Research Article

The Study of Antibiotic Resistance Variations among *Enterobacter spp.* isolated from UTI children

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ABSTRACT

Enterobacter species are important nosocomial pathogens, responsible for variants infections and also cause different community acquired infections. This study was doing to detection the incidence of *Enterobacter spp*. In this study 57(3.9%) *Enterobacter spp*. were isolated from a total of 1474 urine samples collected from patients Suffering from UTI in children (1-7 years) during January-September, 2011 from AL-Hakeem hospital in Nejaf city/ Iraq. The results indicated that *Enterobacter* spp. were isolates from females (68.4%) more than males (13.6%). Two peaks of infection were obtained; the first one occurred in February (6.6%) and the second in July (5.8%). The antibiogram of *Enterobacter spp*. during 9 months. During January and February the isolates are strongly resistant to KF and CTX in (100, 71.4)% and (80, 57.1)%, respectively. In March the isolates shows a strong resistance to CRO, KF and CN in 100% for each one, while in April all isolates show 57.1% resistance to CTX and 42.8% for NA, while AK and CIP is the drug of choice for treatment of *Enterobacter spp*. during May ,since all isolates show high sensitivity(100%) for these antibiotics. CIP, CRO, AK and CTX is the drugs of choice in June (14.2)%. In July the isolates resist AK and CIP in low percentage (0,11)%, respectively. In August and September Enterobacter spp. shows a high resistant to KF in (83.3, 100)%, respectively.

Keywords: *Enterobacter spp*., urine, antibiogram, seasonal variation.

INTRODUCTION

Enterobacter is a gram negative bacillus that belong to the *Enterobacterceae* family (facultative anaerobic gram negative rods). *Enterobacter* Spp. are motile and lactose fermenter similar to *Klebsiella* in that they are usually Voges Proskuar positive. However they are usually urease negative and orthinine positive, characters that distinguish them from *Klebseill.*[1,2] They are negative for phenylalanine chemicals, H_2S production In TSI agar, and gelatin liquification and positive for Citrate, KSN.[3] *Enerobacter* Spp. is bile tolerant organism, grow readily on routine laboratory media, oxidase negative, and sometimes capsulated. The Colonies of *Enterobacer* strains appear large, dull grey, may be slightly mucoid. *Ent.aerogenes* is usually able to express a lysine decarboxylase enzyme but not arginine decarboxylase, whereas *Ent.cloaca* decarboxylates arginine but not lysine. The normal habitat is gut of human and animals and moist environment, especially soil and water.[4]

They are rarely cause primary disease in humans, but frequently colonize hospitalized patients, especially in association with antibiotic treatment, indwelling cathers, or invasive procedures.[1] Other *Enterobacter* spp. like *Ent.amnigenuus*, *Ent.absuviae*, *Ent.gergovia* and *Ent.taylorae* may be isolated from Urine, blood, wounds respectively. Source of infection may be endogenous source (via colonization of the skin), gastrointestinal tract, or urinary tract or exogenous source.[3] Enterobacter species, particularly *Enterobacter Cloaca* and *Eterobacter aerogenes* are important nosocomial pathogens responsible for various infections and also cause various community acquired infections.[5] Urinary tract infections (UTIs) are a significant cause of morbidity and mortality worldwide. Although over 90% of UTIs are relatively Simple infections in anatomically normal patients and caused by Gram negative bacteria in fecal origins (Nickel 1993). In complicated UTIs, renal damage leading to kidney failure and death can occur.[6] Bacteremia, Lower respiratory tract infections, Skin & Soft tissue infections, Endocariatis, Central Nervous System Infections, Bone and joint infections, and Opthalmic infections.

The pathogenecity = of *Enterobacter Spp.* Include Fimbriae (type 1 and type 3),

An aerobectin mediated iron uptake system, Hemolysin, Outer membrane protein (is a pathogenic factor of strains of Ent. cloaca its reduce production of porins, leading to decrease Sensitivity to β-lactam antibiotics and play a role in host cell invasion), Endotoxin (LPS) and Capsule which Inhibit phagocytosis.[3,4] *Enterobacter* was moderately susceptible to the Cephalosporins and Quinolones. It is poorly susceptible to Pencillines (amoxilin clavulanate) and the Aminoglycosides. It is strongly resistant to erythromycin, cloxacillin, cotrimoxaole, tetracycline and chloromiphenicol.[3] *Enterobacter* strains differ from Serratia strains in being sensitive to the polymyxins. The cheep and frequently used antimicrobial agents raises serious concern in increasing rate of drug resistance.[2]

