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Evaluation of Antibiotics Sensitivity for Bacterial Isolated from Urinary Tract Infections of Patients in Tikrit City, Iraq

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Abstract

This study was aimed for isolation and diagnosis of bacterial that contaminated of Urinary Tract Infections (UTIs) samples from both gender patients whom referred of Salah-Adden hospital in Tikrit city, Iraq, to test the bacterial isolates sensitivity toward some antibiotics. The results were investigated that collect 91 samples of UTI from patients, and isolated include 113 isolates bacteria distributed to 51 isolates (45,13%) from gram positive bacteria and 62 isolates (54,87%) from gram negative bacteria. The diagnosis of isolates bacteria dependence to morphological, microscopic, cultural and biochemical tests, distributed isolates of gram positive were appear at 50 isolates from genus *Staphylococcus* which included at 34 isolates (30,08%) from species *S. aureus* and 12 isolates (10,62%) from *S. epidermidis* and 4 isolates (3,54%) back to *S. saprophyticus* and the *S. pyogenes* was isolated to 1 isolate (0,08%). While gram negative bacteria was distributed on 31 isolates (27,43%) back to species *K. pneumoniae* and 26 isolates (23,01%) from *E. coli* and 4 isolates (4,42%) from *P. aeruginosa*. The tests of bacterial isolates sensitivity towards 8 types of antibiotics founded high resistance against Penicillin G, Ampicillin and Neomycin while mostly high sensitivity to Ciprofloxacin and intermediate for other antibiotics.

Keywords: *Urinary Tract Infections; Antibiotics; Sensitivity; Bacterial Isolates*

Introduction

The Urinary Tract Infections (UTIs) were increased between the human populations in the last years, these infections were occur when pathogenic bacteria enter the urinary tract through the urethra. UTIs are considered one of the frequent Community acquired Infections, and it was considered the most infections distribute after the respiratory infections, further its became the more important infections transmitted in hospitals [1]. The UTIs was caused by a variety of bacterial species, whereas the gram negative species the most caused ones, specially the species of enteric bacteria further to the species of Staphylococcus and Enterobacter genes [2]. The hospital infections may be occurred as a result by interaction of multi factors including the microbial species and the patient immune status as well as the microbial transfer factors [3].

The failure to cure the patient in case of use of antibiotics, may be refer to the ability of pathogenic bacteria to resistant of antibiotics used or because the structural defects in the urinary tract, which it were causes in increased the infections. Also the most infection cases were occur as a result for used the hospital systems that contaminations such as cystoscopy system or when occurred the defect in the prostate gland in men's [4].

Aim of the Study

The aims of the research was to diagnosis the bacterial species that most isolated from urine samples from patients referred for teaching Tikrit Hospital, and determine the bacterial resistant for some of antibiotics.

Materials and Methods

Samples collection and preparations

The urine were collected at 91 samples from the patients with UTIs at ages between one to 80 years whom refer the Salah-Adden teaching hospital for the period from first of November 2017 to last of January 2018. The samples were collected in clean and sterile plastic container in quantity not less than 25 ml and ask the patients to takes the samples from the clean-catch midstream urine. each sample was labeling and transferred to microbial laboratory at not more than 30 minutes [5].

Bacterial isolation and identifications

The five millimeters from each sample takes in centrifuge tubes and centrifugation at 5000 cycles/mint., for 5.0 minutes, the precipitation was gated, and 0.5 ml from each precipitation urine sample was inoculated on Blood agar, MacConkey agar and Mannitol salt agar then incubated aerobically on 37ºC for 24h [6]. The isolates were sub-culturing on the same isolation media to get the single and pure of bacterial isolates then complete the identification by the morphology, microscopic and biochemical characterizes [7]. The shape, size and color of each pure colony was depended as the initial identification for the bacterial species [8].

