Development of resistance of some *Klebsiella* species isolated locally from urinary tract infection to some beta – lactam antibiotics

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Date of acceptance 7/10/2006

Abstract

200 urine specimens were collected from patients with urinary tract infection and speculated for *Klebsiella* species. 70 specimens (35%) showed growth and 11 isolates (15.7%) were identified as *Klebsiella species (K. pneumoniae, K. oxytoca and K. ozaenae).*

Most of local isolates revealed high resistance to antibiotics: Penicillin G, Ampicillin, Amoxicillin, Piperacillin, Cephalothin, Cefaclor and Cefotaxime, moderate resistance to cephalexin, Ceftazidime and Cefixim and low resistance to Augmentin, Cefoxitin an Ceftizoxime.

The MICs of Amoxicillin, Piperacillin, Cephalexin and Cephalothin (512-1024) µg/ml were higher than other antibiotics (32-512) µg/ml and 10 isolates (90.9%) produced β-lactamase.

The cations Mg^{2+} and Ca^{2+} were more affective in decreasing the ceftizoxime MICs than other cations. No significance effect of Fe^{3+} and Zn^{2+} on MICs in all concenentrations (1, 2.5, 5, 10) µg/ml while Na^{+} caused increasing in MICs at concentrations (0.5, 1)%.

Introduction

The majority of microorganisms causing UTI (Urinary Tract Infection) are *Escherichia coli.* Other organisms are *Klebsiella, Proteus* and *pseudomonas (1).*

Klebsiella pneumoniae and *Klebsiella oxytoca* are responsible for most human infections. *Klebsiella* species are resistant to many antibiotics, and thought to be extended-spectrum beta-lactamase (ESBL) producer (2).

The amount of minerals in the medium may influences the activity of some drugs, monovalent cations may enhance the activity of penicillin against *Proteus* species while divalent cations reduce the activity of some antibiotics (3).

The aims of this study are detection of *Klebsiella* species associated with suspect UTI, determination of their sensitivity patterns to some penicillins an cephalosporins, detection the production of beta-lactamase and study the effect of some cations on MICs of *Klebsiella* species.

Materials and Methods

Specimens

Two hundred urine specimens were collected from patients admitted to Al-Kindy Teaching Hospital during the period from July to November 2005. The specimens were collected in sterilized containers.

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Isolation and Identification of bacteria

0.5 ml urine specimens were inoculated onto 5% blood agar and McConkey agar, incubated for 24 hrs. at 37°C. Bacteria were identified according to culture characteristics and biochemical tests (4).

Antibiotic sensitivity test

Antibiotic sensitivity test was done for the isolated bacteria by Disc-diffusion method (Kirby-Bauer method) (3) with the following antibiotics described in table (1). Bacteria to be tested were spreaded on Mueller-hinton agar by cotton swab.

Table 1: Antibiotic discs with their concentrations and manufactures used for sensitivity test.

Antibiotic disc	Code	Concentration µg/disc	Manufacture
Penicillin G	PG	25	Al-Razi center
Ampicillin	AMP	10	Al-Razi center
Amoxicillin	AMX	20	Al-Razi center
Piperacillin	PRL.	100	Al-Razi center
Augmentin	AMC.	30	Al-Razi center
Cephalexin	CL.	30	Oxoid
Cephalothin	KL.	30	Oxoid
Cefaclor	CFC.	30	Bioanalyse Co.
Cefoxitin	CFP.	30	Bioanalyse Co.
Cefotaxime	CTX.	30	Oxoid
Ceftazidime	CTZ.	30	Al-Razi center
Cefixim	CFX.	30	Oxiod
Ceftizoxime	CZX.	30	Bioanalyse Co.

Minimum inhibitory concentration (MIC)

The broth dilution method was applied for determination of MIC (3). The same antibiotics except penicillin G and Ampicillin (Table 1) were used in Mueller-hinton broth.

Detection of β-lactamase production

The Acidometric method for detection of β-lactamase described by (Sykes and Matthew 1976) (5) was used to determine β-lactamase producing bacteria.