Enterobacter strains produce a chromosomal β lactamase with cephalosporinase activity and highly resistant to pencillines and cephalosporines. Unlike plasmid mediated β lactamase, these are not normally expressed. It is only under the influence of an inducer or following mutation that the gene becomes activated and the enzyme

expressed. In France, an increasing number of clinical strains have produced plasmidic ESBL, chromosomal cephalosporinase, and aminoglycosides acetyltransferase and remain susceptible only to imipenem and gentamycin. Mallae *et al*, (2002) have described clinical *E.aerogenes* strains presenting a complex resistance associating β lactamase production, porin deficiency, and active efflux. A strong correlation was reported between the presence of the non specific major porin (OMP36), and the β lactam susceptibility of *E.aerogenes* isolates. This study was doing to detect the incidence of *Enterobacter* spp. among children with UTI during January-September, 2010. and determine the antibiotic resistant patterns and variation of the isolates to eight types of Antimicrobial agents.[7,8]

MATERIALS AND METHODS

Collection of samples

About 1474 urine sample were collected from patient with Urinary Tract Infection (UTI) in AL Hakem Hospital /Najaf governorate from the period of January-September (2011) with both sexes in different age groups. Each sample was streaked on MacConkey agar and blood agar and incubated at 37˚C for 24 hr, culture results were interpreted as being lactose fermenting and non-fermenting bacteria. Then the colonies identified using classical morphological and biochemical tests.

Identification of bacteria

The bacteria identify according to the diagnostic procedures recommended by Macfaddin (2000) & Goering et al., (2008).[9] The identification of bacteria was established according to the culture and morphological characteristic, including the shape of colonies, lactose fermentation or non-lactose fermenter, appearance, pigment production ....etc. and the biochemical tests **.**

Antibiotic susceptibility test

The susceptibility test of *Enterobacter spp.* was carried out against 8 types of antibiotic using the disk diffusion method on MHA.[10] Two-ml of brain heart infusion broth have been inoculated with an isolated colony of test bacteria and incubated for 24 hours at 37°C. After that, the turbidity of bacterial suspension has been adjusted turbidity of McFarland (0.5) standard tube. The resulting zone of inhibition have been measured by using a ruler and compared with zones of inhibition determined by CLSI (2011) and to decide the susceptibility of bacteria to antimicrobial agent, whether being resistant or susceptible.

RESULTS AND DISCUSSION

Isolation and Identification of *Enterobacter spp*.

In this study, a total of 57(3.9%) *Enterobacter* spp were isolated from 1474 urine sample collected from patients (both sexes), Suffering from UTI in children (1-7 years) during January-September, 2011 from AL-Hakeem hospital. The result of the most important characteristic and identification test of 57 *Enterobacter* spp. isolates are shown in Table 2. Enterobacter infections are most common in neonates and in elderly individuals, reflecting the increased prevalence of severe underlying diseases at the age extremes. *Enterobacter* Spp. has been associated with many outbreaks due to the contaminated powdered formula for infants.[11]

Table 1: Antibiotic Disks (Bioanalyse, Turkey).

Antibiotic used	Abbreviation	Content (µg)
Cephalothin	KF	30
Cephatoxime	CTX	30
Ceftraiaxone	CRO	30
Nalidixic Acid	NA	30
Ciprofloxacin	CIP	5
Nitrofurantion	F	300
Gentamicin	CN	10
Nitrofurantoin	F	300

Table 2: Biochemical characters of 57 *Enterobacter spp*. isolates

Test	Result
Gram stain	-
Oxidaes	-
Catalase	+
Indole	-
Methyl red	-
Voges proskuar	+
Simone citrate	+
Urease	-
Motility	+
TSIA	A/A

Table (3) indicated that *Enterobacter* spp. were isolates from females (68.4%) more than males (13.6%). These results may be attributed to the anatomical structure of urinary tract of female in comparisons with male which make susceptible to infection with UTI and Enterobacter spp. is the commonest one pathogens. Fraser and Arnett (2010) reported that female predominance in UTI infection in pediatric population.[5]

Figure (1) shows the distribution of *Enterobacter* spp. isolates according to the seasonal variation (9 months), in which the highest month of isolation were recorded during February (6.6%) followed by July (5.8%). In other word, two peaks of infection were obtained; the first one occurred in February and the second one in July. The high percentage of *Enterobacter* spp. were isolated during the coldest weather (February may attributed to the high frequency of UTI which may leads to the endogenous infection. The second (highest) peak of infection presented in wormiest weather (July) may attributed to the increasing rate of swimming in the river and pool bath

which may lead to infection with these organism from external sources.[12]

Table 3: The distribution of *Enterobacter* spp. according to sex during nine months

