Certain virulence characteristics of common bacteria involved in urinary tract infection in Erbil setting

Nabeel Waheda * Pishtewan H. Al-Bazzaz ** Esam Y. Mansoor *** Safaa Toma ****

ABSTRACT

Background and Objectives: Urinary tract infections carry a high risk of recurrence and antibiotic resistance due to biofilm formation. This study aims to identify the common bacterial pathogen responsible for UTI in Erbil setting and identify their pathogenic characteristics and sensitivity to commonly used antibiotics, using the minimal inhibitory concentration (MIC) method.

Methods: 96-flat wells microtiter plate was used for detection of the degree of biofilm formation of *E. coli* strains isolated from patients with urinary tract infection. Standard breakpoint MIC have been compared with MIC results of antibiotics.

Results: Only 5.6% of total samples showed UTI, the most common bacterial isolate was *E. coli* (43.2%). Around 26% of pyuric cases appeared to be of sterile type. The biofilm formation involved 9.4% strong adhesion. Around 50% of isolates showed beta hemolysis. The most sensitive antibiotics include nitrofurantine (81.3%), gatifluxacin (40.6%) and Ciprofloxacine (37.5%).

Conclusions: Most common pathogens in UTI are *E.coli*. There was no correlation between biofilm formation and the presence of any of the other virulence factor such as antibiotic resistants and hemolysis. The more effective antibiotics against *E.coli* in this setting are gatifluxacine, Ciprofloxacin and nitrfuratoin having most MIC fall close to their standard breakpoints.

Key words: Antibiotic, biofilm, hemolysis, UTI.

INTRODUCTION:

Urinary tract infections (UTI) refer to the presence of bacteria in the urinary system. UTIs are one of the most common bacterial infections in medicine today and account for over 7 million patient visits annually. It is estimated that 40-50% of women will have at least one onset of UTI in their lifetime [1, 2]. Asymptomatic bacteiruria describes the presence of bacteria in the urinary tract without symptoms and is diagnosed by bacterial culture [2]. Females are more prone to urinary tract infection than males and they also presents the greater problem in the proper collection of specimens [1]. UTI carries a high risk of recurrence as more than 30% of patients are exposes to recurrent infection within one year after a first UTI [3]. The bacteria most often seen in UTIs are of fecal origin [4]. These organisms are a subset of the organisms found in the feces. Strict anaerobic bacteria rarely cause UTIs. More than 90% of acute UTIs in patients are caused by certain strains of *E.coli.* Around 10-20% are caused by coagulase-negative *Staphylococcus saprophyticus* and 5% or less are caused by other *Enterobacteriaceae* organisms or *Enterococci* [5]. In complicated cases of UTI, such as UTI's resulting from catheterization, the most common causes of UTI are *E. coli, Klebsiella pneumoniae, Proteus mirabilis, Enterococcus sp.*, *Pseudomonas aeruginosa*.In rare cases *Candida albicans* can cause UTI especially among diabetic patients. *Staphylococcus saprophyticus* is the second most common cause of UTI in young sexually active women [4, 5]. *E.coli* is

* MSc, PhD, Department of Medical Microbiology, College of Medicine, Hawler Medical University .
** FICMS, Department of Surgery, College of Medicine, Hawler Medical University.
*** PhD, Department of Medical Microbiology, College of Medicine, Hawler Medical University.
****MSc, Department of Clinical Analysis, College of Pharmacy, Hawler Medical University.

isolated from the urine of UTI patients, like uropathogenic *E. coli* (UPEC) strains that cause cystitis and pyelonephritis. UTI *E.coli* strains possess a range of virulence factors, including adhesions (e.g., P, type 1 fimbriae and Curli), toxins (e.g., hemolysin) and capsule. Adherence is important for the colonization of the urinary tract, and the best-characterized adhesions [6, 7]. A formal definition of biofilm includes three components (1) Adherence of the microorganisms, either to a surface or to each other; (2) a change in gene expression resulting in a different phenotype; and (3) an extracellular matrix [6, 7]. Many clinicians depend on presence of pus on counting high power field (HPF) to determine presence of UTI and they manage the patients accordingly. Previous studies have mainly focused on presence of pus cells without determining bacterial culture. Research is scant on the pathogenic parameters and their correlates of isolates from UTI in Erbil settings. Therefore, this study aims to identify the common bacterial pathogen responsible for UTI in Erbil setting and identify their virulence characteristics and determination of minimal inhibitory concentration to commonly used antibiotics.

