

Detection of some Bacterial Uropathogens in Male Students at the Institute of Medical Technology / Al - Mansour

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Abstract:

Infections of urinary tract are the second most common type of infection in the body. About 80-90% of urinary tract infections are caused by *E.coli*, these bacteria live normally in the human intestine but sometimes get into urinary tract .Some of urinary tract infections are caused by other bacteria like *Proteus* spp., *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Enterococcus faecalis* .This study reports the presence of the major uropathogenic species in urine samples from students, and their resistance patterns to many antibacterial agents may help the clinician to choose the correct empirical treatment against the urinary pathogens isolated from students of Institute of medical Technology /Al-Mansour /Baghdad /Iraq, from March to April 2016. Midstream urine was collected from 400 students, all of them were males. The urine samples were processed with microbial isolates identified by using blood agar and MacConkey agar. Out of (400) urine samples from students at Medical Technology Institute /Al-Mansour,(164) samples showed a positive culture growth ,while (236) samples have no growth. The bacteria are *Escherchia coli* , *Staphylococcus aureus* , *Klebsiella pneumoniae* , *Enterococcus faecalis* and *Proteus mirabilis* were (73.2 , 20.7 , 2.4 , 2.4 and 1.2) %respectively.

Keywords: UTIs, Bacteria, Antibiotics susceptibility.

التحري عن بعض البكتريا المسببة لالتهاب المجاري البولية لدى الطلبة الذكور في المعهد الطبي التقني - المنصور -

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المستخلص:

إن التهاب المجاري البولية يأتي بالمرتبة الثانية بالنسبة للأمراض التي تصيب الجسم. تقريبا من ٨٠ - ٩٠ % من اصابات المجاري البولية سببها بكتريا *Escherchia coli*. بعض التهابات المجاري البولية سببها أنواع أخرى من البكتريا مثل *Proteus spp.*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Enterococcus faecalis*. ويهدف البحث الى التحري عن الانواع البكتيرية الممرضة التي تصيب الجهاز البولي في النماذج المأخوذة من الطلبة الذكور، ودراسة نمط مقاومتها لبعض المضادات الحيوية والتي قد تساعد المعالجين في اختيار العلاج الصحيح ضد الممرضات المعزولة من طلبة المعهد الطبي التقني /المنصور/ بغداد قيد الدراسة، للفترة من شهر مارس الى ابريل ٢٠١٦. تم جمع عينات الادرار من (٤٠٠) طالب، جميعهم كانوا من الذكور. وتم التحري عن البكتريا الممرضة في نماذج الادرار باستخدام اكار الدم واكار الماكونكي. وظهر ان (١٦٤) نموذجا كان موجبا للنمو البكتيري في الاوساط الزرعية، بينما (٢٣٦) نموذجا لم يظهر فيه اي نمو بكتيري. عزلت *Escherchia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Proteus mirabilis* (73.2 % (1.2, 2.4, 2.4, 20.7, على التوالي.

Introduction

Urinary tract infection (UTI) remains a worldwide disease and also as a nosocomial disease [1]. It possesses a significant health risk because it can lead to urosepsis and /or renal scarring, kidney damage and associated with high mortality and morbidity [2]. Early diagnosis and prompt antimicrobial treatment are required to minimize these complications [3].

Gram negative bacteria were the most common of uropathogens responsible for UTI with a 59.3% percentage in comparison to 40.7% for gram positive bacteria. *Escherichia coli* and *Staphylococcus aureus* were highly resistant to all antibiotics (20% resistance) [4,5].

Enterococcus faecalis is the most commonly isolated of the genera. It is associated with 80 to 90 percent of human enterococcal infections. *Enterococcus faecalis*, as the name implies, is found normally in the intestines of humans, animals and birds. It is also found in soil and water in nature. *Enterococcus faecalis* has been implicated in a wide variety of human infections and is a notorious problem in hospital-acquired infections. Urinary pathogens have been known to include strains that are resistant to many antibiotics. The antibiotic resistance in the management of UTI's is a serious public health issue, particularly in the developing countries where apart from high level of poverty, and poor hygienic practices, there is also high prevalence of fake and spurious drugs of questionable quality in circulation [6,7,8].

