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Biochemical and Microbiological Analysis of Urine and Blood in Females with Urinary Tract Infection in Waist Province

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Abstract

Urinary tract infections (UTIs) is one of the most common bacterial ailments and is a significant infection that has a high risk of morbidity., take up a significant amount of the workday in clinical biochemical and microbiological laboratories. This study aimed to evaluate the biochemical parameters in urine and blood and bacteriological aggregation in urine, also looked at the relationship between the bacteria isolated from urinary tract infections and their susceptibility to antibiotics. Between (Jan. and April 2023), 150 female patients (average age of 28.7 (range: 15–45years) with urinary tract infection were enrolled in the study at AL-Suwaira General Hospital. Proteus species, E. coli, Klebsiella pneumonia, Neisseria species, Pseudomonas aeruginosa, and Salmonella species were among the bacterial isolates that were obtained. With a documented prevalence of 66.67%, Proteus spp. was determined to have the highest prevalence of particular bacteria among all female UTI patients, according to the study's results comparing the distribution of isolated bacteria. Two categories of patients were worked: fifty patients had Proteus urinary tract infection, and twenty-five patients did not have Proteus urinary tract infection. The results of a blood test and a urine analysis were examined in these

two groups. By using the disk diffusion test, the isolates' pattern of antibiotic sensitivity was also examined. According to our findings, the Proteus patients had proteinuria, cloudy urine, and a positive nitrite test result and frequently had urine specific gravity ($p \leq 0.05$) .Blood test findings did not significantly differ between Proteus and non-Proteus patients. Additionally, the study's findings demonstrated that Gram-negative bacteria are susceptible Levofloxacin is the most effective antibiotic, and it exhibits resistance to Penicillin but limited sensitivity to Nalidixic acid. For both Proteus and non-Proteus urinary tract infection, an early identification is essential in order to promptly administer the proper empirical antibiotic treatment. This is crucial for preventing long-term treatment, complications, irreversible kidney damage, and the severity and chronicity of the disease.

Keywords: Urine analysis, Urinary tract infection, bacteriuria, urea, antibiotic sensitivity.

Introduction

A urinary calculus was discovered in an Egyptian mummy that was almost 5,000 years old. Hippocrates was the first to recognize the connection between stones and decomposition [1]. Humans and other vertebrates produce urine as a waste product of their metabolism. When it builds up within the body, it is first momentarily held in the bladder before being expelled by the urethra. Normally, the urinary system and bladder are sterile. Urine collection can be contaminated by a wide range of gram-positive and negative organisms found in the perineum and a few commensals that may be present in the urethra [2]. In female patients, germs from the vagina may contaminate the urine. A mixture of bacteria and epithelial cells is often a sign of vaginal contamination [3]. Urine containing bacteria is referred to as bacteriuria. When there are 10^5 organisms or more per milliliter of urine, it is typically considered significant. Consequently, gram-positive with negative microbe that function as normal flora in most areas in human body with may be transfer to lower urine tract to create UTIs are the source of multi-infections[4].The common microbes may be infect UTIs in humans is bacteria. Urinary pathogens are the term for these bacterial organisms. Gram-negative in present oxygen and not present oxygen are the two categories into which the bacterial agents are divided. *Proteus mirabilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Alcaligenes spp.*, *Acinetobacter spp.*, *Citrobacter spp.*, *Klebsiella spp.*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* are a

few of them[5].This infection may cause illnesses like pyelonephritis, urethritis, cystitis, bacteriuria, and prostate problems by affecting the complete urinary system[4]. Females are more likely than male to get urinary tract infections because of the shorter female urethra. Many research have indicated the positive of nitrites in the woman urine indicate for substantial bacteria [6]. The following actions could lower the frequency of urinary tract infections, according to studies. For people who have recurrent UTIs, a prolonged course of low-dose antibiotics—typically nitroforantoin—that lasts six months to a year works well to lower the incidence of UTIs. Topical oestrogen cream applied intravaginally can shield postmenopausal women from developing recurrent cystitis. However, low dose antibiotics are more beneficial than this. Research has indicated that nursing may lower an infant's risk of developing a UTI[7] The continuous modifications the microbes' antibiotic susceptibility leads to the increase in mortality and morbidity by infectious diseases in general, and by urinary tract infections in particular, resulting in the increase of expenses for patient care. This study was carried out to evaluate the biochemical makeup and bacteriological population of urine as well as pre-operative urines. We also looked at the relationship between the bacteria isolated from urolithiasis and urinary tract infections and their susceptibility to antibiotics.

