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Isolation, Identification and Antibiotic Sensitivity Pattern of Bacteria from Urine Samples in Erbil Hospitals

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Abstract: ***Seventy one samples of urine were collected from patients suffering from signs and symptoms of urinary tract infections (UTIs) admitted to Erbil hospitals (Erbil teaching hospital, Maternity teaching hospital, Rizgary teaching hospital and Raparen hospital) and Medya diagnostic center during the period from July 2011 to August 2011. The urine samples were cultured on Blood and MacConkey agar, to detect and identify causative bacteria. The results indicate that the positive samples were 50 (70.40 %), while negative samples were 21 (29.60%), out of this 32 (64 %) represent female while 18(36 %) represent male. The isolated bacteria were identified according to cultural characteristics, microscopical examination and biochemical reaction in addition to Api 20 E system. Escherichia coli was among the most predominate pathogenic bacteria isolated from the urine with a rate of (62%) while other bacteria were Klebsiellapneumoniae(10%), Proteus mirabilis (8%), Pseudomonasaerugenosa(8%), Staphylococcus aureus(6%), Enterobacterspp. (4 %), and Staphylococcus saprophyticus(2 %). Sensitivity test of bacterial isolates to different antimicrobials (Amikacin, Ampicillin, Cefteriaxone, Ciprofloxacin, Nalidixic,Gentamycin and Imipenem) were done. The isolated bacteria showed sensitivity to Imipenem and low resistance to Amikacin, variation in resistance to Ceftriaxone, Ciprofloxacin, Nalidixic and Gentamycin; however, they showed high resistance to Ampicillin.***

Keywords: Urinary tract infections, bacterial pathogen & antibiotic sensitivity

1. Introduction

Antibiotics are low molecular weight, natural product of microorganismand are active against other microorganism. Discovery of antibiotics is one ofthe greatest events in the history of medicine which has a profound effect onhuman life, thus in society as a whole (Bhattacharyya and Sen, 2006). However,the overuse and misuse of antibiotics is leading to the emergence of resistance tothese life-saving drugs. Resistance to a variety of antimicrobial agents isemerging in bacterial pathogens throughout the world (Joshi *et al.*, 2008).Antimicrobial resistance is the best-known example of rapid adaptation ofbacteria to a new ecosystem. The ability of bacteria to expand their ecologicalniche, also in the presence of antibiotics, can be explained by accumulation ofpoint mutations leading to the modification of existing genes and/or by theacquisition of resistance genes by horizontal gene transfer. Resistance genes areoften located on extra chromosomal genetic elements or in segments insertedwithin the chromosome that originate from other genome (Carattoli, 2003).*In-vitro* antibiotic susceptibility testing in clinical microbiologylaboratories is primarily to assist the clinician in the choice of an appropriateantibiotic for the treatment of an infected patient (Gosden*et al.*, 1998).

Urinary tract infections (UTIs) are defined in terms of the inflamedurinary structure, Cystitis: the bladder, Urethritis: the urethra, Pyelonephritis:the renal tubules and interstitium, Prostatitis: the prostate (Wolfsthal, 2008).Urine located within the urinary tract, excluding the distal region of theurethra is considered sterile in healthy individuals, as indicated by the absenceof cultivable bacterial cell. UTI describes a condition in which there are microorganisms established and multiplying within urinary tract. It most often due tobacterial, but may also include fungal, parasitic and viral infection (Chaudhuri*etal.,* 2008). Over 95% of UTIs are caused by a single bacterial species, and 90% ofthese are *E. coli*. Other Enterobacteriaceae, *Pseudomonas*, and Gram-positivebacteria become increasingly frequent with chronic (Ryan and Ray, 2004).

2. Material and Methods

2.1 Sample Collection

Clean catch midstream urine wascollected from each patient into a 20mL calibrated sterilescrew-capped universal container which was distributed tothe patients. The specimens were labeled, transported to the laboratory.

Isolation of uropathogens was performed by surface streak procedure on both blood and MacConkey agar and the plateswere incubated for 24-48 hours at 37ºC. All media were examined aftertimes of incubation, if no growth occurs they were incubated for another 24hours before regarded as negative.

2.2 Identification of the bacteria

- **Morphological Characteristics**

The isolated bacterial colonies were identified according to themorphology, colony pigmentation, fermentation, haemolysis and swarmingon the blood agar (Atlas *et al.,* 1995).

- **Microscopical characteristics**

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The isolated bacteria were further classified by Gram staining toGram-negative and Gram-positive (Atlas *et al.,* 1995).

- **Biochemical tests**

Gram negative bacteria were identified by the standard biochemical testsIMViC test (Indol, methyl red, Voges-Proskauer and Citrate) ,Urease test, Oxidase test and Gram positive microorganisms were identified with the corresponding laboratory tests: catalase, coagulase,mannitol test, novobiocin susceptibility test.

- **API 20E kitApi 20E test**

To support the biochemical tests, Api 20E was performed for Gram negative bacteria.

