Antibiogram of the Common Uropathogenic Bacteria among Yemeni Patients in Sana'a City: A Recent Report

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Authors' contributions

This work was carried out in collaboration between all authors. Author HA designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors TKA and MB managed the analyses of the study. Author TA performed the statistical analysis and managed the literature searches. All authors read and approved the final manuscript.

Article Information

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ABSTRACT

Aims: This study aimed to identify the prevalence and antimicrobial susceptibility of the commonly isolated uropathogens in Sana'a city, Yemen.
Study Design: A cross-sectional and descriptive study.
Place and Duration of Study: The study was carried out at the hospitals and clinics of Sana'a city, Yemen between October 2016 and March 2017.
Methodology: Clean-catch mid-stream urine samples were collected to detect the most common uropathogenic bacteria and their antibiotic susceptibility using Kirby Bauer standardized method.

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Results: Urine cultures yielded 170 significant bacterial growths of uropathogens. *Escherichia coli* was the most often isolated pathogen (43.5%), followed by *Klebsiella pneumoniae* (24.7%), *Pseudomonas aeruginosa* (20.0%) and *Staphylococcus aureus* (11.8%). The overall sensitivity was high to an excellent pattern for Carbapenems, Nitrofurantoin, Amikacin, and Piperacillin-Tazobactam. *Escherichia coli* shows an excellent sensitivity (88%) for Nitrofurantoin and Imipenem, followed by (85%) Ertapenem. *Pseudomonas aeruginosa* exhibited moderate resistance to Carbapenems, Moxifloxacin, and Piperacillin-Tazobactam in this study. *Staphylococcus aureus* was more vulnerable to all Quinolones except Nalidixic acid and it displays a high sensitivity pattern, 90% for both Nitrofurantoin and Gentamicin, 83% for Penicillin, 80% for both Minocycline. Antibiogram of isolated organisms revealed that there was resistance to two and more antimicrobials.
Conclusion: In this study, we observe a high resistance rates to Beta-lactam, Quinolones, and Macrolides antibiotics. Nevertheless, most uropathogenic isolates were still sensitive to Nitrofurantoin, Imipenem, Ertapenem, and Amikacin, they considered as a proper antibiotics for empirical therapy of UTIs. Establishment of antibiogram of locally isolated organisms is necessary to avoid indiscriminating use of antibiotic and to decrease the resistance rate in our community.

1. INTRODUCTION

Urinary tract infections (UTIs) are one of the most common human diseases caused by bacterial infections faced by clinicians in developed as well as in developing countries, affecting all ages and gender groups [1]. Gram-negative bacteria, for instance, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa* are the major cause of UTI while *Staphylococcus aureus* represents the Gram-positive bacteria [2]. *Escherichia coli* still the most causative agent among all bacterial species particularity among female due to anatomical reasons, pregnancy, and sexual activities [3,4]. It turns out to be more common in older males because of the obstruction of the urinary system in case of prostatic hypertrophy [5]. Urinary tract infections range from mild asymptomatic to severe type of infection like pyelonephritis according to the presence of risk factors and immune status as well as the location of infection and the type of pathogenic organism [6].

One of the obstacles confronting the clinician of UTIs is the antibiotic resistance that increases dramatically [2]. This public health problem remains of cosmopolitan distribution especially in developing countries like ours [4,5]. Illogical and haphazard use of antibiotics besides fake drugs is common in these countries [6]. Previous studies noticed the tendencies of antibiotic susceptibility change patterns [6,7]. The antibiogram is an intervallic summary of antibiotic susceptibilities of local bacterial isolates submitted to the clinical microbiology laboratory which, used by doctors to judge local susceptibility degrees, as a help in selecting an empiric antibiotic treatment, and in observing resistance tendencies over time within an organization [8].