Material and Methods

Microbiological analysis:

One microliter was spread on a solid nutritional agar medium. After a 24-hour incubation period at 37C°, the plates were checked for bacterial growth. The isolates were sub cultured on hearts infusion agar, MacConkey agar, blood agar, and nutritional agar medium. Next, use streaking method to characterize just one colony of suspension. Then incubated at 37C° for a 1 day. They were kept cold until they were needed for the next test.

Characterization with identification:

After a 24-hour incubation period at 37C°, the plates were checked for growth. Based on their growth pattern, color, size, and shape, the morphological traits of the isolated colonies were noted. Additional tests performed on the isolates include the detection of biochemical characteristics, the Gram stain, oxidase, catalase, and urease and motility test.

Patients:

A doctor's diagnosis indicated that 150 of the female specimens were likely UTI sufferers. Seventy-five female patients (average age of 28.7 (range: 15–45 years) were selected; 25 of them had non-Proteus UTIs, and the remaining 50 had Proteus UTIs, serious clinical presentations, and unsuccessful prior outpatient treatment. All of the patients had UTI-related laboratory and clinical findings, as well as significant bacteriuria in the performed urine culture obtained in an ambulatory or hospital setting. The parameters measured in blood (Hb), (WBC), (ESR), (Neu.) and (RBC).While in serum (urea, creatinine and C-Reactive protein) by using kits specific for all test ,and urine was used to measure some physical and chemical factor by using urine strip .

Methods

The AL-Suwaira Technical Institute's chemical and serology laboratory, part of the medical laboratory department, performed biochemical analysis on blood and urine samples. Following the collection of the patients' symptoms, the physical examination for signs of the disease, the results of the urine culture, blood test, and laboratory analysis, the patients were separated into two groups: female patients with non-Proteus UTIs and female patients with Proteus UTIs. The following laboratory results were found in these two groups: a) Blood test results using kits designed specifically for each test, including urea, creatinine, and C-reactive analysis in serum were carried out on Roche cobas® c 311 analyzer utilizing photometric analysis [8], as well as (Hb), (WBC), (ESR), (Neu.), and (RBC) in blood were carried out on Roche Cobas m 511 integrated hematology analyzer [9]. b) Urine analysis was used to measure some physical and chemical factor by using urine strip (Dipstick test) such as: hematuria, cloudy urine, glycosuria, urine specific gravity, proteinuria, and nitrites in the urine, and urine.

Antibiotic sensitivity test:

The Disc diffusion method was used to conduct the antibiotic susceptibility test. It offers a quick and accurate test that is particularly useful for standard clinical bacteriology procedures. The isolates' pure cultures were utilized to test their susceptibility to antibiotics. Using a sterile spreader, a distinct colony of each was cultured uniformly on the Muller Hinton agar media surface. A commercially prepared selection of gram-positive discs was made using

Ciprofloxacin Norfloxacin, Gentamycin and Cefalexin are 10 µg , Ampiclox , Levofloxacin Cefixime are 20µg , 30 µg. Gram-negative antibiotic discs were made using Nitrofurantoin , Ciprofloxacin ,and Gentamycin, Cefuroxime ,and Ceporex are 10 µg, Amikacin , Naldixic acid , Ceftriaxone ,and Augmentin are 30 µg, Methoprim , Ampicillin ,and Cefotaxime are 30 µg. Every infected plate's surface has them on it. After a 24-hour incubation period at 37°C, the plates were checked for signs of growth inhibition. Antibiotic-sensitive bacterial isolates were identified as having an inhibition zone, but antibiotic-resistant isolates lacked.

Statistical methods

The acquired data were loaded into SPSS version 2020, a statistical application. The test of proportions was used to analyze the data, and a p value of less than 0.05 was deemed statistically significant for the purposes of the hypothesis.