Effect of cations on MIC

Different concentrations of cations (Calcium, Magnesium, Zinc, Ferric and Sodium) were used at the following subinhibitory concentrations (6):

Na^{+}:	0.5, 1, 2%
Fe^{3+}:	1, 2.5, 5, 10 µg/ml
Ca^{2+}:	1, 2.5, 5, 10 µg/ml
Mg^{2+}:	1, 2.5, 5, 10 µg/ml
Zn^{2+}:	1, 2.5, 5, 10 µg/ml

Supplemented Mueller-hinton broth media were prepared by addition sterile stock solutions of $MgCl_2.6H_2O$, $CaCl_2.2H_2O$, $ZnSo_4$, NaCl and $FeCl_3$ to volumes of sterile broth. Bacteria were inoculated in the media, incubated at 37°C for 18 hr. and MIC was determined.

Results and Discussion

From 200 urine specimens, 130 (65%) specimen showed no growth. 70 specimen (35%) showed growth for specific microorganisms. 11(15.7%) isolates were identified as *Klebsiella* species according to Baron *et al.* (7) (table 2).

Table 2: Number and percentage of *Klebsiella* species isolated from urine specimens.

Klebsiella species	Number of isolates	Percentage of isolates
K. Pneumoniae	5	7.1%
K. Oxytoca	3	4.3%
K. Ozenae	3	4.3%

The percentage of *Kelbsiella* species in this study (11%) was different than that reported by Ghiro *et al.* (8), (2.1%) and Kevin *et al.* (9),

(20%), this may be due to the different in sampling time and geographic location.

K. pneumoniae is known as urinary tract pathogen and is medically more important than other *Klebsiella* species, specially in hospitals where they cause pneumonia and UTI in catheterized patients (10).

The antibiotic resistance patterns of *Klebsiella* species isolates were shown in table 3.

Table 3: Resistance* of *Klebsiella* species isolates to *β-lactam antibiotics.*

	No. of resistant isolates			No. of all resistant isolates	Percentage of all resistant isolates
Antibiotic	K. *pneumoniae*	K. *oxytoca*	K. *ozaenae*		
Penicillin G	5	3	3	11	100%
Ampicillin	5	3	3	11	100%
Amoxicillin	4	2	1	7	63.6%
Piperacillin	4	1	2	7	63.6%
Augmentin	3	0	1	4	36.4%
Cephalexin	3	1	2	6	54.5%
Cephalothin	3	2	2	7	63.6%
Cefaclor	4	3	1	8	72.7%
Cefoxitin	2	0	1	2	27.3%
Cefotaxime	3	3	1	7	63.6%
Ceftazidime	4	1	1	6	54.5%
Cefixim	2	1	2	5	45.5%
Ceftizoxime	1	1	0	2	18.2%

* The results of sensitivity to β-lactam antibiotics were performed in accordance with NCCLS guidelines (11).

All *Klebsiella* species revealed resistance to β-lactam antibiotics (Penicillin G and Ampicillin), most of *Klebsiella* species showed high reistance to Amoxicillin, Piperacillin, Cephalothin, Cefaclor and Cefotaxime (63.6-72.7%), moderate resistance to Cephalexin Ceftazidime and Cefixim (45.5-54.5%) and low resistance to Augmentin, Cefoxitin and Ceftizoxime (18.2-36.4%). *Klebsiella* species are resistant to multiple antibiotics, this is thought to be a plasmid-mediated property (12).

The resistance to cephalosporins implies extended-spectrum β-lactamase (ESBL) production in *E.coli* and *Klebsiella* species (13).

Most isolates showed high degree of resistance to Amoxicillin, Penicillin G and Piperacillin, such findings are in agreement with those reported by Alain *et al.*, (14) and Kevin *et al.*, (9). The resistance of Cefotaxime and Ceftazidim are higher than those indicated by Hanan (15).

5 (45.5%) isolates of *Klebsiella* species were resistant to all β-lactam antibiotics (Table 4), these isolates were used for MIC determination.

Table 4: Resistance of *Klebsiella* isolates to β-lactam antibiotics.

Klebsiella species	Number of isolates	Number of resistant isolates	Percentage of resistant isolates
K. pneumoniae	5	2	40%
K. oxytoca	3	1	66%
K. ozaenae	3	1	33.3%

Table 5 give the MICs of β-lactam antibiotics for *klebsiella* species.

Table 5: MICs (μg/ml) for some *Klebsiella* species isolated form UTI.