Months	Total no. of samples	Isolates in Male		Female Isolates in	
		No. %		No. %	
January	131	1	20	4	80
February	105	2	28.5	5	71.4
March	123	0	0	4	100
April	226	4	57.1	3	42.8
May	256	3	37.5	5	62.5
June	204	2	28.5	5	71.4
July	155	3	33.3	6	66.6
August	161	3	50	3	50
September	113	0	0	4	100
Total	1474	18	13.6	39	68.4

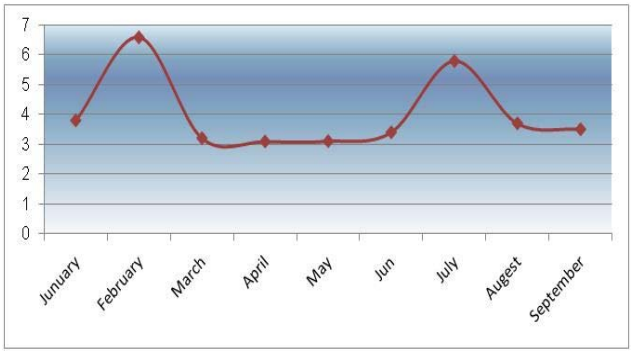

Figure 1: Distribution of *Enterobacter* spp.isolates during 9 months

Table (4) and Figure (2) shows the antibiogram of *Enterobacter* spp. during 9 months. During January and February the isolates are strongly resistant to KF and CTX in (100,71.4)% and (80,57.1)%, and moderately resistance to CRO in (60,57)%, respectively, and show less resistant to others antibiotics. In March *Enterobacter* spp. shows a strong resistance to CRO, KF and CN in 100%, while in April all isolates show 57.1% resistance to CTX and 42.8% for NA and CRO.

AK and CIP is the drug of choice for treatment of *Enterobacter* spp. during May, since the isolates resist to this antibiotics in 0% for each one. While CIP, CRO, AK and CTX is the drugs of choice in June (14.2)%. In July the isolates resist AK and CIP in low percentage (0,11)%, respectively. In August and September Enterobacter spp. shows a high resistant to KF in (83.3, 100)%, respectively.

The resistance to CRO, CTX, KF in this study strongly suggest that *Enterobacter* spp. strains produce ESBL as reported by Arikan and Aygan (2009).[13] Mordi & Momoh (2008) are reporters in literature that *Enterobacter* spp. carry a gene for chromosomally encoded β-lactamase that can be induced by certain antibiotics, amino acid, or body fluids. Fraser and Arnett (2010) reported that resistant mutants can quickly appear in *Enterobacter* spp. and antibiograms must be interpreted with respect to the different resistance mechanisms.[5]

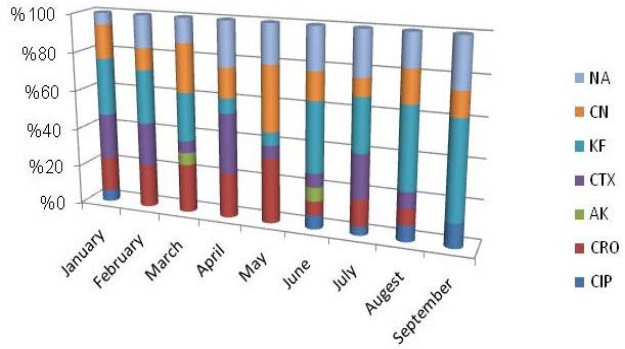

Figure 2: The antibiogram of *Enterobacter* spp.during 9 months

Table 4: The antibiogram of *Enterobacter* spp.during 9 months

Antibiotics → Months ↓	Total no. of samples	CIP		CRO		AK		CTX		KF		CN		F		NA	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
January	5	1	20%	3	60%	0	0%	4	80%	5	100%	3	60%	2	40%	1	20%
February	7	0	0%	4	57%	0	0%	4	57.1%	5	71.4%	2	28.5%	1	14.2%	3	42.8%
March	4	0	0%	4	100%	1	25%	1	25%	4	100%	4	100%	2	50%	2	50%
April	7	0	0%	3	42.8%	0	0%	4	57.1%	1	14.2%	2	28.5%	0	0%	3	42.8%
May	8	0	0%	5	62.5%	0	0%	1	12.5%	1	12.5%	5	62.5%	2	25%	3	375%
June	7	1	14.2%	1	14.2%	1	14.2%	1	14.2%	5	71.4%	2	28.5%	2	28.5%	3	42.8%
July	9	1	11%	3	33.3%	0	0%	5	55.5%	6	66.6%	2	22.2%	0	0%	5	55.5%
August	6	1	16.6%	1	16.6%	0	0%	1	16.6%	5	83.3%	2	33.3%	1	16.6%	2	33.3%
September	**4**	**1**	**25%**	**0**	**0%**	**0**	**0%**	**0**	**0%**	**4**	**100%**	**1**	**25%**	**1**	**25%**	**2**	**50%**

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