycline (30 µg); TGC, tigecycline (15 µg); E, erythromycin (15 µg); CD, clindamycin (2 µg); PTN, pristinamycin (15 µg); LZD, linezolid (10 µg); TEI, teicoplanin (30 µg); VAN, vancomycin (5 µg); NXM, norfloxacin (10 µg); LVX, levofloxacin (5 µg); FUS, fusidic acid (10 µg); FSF, fosfomycin (200 µg); RIF, Rifampicin (5 µg); SXT, trimethoprim/sulfamethoxazole (1,25/23,75 µg); FUR, nitrofurantoin (100 µg).

(MRSA), based on cefoxitin resistance was higher than that reported in nasal secretions of healthy school children in Tripoli, north of Lebanon [33]. In addition, Chamoun *et al.* [8] reported an increase in the prevalence of MRSA between 2011 and 2013 in Lebanese hospitals. Therefore, there is urgent

need to control and prevent transmission of MRSA infections in hospitalized patients. Contamination through MRSA frequently occurs by direct skin-to-skin contact or shared items and is often associated with poor hygiene practices in hospitals [34]. However, all *S. aureus* isolates in our study were susceptible to vancomycin, teicoplanin and linezolid. Concerning *S. agalactiae* isolates, 100% susceptibility rate was observed to penicillin, vancomycin, teicoplanin, pristinamycin, tigecycline and nitrofurantoin, and high susceptibility rates were demonstrated to fluoroquinolones and rifampicin, respectively (**Table 5**). High levels of resistance were also recorded to tetracycline, trimethoprim/sulfamethoxazole, and less to erythromycin and clindamycin (**Table 5**). These findings are higher than the respective rates reported previously in our country [31].

Overall, our data indicate that antibiotic resistance rates of bacterial uropathogens are relatively high in the Akkari patients, and are much comparable to previous community based studies conducted in Lebanon. The increase in antibiotic resistance rates is now becoming a major problem in the Akkar Governorate as well as in the whole country. High resistant rates among uropathogenic bacteria will certainly contribute to antibiotic selective pressures, maintain and spread of resistant strains in our community. Additionally, antibiotic selective pressure is usually linked to high spread of counterfeit medicines, misuse and overuse of antibiotics.

In conclusion, a national strategic plan is urgently needed to combat antibiotic resistance in all regions of Lebanon. A good antibiotic stewardship plan should aim to ensure the appropriate use of antibiotics and reduce the risk of infection with drug-resistant organisms.

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Competing Interests

The authors declare that they have no competing interests.

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