PATIENTS METHODS:

The study included screening of 1,296 people who visited Urology Consultation Unit at Rizgari Hospital/ Erbil city and were suspected of having UTI from January to July 2009. Aseptic collection of urine was achieved with morning midstream, a urine slide were prepared for counting leukocyte. A more than 5-8 leukocyte/ HPF is considered to be the cut-off for defining pyuria [8]. Less than five leukocyte/HPF was excluded from the study, while the pyuric samples (>5 leukocyte/HPF) were immediately cultured on the three type of culture media MacConkey agar, blood agar and nutrient agar, all plate incubated aerobically at 37C° for 24 - 48 h. The samples were grown on the plates, the case indicted as UTI, when the plate count symptoms and signs is suggested UTI as well [1]. The identification was done by standard bacteriological methods and when findings were dubious API-20E (bioMerieux) was used. Patients already on treatment were excluded from the study [9].

Evaluation of biofilm formation of *E coli* isolates was carried out by growing bacterial isolates on 96-flat well microtiter plates [10]. Each sample was repeated for five times and the average reading was calculated. Briefly, cells were grown for 18h in at 37 C°, washed by PBS to remove unbound cells, and stained with crystal violet 0.1%. Bound bacteria were quantified by addition of ethanol (70%) and measurement of the dissolved crystal violet at an optical density OD of 590nm using 96-flat wells microtiter plate Elisa reader. For calculation of adhesion degree, we used classification based on OD values obtained for individual strains.[11] The method used for evaluation of hemolysin assay of *E coli* included detection of hemolysin by isolates growing on the blood agar plates [12]. Strains that produced a clear zone of lysis around colonies after incubation for 24 h at 37 C were considered as positive hemolysis; either beta as complete hemolysis or alpha as partial hemolysis. Saginur method used for determination MIC[13.] of commercial antibiotics produced by Ajanta / India. A 96 -flat wells microtiter MIC tests were done according to National Committee for Clinical Laboratory Standards. Briefly, serial twofold dilutions of antibiotic were performed in Mueller-Hinton broth. A suspension of the organism was added to wells at a concentration of 10^6cfu/ml that adjusted with McFarlan tube 0.5 (bioMerieux), and the microtiter plates were incubated aerobically at 37C°. The MIC was defined as the lowest concentration of antibiotic in which there

RESULT:

Out of 1,296 persons with suspected UTI, 719 (55.47%) were females and 577 (44.5%) were males. Only 99 (7.6%)

showed pyuria on general urine examination, that were delivered for bacterial culture. The mean± SD of these 99 persons was 33.2± 14.7 while their age ranged from 5 to 70 years. The 99 (7.6) pyuric sample represented 73 (5.6%) with UTI, as they revealed 10^4 - 10^5 cfu/ml, while 26 (2%) with sterile pyuria, showed no growth (Table 1&2). Among the 73 culture samples of UTI, one sample showed two types of bacteria, resulting in a total 74 bacterial strains (Table 3). Out of 73 patients, 56 (76.7%) were female and 17 (23.3%) male. As the highest isolates were *E. coli*, the study focused specifically on this bacterium. *E.coli* isolates showed different degree of biofilm formation that ranged between no biofilm, weak, moderate and strong. This adhesion degree depended on the optical density that compared with control negative. Table (4) and (5) showed that out of 32 isolates (9.4%) have a strong adhesion, 34.4% weak, 31.3% showed moderate and only 25% showed no biofilm formation. About 50% of the isolates revealed beta hemolysis and only 6.3% had alpha hemolysis, whereas 43.8% showed no hemolysin formation (Table 4).The MIC for seven types of antibiotics was determined as shown in the (Table 6). The sensitivity rate was dependent according to the standard breakpoint (cut-off value) for each antibiotic type. It is found that the best effective antibiotics that resulted high percentage with MIC close to their breakpoints are gatifloxacin and ciprofloxacin as they showed 40.6% and 37.7% of MIC respectively within 0.5µg/ml, followed by nitrofurantoin 37.5% with MIC 64 µg/ml, then nalidixic acid 43.8% with MIC1024 µg/ml followed by cefixime 40.6% with MIC 512 µg/ml and finally ceftriaxone and cefotaxime as they showed 65.6% and 68.8% with MIC 1024 µg/ml. Moreover, for comparison the MIC results in this study with breakpoints MIC for each antibiotic, it revealed that *E.coli* strains resulted a high sensitivity percentage with 81.3% against nitrofurantoin since most MIC rates showed breakpoint MIC, followed by gatifloxacin 40.6%, then ciprofloxacin 37.5% and third generation of cephalosporin (cefotaxime, ceftriaxone and cefixime) showed 31.3%, while just the 28.1% showed sensitivity for nalidixic acid as a weak one in this study. (Details in table 6).