The pure bacterial isolates were tests of gram stain response, followed by the optimal biochemical tests for completed the identification of bacterial species levels, which consist to test the oxidase, catalase and coagulase tests according [9]. Also tested the ability of isolates to resistant the novobiocin [10]. Further to use the culturing on Eosin Methylene Blue and on the Kligler Iron Agar [11] and the Methyl red, Indole and Voges-Proskauer, also the citrate consumption and the sugar fermentation of glucose, lactose, fructose, sucrose, maltose, mannose, raffinose, xylose and mannitol fermentation [7].

Antibiotic susceptibility test of bacterial isolates

Antimicrobial Susceptibility Test was done by Kirby-Bauer method according to Kirby., *et al*. [12] which followed by adjusted the turbidity of overnight incubated bacterial suspension at 37ºC with the 0.5 concentration tube of McFarland, then dipping a sterile cotton-wool swab into the bacterial suspension of each isolates and removing the excess liquid by turning the swab against the side of the container above the level of the liquid. The swab was streaked evenly over the entire surface of the Muller Hinton Agar plate by swabbing in three directions, rotating the plate through an angle of 60º after each application. Finally, the swab was pass round the edge of the agar surface. The plate was allow to dry with the lid closed before applying discs. The antimicrobial discs were place on the inoculated plates using a pair of sterile forceps. Plates were incubated at 37ºC for 24 hrs. After overnight incubation, the diameter of each inhibition zone (including the diameter of the disc) was measured and record in millimeter unit and compared to the standard vales in CLSI [13]. The antibiotics used in this experiment were Gentamycin (GN), Penicillin (P), Vancomycin (VA), Nitrofurantoin (NF), Ciprofloxacin (CIP), Tetracycline (TE), Ampicillin (Am), Neomycin (Ne).

Results and Discussion

The bacterial isolates that caused the UTIs has been diagnosed after purifying them on the optimal media, These were made by clarifying the morphological and cultural characterizes on media agar and the microscopic characterizes to detect the shape, color and arrangement of cells further the gram stain responses types (Table 1). The microscopic exam for the staining cells were appear the both of gram positive and negative species. The gram positive species were distinguish the cells shape at cocci and its arrangement at the cluster or diploid shape, further to appear as catalase positive, these characterizes can be inferred those cells are from the species of Staphylococcus genes. The coagulase and novobiocin sensitivity tests with the ability of sugar fermentations were determine the species of each isolates [14]. While the gram positive cells of isolates those, appear as negative of catalase test, and cocci shapes with strep arrangement and violet or blue color of microscopic film test, can be point out to its as one of the species of Streptococcus genus. The other isolates those appear as gram negative response and the cells at rod shapes with the multiple arrangements were diagnosis according the morphological, catalase, oxidase, sugar fermentation and the IMVIC tests [14].

The results in table 2 were investigate the number and percentage of bacterial species isolates from the UTIs samples. The total bacterial isolates were appear at 113 isolates, and the S. aureus was found at a high isolation numbers 34 isolates (30.08%), follow by K. pneumoniae at 31 isolates (27.43%), and E. coli at 26 isolates (23.01%).

Bacterial Isolates	Tests used in diagnose isolated bacteria from UTIs									
	G-stain	Oxidase	Catalase	Coagulase	Mannitol	KIA	Indole	Citrate	MR	VP
S. aureus	+	-	+	+	+	*	*	*	*	*
S. epidermidis	+	-	+	-	-	*	*	*	*	*
S. saprophyticus	+	-	+	-	+	*	*	*	*	*
S. pyogenes	+	-	-	-	-	*	*	*	*	*
K. pneumoniae	-	-	+	*	*	-	+/-	+	-	+
E. coli	-	-	+	*	*	+	+	-	+	-
P. aeruginosa	-	+	+	*	*	+	-	+	-	-

Table 1: *Parameters used to diagnosis of bacterial isolates from UTIs.*

*+: Positive, -: Negative, -/+: changeable; *: Not detected.*

Bacterial Isolates	Number	%
S. aureus	34	30,08
S. saprophyticus	4	3,54
S. epidermidis	12	10,62
S. pyogenes	1	0,88
K. pneumoniae	31	27,43
E. coli	26	23,01
P. aeruginosa	5	4,42
Total	113	100

Table 2: *The number and percentage of bacterial isolates from the UTIs samples.*

While the *S. epidermidis* at 12 isolates (10.62%), and each of *S. saprophyticus* and *P. aeruginosa* at 4 and 5 isolates (3.54 and 4.42%) respectively, and one isolate from *S. pyogenes* (0.88%).

