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ISOLATION OF BACTERIAL PATHOGENS FROM PATIENTS WITH URINARY TRACT INFECTION IN ERBIL CITY AND DETERMINATION OF THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERNS

May Ali Saleh	Dr. Sabahattin COMERTPAY
College of Science / Biology Department	Assoc. Prof
KAHRAMANMARAŞ SÜTÇÜ İMAM UNIVERSITY	KahramanmarasSutcu Imam University
Erbil /Iraq	Kahramanmaras /Turkey

ABSTRACT: Urine is a liquid secreted out of the body after the blood stream is filtered by the kidney, and carries with it some of the salts and waste, but must be known the urine in healthy person is sterilized doesn't contain any type of bacteria or microorganisms.

The aim of this study was to identify the etiologic agents and antibiotic resistance on samples taken from the patients living in Erbil City of Iraq between. In this study we compare the effect of sex, age and other factors on the prevalence of urinary tract infection in the Erbil city and have examined their susceptibility profile antibiotics is commonly used on different types of bacteria causing the disease.500 urine specimens were collected from patients clinically suspected cases of UTIs. The bacterial pathogens were isolated by standard microbiologic methods and antimicrobial susceptibility testing was detected by the Kirby-Bauer disk diffusion method.

The results showed significant bacterial pathogens were found in (71.2%) the final results of patient. The ultimate widespread pathogens isolated were E. coli(44.38%) while other bacteria was Staphylococcus aureus (19.10%), Staphylococcus epidermidis (10.39%),Klebsiella pneumonia (6.17%),Pseudomonas aeruginosa (5.05%),Proteus mirabilis (3.37%),Staphylococcus haemolyticus (1.96%),Staphylococcus saprophyticus (1.96%), Enterobacteraerogenes (1.68%),Klebsiellaoxytoca (1.12%),Citrobacterkoseri (0.84%),Morganellamorganii (0.84%),Enterobacter cloacae (0.56%),Enterococcus faecalis (0.56%), Proteus vulgaris (0.56%),Serratiafonticola (0.56%),Streptococcus agalactiae (0.28%), Citrobacterfreundii (0.28%), and Salmonella typhimurium (0.28%).

INTRODUCTION

Urinary tract infection is the most widespread micro bacterial infection in the all around the world and accounts for 1-3% of consultation. Up to half of females have UTI. 3% of women have UTI at the age 20, increasing by about 1% in each following decade. (Davidson practical medicine, 21st Edition) In male urinary disease and infection is infrequent but that doesn't include the first year of the life and in above the age of 60, Rectal flora may enter the urinary tract and case UTI in healthy person (Handley et al. 2002).

UTI is a kind of infection that is resulted from any kind of bacteria going into the urinal system and growing anywhere in the urinary tract. The urinary tract includes organs which collect and keeps the urine and release it outside of the body, and that system comprises the kidneys, ureters, bladder and urethra. UTIs are among the most widespread micro bacterial infections in human beings of both the community and hospital have been reported in all age groups in both male and female and young patients (Faried, 2012).

STERILIZATION OF MEDIA AND MATERIALS

Autoclaving: All media were sterilized by autoclaving at 121°C and under 1.5 bar for 15-20 minutes, except for sugar containing media which were sterilized just for 10 minutes.

Dry sterilization: All glassware's were washed then the glassware's were sterilized by a hot air oven at 200°C for two hours.

Processing of urine samples:

Urinalysis: Urinalysis is very important in the diagnosis of urologic conditions can alert and worn the physician to the presence and appearance of systemic disease that's affecting the kidneys such as calculi, urinary tract infection (UTI), and malignancy.

General urine examination: The microscopic exam is done on the urine that has been centrifuged to focus the substances in it at the bottom of a tube. They discarded the liquid at the top of the tube and then the drops of fluid remaining are examined by a microscope for detection of pus cells, erythrocytes, casts, crystals and other such as mucous, bacteria and parasites.

Gram stain smear: A staining technique used to classify bacteria. they Place a drop of an uncentrifuged urine that is well mixed on slide and then allow the drop to dry and should not be spread, heat fix and stain. They Examineit under an oil-immersion lens for the presence or absence of microorganisms and polymorphonuclear leukocytes. One or more microorganisms cells per oil-immersion field normally implies that there are 10 or more micro bacteria per milliliter in the specimen and presence of one or many leukocytes per oil-immersion is another indication of UTI (Vandepitte*et al.*, 2003).

Urine culture: Prepare the agars to the bacteriological and biochemical tests using a standard procedure. That happens after the plates were kept in the incubator at 35-37°C for 18-24 hours. The number of isolated bacterial colonies was multiplied by 1000 for the estimation of bacterial colony forming/ml of the urine samples. A specimen was considered positive for UTI if an organism was cultured at a concentration of 104 CFU/ml.Ifno growth appeared they were further incubator for another 24 hours before regarded as a negative.

IDENTIFICATION OF PATHOGENS:

Morphology: The isolated bacterial colonies were identified according to the morphology, pigment production, fermentation and haemolysis on the blood agar.

Microscopically: The bacterial isolates were further classified by Gram-staining to Gram-negative and Gram-positive bacilli and cocci.