	MICs (μg/ml) of *Klebsiella* species		
Antibiotic	*K. pneumoniae*	*K. oxytoca*	*K. ozaenae*
Amoxicillin	512-1024	512	512
Piperacillin	512	512	512
Augmentin	64-128	64-128	128
Cephalexin	512	512	512
Cephalothin	1024	1024	1024
Cefaclor	256-512	256-512	512
Cefoxitin	32-64	64-128	64
Cefotaxime	128-256	512	256
Ceftazidime	64-128	128	64
Cefixime	64-128	64-128	128
Ceftizoxime	32	32-64	32

The MICs of Amoxicillin, Piperacillin, Cephalexin and Cephalothin (512-1024 µg/ml) were higher than other antibiotics (32-512 µg/ml), the increasing MIC of Cephalosporins specially first generation indicate decreasing the susceptibility of these types of β-lactam antibiotics due to production of extended-spectrum β-lactamases (ESBLs) (14).

High level of resistance to β-lactam antibiotics may be due to the source of isolates from patients at the extremes of ages where antimicrobial usage is likely to be higher (16). This study revealed no significance differences among *Klebsiella* species in their MICs.

All isolates of *Klebsiella* species were examined for β-lactamase production. It was found that 10 (90.9%) produce β-lactamase (Table 6).

Table 6: *Klebsiella* isolates with positive β-lactamase production.

Klebsiella species	Number of isolates	Number of isolates with positive β-lactamase	%
K. pneumoniae	5	5	100%
K. oxytoca	3	3	100%
K. ozaenae	3	2	66.6%
Total	11	10	90.9%

Zaman *et al.*, (17) reported highest frequency of ESBL production in *Klebsiella* species followed by *E. coli.* Mathur *et al.*, (18) have reported *Klebsiella* species as the first ESBL producing microorganism, while in Europe, the prevalence of ESBLs production among isolates of enterobacteriaceae members varies greatly from country to country (19).

Effect of some cations on MICs of Ceftizoxime against 3 isolates of *Klebsiella* species has shown that Ca^{2+} (Fig. 1) and Mg^{2+} (Fig. 2) were more affective in decreasing the Ceftizoxime MIC than other cations.

The effect of cations on the resistance of *Klebsiella* to ceftizoxime may be due to changing structural target for the antibiotic (20). The mechanism of the increase in resistance of *Pseudomonas aeraginosa* to aminoglycosides when calcium and magnesium concentrations increased is uncertain, although the interaction of cations with *P. aeruginosa* appears to occure at alocus on the cell wall (21).

It was found that there is no significance effect of Fe^{3+} (Fig. 3) on MICs in all concentrations while Zn^{2+} (Fig. 4) showed increasing resistance in the highest concentration (10 µg/ml).

Some antibiotics chelate divalent cations, the chelation may have significant effect on susceptibility (22). Na^{+} caused decreasing in MICs at concentrations (0.5%, 1%) while caused increasing at (1.5%, 2%) (Fig. 5), some studies revealed that the monovalent cations may enhance the activity of penicillin against *Proteus* species (3).

George *et al.*, (23) reported addition of zinc to Mueller-hinton agar resulted in increase in MICs of imipenem for *P. aeruginosa* but not in the MICs of ceftazidime for *P. aeruginosa,* alesser zinc effect was seen on the activity of imipenem against *K. pnenmoiae.*