In Yemen, few studies that mainly focused on specific groups as pregnant women have been carried out on the occurrence and antimicrobial resistance patterns of UTIs [9–15]. The significance of this study is to rationalize the use of antibiotics based on the proper selection of empirical for UTI treatment, hence to avoid spreading of the resistant strains, reducing the complications and additional costs among the community and each patient. Continuous checking of the susceptibility pattern is of vital importance [4]. In addition, this can enhance the therapeutic guidelines development at the local level [8]. Therefore, the recent study aimed to identify the prevalence and antimicrobial susceptibility of the commonly isolated uropathogens in Sana'a city, Yemen.

2. MATERIALS AND METHODS

2.1 Study Design

Cross-sectional and descriptive study.

2.2 Location, Target Population, Study Period & Ethics

This study was conducted, among patients presenting with UTI and have significant bacteriuria, on bacterial isolated from urine

cultures that referred for routine microbiological examining along with clinician orders in the hospitals or clinics of Sana'a city, Yemen during October 2016 to March 2017. Related information for all selected isolates retrieved from request forms and medical records. Consent to do this study was achieved from the management of Sana'a city hospitals and clinics. All of these specimens were a part of the routine diagnosis. We consider all related ethics.

2.3 Target Pathogenic Bacteria

All common uropathogens isolated from urine specimens were undergone complete microbial investigation during the period of the study, beginning with pyuria and bacteriuria detection microscopically then culturing, full identification and antibiotic susceptibility testing according to the standardized technique. Briefly, 40 µl of urine specimens were inoculated aseptically using a calibrated wire loop on Cystine Lactose Electrolyte-Deficient medium, Blood agar and MacConkey agar (Oxoid, Basingstoke, UK) and incubated at 37°C, aerobically overnight [16–18]. Only aerobic and/or facultative anaerobic Gram-negative *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and Gram-positive *Staphylococcus aureus* were included in this study. Single urine sample yielded a significant growth was obtained from each patient was included in this study.

2.4 Methods

2.4.1 Laboratory identification of isolates

A total of 170 microorganisms were isolated, identified and antimicrobial susceptibility tested from midstream clean-catch urine specimens during the five months of study period using standard microbiological techniques. Confirmation of the species level was done by using API 20 E diagnostic system (BioMerieux, France) and according to manufacturer directions [2].

2.4.2 Antimicrobial susceptibility test

After complete identification using standard microbiological technique was done for all isolates, their antibiotic susceptibility pattern was detected on Muller Hinton agar (Oxoid, UK), by disk diffusion method according to the Kirby–Bauer method [2]. Thirty antimicrobial agents were tested and standardized according to the MIC break-points recommended by the National

Committee for Clinical Laboratory Standards with CLSI guidelines [16,17] susceptibility was defined as sensitive and resistant based on the diameter of the zone of inhibition. Using the following antimicrobial drugs; Penicillin 10 U, Ampicillin 10 µg, Amoxicillin 10 µg, Piperacillin 100 µg, Ticarcillin 75 µg, Carbenicillin 100 µ, Amoxiclav 20/10 µg, Piperacillin-Tazobactum 100+10 µg, Aztreonam 30 µg, Imipenem 10 µg, Ertapenem 10 µg, Cefradine 30 µg, Cefazolin 30 µg, Cephalothin 30 µg, Cefaclor 30 µg, Cephalexin 30 µg, Cefpirome 30 µg, Cefuroxime 30 µg, Ceftizoxime 10 µg, Cefoxitin 30 µg, Cefotaxime 30 µg, Ceftazidime 30 µg, Cefepime 30 µg, Nalidixic acid 30 µg, Nitrofurantoin 300 µg, Ciprofloxacin 5 µg, Norfloxacin 10 µg, Ofloxacin 5 µg, Pefloxacin 5 µg, Lomefloxacin 10 µg, Levofloxacin 5 µg, Moxifloxacin 5 µg, Erythromycin 15 µg, Azithromycin 15 µg, Roxithromycin 15 µg, Amikacin 30 µg, Kanamycin 30 µg, Gentamicin 10 µg, Tobramycin 10 µg, Tetracycline 30 µg, Minocycline 30 µg, Doxycyline30 µg, Co-trimoxazole 25 µg, Chloramphenicol 30 µg. Oxoid Ltd. Hampshire, United Kingdom, manufactured all the antibiotic discs used in this study.

