

A Comparative Study of Antibiotic Resistance of Bacteria That Cause Urinary Tract Infection in Diabetic and Non-Diabetic Patients in Najaf City

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Abstract

Diabetes mellitus (DM) has been long considered to be associated with an increase prevalence of urinary tract infection (UTI) causing bacteria in comparison with patients without diabetes. One hundred and five urine samples were collected in the study. These samples distributed unequally (23 diabetic males and 14 diabetic females and also 46 non-diabetic males and 22 non-diabetic females). Ten antibiotic were tested in this study Ciprofloxacin 5µg, Cefuroxime 30µg, Amoxicillin 75µg, Chloramphenicol 30µg, Ampicillin 2µg, Cephalothin 30µg, Gentamicin 10µg, Tetracycline 30µg, Vancomycin 30µg, Nalidixic acid 30µg. Results showed that *Escherichia coli* was the most pathogen present in UTI (27.61%) from Gram negative bacteria followed by *Pseudomonas aeruginosa* (17.14%), *Proteus mirabilis* (15.23%) and *Klebsiella pneumonia* (13.33%). Whilst *Enterococcus faecalis* was the most pathogen from gram positive bacteria presented in UTI (11.42%) followed by *Staphylococcus aureus* and *Staphylococcus saprophyticus* (4.76%) for both and *Streptococcus pyogenes* and *Streptococcus epidermidis* (2.85%) for both. Ciprofloxacin (82.85%) and Chloramphenicol (76.19%) were the most effective antibiotic against UTI causing bacteria, while the least effective antibiotic was Cefuroxime (22.85%) followed by Cephalothin (25.71%). Conclusion: *E. coli* was the most dominant pathogen in UTI in both diabetic and non-diabetic patients and the sensitivity of isolated bacteria for used antibiotics was lower in diabetic patients compared with non-diabetic patients.

Keywords: Diabetes, UTI, Antibiotics, *E. coli*.

Introduction

Urinary tract infection (UTI) are considered as a common infectious disease and infected all ages, Although the anyone can infect with UTI, however, the people with other health problems are more susceptible to infect with UTI, examples of these groups of people are elderly, and those with diabetes (FazlyBazzaz et al., 2021; Sheerin and Glover, 2019). *E. coli* are the most common bacteria that cause UTI from gram negative bacteria. Clinically, UTI may be uncomplicated or complicated. Uncomplicated UTI generally use to describe individuals who do not suffer from any

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diseases (just UTI) and have no structural or neurological urinary tract irregularities, these infections are separated into lower UTI (cystitis) and upper UTI (pyelonephritis). Complicated UTI are defined as UTI related with other medical issues that compromise the urinary tract or host defense. (Alsadiet *al.*, 2019; Stormeet *al.*, 2019; Lane and Takhar, 2011).

Diabetes mellitus (DM) is a metabolic disease which is defined by disordering the glucose level in blood due to lacking or missing insulin. The bladder of diabetic patients has malfunction in which urine is accumulated and it serves as favorable environment for microbes to develop and cause UTI (Fünfstück *et al.*, 2012; Kofteridis *et al.*, 2009).

The resistance of bacteria to antibiotics is a major global health problem, caused in partiality by the overexertion of antimicrobials in clinical disease where they are not necessary or in prolonged courses of therapy when shorter durations are as effective (Dryden *et al.*, 2011; DucVolluz *et al.*, 2010; Tremolieres, 2002). Antimicrobial prescribing have to be sensible, thoughtful and rational. The alternative of antimicrobial agents necessity be personalized based on the patients allergy suitably, native practice patterns, prevalence of resistance, availability, expense and deference (Fitzgerald *et al.*, 2012). Several examinations in adults and children have shown that short-term antimicrobial courses are as viable as longer ones for the treatment of uncomplicated UTI and many complicated UTI, despite the fact that there still stay numerous inquiries regarding the ideal term of treatment for some kinds of complicated UTI (Klausner *et al.*, 2007; Talan *et al.*, 2000;). It is the obligation of all medical services suppliers to rehearse antimicrobial stewardship and to keep away from the superfluous utilization of antimicrobials (Herr, 2013; Hyun *et al.*, 2013).

The current study was aimed to determine the most UTI-causing bacteria in both diabetic and non-diabetic patients and to test the antibiotics susceptibility in hospitalized patients in Najaf city.