Table 1: Bacterial count

cfu/ml	No. of bacteria	% of isolates
Nil	26	26.3
>10,000	13	13.1
>100,000	60	60.6

Table 2: UTI and sterile pyuria percentage

Total sample	Pyuria sample	73 UTI sample		26 Sterile pyuria sample	
		% of Total	% of Pyuric	% of Total	% of Pyuric
1296	99 (7.6%)	5.6%	73.7%	2%	26.3%

Table 3: Cultures identification

Isolated bacterial	Number of isolates	%
E.coli	32	43.2
Enterobacter aerogenes	4	5.4
Klebsiella pneumoniae	4	5.4
Proteus mirabils	1	1.4
Serratia odorifera	3	4
Staphylococcus aureus	4	5.4
Staphylococcus epidermidis	17	23.3
Staphylococcus saprophyticus	5	6.8
Enterococcus faecalis	4	5.4
Total	**74**	**100**

Table 4: Biofilm and hemolysis characteristics of 32 *E. coli* isolates

BacterialStrain	Average biofilm formation ODat 500 nm		Hemolysin
	Sample	Control	
1	0.051	0.051	Nil
2	0.052	0.054	Beta
3	0.053	0.054	Nil
4	0.054	0.055	Nil
5	0.055	0.057	Nil
6	0.055	0.055	Alpha
7	0.057	0.053	Nil
8	0.058	0.048	Beta
9	0.061	0.053	Nil
10	0.062	0.051	Beta
11	0.062	0.051	Beta
12	0.063	0.054	Nil
13	0.063	0.057	Nil
14	0.068	0.05	Beta
15	0.071	0.051	Nil
16	0.073	0.056	Nil

BacterialStrain	Average biofilm formation ODat 500 nm		Hemolysin
	Sample	Control	
17	0.086	0.054	Beta
18	0.087	0.057	Beta
19	0.091	0.052	Beta
20	0.101	0.055	Beta
21	0.101	0.052	Nil
22	0.105	0.051	Beta
23	0.108	0.058	Beta
24	0.109	0.043	Nil
25	0.110	0.055	Nil
26	0.112	0.058	Beta
27	0.118	0.055	Alpha
28	0.131	0.057	Beta
29	0.133	0.057	Beta
30	0.202	0.057	Beta
31	0.209	0.054	Beta
32	0.295	0.053	Nil

* ***E.coli* ATCC 25922 showed weak adhesion 0.086 and no hemolysin formation**

Table 5: Detection of biofilm and hemolysin formation among 32 *E. coli* isolates

Biofilm formation (O.D 590 nm)	Adhesiondegree	No.of Bacteria	%
0.04 - 0.05	No biofilm	8	25
0.06 - 0.09	Weak	11	34.4
0.1 - 0.2	Moderate	10	31.3
>0.2	Strong	3	9.4
Total		**32**	**100**
Hemolysin type			
No hemolysin		14	43.8
Alpha		2	6.3
Beta		16	50
Total		**32**	**100**

Table 6: Determination of MIC, sensitivity and resistance rate among 32 *E. coli* strains

Antibiotic type	MIC µg/ml	No. of total isolates	%	* Breakpoint MIC µg/ml	No. of Resistant isolates	%	No. of Sensitive isolates	%	MIC of *E.coli* ATCC 25922
Cefotaxime	1	10	31.3	≤ 1	22	68.8	10	31.3	< 0.5
	1024	22	68.8						
Ceftriaxone	1	10	31.3	≤1	22	68.8	10	31.3	< 0.5
	512	1	3.1						
	1024	21	65.6						
Cefixime	1	10	31.3	≤ 1	22	68.8	10	31.3	< 0.5
	512	13	40.6						
	1024	9	28.1						
Gatifloxacin	0.5	13	40.6	≤ 1	19	59.4	13	40.6	< 0.5
	2	2	6.3						
	4	3	9.4						
	8	2	6.3						
	16	9	28.1						
	32	2	6.3						
	64	1	3.1						
Ciprofloxacin	0.5	12	37.5	≤ 0.5	20	62.5	12	37.5	< 0.5
	2	1	3.1						
	4	2	6.3						
	16	1	3.1						
	64	7	21.9						
	128	2	6.3						
	256	5	15.6						
	1024	2	6.3						
Nalidixic acid	1	7	21.9	≤ 16	23	71.9	9	28.1	< 0.5
	2	2	6.3						
	32	2	6.3						
	256	3	9.4						
	512	4	12.5						
	1024	14	43.8						
Nitrofurantoin	1	3	9.4	≤ 64	6	18.8	26	81.3	< 0.5
	2	2	6.3						
	32	9	28.1						
	64	12	37.5						
	256	6	18.8						

