Urinary PH and crystals association with bacterial isolates in patients with urinary tract infection

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Abstract:

Urinary Tract Infection (UTI) is among the most community infections worldwide. Host factors such as patients age and gender may influence the prevalence of the infection. In this study a total of 186 urine samples from patients (1–79 years) whom attended Azady Teaching Hospital at Kirkuk province, for UTIs disorders, were examined microscopically and cultured on suitable medias .The age group which were significantly more infected was 20 – 40 years with rate 21.1-34.9 %. The most common bacteria was *Escherichia coli* with rate of 60.3, 42.3% for each of females and males respectively. Followed by *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Among 13 antibiotics used for sensitivity test *S. aureus* and *K. pneumonia* were more resistance comparing with other bacteria, they were resistant to 6 antibiotics, followed by *Streptococcus sp*. which was resistant for five antibiotics. *Proteus mirabilis* was resistant for four antibiotics, each of *E. coli*, *P. aeruginosa* and *Serratia sp*. were resistant for only three antibiotics. The majority of isolates were sensitive to tobramycin ceftizoxime and ntrofurantion, followed by chloramphenicol and ampcillin.whereas, high level resistance was seen to augmentin amoxicillin and nalidixic acid.

Pus, epithelia and R.B.C cells were more seen in samples infected with *E. coli* with rate of 80.3, 83.6, 45.9 % for each type of cells respectively, followed by *S. aureus* and *P. aeruginosa*. The most urine crystals was appeared in urine samples of patients infected with *E. coli* followed by *P. aeruginosa K. pneumonia S. aureus*. In urine samples with specific gravity 1.020 – 1.025 the most crystals seen was calcium oxalate followed by amorphus urate, calcium phosphate , while in urine samples with specific gravity 1.030 the only crystal seen was calcium carbonate . The PH of most urine samples were acidic (5.0 – 5.5, 6.0 – 6.5) with rate of 56.5, 43.5 % for each PH respectively. The PH of 70.5 % of the samples were acidic and 29.5% of them were 8.0 – 8.5. Calcium oxalate, amorphus urate and uric acid were seen in acidic urine samples, while in alkaline one calcium carbonate, calcium phosphate, triple phosphate and amorphus phosphate were seen.

Key words: UTIs, Crystals, PH, Bacterial isolates.

Introduction

Urinary tract infection is extremely common clinical problem, it is important because it may involve the urethra, bladder, ureter and kidney [1]. UTI affects all age groups, women are more susceptible than men due to short urethra, pregnancy and easy contamination of the urinary tract with fecal flora [1, 2]. The *Enterobacteriaceae* particularly *Escherichia coli* is consider the principle pathogen causing UTI and responsible for 75 – 90% of all UTIs infections[3]. *klebsiella sp, staphylo coccus, Enterobacter sp, proteas sp, pseudomonas sp*. and *Enterococcus sp*. were more often isolated from patients [4]. Crystalluria is the excretion of crystals in urine, its present in normal and in pathological conditions like urolithiasis, uric acid nephropathy. Crystalluria depends on the saturation of salts, ingestion of certain foods, changes of urine pH and drugs, crystal inhibitor and promoter and crystal morphology [5]. Most crystals related to urolithiasis are calcium which is found in 80 – 95% of kidney stones as oxalate or phosphate [6]. Renal stone disease has been recognized in many parts of the world. In most instances the precipitation of crystals occurs in little quantities. Hypercrystaluria can be caused by an excessive bone resorbtion, a primary hyperparathyroidia or a combination of other causes, polycrystalline aggregates and urinary tract infection led to renal stones. [7]. The metabolism (glyoxitic acid cycle) produce the majority of the urinary oxalates, only 10 – 15% of urinary oxalate is directly related to diet [8].Citrate urea may be seen in conditions like renal tubular acidosis, chronic diarrhea and excessive animal protein in takes [9]. The sharp edges of some crystals like oxalate calculi can damage the urinary tract epithelium and enhance the growth of the pathogen by forming the nidus to the infectious agents, on the other hand urea splitting organism promotes precipitation of phosphates and oxalates in alkaline media [10]. Persistent infection with urea splitting or non- splitting bacteria may be the initial factors in the synthesis of renal stones. bacterial superimposition in metabolic stones may be responsible for the recurrent urinary tract infections. A definite association is seen between urinary stones and urinary tract infection, positive cultures are not only found with struvite stones, but also with apatite and calcium oxalate stones [11].