Materials and Methods

Urine Samples collection

One hundred and five patients were volunteered in the current study from Alsader hospital from Najaf city. The common factor among these patients was the present of symptoms of urinary tract infection. Four months were the period of sample collection from August 7th to December 10th 2020. 5 to 54 years old were the ages of patients, both male and female, diabetic and non-diabetic were included in current study (23 diabetic males and 14 diabetic females and also 46 non-diabetic males and 22 non-diabetic females). All samples were collected at morning under sterile conditions by using sterile containers. The samples were directly transported to the microbiology laboratory to carry out the necessary operation on them. Suitable media was used to culture the sample for 24h in aseptic conditions.

Isolation and Identification of Bacteria

When the samples were arrived to the laboratory, it were directly cultured on nutrient agar and incubated for 24h at 37° C. Pure colonies were selected to subculture on maccokey agar, mannitol salt agar, blood agar, eosin methylene blue (Himedia, India) (three petri dishes for each sample). Gram's stain and biochemical tests such as oxidase, catalase, Indole, methyl red, vogasproskaur, simmons citrate, gelatin and sugar fermentation besides colony morphology were achieved to complete the identification of bacteria (Forbes *et al.*, 2007; MacFaddin, 2000). To emphasize the manual identification of bacteria, Vitek 2 (Biomérieux\ USA) is applied for all pure colonies.

Antibiotics Test

The method of antibiotics test was achieved by diffusion method, which was stated by Kirby-Baucer 1966. Pure colonies from each agar were streaked by mat like method on Mueller Hinton agar (Himedia-India) and incubated for 24h at 37° C. Formation of clear zone around the antibiotic disc, it mean bacteria susceptible for that antibiotic. The inhibition zone that formed was compared with CLSI 2017. The following antibiotics were used in the current study: Ciprofloxacin 5 µg (Cip 5 µg), Cefuroxime 30 µg (Ceu 30 µg), Amoxicillin 75 µg (Am 75 µg), Chloramphenicol 30 µg (C 30 µg), Ampicillin 2 µg (Amp 2 µg), Cephalothin 30 µg (Cep 30 µg), Gentamicin 10 µg (G 10 µg), Tetracycline 30 µg (TE 30 µg), Vancomycin 30 µg (Va 30 µg), Nalidixic acid 30 µg (Na 30 µg) (Himedia, India).

Statistical analysis

The percentages of the number were achieved by SPSS version 17 windows7.

Results

Groups of study

In the current study, One hundred and five patients were volunteered, 23 diabetic males and 14 diabetic females and also 46 non-diabetic males and 22 non-diabetic females. Patients were divided into six groups depending on their ages. The most participated group in diabetic patients was group 5 (41-50 years) in female (50%), whilst The most participated group in non-diabetic patients was also group 5 (41-50 years) but in male (41.3%) table 1.

Table 1: Number of diabetic and non-diabetic patients for both male and female and their groups that participated in the current study

No. of groups	Groups Years	Diabetic patients				Non-Diabetic patients			
		Male n=23		Female n=14		Male n=46		Female n=22	
		No.	%	No.	%	No.	%	No.	%
1	5-10	3	13.03	0	0.0	0	0.0	0	0.0
2	11-20	2	8.69	1	9.09	0	0.0	1	4.54

3	21-30	6	26.08	0	0.0	13	28.26	9	40.9
4	31-40	8	34.78	6	42.85	8	17.39	5	22.72
5	41-50	0	0.0	7	50	19	41.3	4	18.18
6	51-54	4	17.39	0	0.0	6	13.04	3	13.63

Identification of bacteria

The identification of UTI causing bacteria revealed that *Escherichia coli* was the most pathogen present in UTI (27.61%) from Gram negative bacteria followed by *Pseudomonas aeruginosa* (17.14%), *Proteus mirabilis* (15.23%) and *Klebsiella pneumonia* (13.33%). Whilst *Enterococcus faecalis* was the most pathogen from gram positive bacteria presented in UTI (11.42%) followed by *Staphylococcus aureus* and *Staphylococcus saprophyticus* (4.76%) for both and *Streptococcus pyogenes* and *Streptococcus epidermids* (2.85%) for both. Table 2.