Antibiotic sensitivity

Antibiotic sensitivity test was performed for each isolate utilizing the method of Kirby-Bauer (disc diffusion method) This was performed on Mueller–Hinton agar with the following antibiotic discs (Ampicillin AMP 10µg, Amikacin AK 30µg, Ceftraxon CTR 30µg, Ciprofloxacin CIP 5µg, Gentamicin CN 10µg, Nalidixic acid NA 30µg, and Imipenem IMI 10 µg,). Sensitivity was read after incubation for 24 hrs. at 35ºC.

3. Results

3.1 Incidence of urinary tract infection

Out of 71 urine specimens collected from patients complaining of signs and symptoms of UTIs, attended to four hospitals in Erbil city(Maternity teaching hospital, Erbil teaching hospital, Rizgary teachinghospital and Raparen hospital) and Medya diagnostic centre.In the periodfrom July 2011 till August 2011. Fifty samples (70.40%) were positive forbacterial infection .

3.2 UTIs related to sex

Figure (3-1) represent that out of 50 patients, 32 (64%) of bacterialisolates obtained from female and 18 (36%) isolates from male.

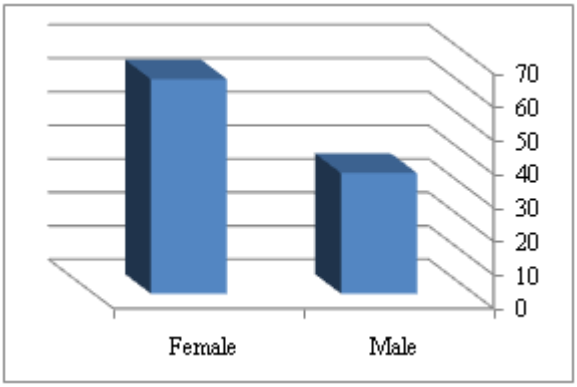

Figure (3-1) the relationship between sex and UTIs.

3.3 Incidence of bacterial isolates associated with UTIs

Table (3-1) shows the percentage of bacterial isolates from 50 positive urine culture specimens, the percentage of isolateswere as follows: *E. coli* (62%), *Klebsiellapneuomoniae*(10%),*Pseudomonas aerugnosa*(8%), *Proteus mirabilis* (8%), *Staphylococcusaureus*(6%), *Enterobacter*spp. (4%) and *Staphylococcus saprophyticus*(2%) respectively.

Table (3-1): Percentage of bacterial isolates from 50 cultured urine samples.

Bacterial isolates	No	%
Escherichia coli	31	62
Klebsiella pneumonia	5	10
Staphylococcus spp	4	8
Proteus mirabilis	4	8
Pseudomonas aeruginosa	4	8
*Enterobacter*spp.	2	4
Total	50	100

3.4 Identification of Gram-negative bacteria

Table (3-2): Represent some biochemical tests for gram negative bacteria

Species	Tests						
	I	Mr	Vp	Si	Oi	Mot	Ur
Escherichia coli	+	+	-	-	-	+	_
Klebsiella pneumonia	-	-	+	+	-	-	+
Proteus mirabilis	-	+	D	D	-	+	+
Pseudomonas aeruginosa	-	-	-	+	+	+	-
*Enterobacter*spp.	-	-	+	+	-	+	-

Abbreviation: I: Indole test, Mr: Methyl red test, Vp: VogesProskauer test, Si: Simmon citrate test, Oi: Oxidase test, Ur: Urease, Mot: Motility, D:Different strains gave different results, +: Positive, - : Negative.

3.5 Identification of Gram-positive bacteria

Table (3-3): Represents some biochemical tests for Staphylococci.

Tests	*Staphylococcus* spp.	
	S. aureus	*S. saprophyticus*
Catalase	+	+
Mannitol fermentation	+	+
Coagulase	+	-
Hemolysis	+	-

Staphylococcus saprophyticus

Most strains of *S.saprophyticus*mannitol fermented, coagulase negative, do not exhibit hemolysis on blood agar. The bacterial isolate was resistant to Novobiocin (Benson, 2001).

3.6 Antimicrobials resistance of bacterial UTIs

Table (3-4): Resistance percentage of UTI bacterial isolates to different antimicrobials under study

Species	No	Antimicrobials						
		AK	AMP	CTR	CIP	GN	NA	IMI
Escherichia coli	31	9.6	90.3	61.2	51.6	45.1	25	0
Klebsiella pneumonia	5	0	100	60	20	20	75	0
Proteus mirabilis	4	0	50	50	0	0	0	0
Pseudomonas aeruginosa	4	25	100	75	25	70	40	0
Staphylococcus spp.	4	0	70	50	25	0	75	0
*Enterobacter*spp.	2	0	100	50	50	0	50	0

Ampicillin AMP 10µg, Amikacin AK 30µg, Ceftraxon CTR 30µg, Ciprofloxacin CIP 5µg, Gentamicin CN 10µg, Nalidixic

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acid NA 30µg, and Imipenem IMI 10 µg,). Sensitivity was read after incubation for 24 hrs. at 35ºC. The bacteria isolates were regarded as sensitive or resistant according to CLSI criteria.