2.5 Statistical Analysis

A bacterial resistance pattern of all uropathogenic isolates was determined and expressed as frequencies with percent. Descriptive statistics of antibiotics activity and other characteristics of the isolated bacterial population were calculated. The Statistical Package for Social Sciences (SPSS) software package version 22 did all the statistical analysis. (SPSS Inc. Chicago, Illinois, USAT).

3. RESULTS AND DISCUSSION

Distribution of the uropathogenic isolates is presented in Table 1. One hundred and seventy urine samples yielded significant bacterial growth was obtained from Yemeni patients predicted of having UTI, according to clinical presentation and laboratory findings, during 5 months study period. Gram-negative isolates were 150 (88.2%), whereas Gram-positive isolates were 20 (11.8%). Overall isolates the *Escherichia coli* was the predominant one 74 (43.5%), followed by *Klebsiella pneumoniae* 42 (24.7%) and none fermenting Gram-negative bacteria *Pseudomonas aeruginosa* 34 (20.0%). The last prevalence rate was Coagulase positive *Staphylococcus aureus* 22 (11.8%).

Table 1. Frequency and percentage of the resistant pattern of commonly isolated UTI pathogens

Antibiotics classes	Antibiotic sub-classes	*E. coli* (N=74)		*K. pneumoniae* (N=42)		*P. aeruginosa* (N=34)		*S. aureus* (N=20)		Total (N=170)	
		N	%	N	%	N	%	N	%	N	%
Penicillin	Penicillin	73	98.6	42	100.0	34	100.0	14	70.0	163	95.9
	Ampicillin	72	97.3	41	97.6	31	91.2	9	45.0	153	90.0
	Amoxicillin	68	91.9	40	95.2	33	971.	8	40.0	149	87.6
	Aztreonam	44	59.5	29	69.0	16	47.1	NT	0.0	89	52.4
	Ticarcillin	61	82.4	36	85.7	31	91.2	NT	0.0	128	75.3
	Carpanicillin	61	82.4	36	85.7	25	73.5	7	35.0	129	75.9
	Pipracillin	43	58.1	37	88.1	30	88.1	NT	0.0	110	64.7
Beta-lactam inhibitors	Piperacillin-Tazobactam	15	20.3	19	45.2	14	41.2	7	35.0	55	32.4
	Amoxiclave	37	50.0	26	61.9	24	70.6	8	40.0	95	55.9
Carbapenems	Imipenem	9	12.2	6	14.3	14	41.2	NT	0.0	29	17.1
	Ertapenem	11	14.9	10	23.8	17	50.0	12	60	50	29.3
Cephalosporins	Cefradine	66	89.2	41	97.6	34	100.0	15	75.0	156	91.8
	Cefazolin	59	97.7	41	97.6	31	91.2	13	65.0	144	84.7
	Cephalexin	64	86.5	40	95.2	31	91.2	13	65.0	148	87.1
	Cephalothin	63	85.1	38	90.0	31	91.2	13	65.0	145	95.3
	Cefaclor	61	82.4	39	92.9	31	91.2	10	50.0	141	82.9
	Cefpirome	35	47.3	26	61.9	28	82.4	8	40.0	97	57.1
	Cefuroxime	60	81.1	32	76.2	29	85.3	11	55.0	132	77.6
	Cefoxitin	57	77.0	31	73.8	28	82.4	10	50.0	126	74.1
	Ceftizoxime	51	68.9	33	78.6	28	82.4	14	70.0	126	74.1
	Cefotaxime	57	77.0	32	76.2	30	88.2	8	40.0	127	74.7
	Ceftazidime	62	83.8	35	83.3	32	94.1	8	40.0	137	80.6
	Cefepime	35	47.3	26	61.9	28	82.4	8	40.0	97	57.1
Quinolones	Nalidixic acid	55	74.3	40	95.2	33	97.1	14	70.0	142	83.5
	Ciprofloxacin	45	60.8	37	88.1	26	76.5	9	45.0	117	68.8
	Norfloxacin	46	62.2	36	85.7	24	70.6	9	45.0	115	67.6
	Ofloxacin	49	66.2	30	31.4	22	64.7	7	35.0	108	63.5
	Pefloxacin	40	54.1	26	61.9	30	88.2	10	50.0	106	62.4
	Lemefloxacin	50	67.6	30	71.4	25	73.5	5	25.0	110	64.7
	Levofloxacin	29	39.2	28	66.7	28	82.4	8	40.0	93	54.7
	Moxifloxacin	34	45.9	24	57.1	15	44.4	4	20.0	77	45.3
Macrolides	Erythromycin	71	95.9	40	95.2	30	88.2	11	55.0	152	89.4
	Azithromycin	48	64.9	21	50.0	24	70.6	18	90.0	111	65.3