Table 2: The type and percentage of UTI-causing bacteria in diabetic and non-diabetic patient which identified in the study

Bacteria	Diabetic males N= 23	Diabetic female N= 14	Non-diabetic males N= 46	Non-diabetic females N= 22	Percentage %
Gram negative					
<i>E. Coli</i>	8 (39.1%)	5 (35.7%)	11 (23.9%)	5 (22.7%)	27.61
<i>P. aeruginosa</i>	4 (17.3%)	2 (14.2%)	7 (15.2%)	5 (22.7%)	17.14
<i>P. mirabilis</i>	3 (13.04%)	2 (14.2%)	8 (17.3%)	3 (13.6%)	15.23
<i>K. pneumonia</i>	3 (13.04%)	3 (21.4%)	6 (13.04%)	2 (9.09%)	13.33
Gram Positive					
<i>E. faecalis</i>	1 (4.3%)	1 (7.1%)	6 (13.04%)	4 (18.1%)	11.42
<i>S. aureus</i>	1 (4.3%)	1 (7.1%)	2 (4.3%)	1 (4.5%)	4.76
<i>S. saprophyticus</i>	1 (4.3%)	0 (0%)	3 (6.5%)	1 (4.5%)	4.76
<i>S. pyogenes</i>	1 (4.3%)	0 (0%)	2 (4.3%)	0 (0%)	2.85
<i>S. epidermids</i>	1 (4.3%)	0 (0%)	1 (2.17%)	1 (4.5%)	2.85

Antibiotics sensitivity

The results in table 3 showed that the least effective antibiotic was Cefuroxime (22.85%) followed by Cephalothin (25.71%). While the most effective antibiotic against UTI causing bacteria was Ciprofloxacin (82.85%) followed by Chloramphenicol (76.19%). The percentage of remaining antibiotics was listed in the table 3.

Table 3: the number of sensitive and resistance of UTI causing bacteria to different antibiotics

Antibiotics	No. of Sensitive	No. of Resistance	Percentage of
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			sensitive
Cip 5 µg	87	18	82.85
Ceu 30 µg	24	81	22.85
Am 75 µg	62	43	59.04
C 30 µg	80	25	76.19
Amp 2 µg	54	51	51.42
Cep 30 µg	27	78	25.71
G 10 µg	43	62	40.95
TE 30 µg	33	72	31.42
Va 30 µg	69	36	65.71
Na 30 µg	71	34	67.61

In the table 4 the results showed that the percentage of sensitive in diabetic patients was less than the percentage of sensitive in non-diabetic patients for all antibiotics except gentamicin (20% and 20.95%) and vancomycin (31.42% and 34.29%) respectively.

Table4 :Comparative between percentage of sensitive for different antibiotics in diabetic and non-diabetic patients

Antibiotics	Diabetic patients			Non-diabetic		
	No. of sensitive	No. of resistance	Percentage of sensitive	No. of sensitive	No. of resistance	Percentage of sensitive
Cip 5 µg	41	10	39.04	46	8	43.81
Ceu 30 µg	11	45	10.47	13	36	12.38
Am 75 µg	27	22	25.71	35	21	33.33
C 30 µg	39	12	37.14	41	13	39.05
Amp 2 µg	24	28	22.85	30	23	28.57
Cep 30 µg	15	42	14.28	12	36	11.43
G 10 µg	21	33	20	22	29	20.95
TE 30 µg	17	36	16.19	16	36	15.23
Va 30 µg	33	19	31.42	36	17	34.29
Na 30 µg	30	18	28.57	41	16	39.04

Discussion

This study revealed that gram positive bacteria and gram negative bacteria were associated with UTI with different percentages. Diabetic females were exhibited a high percentage of UTI (50%) while non-diabetic males were exhibited a high percentage of UTI (41.3%). This may be belong to lack of space between vagina and anal region in females. 14-15. *E. coli* was most dominant in UTI (27.61%) in both diabetic and non-diabetic patients for both genders, this could be attributed to ability of this bacteria to adhere rabidly on the surface of epithelial cells, because *E. coli* has a lot of virulence factors such as fimbriae with help it to invade the urinary tract cells (Bazzaz *et al.*, 2019 Althunibat *et al.*, 2016; Mnifet *et al.*, 2013).

P. aeruginosa was identified as a second pathogen (17.14%) in UTI in diabetic and non-diabetic patients. *P. aeruginosa* is considered a hospital-contaminated pathogen that can easily spread to contaminate the burn and injuries (Alwaeli, 2020). This feature facilitates bacteria to cause UTI, and another reason is the ability of *P. aeruginosa* to resist phagocytosis because it is able to produce a capsule. The presence of peritrichous flagella which help bacteria to move toward urinary tract cells. [Mnif et al., 2013].