Thus, the aims of our study are to determine the prevalence of urinary tract infection in male students at Institute of Medical Technology /AL-Mansour –Baghdad as well as the susceptibility of uropathogens to antibiotics and to determine the bacterial etiologic agent of uropathogens and evaluate them in vitro susceptibility pattern to commonly used antimicrobial agents.

Methods and Materials:

1- Collection of samples:

Four hundred urine samples were collected from male students at the Institute of Medical Technology, Al-Mansour / Baghdad, aged between (19-24) years old. During the study from March, 2016 to April, 2016. Mid-stream urine sample was taken from each student in sterilized cup. Before collection of a sample, male subjects were asked to clean the genital part with soap and water.

2-Urine analysis:

A-Macroscopic and microscopic examinations:

Wet preparations were made from centrifuged samples (3000 round per minute for 5 minutes). Leukocytes and erythrocytes per high power field were looked for. Specimens of urine containing ≥ 5 pus /cells power field were considered as having significant bacteriuria . A rapid dipstick (Combur 10 test, Boehringer Manhim, Diagnostics and Biochemicals, East Sussex, United Kingdom) was also used to detect pyuria via leukocyte esterase activity [9].

B- Gram's staining:

Loopful urine smears of uncentrifuged urine were stained by Gram's stain and examined microscopically under oil immersion. The presence of more than one bacteria of oil immersion field in 20 correlated with significant bacterial culture of more than 10^5 CFU/ml urine [10].

C-Urine culture:

All urine samples were processed within one hour after arriving at the laboratory. A standard calibration platinum wire loop delivering 0.01 ml of uncentrifuged urine was to inoculate blood agar (Oxoid) and MacConkey agar (Oxoid). These plates were incubated aerobically at 37C° for 18-24 hour. All positive samples for one or both leukocyte and nitrite positive for one or both WBCs and bacteria were inoculated on blood agar in (5-10%) CO₂ and aerobic conditions, and MacConkey agar in aerobic condition only [11].

D- Identification of bacterial isolates:

Bacterial colonies were identified on the basis of Gram's reaction, morphology, biochemical characteristics features. API-Staph and API 20 (bioMerieux,France) were used as reference identification system to identify *Staphylococcus spp.* and enterobacteraceae after culturing on culture media [9,12,13].

E-Antibacterial susceptibility:

Antibacterial susceptibility test was performed by the Kirby Bauer disc diffusion technique [14, 15] using antibiotic discs containing the following concentrations: Amikacin (AN,30µg), chloramphenicol (C,30 µg),Trimethoprim +Sulphamethazole (SXT ,25 µg), Ampicillin (Am, 10 µg). Nalidixic acid (AN, µg) , Gentamycin (CN, 10 µg), Nitrofurantoin (F,300 µg), Cefotaxime (CTX, 30 µg) , Amoxicillin (AMX,25 µg), Tetracyclin (TE, 10 µg), Oxacillin (OX, 10 µg), Vancomycin (VA, 10 µg), Co – Trimoxazole (CT,25 µg), Norfloxacin (NOR ,10 µg), penicillin (G , 10 U)

,Ceftriaxone (CRO,30 μ g) and Erythromycin (E , 10 μ g). These antibiotics were chosen as they are the antibiotics of choice in the treatment of UTI. The diameters on inhibition zones were measured by mm and then compared with the standard diameters installed in the standard scales. All the antimicrobials used for the study were obtained from Oxoid Ltd. Basingstore Hampaire, UK. A standard inoculum adjusted to 0.5 McFarland was swabbed on to Muller-Hinton agar (Oxoid Ltd. Basingstore Hampaire, UK); antibiotic discs were dispensed with after drying the plate for 3–5 min and incubated at 37°C for 24 hours [14,15].