Antibiotics classes	Antibiotic sub-classes	*E. coli* (N=74)		*K. pneumoniae* (N=42)		*P. aeruginosa* (N=34)		*S. aureus* (N=20)		Total (N=170)	
		N	%	N	%	N	%	N	%	N	%
	Roxithromycin	73	98.6	41	97.6	32	94.1	16	80.0	162	95.3
Aminoglycosides	Amikacin	17	23.0	10	23.8	20	58.8	9	45.0	56	32.9
	Kanamycin	38	51.4	24	57.1	27	79.4	13	56.0	102	64.4
	Gentamicin	32	53.2	20	47.6	22	64.7	2	10.0	76	44.7
	Tobramycin	56	75.7	28	66.7	23	67.6	13	65.0	120	70.6
Tetracycline	Tetracycline	56	75.7	33	78.6	28	62.4	13	65.0	130	76.5
	Minocycline	30	40.5	22	52.4	22	64.7	4	20.0	78	45.9
	Doxycyline	43	58.1	29	69.0	20	58.8	6	30.0	98	57.6
Others	Cotrimoxazole	39	52.7	33	78.6	25	73.5	10	50.0	107	62.9
	Chloramphenicol	21	28.4	21	50.0	17	50.0	9	45.0	68	40.0
	Nitrofurantoin	9	12.2	13	31.0	23	67.6	2	10.0	47	27.6

E. coli: Escherichia coli, K. pneumoniae: Klebsiella pneumoniae, P.aeruginosa: Pseudomonas aeruginosa, S. aureus: Staphylococcus aureus N: number, Nt: Not tested.

The antibiotic resistance pattern of Gram-negative bacilli and Gram‑positive coccus is shown in Tables 1. The overall sensitivity was high to an excellent pattern for Carbapenems, Nitrofurantoin, Amikacin and Piperacillin-Tazobactam. Among Quinolones Gram-negative bacilli, isolates showed a high resistance rate range (74.3% to 97.1%) to Nalidixic acid, and reach to 88.1% for Ciprofloxacin, and Norfloxacin. On other hand, a good sensitivity about (67%, 61% and 56%) for *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa* was observed against Ofloxacin, Levofloxacin and Moxifloxacin. *Staphylococcus aureus* was more vulnerable to all Quinolones except Nalidixic acid. *Escherichia coli* shows an excellent sensitivity (88%) for Nitrofurantoin and Imipenem, followed by (85%) Ertapenem, and moderate to high sensitivity (72%, 77% and 80%) for Chloramphenicol, Amikacin and Piperacillin-Tazobactam respectively. Similar to *Escherichia coli* findings were seen for *Klebsiella pneumoniae* sensitivity in relation to Carbapenems and Amikacin. However, *Pseudomonas aeruginosa* expresses a high resistant pattern against almost all examined antibiotics except for Carbapenems, Piperacillin-Tazobactam, Moxifloxacin, Amikacin, and Chloramphenicol that shows intermediate resistant pattern against them. On the other side, *Staphylococcus aureus* displays a high sensitivity pattern, 90% for both Nitrofurantoin and Gentamicin, 83% for Penicillin, 80% for both Minocycline and Moxifloxacin and 75% for Lemefloxacin and a very high resistant pattern (90%) was detected against Azithromycin.