Proteus mirabilis isolated by 15.23% as a third pathogen causing urinary tract infection, like *E. coli*, *P. mirabilis* has adherence factors such as fimbriae (express four types of fimbriae). The presence of flagella which help bacteria to move toward urinary tract cells. *Klebsiella pneumoniae* was isolated as a fourth pathogen (13.33%) which caused UTI. This bacteria has a capsule to prevent phagocytosis and has fimbriae to facilitate the adherence to uro-epithelial cells (Clopés and Winkler, 2021). In the current study, gram positive bacteria were isolated which included *E. faecalis*, (11.42%) *S. aureus*, *S. saprophyticus* (4.76% for both), *S. pyogenes* and *S. epidermidis* (2.85% for both), however, *E. faecalis* was identified as a fifth pathogen causing UTI and the first in gram positive bacteria, this result could be returned to the ability of bacteria to adhere to the surface of epithelial cells and their ability to escape and evade the immune system. This result was agreed with many investigations that stated newly from different geographical areas (Ummeet et al., 2019; Al-soufi et al., 2017). The virulence factors of *E. faecalis* include EbpA, Ace adhesion and Esp adhesion which use for adhesion in addition to Epa that use for invasion of uro-epithelial cells (Nielsen et al., 2013; Arias et al. 2012; Caparon and Hultgren 2010).

Although in diabetics, the relationship between the level of sugar in urine and the risk factor for UTI is debatable, DM has been long considered to be associated with an increased prevalence of bacteria in comparison with patients without diabetes (Bonadio et al., 2004; Goswami et al., 2001). In the current study, the prevalence of gram positive bacteria was high in non-diabetic patients in comparison with diabetic patients, this may be attributed to small size of samples in the study, however, the detailed studies which included morphological, Immunohistochemistry and biochemical tests have shown that the behavior of bacteria that isolated from diabetic and non-diabetic patients in UTI was the same. Many studies tried to explain the mechanisms by which DM increases the risk of UTI, such as glycosuria mechanism that enhances the growth of bacteria. Or decrease the function of polymorphonuclear cells (Nitzan et al., 2015). Or decrease the level of cytokines (Bonadio et al., 2004).

In the current study, all isolated bacteria were submitted for various antibiotics to test the sensitivity and resistance of bacteria in order to choose the appropriate treatment for UTI patients.

From the table 4 the results showed that the percentage of sensitive in diabetic patients was less than the percentage of sensitive in non-diabetic patients, this may be attributed to the presence of bacteria for a longer period of time in diabetic patients

due to lack of cellular and humoral immunity and this period let the pathogen to develop some kind of resistance against antibiotics. A lot of studies revealed that the function and number immune cells were decreased in DM patients with UTI (Nitzan et al., 2015). Other study found that the cytokines level (IL-6 and IL-8) were lower in DM patients (Bonadio et al., 2004).

The most isolated bacteria have a resistance against cefuroxime, cephalothin and ampicillin due to their ability to produce beta lactamase enzyme that destroyed the beta lactam ring in these antibiotics. So use beta lactamase enzyme inhibitor like clavulanic acid is necessary to continually give these antibiotics. Amoxicillin is good choose for *K. pneumoniae*. It is inactive alone to treat UTI but when it combine with clavulanic acid it will be so (Takahashi et al., 2004; Bonadio et al., 2004). Nalidixic acid, Tetracycline, gentamicin and vancomycin have effect on some isolated bacteria with good result but it cannot be used for all isolated bacteria. Ciprofloxacin and chloramphenicol were the most effective antibiotics for all isolated bacteria in diabetic and non-diabetic patients with UTI, although both of them have different antibiotic action, ciprofloxacin prevents DNA synthesis while chloramphenicol inhibits the protein synthesis. these results were in agreement with Al-Asoufi et al. Generally gram negative bacteria are a common bacteria that cause UTI. patient with UTI could treat with Ciprofloxacin in both diabetic and non-diabetic, male or female (Khleifat et al., 2006).

Conclusion

The conclusion of the current study is: *E. coli* was the most dominant pathogen in UTI in both diabetic and non-diabetic patients and the sensitivity of isolated bacteria for used antibiotics was lower in diabetic patients compared with non-diabetic patients. The study suggests to use ciprofloxacin in the treatment of UTI in both diabetic and non-diabetic patients.

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