Results and Discussion:

During the study period (March-April, 2016), out of (400) urine samples from students at Medical Technology Institute /Al-Mansour, the microscopic examination of all male students urine samples with urinary tract infection showed pus cells percentage of (100%) , and all of those patients were positive result for leukocyte esterase. The results showed also that the other organized deposits were epithelial cells, bacteria, red blood cells and monilia (73.1, 48.8, 35.9, and 30.5) % respectively. Whereas the unorganized deposits were calcium oxalate, amorphous urate, uric acid, amorphous phosphate and triple phosphate were (78.04, 74.4, 3.04, 1.2, and 1.2) % respectively (Table 1).

Table 1: The deposits finding in general urine examination in male students with UTI in positive culture

	Findings	Case no.	%
Organized deposits	Pus cells	164	100
	Epithelial cells	120	73.1
	Bacteria	80	48.78
	RBCs	59	35.9
	Monilia	50	30.5
Unorganized deposits	Calcium oxalate	128	78.04
	Amorphous urate	122	74.4
	Uric acid	5	3.04
	Amorphous phosphate	2	1.22
	Magnesium Ammonium phosphate (Triple phosphate)	2	1.22

164 (41%) urine samples showed a positive culture growth, while 236(59 %) samples had no growth. In positive culture growth ,*Escherchia coli* , *Staphylococcus aureus* , *Klebsiella pneumoniae* ,*Enterococcus faecalis* and *Proteus mirabilis* were 73.2%,20.7%,2.4%,2.4% and 1.2% respectively [Table2].Regarding other study included 1120 urine samples were collected from people. Diagnoses tests showed that only 106 (9.4%) isolates were *E. coli* [10].

Table 2 : numbers and percentages of uropathogens isolated from patients with UTI

Pathogens	Isolation no.	%
Escherichia coli	120	73.2
Staphylococcus aureus	34	20.7
Klebsiella pneumoniae	4	2.4
Enterococcus faecalis	4	2.4
Proteus mirabilis	2	1.2
Total	164	100

Gram's stain of uncentrifuged urine revealed highest number of Gram positive cocci and Gram negative bacilli. The colonies of *Escherichia coli* on MacConkey agar appeared pink smooth and white colour without haemolysis on blood agar. The morphological study of *Escherichia coli* under light microscope revealed that the shape of these bacteria was bacilli and their reaction with Gram stain was red colour. The biochemical positive reaction by API-20E system was O-nitrophenyl - β - galactosidase, lysine decarboxylase, Ornithine decarboxylase, Indol, Glucose fermentation in addition to other tests appearing in [table 3]. Other Gram negative bacilli isolated from urine samples were *Klebsiella pneumoniae* [table 2]. Oxidase test was negative for *E. coli* and *Klebsiella pneumoniae*.

The colonies of *Staphylococcus aureus* appeared golden to yellow colour and smooth with beta haemolysis on blood agar. The morphological study of *Staphylococcus aureus* under light microscope revealed that the shape of this bacteria was cocci and their reaction with Gram stain was blue colour. The biochemical positive reactions by using API Staph system were D-mannose, D-Lactose, D-mannitol, voges proskauer

test .Catalase and coagulase tests were positive but Oxidase test was negative .

In our study , *Klesiella pneumoniae* colonies appeared pink smooth and mucoid on MacConkey agar , Gram-negative rods under light microscope , acids were produced from lactose , glucose , mannose ,rhamnose fermenting, oxidase test was negative , voges proskauer test was positive. As a general rule, *Klebsiella* infections tend to occur in people with a weakened immune system from improper diet (alcoholics and diabetics). Many of these infections are obtained when a person is in the hospital for some other reason (a nosocomial infection). New antibiotic resistant strains of *K. pneumoniae* are appearing, and it is increasingly found as a nosocomial infection. *Klebsiella* ranks second to *E. coli* for urinary tract infections in older persons [16].