4. Discussion

4.1 Incidence of bacterial isolates associated with UTIs.

*E.coli*was the most prevalent bacterial UTIs in this study, it constitutes about (62%). This result was similar to those recorded by Barrett *et al.,* (1999) and Gluhovschi*et al.,* (1998), they revealed that the isolates percentage of *E.coli*in UTIs were (65.1%) and (58.06%) respectively; However, Al-Hemidawi (2005) and Kose*et al.,* (2007)demonstrated that *E.coli*was most common isolated bacteria from UTIs (42.1%) and (73.6%) respectively, These fluctuations might be attributed to the social habits, hygienic status in different communities and the difference in the time of the study.

The recovery rate of *Klebsiellapneumoniae*from cases of UTIs in this study was (10%). This finding was in agreement with the research results of Iris *et al.,* (2006) and AL-Hemidawi (2005), in which the percentage of *Klebsiellapneumoniae*in UTIs were (9 %) and (8.7 %) respectively. In the present study the percentage of *Pseudomonas aeruginosa*isolates were (8%), the same result was obtained by Al-Hemidawi (2005) and Jarjees (2006), they recorded the isolation percentage of *Pseudomonas aeruginosa*in UTIs were (7%) and (7.39%) respectively. The frequency of *Proteus mirabilis* was (8%), this result was near to those reported by Memon (2007) and Jarjees (2006) that the frequency of *Proteus mirabilis* isolated from UTIs were (4.9%) and (6.78%) respectively. The percentage rates of *Staphylococcus* spp. from cases of UTIs in this study were (6%) for *Staphylococcus aureus*and (2%) for *Staphylococcus saprophyticus*. Al-Hemidawi (2005) and Ameen (2002) revealed that *Staphylococcus aureus*isolated from UTIs were (10.1%) and (11.36%) respectively, these differences in the rate are due to size of sample, social habits and hygienic status in different communities. The percentage rate of *Staphylococcus saprophyticus*was in agreement with Barrett *et al.,* (1999) who reported (1.5%) for coagulase negative*Staphylococcus* isolated from UTI. Kolawole*et al.,* (2009) recorded (7.22%) for *Staphylococcus saprophyticus*isolated from UTIs and that is disagreement with our result. The results in the present study showed that the percentage of *Enterobacter*spp. in UTIs was (4%). This findings are supported by Ameen (2002) and Jarjees (2006) who demonstrated (3.78%) and (2.60%) respectively for *Enterbacter*spp. isolated from UTIs.

4.2 UTIs related to sex

In this study out of 50 patients, 32 (64%) of bacterial isolates obtained from female and 18 (36%) isolates from male. Kolawole*et al.,* (2009) investigated a high prevalence of bacteriuria in femal (66.67%) than male (33.33%), also Al-Hemidawi (2005) recorded high incidence of UTIs infection in female (60.8%) than male (39.1%). Women are more susceptible to UTI because a woman's urethra is short, allowing quick access of bacteria to the bladder. Also a woman's urethral opening is near sources of bacteria from the anus and vagina. The incidence increases with age and sexual activity. Rate of infection are high in postmenopausal women, because of bladder or uterine prolapse causing incomplete bladder emptying, loss of estrogen with attendant changes in vaginal flora, loss of *Lactobacilli*, which allows periurethral colonization with gram negative aerobes (Chaudhuri*et al.,* 2008).

4.3 Antimicrobials resistance of bacterial UTIs

Sensitivity test for fifty bacterial isolates done against six common widely used antimicrobials for UTIs which includes (AK, AMP, CIP, GN, CTR, NA.IMI). In general, the bacteria investigated in the present study showed sensitivity to Imipenem and low resistance to Amikacin, variation in resistance to Ceftriaxone, Nalidixic, Ciprofloxacin and Gentamycin; however, they showed high resistance to Ampicillin.
These results are in agreement with Parvin*et al.,* (2009) and Jarjees (2006). Chigbu and Ezeronye (2003) reported that higher prevalence of resistance of antimicrobial agent such as Ampcillin could be due to widespread and discriminate use of this antibiotic and production of B- lactamases by most bacteria. Imipenem is carbapenem antibiotic with a broad spectrum of activityon Gram-positive and Gram-negative bacteria. It is a potent inhibitor ofplasmid and chromosomally mediated B- lactamases (Acar*et al.,* 1983).

Increasing bacterial resistance in Erbil city may be due to**:**

- Most antibiotics prescription in hospitals are given without clear evidence of infection or adequate medical indication.
- Many physicians have administered antibacterial drugs to patients with colds, Influenza, viral pneumonia and other viral diseases.
- Antibiotics are prescribed without culturing and identifying the pathogen or without determining bacterial sensitivity to the drug.
- The patient not completing their course of medication.
- Drugs are available to the public.

5. Conclusion

During the course of this study the rate of incidence of UTIs inHawler Hospitals among female was more than male.*E.coli*was among the commonest pathogenic bacteria isolated fromUTI.Amikacin and Imipenem were the most active antibiotics againstbacteria causing UTI. Most of the isolates causing UTI seem to be highly resistance toAmpicllin.

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