The aim of this study was to find out the prevalence of UTI in different age groups and detect the causative bacteria with sensitivity test to some antibiotics, and also to find out the relationship of the microscopic analysis with the causative bacteria and the association of the urine PH and specific gravity with crystal types.

Methodology:

Samples collection:

One hundred eighty sex mid-stream urine samples were collected in sterile universal containers, from 130 femal and 56 male patients whom attended Azady Teaching Hospital in Kirkuk province, Iraq, from July to December 2010. The patient ages were 1-79 years old.

Urine PH measurement:

The urine PH was measured by immersing litmus paper dipstick in the urine and comparing the color change with the standard within 30 sec.[12].

Specific gravity :

Urinometer technique was used to perform the urine specific gravity measurement. 40 ml of urine was put in the urinometer cylinder tube, the urinometer then was let to flout. The specific gravity of urine was determined by the value of urine surface reached the urinometer. .[12]

Microscopy:

The urine samples (3-5ml) were centrifuged at 5000 rpm for 5 min. The deposits were examined using high power objective lens ×40 [13].

Culture media :

The media used were nutrient agar, nutrient broth, macconkey agar, blood agar , cysteine lactose electrolyte deficient agar and muller hinton agar media. All media were supplied by Oxoid Limited. Media were prepared according to manufacturer's specifications and sterilized by autoclaving at 121oC and 1.5 atm for 20 min. Disposable dishes were used for culturing.[14]

Culturing of bacteria from urine samples:

This was done as described by Prescott *et al*. (2008) [14].Ten-fold serial dilutions were made by transferring 1ml of the sample in 9 ml of sterile physiological saline.1 ml was then poured into molten nutrient agar in Petri dishes and rotated gently for proper homogenization. The contents were allowed to set and the plates were then incubated at 37 oC for 24 hr. Bacterial colonies growing on the agar after the incubation period were enumerated to determine urine samples with significant bacteriuria. A loopful of each urine sample was also streaked on MacConkey agar, Blood agar plate for the isolation of the bacteria present in the urine. After incubation, plates with growth were selected, the colonies were isolated using an inoculating loop and subsequently sub-cultured on agar slants for use in further tests.

Identification of isolates:

The methods used in the identification and characterization of isolated bacteria included Gram stain followed by microscopic examination, motility test and biochemical tests according to Cheesbrough [15,16]. The isolates were identified by Bergey's manual for determinative bacteriology [17]. Identification was confirmed with the API 20E system (BioMereiux).

Antibiotic susceptibility test:

Bacterial isolates were tested for their susceptibility to some antibiotics (Ciprofloxacin, Cetazidime, Augmentin, Amoxicillin, Nalidixic acid, Ceftizoxime, Ntrofurantion, Chloramphenicol, Tetracycline, Tobramycin, Amikacin, Trimoxazole, Ampcillin) by modified disc diffusion technique Kirby-Bauer according to the National Committee for Clinical Laboratory Standards [18].

2.9- Statistical analysis:

Statistical analyses were performed using Chi-square to compare categorical variables. A p-value less than 0.05 were considered significant.

Results :

One hundred eighty six urine samples were eligible for this study, 56 samples for males and 130 samples for female their ages were 1 – 79 years old, the UTIs was significantly more prevalent in adult than in children (table1).The age groups which were most infected were 30-39, 20-29 and 40-49 years old, with rate of 38, 23, 15% for each age group respectively. Regarding the sex of the patients, the females were significantly had bacteriauria more than males, with rate of 76.1, 23.9% for each of females and males respectively. The significantly highest bacterial infection was for *E. coli* with rate of 55.9% followed by *S. aureus* with rate of 18.4%. The lowest rate (1.8%) was for *Serratia sp.*

Table (1) UTI bacterial infection according to age group

Age group	Samples examined	%	Bacteria ve+	%	Bacteria ve -	%
1-9	23	12.3	10	9.2	13	16.9
10-19	13	6.9	5	4.6	8	10.4
20-29	47	25.4	23	21.1	24	31.2
30-39	51	27.4	38	34.7	13	16.8
40-49	26	13.9	15	13.8	11	14.3
50-59	14	7.5	9	8.3	5	6.5
60-69	6	3.3	3	2.8	3	3.9
70-79	6	3.3	6	5.5	0	0
Total	186	100	109	100	77	100