Table 3: biochemical reaction profiles in API 20E test :

Identification code	Reaction	<i>E. coli</i>
OPNG	o-nitrophenyl- β - galactosidase	+
ADH	Arginiedehydrolase	-
LDC	Lysinedecarboxylase	+
ODC	Ornithinedecarboxylase	+
CIT	Citrate	-
H ₂ S	H ₂ S	-
URE	Urease	-
TDA	Tryptpphanedeaminase	-
IND	Indole	+
VP	Voges – Proskauer reaction	-
GEL	Gelatinase	-
GLU	Glucose	+
MAN	Mannitol fermentation	+

INO	Mannitol fermentation	-
SOR	Inositol fermentation	-
RHA	Sorbitol fermentation	+
SAC	Rhamnose fermentation	+
MEL	Saccharose fermentation	+
AMY	Amygdalin fermentation	-
ARA	Arabinose fermentation	+

Biochemical reactions of with API 20E strips. Positive reactions are indicated with a "+" sign, negative ones with a "-".

Because *Enterococcus faecalis* is normally found in the intestinal tract of people, it easily becomes a contamination problem in hospital and institutional settings. Because of the debilitated status of hospitalized patients and the antibiotic resistance of *Enterococcus faecalis*, it can cause many types of infections. The infection most commonly caused by *E. faecalis* is urinary tract infections (UTI). Most enterococcal UTI's are nosocomial in nature or related to abnormalities of the urinary tract. Bacteremia with *E. faecalis* is usually the result of infections from other sites, like the urinary tract. Wound infections, particularly in the abdominal area, are frequently seen. It is also commonly seen in cases of endocarditis. One of the most important and serious factors about *Enterococcus faecalis* and the other enterococci is its resistance to a variety of antibiotics. This also contributes to its ability to cause disease. Most antibiotics used for systemic infections singly that are effective against other types of Gram-positive cocci (staphylococci and streptococci) are ineffective against *E. faecalis*. Treatment typically involves the synergistic treatment of an antibiotic that attacks the cell wall like penicillin or vancomycin and aminoglycoside like gentamicin. Resistance to vancomycin is becoming increasingly common.

Treating *E. faecalis* UTI's are usually easier to treat with antibiotics [17, 18].

In our study, seventeen (17) antibiotics were tested against the isolated uropathogens among which Co-Trimoxazole, Amikacin , Cefotaxime , Nitrofurantoin and Oxacillin were more effective than other antibiotics against *Escherichia coli*. *E. coli* as predominant cause of UTI, showed the highest percentage of resistance to Ampicillin, Erythromycin and Amoxcillin (100%, 98.3% and 66.7% respectively). *Staphylococcus aureus* as the second most prevalent pathogen of UTI displays a resistance pattern and it was 100% resistant to Co- Trimoxazole, Nalidixic acid and Nitrofurantoin and susceptible to Tetracyclin, Cefotaxime, Chloramphenicol and Erythromycin, *Klebsiella pneumoniae* and *Proteus mirabilis* showed the highest antibiotic resistance rate and was significantly resistant to most antibiotics. Our study showed that *E. coli* was the predominant isolate causing UTI and this agreed with previous researches [19,20]. Nalidixic acid , Nitrofurantoin, Sulphamethoxazole - trimethoprim and Amoxicillin showed very poor activity, this may be due to a long term use of these drugs over the , also prescription of antibiotics with lab guidance as well as over the counter sales of antibiotics without prescription are rife in Kikuk [21]. In Ahvaz city, Iran, a total of 475 children were screened by collecting mid- stream urine samples for the presence of causative organisms. The isolated pathogens included *Escherichia coli* (50%), *Proteus mirabilis* (25%) and coagulase negative *Staphylococcus* (25%) .The antibiograms indicated that Gentamicin (100%), Nalidixic acid (100%), Amikacin (75%) , Nitrofurantoin (62.5 %), and Chloramphenicol(50%) [22].