***Reference [14] showed the standard breakpoints MIC of antibiotics**

DISCUSSION:

As revealed in the results of bacterial isolates that most common pathogens was *E.coli.* This might be referred to be a very diverse species of bacteria found naturally in the human intestinal tract. A subset of *E.coli* are capable of causing enteric disease and a different subset extra intestinal disease, including UTI [15]. It is believed that Uropathogenic *E. coli* reside in the colon, and are later introduced through the urethra to the bladder and further to the kidney [16]. Around 26% of the pyuria samples, which represents (2%) of total sample showed no growth. These cases can be described to be a sterile pyuria [8], which might be due to the one or more of the following: treated UTI within two weeks of treatment, inadequately treated UTI, UTI with fastidious culture requirement, renal stones, prostatitis, chlamydia urethritis, renal papillary necrosis, genitourinary tuberculosis, dehydration, urinary tract neoplasm, polycystic kidney [8, 17]. Regarding the biofilm formation, specific binding to the uroepithelium is a key event in the pathogenesis of UTI. UPEC express several different classes of adhesions that are defined by their receptor specificity. The most common fimbriae on UPEC are type1, P fimbriae and curli fimbrae. Around 80% of strains are able to express either one or all types of fimbrae [9]. The ability of each *E.coli* strain into biofilm formation was assessed using standard microtiter tray assay. Overall, the strains displayed a significant difference in biofilm formation; only 25% showed no biofilm formation, around 34.4% showed a weak biofilm, while only 31.3% revealed a moderate biofilm formation and 9.4% showed a strong biofilm [11]. Some 25% of the *E.coli* strains in this collection failed to adhere to microtitter plate; however, strong adhesion was noticed in three strains only. These three strains might possess different genotypic and phenotypic properties, including ability to produce type 1, P or curli undefined. Previous studies have suggested that certain commensal strains produce factors that actively inhibit the pro -inflammatory cytokine response [18]. The three strongly adherent strains might produce such inhibitory factors. Alternatively, the strains may bind to a class of receptors that do not participate in signaling and host cell activation, thus benefiting from adherence without provoking the antibacterial defense [9]. On the other hand, there was no correlation between biofilm formation and the presence of any of the other virulence factor examined in the study such as antibiotic resistance and hemolysis, since five isolates with negative hemolysin showed a strong and moderate adhesion and three positive hemolysin showed no biofilm formation. These results agreed with what Mabbetta *et al* 2009 found [9]. Biofilm formation by UPEC has been linked to chronic and recurrent infection of the bladder [19]. Our analyses revealed that most isolates produced better biofilms, indicating to the pathogenic *E.coli*. The correlation between these results and in vivo biofilm growth remains to be determined. However, recent studies have proposed that UPEC may persist in the bladder as intracellular bacterial communities in a quiescent state [20, 21]. Bacterial resistance to the antibiotics became a complicated issue in the UTI treatment, especially with emergence of new strains resistant to high range of antibiotics. Furthermore, frequent use of one type of antibiotic with high doses might lead to increasing this resistance. As showed in the results that the third generation of cephalosporin became weak effective, as its MIC was 1024 for about 22 strains out of 32, while their breakpoints are 1 µg/ml. The study revealed that gatifluxacin and ciproflxacin could be considered suitable choices for treatment as their MIC was fit and close to their breakpoints. Nitrofurantion is revealed as well to be effective even with a high breakpoint MIC, since around 26 strains

CONCLUSION

Most common pathogens in UTI are *E.coli* followed by *Staphylococcus epidemidis*. Pyuria is not always related to UTI. Bacterial culture is the golden method for detection of UTI. There was no correlation between biofilm formation and the presence of any of the other virulence factor such as antibiotic resistant and hemolysis. The more effective antibiotics against *E.coli* in this setting are gatifluxacine, Ciprofloxacin and nitrfuratoin having most MIC fall close to their cut-off value.

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