The results for isolation of *Staphylococcus* bacteria at 50 isolates which distributed for *S. aureus*, *S. epidermidis* and *S. saprophyticus* at 30.08, 10.26 and 3.54% respectively. These results were agreement with results of Hummer and Caraon [15], but them were isolation its at 5.2%. These bacterial species were conducted to be as important pathogenic that causes of UTIs especially with female and the pregnancy from them, compared with the male infection, which referred to the physiological structures differences. The gram negative bacteria were isolated at 62 isolates distributed for *K. pneumoniae*, *E. coli* and *P. aeruginosa*, the differences in number and the species of bacterial isolations from the UTIs samples may be referred to multiple factors such as that relation with samples preparation and the media types used for isolation, further to the patients condition and the drug type takes, also for the differential at the physiological and structural factors, those were causes a different in types and the numbers of bacterial isolates from patient samples [16].

Antibiotic susceptibility from bacterial isolates

The eight antibiotic types were used to determine the ability of bacterial isolates for it resistant (Table 3).

The results were illustrated that bacterial isolates were has a clear differ in the antibiotics resistant ranges. The *S. aureus*, *S. epidermidis* and *S. saprophyticus* were appear to has a highly resistant for the penicillin G at 81, 82 and 71.4% respectively, while the *S. pyogenes* was completely resistant of its at 100%. The bacterial species isolates from *K. pneumoniae*, *E. coli* and *P. aeruginosa* were resistant this antibiotic at 87.7, 71.8 and 57.14% respectively.

The tetracycline antibiotic was resistant from *S. aureus, S. epidermidis, S. saprophyticus* and *S. pyogenes* at 43.93, 58.80, 42% and 50% respectively, while the *K. pneumoniae*, *E. coli* and *P. aeruginosa* at 71.4, 78.0, and 42.8% respectively. The gentamycin was resistant from the above mention of gram positive bacteria at 48.4, 29.4, 57.1 and 50.0% respectively, while the vancomycin were resistant from those isolates at 56, 47, 57 and 50% respectively, and the gram negative isolate species capable to resistant the vancomycin at 38.7, 75.0 and 8.42% respectively.

Antibiotics	Response	*S. aureus*		*S. epidermidis*		*S. saprophyticus*		*S. pyogene*s	
		Number	%	Number	%	Number	%	Number	%
GN	R	32	48,48	5	29,41	4	57,14	1	50
	S	24	36,36	8	47,05	3	42,85	1	50
	I	10	15,15	4	32,52	0	0	0	0
P	R	53	80,30	14	82,35	5	71,42	2	100
	S	5	7,57	0	0	2	28,57	0	0
	I	8	12,12	3	17,64	0	0	0	0
Va	R	37	56,06	8	47,05	4	57,14	1	50
	S	21	31,81	4	32,52	0	0	1	50
	I	8	12,12	5	29,41	3	42,85	0	0
Nf	R	31	46,96	7	41,17	1	14,28	2	100
	S	17	25,75	6	35,29	3	42,85	0	0
	I	18	27,27	4	23,52	1	14,28	0	0
CIP	R	19	28,78	3	17,64	2	28,57	0	0
	S	35	53,03	6	35,29	2	28,57	2	100
	I	12	18,18	8	47,05	3	42,85	0	0
TE	R	29	43,93	5	29,41	3	42,85	1	50
	S	27	40,90	8	47,05	1	14,28	1	50
	I	10	15,15	4	23,52	3	42,85	0	0
Am	R	40	60,60	10	58,82	4	57,14	1	50
	S	15	22,72	5	29,41	3	42,85	1	50
	I	11	16,66	2	11,76	0	0	0	0
Ne	R	36	54,54	9	52,94	4	57,14	1	50
	S	28	42,42	8	47,05	2	28,57	0	0
	I	2	3,03	0	0	1	14,28	1	50

Table 3: *Antibiotic susceptibility from gram positive bacterial isolates.*

GN: Gentamycin; P: Penicillin; VA: Vancomycin; NF: Nitrofurantoin; CIP: Ciprofloxacin; TE: Tetracycline; Am: Ampicillin; Ne: Neomycin.