In Diyala city ,the number of patients with urinary tract infection who yielded positive cultures from their mid- stream urine specimens were 39 out of 150 patients (26%).

Escherichia coli was the main pathogen, it was (56%), followed by *Klebsilla pneumoniae* , (10%) and *Proteus* species (5%). The other pathogen *Staphylccoccus aureus* was (10%) [23]. In Babylon city, the number of males with UTI who yielded positive cultures of urine was (14) out of (31) patients (11.7%) [18]. Abbas and Naji in Diyala found that the reason for the highest prevalence of *Staphylococcus ureus* in males is not clear and the most frequently encountered organism was *Escherichia coli* ,it was (56.4%) of the isolates followed by *Staphylococcus aureus* (17%) [25].

Enterococcus faecalis is resistant to many commonly used antimicrobial agents like Cephelosporins , Clindamycin, Penicillin, Oxacillin , Trimethoprim– sulfamethoxazole and Vancomycin .Treatment options for *E. coli* include Linezolid and Ampicillin [26 ,27] .Our study is in agreement with Zhanel *et al.* in which the most active agents were Nitrofurantion and Chloramphenicol, with 0.3% and 2.4% resistance , respectively. (77.8%) of Vancomycin resistant *E. faeclum* isolates displayed the Van A phenol type [28]. In India, the urinary pathogens isolated from patients with urinary tract infection were *Escherichia coli* (21.95%), *Klebsilla spp.* (12.19%) and *Pseudomonas aeruginosa* (9.75%), *Staphylococcus aureus* (17.07%) . Among the drugs used for testing the antimicrobial susceptibility, are Amikacin, Gentaycin, Norfloxacin and Nitrofurantoin [19].Sekowska *et al.* showed that *Klebsiella pneumoniae* isolated from patients with UTIs were (86.7%) susceptibility to Gatifloxacin , (76.7%) to Norfloxacin and (51.2%) to Ciprofloxacin [29]. Another study in Nigeria , the susceptibility profile of the bacterial isolates , *E. coli* , *Klebsiella pneumoniae* , *Proteus spp.* , *Pseudomonas aeruginosa* and *Staphylococcus areus* ,showed that Ciprofloxacin was the most active antibacterial agents while Nalidixic acid , Nitofurantoin ,Sulphamethoxazole – trimethoprim ,Amoxicillin and

Amoxicillin-clavulanate were poorly active against the bacterial isolates ,also it was found that *E. coli* was the most prevalent isolate generally in females , while *Staphylococcus aureus* was the predominant isolate causing UTI in males [30,31,32] .In Tikrit city , 45% (45/160) of patients had UTI's, (6) Patients revealed mixed bacterial growth. 84 % (38/45) of patients were infected with gram negative organisms whereas only 16% (7/45) of patients were infected with gram positive organisms. *E. coli* was isolated from 14 (31%) cases [33]. In Karbala, 123 cases out of 300 showed positive culture of pathogenic bacteria; those were *E. coli* (39.02%), *Proteus mirabilis* (16.26) % and *Enterobacter* (6.50%) [34]. An Egyptian study was conducted on 5% of primary school children aged (6-12) in Ismailia governorate to measure the prevalence of UTI in primary school children showed 2.4% overall prevalence of UTI 1.7% among males and 2.7% among females, they found also that the prevalence of UTI in urban areas was 1.8% in comparison to that in rural areas which was 2.4% [4,5].In Erbil, *Escherichiacoli* is considered the predominant organism and it was responsible for 41 (58.57%) of the cases of UTI. This was followed by *Staphylococcus aureus* 10 (14.29%), *Klebsiella pneumoniae* 6 (8.57%), and *Proteus mirabilis* 2 (2.86%) [6].

Conclusion:

Escherichia coli was the most common pathogen causing urinary tract infection in male students. The most of urinary isolates had high level of resistance to antibiotics.

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