UTI is the most common community and nosocomial infections and the second most common reason for empirical antibiotic treatment [19]. Continuous extensive surveys are required to eradicate the pathogen and to cut complicated consequences due to these infections [20].

In the present study, 170 urine samples yielded a significant growth of four species of bacteria. Gram-negative isolated bacteria in this study were more accountable for UTI than Gram-positive bacteria and this result is in agreement with the conclusions of earlier studies [1,3,6,9, 14,15,19,21–25,25]. Conversely, Kyabaggu, et al. [5] reported a high incidence if Gram-positive among UTI patients in Uganda compared to Gram-negative bacteria [5].

In this study, the most frequently isolated microorganism was *Escherichia coli* with a rate of (43.5%), followed by *Klebsiella pneumoniae* with (24.7%) and this result is consistency with other studies from other Arabic and non-Arabic countries across the world such Egypt, Sudan, Libya, Ethiopia, Nepal, India, China and Turkey [1,3,6,19,21–26,26–29]. Alkhyat and Al. Maqtari (2014) previously published similar finding to our result in Yemen [13]. On contrary regarding the second most common uropathogenic bacteria in Yemen, Gondos, et al. [14], on his study, of UTIs among renal transplants patients, and Mohanna, and Raja'a [9], in their study about urinary tract infection in children, were documented that *Staphylococcus saprophyticus* was the second most commonly isolated bacteria [9,14]. *Staphylococcus saprophyticus* and *Staphylococcus aureus* were also reported as the second uropathogenic other studies carried out in Bangladesh by Haque, Akter and, Salam [4] and in Nigeria by (Oli et al) [4,6]. The present result stated that *Pseudomonas aeruginosa* and *Staphylococcus aureus* were the subsequent predominant (20% and 11.8%) uropathogenic isolated bacteria after *Escherichia coli* and *Klebsiella pneumoniae*. Variance in identification techniques may affect the relative occurrence of bacteria, which makes a difficult comparison [30]. In addition to that, geographic variations were shown to make an impact on different results [2].

The current study also detects that Gram-negative bacteria were very good sensitivity to Carbapenems (mainly Imipenem) except for *Pseudomonas aeruginosa* that show an intermediate susceptibility which was consistent with many previously reported studies by Indian researchers [7,20,22,23]. This finding was confirmed also by recent Elzayat et at. an Egyptian researcher [29] and Yemini study by AlZubiery et al. [7] which declared that Carbapenems were highly active against Gram-negative bacteria which are highly resistant to the 1st, 2nd and slightly 3rd generations Cephalosporins and Penicillins. Therefore, doctors to be guided to discontinue prescription Cephalosporins and Penicillins as an empiric therapy for UTIs. The incidence of Carbapenems resistance is low in our situation in contrasting to increasing trends of Carbapenems resistance revealed lately by Sudanic investigator [19].

Concerning Nitrofurantoin, it has been recommended for the treatment of UTI that caused by MDR strains [31]. High sensitivity pattern (72.4%) among our bacterial isolates suggested its inclusion in the empirical treatment strategy as well as it is the most suitable first-line

antibiotic for UTI therapy. Our finding was in agreement with that reported by other studies from Egypt, China, Ethiopia, Uganda, Bangladesh and India [4,5,22 and 25], and it is comparable with recent guidelines on the antibiotic recommendation for UTI by Infectious Diseases Society of America guidelines [32]. Locally, Al-Haddad et al. [10] documented similar report to our finding. In contrast, Mohanna and Raja'a (2005) detect a very low sensitivity for Nitrofurantoin (15.9%) [9]. Nevertheless, there may be non-compliance to Nitrofurantoin because of its bitterness and there have been worries over its possible effects on the fetus [24]. Alternatively, an intermediate to low sensitivity pattern to Nitrofurantoin has been reported from an earlier study in Ethiopia and Nigeria that could be because of the previous exposure to these antibiotics [33,34].