Results

A physician diagnoses showed growth of bacteria in urine 150 of the female UTI specimens; out of 45 (30%) had a confirmed bacterial infection with a gram positive bacteria (*Staphylococcus saprophyticus* and *Enterococcus faecalis*) ,while 75 (50%) had a confirmed bacterial infection with a gram negative bacteria (*E. coli, Pseudomonas aeruginosa, Salmonella spp.*,and *Neisseria spp.*), and the remaining 30 (20%) had an no growth of bacteria.

Table1: Percentage of gram positive and negative bacteria in UTI

Result of urine culture	Females with UTI	
	No.	Percentage
Gram positive bacterial	45	30%
Gram negative bacterial	75	50%
No growth of bacteria	30	20%
Total	150	100%

In the current study a total 75 gram negative bacterial isolates 50 of which were from Proteus isolates as percentage (66.67%) and 25 of other gram negative bacterial in percentage (33.33%), as show in Table2.

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Table 2: Percentage of Proteus and other gram negative bacteria isolated in study groups

Urine culture results	Females with UTI	
	NO.	Percentage
Proteus *spp.*	50	66.67%
Other gram negative Bacteria (*E. coli*, *Klebsiella pneumonia*, *Neisseria species*, *Pseudomonas aeruginosa*, and *Salmonella species*)	25	33.33%
Total	75	100%

Proteus spp. was found to have the highest prevalence of specific bacteria among all female UTI cases, with a recorded prevalence of 66.67%. Other bacteria found to be present were *E. coli*, *Pseudomonas aeruginosae*, *Salmonella spp*., and *Neisseria spp*. with respective prevalences of 10.67%, 9.33%, 6.67%, 4%, and 2.66%.

Table 3: Distribution of isolated bacteria in the study groups and diagnostic test for gram negative bacteria**.**

Isolated	**Female cases of positive UTI**			
	No.		**Percentage**	
Proteus spp.	50		66.67 %	
E.coli	8		10.67%	
Klebsiella pneumonia	7		9.33%	
Neisseria spp.	2		2.66%	
Pseudomonas aeruginosa	5		6.67%	
Salmonella spp.	3		4%	
Total	75		100%	
Microbis	Catalase	Oxidase	Urease	Gram stain
Proteus spp.	+	-	+	-
E.coli	+	-	-	-
Klebsiella spp.	+	-	-	-
Neisseria spp.	+	-	-	-
Pseudomonas aeruginosa	+	+	+	-

Biochemical test:

The most frequent abnormal results from blood tests in patients with UTIs were: leukocytosis in 20 (40%), erythrocytopenia in 28 (56%), neutrophilia in 22 (44%), high erythrocyte sedimentation rate in 42 (84%), high C-reactive protein in 34 (68%), low serum hemoglobin level in 26 (52%) and high urea in 32 (64%). Between the two groups of patients, there were no statistically significant variations in the frequency of abnormal laboratory test results ($p>0.05$) (Table 4). The most frequent aberrant outcomes from urine analyses were: hematuria in 14

(28%), proteinuria in 36 (72%), cloudy urine in 14 (28%), and nitrites in 40 (80%). Urine cloudiness (p=0.00), proteinuria (p=0.01), and urine nitrites (p=0.03) were shown to be substantially more common in female Proteus UTI patients. Urine specific gravity was more frequently (p=0.02) in the female patients with non-Proteus UTIs as show in (Table 5).

Table 4: Results of blood biochemical parameters in female patients with urinary tract infections, including Proteus and non-Proteus

Biochemical parameters	Proteus UTI			Non- Proteus UTI			P-value
	NO.	Percent	Mean ±SD	NO.	Percent	Mean ±SD	
Urea (mmol/L)	32	64%	4.82±1.21	10	40%	3.12±1.19	0.15
Creatinine (µmol/ L)	24	48%	23.11±6.20	8	32%	22.12±5.99	0.32
Hemoglobin(g/L)	26	52%	10.42±1.85	15	60 %	10.85±1.04	0.10
Erythrocytes sedimentation rate (mm/h)	42	84%	2.5±086	19	76%	1.58±0.95	0.09
Leukocytes number ($\times10^9$/L)	20	40%	15.1±3.526 45	9	36%	15.425±4.75	0.08
Neutrophils Number($\times10^9$/L)	22	44%	8.558±2.86	16	64%	7.895±1.74	0.11
Erythrocytes Number$\times10^{12}$/L)	28	56%	4.8±1.04	12	48%	4.3±0.95	0.21
C-reactive protein (mg/L)	34	68%	20.84±8.99	19	76%	11.65±2.95	0.19