Biochemical tests: The predominant isolated bacterial colonies were testing for their biochemical characteristics as follows: Indole test, Methyl Red-Voges- Proskauertests, Citrate utilization test, Urea utilization ,Motility test, Oxidise test, Catalase test, Coagulase test, Identification of isolated bacteria by Vitek 2 Compact System, Suspension preparation, Antimicrobial sensitivity testing

TEST RESULTS AND DISCUSSIONS

Our result shown that, out of 500 urine specimen collected from patients complaining of signs and symptoms of UTIs attending Erbil general hospital laboratory in Erbil city, 356 (71.2%) were positive for bacterial infections (their colony count was equal or more than 104 CFU/ml), while 144 (28.8%) samples showed culture negative, as shown in table (1.1).

Table	Error! No	text of s	pecified	style	in doc	ument. .1)	Distribution	of j	patients	with	UTIs

	Cultures results				
Samples (No. =500)	Positive No. (%)	Negative No. (%)			
	356 (71.2%)	144 (28.8 %)			

The pattern of pathogens encountered in this study correlates well with many studies conducted in different countries either in the regional or international settings. For example, Mezal*et al.* (2011) in Basrah, Iraq, reported that only (72.6%) of samples showed positive culture. Ali *et al.*(2014) in Kalar, Iraq reported

that only (69.8%) of samples is positive culture. Kolawole*et al.* in (2009) studied that among 300 urine samples only (60%) showed culture positive. Mishra *et al.* in (2013) reported that among 1245 samples only (80%) of samples were positive for bacterial infections.

The percentage variable differences could be assigned to the variation in the size of the sample or in the technical procedure or in relation to the survey area, the remaining 144 specimens (28.8 %) as shown in table (1.1), did not give any sign of the growth of bacteria even after 48 hours of incubation, this could be due to UTI caused by agents other than bacteria such as viruses, fungi, anaerobic bacteria and other bacterial causes that cannot be isolated by traditional methods used in this study and may need special media for their growth or improper use of antibiotics.

We showed in the Table (1.2) , the antibiotic susceptibility tests for isolated bacterial pathogens are found that Staphylococcus haemolyticus (42.85%), Enterobacter cloacae (50%), Proteus vulgaris (50%) were resistance for ciprofloxacin, but Escherichia coli (62.02%), Staphylococcus aureus (57.35%), Staphylococcus epidermidis (56.75%), Klebsiella pneumonia (86.36%), Pseudomonas aeruginosa (55.55%), Proteus mirabilis (50%), Staphylococcus saprophyticus (71.42%), Enterobacteraerogenes (83.33%), Klebsiellaoxytoca (75%), Citrobacterkoseri (66.66%), Enterobacter cloacae (50%), Enterococcus faecalis (100%), Proteus vulgaris (50%), Serratiafonticola (100%), Streptococcus agalactiae (100%), Citrobacterfreundii (100%), Salmonella typhimurium (100%) were sensitive for the same antibiotic.

No	Isolated notherang	Number	Amoxicillin No. (%)			
NO.	Isolated pathogens	Inumber	Resistant	Intermediate	Sensitive	
1	Escherichia coli	158(44.38%)	55(34.82%)	5(3.16%)	98(62.02%)	
2	Staphylococcus aureus	68(19.10%)	13(19.11%)	16(23.52%)	39(57.35%)	
3	Staphylococcus epidermidis	37(10.39%)	9(24.32%)	7(18.92%)	21(56.75%)	
4	Klebsiella pneumonia	22(6.17%)	1(4.45%)	2(9.09%)	19(86.36%)	
5	Pseudomonas aeruginosa	18(5.05%)	4(22.22%)	4(22.22%)	10(55.55%)	
6	Proteus mirabilis	12(3.37%)	3(25%)	3(25%)	6(50%)	
7	Staphylococcus haemolyticus	7(1.96%)	3(42.85%)	1(14.28%)	3(42.85%)	
8	Staphylococcus saprophyticus	7(1.96%)	2(28.57%)	0(00)	5(71.42%)	
9	Enterobacteraerogenes	6(1.68%)	1(16.66%)	0(00)	5(83.33%)	
10	Klebsiellaoxytoca	4(1.12%)	0(00)	1(25%)	3(75%)	
11	Citrobacterkoseri	3(0.84%)	1(33.33%)	0(00)	2(66.66%)	
12	Morganellamorganii	3(0.84%)	1(33.33%)	1(33.33%)	1(33.33%)	
13	Enterobacter cloacae	2(0.56%)	1(50%)	0(00)	1(50%)	
14	Enterococcus faecalis	2(0.56%)	0(00)	0(00)	2(100%)	

Table (1.2) Antimicrobial susceptibility of isolates pathogens to ciprofloxacin

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	15	Proteus vulgaris	2(0.56%)	1(50%)	0(00)	1(50%)	
	16	Serratiafonticola	2(0.56%)	0(00)	0(00)	2(100%)	
	17	Streptococcus agalactiae	1(0.28%)	0(00)	0(00)	1(100%)	
	18	Citrobacterfreundii	1(0.28%)	0(00)	0(00)	1(100%)	
	19	Salmonella typhimurium	1(0.28%)	0(00)	0(00)	1(100%)	

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