Table (2) UTI bacterial infection according to sex and type of bacteria

Bacterial type	Female samples	%	Male samples	%	Total	%
E. coli	50	60.3	11	42.3	61	55.96
S. aureus	14	16.9	6	23.2	20	18.4
P. aeruginosa.	9	10.8	4	15.4	13	11.9
K. pneumonia	5	6.1	3	11.5	8	7.4
Proteus mirabils	2	2.4	1	3.8	3	2.7
Streptococcus sp.	2	2.4	0	0.0	2	1.8
Serratia sp.	1	1.1	1	3.8	2	1.8
Total	83 =76.1%	100	26 =23.9%	100	109	100

The susceptibility test (table 3) of the bacterias, indicated that the *S. aureus* and *K. pneumonia* were more resistant to antibiotic comparing with the other bacterial isolates. They were resistant to 6 antibiotics, followed by *Streptococcus sp.* which was resistant for 5 antibiotics. *E. coli*, *Pseudomonas* and *Serratia sp.* were resistant for only 3 antibiotics.

Table (3) Susceptibility of isolated bacteria to antibiotic

Bacteria	Antibiotics												
	CF	CAZ	AUG	AMX	NA	CI	NF	CH	TE	TM	AK	TR	AMP
E. Coli	++	++	R	R	+++	+++	++	+++	R	+++	++	++	+++
S. aurius	+++	R	R	R	R	R	++	++	++	++	+++	R	++
P. aeruginosa	+++	+++	++	R	++	+++	R	+++	+++	+++	R	++	R
K. pneumonia	R	+++	R	R	R	+++	+++	R	R	++	++	++	++
Proteus mirabils	R	+++	R	++	R	+++	++	+++	++	++	R	++	++
Streptococcus sp.	++	R	R	+++	R	++	++	R	++	++	R	++	+++
Serratia sp.	++	R	R	++	++	++	++	++	R	+++	+++	++	++

CF = Ciprofloxacin, CAZ = Cetazidime, AUG = Augmentin , AMX = Amoxicillin ,NA= Nalidixic acid,CI = Ceftizoxime, NF = Ntrofurantion, CH = Chloramphenicol, TE=Tetracycline, TM = Tobramycin , AK = Amikacin , TR = Trimoxazole, AM P = Ampcillin. R=Resistance, ++ =Moderate sensitive , +++ =High sensitive.

E. coli positive urine samples showed significantly high rate of pus, epithelia and RBC cells (table 4). The rates were 80.3, 83.6, 45.9 % for each type of cells respectively, followed by *S. aureus* and *Pseudomonas* , no significant differences was appeared between the other types of bacteria. Significant association was appeared between *E. coli* and urine crystals. But no association were found with other types of bacteria.

Table (4) frequency of bacteria in relation to urine analysis

Bacterial type	Positive samples	Puss cell	%	Epithelial cell	%	R.B.C	%	Crystals	%
E. Coli	61	49	80.3	51	83.6	28	45.9	39	82.9
S. aurius	13	10	76.9	9	69.3	3	23.1	2	4.2
P. aeruginosa	8	8	100	8	100	6	75	2	4.2
K. pneumonia	3	2	66.7	2	66.7	1	33.4	0	0
Proteus mirabils	20	18	90	15	75	13	65	4	8.7
Streptococcus sp.	2	2	100	2	100	0	0.0	0	0
Serratia sp.	2	2	100	2	100	0	0.0	0	0
Total	109	91		89		51		47	100

Most crystal types (97.9%) were appeared in urine samples with specific gravity ranged between 1.020 to 1.025 (table 5). Only 2.1% of the crystals were seen in urine samples with 1.035 specific gravity. The crystal types appeared in 1.020-1.025 specific gravity were calcium oxalate, amorphous urate, calcium phosphate and other types of crystals. While the crystal which was found in urine with1.035 specific gravity was calcium carbonate. A rate of 70.5% of the examined samples were acidic (PH 5 – 6.5) and

29.5% of them were alkaline (PH 8 - 8.5). In acidic urine samples calcium oxalate, amorphus urate and uric acid were seen. Calcium carbonate, calcium phosphate, triple phosphate and amorphus phosphate were seen in alkaline urine samples.