Antibiotics Type	Response	*E. coli*		*K. pneumonia*		*P. aeruginos*a	
		Number	%	Number	%	Number	%
GN	R	11	34,37	15	30,61	3	42,85
	S	16	50	25	51,02	3	42,85
	I	5	15,62	9	18,36	1	14,28
P	R	23	71,87	43	87,75	4	57,14
	S	5	15,62	4	8,16	1	14,28
	I	4	12,5	2	4,08	2	28,57
Va	R	24	75	19	38,77	3	42,85
	S	3	9,37	10	20,40	2	28,57
	I	5	15,62	20	40,81	2	28,57
Nf	R	23	71,87	23	46,93	2	28,57
	S	4	12,5	20	40,81	2	28,57
	I	5	15,62	6	12,24	3	42,85
CIP	R	6	15,75	7	14,20	1	14,28
	S	21	65,62	30	61,22	4	14,57
	I	5	15,62	12	24,48	2	28,57

TE	R	25	78,1	35	71,42	3	42,85
	S	3	9,37	8	16,32	3	42,85
	I	4	12,5	6	12,24	1	14,28
Am	R	26	21,25	24	48,8	1	14,28
	S	1	3,12	11	22,44	4	14,57
	I	5	15,62	14	28,57	2	28,57
Ne	R	25	78,12	29	59,18	3	42,85
	S	3	9,37	13	26,53	2	28,57
	I	4	12,5	7	14,28	2	28,57

Table 4: *Antibiotic susceptibility from gram negative bacterial isolates.*

GN: Gentamycin; P: Penicillin; VA: Vancomycin; NF: Nitrofurantoin; CIP: Ciprofloxacin; TE: Tetracycline; Am: Ampicillin; Ne: Neomycin.

The gram positive isolates were capable for resistant of nitrofurantoin at 46.9, 41.1, 14.28 and 100% respectively and the gram negative isolates at 46.9, 71.8 and 28.2% respectively. The ciprofloxacin antibiotic was appear as high active against the bacterial isolates species and the gram positive isolates were resistant this antibiotic at 28, 17, 14.28 and 0.0% respectively, while the gram negative isolates were resistant at 14.28, 18.75 and 14.28% respectively. The gram positive isolates appear the intermediate resistant ability against ampicillin at 60.6, 58.8, 57.14 and 50% respectively, and the gram-negative isolates were resistant at 48.8, 81.2 and 14.28% respectively. While the neomycin was resistant, the gram-positive isolates at 54.54, 9.0, 4.0 and 50% respectively, and the resistant ability of gram-negative isolates were at 59.18, 78.12 and 42.85% respectively.

Some isolates were modified in the sensitive to antibiotics, which may be refer to has a multiples mechanism for antibiotics resistant, such as ability to produce the enzymes which has able to lysis the antibiotics, example β-lactamase that capable to lysis the β-lactam ring in the penicillin antibiotic, or from the ability of bacterial to modified the local target of antibiotics action [17]. Also, may be refer to the bacterial content of Efflux System which caused to un allowed for the antibiotics to entrance to the cells [18]. These mechanisms were gets from the genetic modified of bacterial isolates that causes for the antibiotics resistant changed [19].

Conclusion

These cases were causes to increase of the bacterial isolates for infection causes and its dangers effects increased which distributed in hospitals and became difficult in inhibited with antibiotics because its has the multi-mechanism for antibiotic resistant's [20].

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