Table 5: Abnormal urine analysis results in in female patients Proteus and non- Proteus urinary tract infections

Abnormal urine test	Proteus UTI			Non- Proteus UTI			P-value
	NO.	Percent	Result	NO.	Percent	Result	
Hematuria	14	28%	+25 RBC/µL	2	8%	+25 RBC/µL	0.14
Cloudy	46	92%	Very cloudy	22	88%	Cloudy	0.00
Proteinuria	36	72%	++100 mg/dL	17	68%	+30 mg/dL	0.01
Specific gravity	12	24%	1.030	20	80%	1.025	0.02
Nitrites	40	80%	+ Pink rose rasdo	13	52%	+ pink	0.03
Glucosuria	22	44	+250 mg/dL	4	16	±100 mg/dL	0.13

Table (6) demonstrates the pattern of antibiotic sensitivity seen in the urine isolates from 75 females UTI patients. As may be necessary, gram negative antibiotic disks were used to test the isolates. It was revealed that *Proteus species* were extremely sensitive to Levofloxacin, Cefotaxime, Methoprim and Amikacin , and low sensitivity to Ciprofloxacin, Nitrofurantoin, Ofloxacin, Ceftriaxone and Naldixic acid, while it show resistance to Penicillin, while *E.coli*

was highly sensitive to Methoprim and there was visible resistance to the remain antibiotic used in this study. As well as *Klebsiella spp.* sensitive just for Levofloxacin, Cefotaxime and Cephalexin. Additionally *Neisseria ssp.* showed sensitivity to Amikacin, while *Pseudomonas spp.* sensitive to Naldixic acid , Levofloxacin, amikacin and ciprofloxacin, while *salmonella spp.* was resistance for all antibiotics except Levofloxacin, Ceftriaxone and Nitrofurantoin. As compared with other studies for potency of antibiotics revealed that Levofloxacin appeared higher activity against all types of used bacteria except *Neisseria spp.*

.**Table 6:** Profile antibiotic sensitivity test for gram negative bacteria.

Organisms		Antibiotic												
		N	**L**	**CX**	**AM**	**P**	**CXN**	**CX**	**OX**	**MO**	**CIP**	**CN**	**NIT**	**NAL**
Proteus spp.	**No.**	50	10	9	5	0	1	8	2	6	3	2	3	1
	Test	S	S	S	S	R	S	S	S	S	S	S	S	S
E.coli	**No.**	8	0	0	0	0	0	0	0	8	0	0	0	0
	Test	S	R	R	R	R	R	R	R	S	R	R	R	R
Klebsiella spp.	**No.**	7	5	0	0	0	0	1	0	0	0	1	0	0
	Test	S	S	R	R	R	R	S	R	R	R	S	R	R
Neisseria spp.	**No.**	2	0	0	1	0	0	0	0	0	0	0	0	1
	Test		R	R	S	R	R	R	R	R	R	R	R	S
Pseudomonas spp.	**No.**	5	2	0	1	0	0	0	0	1	1	0	0	0
	Test	S	S	R	S	R	R	R	R	S	S	R	R	R
Salmonella spp.	**No.**	3	3	0	0	0	2	0	0	0	0	0	1	0
	Test	S	S	R	R	R	S	R	R	R	R	R	S	R

L: Levofloxacin; CX: Cefotaxime; AM: Amikacin; P:Penilline;CXN:Ceftriaxone; ox: Ofloxacin; MO: Methoprim; cip: Ciprofloxacin; CN: Cephalexin; NIT: Nitrofurantoin; NAL: Nalidixic acid; CX, Cefotaxime, S: Sensitive, R:Resistance