Table (5) Urine crystals occurrence in relation to specific gravity

Specific gravity	Type of crystal seen	Samples No.	Occurrence in %
1.020 – 1.025	Calcium oxalate	38	31.9
	Amorphus urate	24	20.1
	Calcium phosphate	20	16.8
	Calcium oxalate with other crystal	17	14.2
	Amorphus phosphate	6	5.04
	Triple phosphate	2	1.6
	Tyrosilic needle	1	0.83
	Uric acid	9	7.6
		Total 117	97.9
1.035	Calcium carbonate	2	2.1
		Total 119	100

Table (6) Crystal association with PH

PH		Samples				Type of crystal
		Number examined	%	Crystal positive samples	%	
Acidic	5.0 – 5.5	74	56.5	56	62.9	Calcium oxalate, amorphus urate, uric acid
	6.0 – 6.5	57	43.5	33	37.1	Amorphus urate, calcium oxalate, Tyrosilic needle
		Total =131	70.5	89	100	
Neutral	7.0	-	-	-	-	-
Alkaline	8.0 – 8.5	55	29.5	30	54.5	Calcium carbonate, calcium phosphate, triple phosphate, amorphus phosphate
Total		186	100	119	64.8	

Discussion:

Urinary infections frequently occur in both genders and across all age groups [2, 19].The results of this study showed that the UTI is prevalent in adult more than in children. Adult infection were concentrated in age groups of 20-50 years old. Similar results were found in a study on age dependent etiology of UTI, they indicated high prevalent of UTI in adults, 4167 patients 30-59 years and 8109 patients ≥60 years were found to had bacterial UTI compared with only 703 patients ≤14 years [20] The prevalence of urolithiasis among patients was highest among 40-49 age group (34%) and least at 17 (1%) and 70 years old (1%) [21]. Adult sexual activities may be the main causative for this occurrence or because of the hormonal stats in adults that may influence bacteria adherence and colonization [22]. The sex of the patients in the present stduy had significantly effected the prevalence of bacteriauria. The infection in the females were significantly more than males, with a rate of 76.1, 23.9% for each of females and males respectively. Kolawole *et al.* conducted a study comparable to ours, in terms of patients sex women tend to have UTIs more often than men because of absence of prostatic secretion, pregnancy and bacteria can reach the bladder more easily in women. This is partially due to the short and wider female urethra and its proximity to anus. Bacteria from the rectum can easily travel up the urethra and cause infections [1, 2, 20]. The significantly highest bacterial infection in this study was for *E. coli* with rate of 55.9% followed by *S. aureus* with rate of 18.4%. The lowest rate (1.8%) was for *Serratia sp.* Similar result revealed that the *Escherichia coli* is the predominant uropathogen responsible for roughly 80% of all UTI cases, followed by *Staphylococcus*, *Klebsiella*,

Enterobacter, *Proteus* and *Enterococci* species [4, 23]. These isolated bacteria have been reported as agents of UTIs, their presence in the sample is not unusual. This high frequency of *E. coli* may be due to the fact that strains of *E. coli* possess high virulence factors that promote their persistence, adherence, colonization and invasion of the urinary tract [24]. but the variably in the bacterial distribution pattern among different areas in the world may be explained by the geographical differences which affect the types of bacterial isolates as well as the new strains production that occur within bacterial isolates over years [4].

The susceptibility test showed that *S. aureus* and *K. pneumonia* were more resistance to the antibiotics used comparing with other bacteria, followed by *Streptococcus sp*. The majority of isolates were sensitive to tobramycin ceftizoxime and ntrofurantion, followed by chloramphenicol and ampcillin. whereas, high level resistance was seen to augmentin amoxicillin and nalidixic acid. Studies result about sensitivity of UTIs bacteria to antibiotics have demonstrated that the geographical variability of pathogens occurrence is limited by the predominance of Gram negative, usually Enterobacteriaceae and particularly *E. coli* and *Enterobacter* spp., in various regions of the world and the resistance patterns of these organisms can vary significantly between hospital, countries and continents [4, 19, 25].

High rate of Pus, epithelial, RBC and crystals was noted in urine samples infected with *E. coli* bacterial followed by staph and pseudomonas. This may because of the toxic product and prototyping enzymes produced by these bacteria's which led to inflammation and heamorrgge and exclusion of these cells in urine. This result was comparable to what found by Mohamed [25] whom indicated high frequency of Pus, epithelial, RBC and crystals in urine contained *E. coli* and staph bacteria.

The specific gravity (measures how dilute urine is) of urine were differed according to crystal types appeared in urine. The most crystal type was noted in 1.02 - 1.025 degree of gravity and the only crystal noted in 1.030 was Calcium carbonate. Specific gravity takes into account the weight of the urine and particle size. Most human urine is around 1.010, but it can vary greatly depending on when one drank fluids last, or if he are dehydrated. In end-stage renal disease, specific gravity tends to become 1.007 to 1.010. Any urine having a specific gravity over 1.035 is either contaminated, contains very high levels of glucose, or the patient may have recently received high density radiopaque dyes intravenously for radiographic studies or low molecular weight dextran solutions [26].