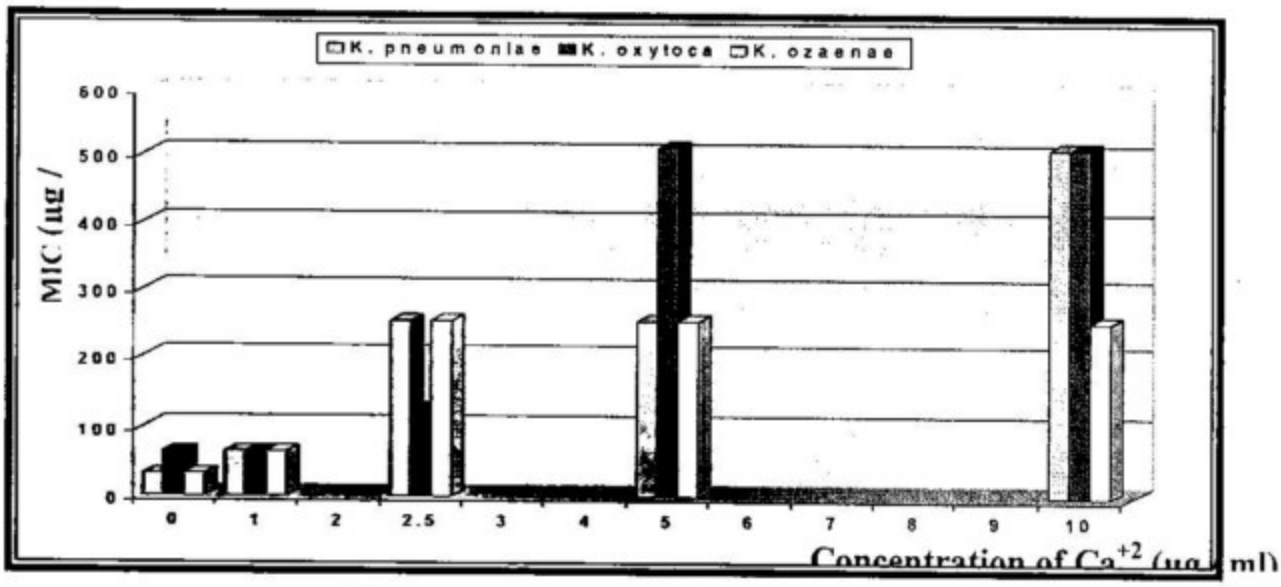

Figure 1: Effect of Ca^{2+} ion on MICs of Ceftizoxime against *Klebsiella* species

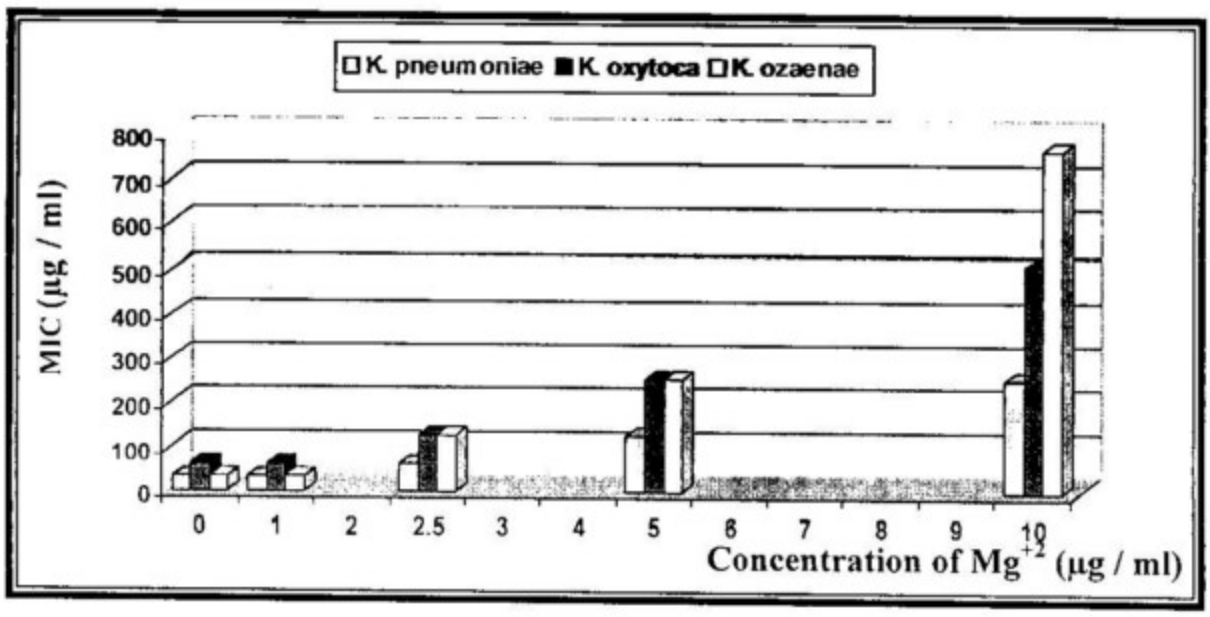

Figure 2: Effect of Mg^{2+} ion on MICs of Ceftizoxime against *Klebsiella* species