Enterobacteriaceae are well known vulnerable to Amikacin [8]. A good sensitivity pattern for Amikacin and Piperacillin-Tazobactam was seen in the current study particularity among the most common isolated *Escherichia coli* strains (79.7% and 77% respectively). Our result was in alignment line with recent studies in India [22–24], and in Egypt for Amikacin only [29]. In Yemen, similar and an excellent finding was observed by Gondos et al., Al-Haddad [10,14] for Amikacin since Piperacillin-Tazobactam antibiotic was not tested in the later study. Subedi N et al (2014) noted moderate sensitivity pattern for Amikacin with a high pattern of sensitivity to Piperacillin-Tazobactam in Nepal [21]. On other hand, Badri and Mohamed Sudanese researchers reported a very high resistance pattern against Amikacin [19].

The frightening finding in the existing study is the decreased sensitivity of isolated uropathogens to Quinolones mainly for Nalidixic acid followed by Ciprofloxacin. This finding is consistent with that previously published in Yemen by Al-Haddad et al., AlZubiery et al. [7,12] Similar finding were observed in southeastern Nigeria among female patients to Levofloxacin [6]. On the contrary, Wariso KT et al. [34] in Nigeria stated, that Quinolones still principally efficient for empirical therapy in UTIs except for Nalidixic acid [34]. The long period of using Quinolones without a proper restriction is the possible reason for this condition [26].

Multidrug-resistant pathogen (MDR) like *Pseudomonas aeruginosa* causing various chronic biofilm-associated infections including UTI [31]. The *Pseudomonas aeruginosa* showed moderate sensitivity to Carbapenems, Moxifloxacin, and Piperacillin-Tazobactam in this study. Recently, researchers from India documented extensive very high resistance pattern [22,23]. On other hand, a lower resistant rate was documented in India [20]. Selective stress due to extreme exposure of bacteria to antibiotics is usually the source of such high incidence of resistance especially in the hospitals setting [31].

The isolated uropathogenic *Staphylococcus aureus* in this study was found extremely resistant to Azithromycin (90.0%) and Roxithromycin (80.0%) but highly sensitive for Penicillin (83.0%), and moderate sensitivity to both aminopenicillins Ampicillin (55.0%) and Amoxicillin (60.0%). Gentamicin shows an excellent sensitivity (90.0%) against uropathogenic isolates. This was similar to the result documented recently in Yemen by AL-Kadassy et al. [15] except for Amoxicillin, hence it is not included in her study. Our results about Ampicillin and Gentamicin were similar to previously reported studies in Nigeria [35]. Kotgire Santosh et al. and Derese et al. in their studies in India and Ethiopia showed that a comparable finding for Gentamicin [23,33] however, Wariso KT et al. in Nigeria reported a high resistance rate against Gentamicin [34]. In Yemen, AL-Kadassy et al. documented similar finding for Gentamicin (100.0%) but high sensitivity pattern for Ampicillin (75.0%) [15]. Our result concerning Gentamicin was in accordance with the previous recommendation [30].

4. CONCLUSION

Although Gram-negative bacilli were the most common isolated uropathogens, the majority of them was *Escherichia coli*. There is a decrease in sensitivity pattern that was observed in our study with an increased resistance against the commonly used UTI antibiotics. Therefore, antibiograms establishment for uropathogenic isolates introduce a guide to the doctors starting proper empirical antibiotic treatment.

5. RECOMMENDATION

Individual antibiograms to detect trends are useless unless they are done at a fixed interval at least one each year. An antibiotic strategy is one of the obligatory necessities for accreditation, and creation of an antibiogram is the first stage before bordering the antibiotic

strategy. Reference strains such as ATCC should be included in the future study for antibiogram detection based on Clinical and Laboratory Standards Institute.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors. The data that support the findings of this study are available from the hospitals or clinics of Sana'a city but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of the hospitals or clinics of Sana'a city.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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