The PH of most urine samples were acidic which indicate a normal condition. Low number of urine samples were alkaline. In acidic samples calcium oxalate, amerphas urate and calcium pholate were seen. This result is in agreement with [27] whom revealed the presence of calcium oxalate, amerphasurate and calcium pholate in acidic urine and carbonate in alkaline samples. However, depending on the acid-base status, urinary pH may range from as low as 4.5 to as high as 8.0. The change to the acid side of 7.4 is accomplished in the distal convoluted tubule and the collecting duct. Some stones, such as those composed of uric acid or cystine, are pH-dependent, meaning that they can form only in acidic conditions. Calcium phosphate and struvite only form when the urine pH is alkaline. Although the other parameters in the 24-hour urine usually identify patients at risk of forming these stones, pH studies can be important in monitoring these patients, in optimizing therapy with citrate supplementation, and in identifying occult stone disease in some patients [26].

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علاقة الاس الهيدروجيني و البلورات في البول مع العزلات البكتيرية في مرضى التهاب المجاري البولية

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الملخص

التهاب المجاري البولية هو من احد امراض المجتمع السائدة عالميا. انتشار المرض من الممكن ان يؤثر فيه بعض العوامل مثل العمر والجنس للشخص المصاب. اخضعت هذه الدراسة 186 نموذج ادرار للمرضى الوافدين الى مستشفى ازادي التعليمي في محافظة كركوك للفحص المجهري و الزرع في الاوساط البكتيرية. 130 نموذج كان من الاناث و 56 نموذج كان للذكور تراوحت اعمارهم بين 1– 79 سنة. المرحلة العمرية التي كانت معنويا اكثر عرضة للاصابة هي الفئات 20–40 سنة وبمعدل21,1–34,9 %. نسبة تردد بكتريا *E. Coli* كان معنويا اكثر مقارنة بالانواع الاخرى من البكتيريا وبنسبة 60,3, 42,3% لكل من الاناث و الذكور بالتعاقب. وتبعها بكتريا *S. aureus* و *P. aeruginosa*. بين 13 مضادا حيويا استخدمت, كانت بكتريا *S. aureus* و *K. pneumonia* الاكثر مقاومة, حيث اظهرت مقاومة لستة مضادات حيوية. وتبعت ببكتريا *Streptococcus sp.* و*Proteus sp* حيث اظهرتا مقاومة لخمسة مضادات. بينما كل من بكتريا *E . Coli* و *Serratia sp.* اظهرتا مقاومة لثلاث مضادات فقط . نسبة كبيرة من البكتريا اظهرت حساسية لمضاد توبرامايسين و سفتيزومكس ونايتروفيورشن وتبع بمضاد كلورومفينيكول والامبيسيلين. بينما شوهد مستويات عالية من المقاومة لمضاد اكومنتين والاموكسيلين وحامض ناليدكس.

تم ملاحظة الخلاية الالتهابية و الظهارية و خلايا الكريات الحمراء بنسبة تردد اعلى في النماذج الحاوية على بكتريا *E.Coli* و بنسبة 45,9 ,83,6, 80,3 % لكل من الخلايا بالتوالي. ظهر الكريستالات بنسبة اكبر في العينات الحاوية على بكتريا *E.Coli* وتبع بكتريا *S. aureus* و *P. aeruginosa* و*K. pneumonia*.

شوهد املاح اوكسالات الكالسيوم وامورفس يوريت وفوسفات الكالسيوم في نماذج الادرار ذات الوزن النوعي1,025–1,020.بينما في نماذج الادرار ذات الوزن النوعي1,030الكريستال الوحيد التي شوهدت هي كاربونات الكالسيوم. الغالبية العظمى(86,2%) من عينات الادرار كان حامضيا (5–5,5 ,6–6,5) وبنسبة 58,28,4% لكل اس هيدروجيني على التوالي. الاس الهيدروجيني ل13,8% من العينات فقط كان8–8,5. لوحظ اوكسالات الكالسيوم وامورفس يوريت وحامض اليورك في عينات البول الحامضي. بينما في العينات القاعدية سجل كل من كاربونات الكالسيوم وفوسفات الكالسيوم الفوسفات الثلاثي و والامورفس فوسفيت.

الكلمات المفتاحية: التهاب المجاري البولية, الكريستال, الاس الهدروجيني, العزلات البكتيرية.