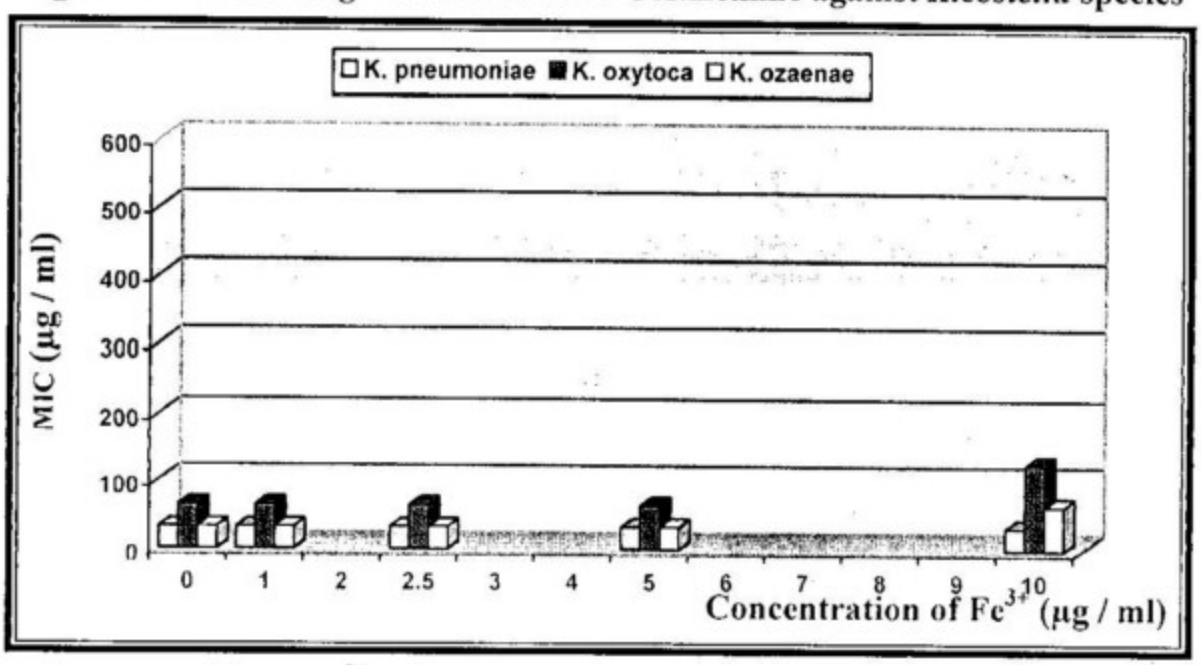

Figure 3: Effect of Fe^{2+} ion on MICs of Ceftizoxime against *Klebsiella* species