Discussion

High erythrocyte sedimentation rate, high C-reactive protein, low serum hemoglobin level, high urea, and leukocytosis patients were the most often occurring abnormal blood test results in UTI patients. Based on the literature study, it has been shown that the systemic inflammatory response associated with UTIs is limited to upper urinary tract infections. In these cases, elevated blood test results, including elevated C-reactive protein, elevated erythrocyte sedimentation rate, and leukocytosis, may be observed [10,11].The blood test findings of patients with Proteus and non-Proteus UTI no statically significant differ from one another, according to this study ($p>0.05$). Analogous research has demonstrated that individuals with neutrophilia and UTI experienced a markedly severe course of illness, leading to a fatal [12].

Female patients with non-Proteus UTIs showed significantly different urine analysis results, but female patients with Proteus UTIs frequently had higher specific gravity, cloudy urine ($p<0.05$) and proteinuria ($p=0.02$) were more common in female patients with non-Proteus UTIs. These findings may be attributed to the significant effects of Proteus species on the glomerular membrane, which increase the permeability of the membrane for proteins, and the effect of non-Proteus species on renal tubules, which reduce renal tubular re-absorption. Additional research is required to validate these results [13]. Leibovici *et al.* examined 247 elderly patients (median age: 75 years) and discovered that patients with bacteremic UTIs caused by different types of bacteria are more likely to have high serum creatinine, high WBC count, hyperthermia, diabetes mellitus, and low serum albumin levels [14]. Proteus species-caused UTIs are frequently more severe and linked to a higher incidence of pyelonephritis than *E. coli*-caused UTIs [15].Other studies showed urine pH in *Proteus spp.* in UTIs is frequently higher than 7.2 most bloodstream infections caused by Proteus species result from (UTIs), which are frequently linked to urinary catheter use. [15,16]. Our research indicates that *P. mirabilis* bacteremic UTIs are more likely to have hydronephrosis, band neutrophils that account for more than 10% of the WBC count, hyper- or hypothermia, and a high concentration of C-reactive protein. Significantly fewer negative nitrite test results were observed in female patients with non-Proteus urinary tract infections. However, it is important to note that some of the bacteria in these female patients' Non-Proteus group are incapable of converting nitrates to nitrites, and they may also be slow-growing bacteria found in urine media, such as *E. faecalis, S.aureus,* and *S. saprophyticus* [17]. Leukocyturia, a positive nitrite test, and UTI have a strong association, according to similar research [18,19]. On other hand about antibiotic used in this study showed that a significant proportion of the bacteria deals with sensitive against Levofloxacin. These results suggest that these medications are still effective urinary antiseptics in our environment. Furthermore, this result is consistent with data on the pattern of antibiotic sensitivity of isolates from different national [20]. Due to its high cost, Levofloxacin is unlikely to be bought over-the-counter without a prescription, while Amikacin is affordable and efficient, but it must be administered parenterally; as a result, it cannot be used to treat outpatients. As well as Methoprim is likewise inexpensive, but it needs an alkaline pH environment to work, so it's only appropriate for people

without stomach issues. The susceptible cell wall of the isolates, which is shielded from the antibiotics by an outer membrane, may also be the cause of their resistance to some antibiotics, in addition to drug addiction [21]. We will be able to evaluate whether this is a case of Proteus or non-Proteus UTI based on these clinical and laboratory characteristics, which can be obtained in a few hours. This would allow us to prevent major illness consequences by providing early and sufficient empirical therapy.

Conclusion

Our investigation has shown that lower urine specific gravity is suggestive of a non-Proteus UTI, as well as cloudiness of urine samples, proteinuria, and positive nitrite tests being much more common in female patients with non-Proteus UTIs. Proteus species were the most sensitive isolate, whereas Neisseria species were the least sensitive. Levofloxacin is the most effective antibiotic, and it exhibits resistance to Pencillin but limited sensitivity to Nalidixic acid. For both Proteus and non-Proteus UTIs, an early etiological identification is essential in order to promptly administer the proper empirical antibiotic treatment. This is crucial for preventing long-term treatment, complications, irreparable kidney damage, recidivism, and the illness's chronicity.

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