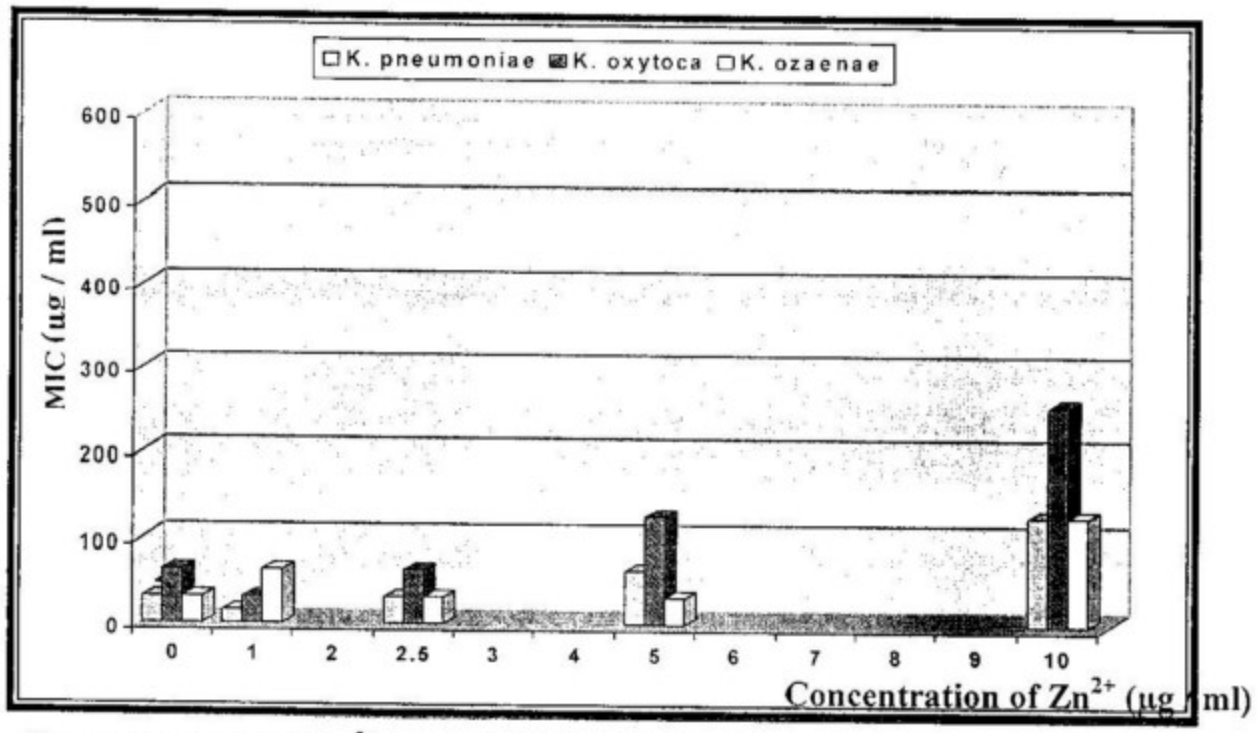

Figure 4: Effect of Zn^{2+} ion on MICs of Ceftizoxime against *Klebsiella* species

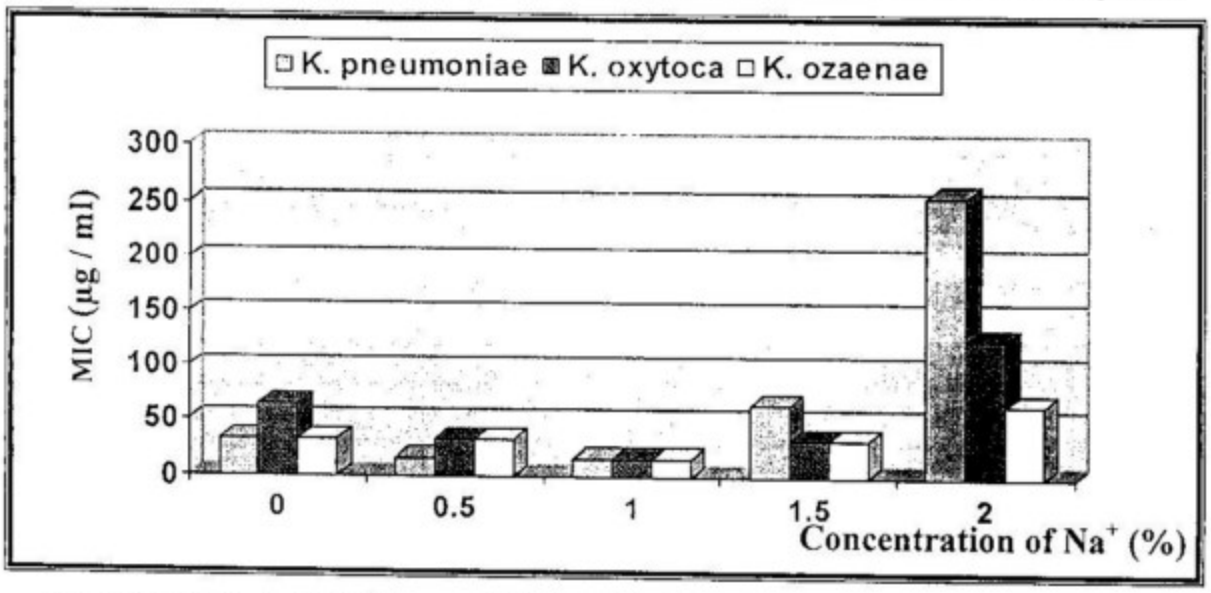

Figure 5: Effect of Na^{+} ion on MICs of Ceftizoxime against *Klebsiella* species

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تطور مقاومة بعض أنواع جرثومة *Klebsiella* المعزولة محلياً من التهاب المجاري البولية لبعض مضادات البيتالاكتام

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المستخلص

جُمعت 200 عينة إدرار من مرضى مصابين بألتهاب المجاري البولية وتم التحري عن أنواع جرثومة *Klebsiella*. 70 عينة (35%) أظهرت نموا وأن ١١ عزلة *(15.7%)* شخصت أنها تعود لأنواع الجنس *Klebsiella (K. pneumoniae, K. oxytoca and K. ozaenae).*

معظم العزلات المحلية أظهرت مقاومة عالية للمضادات: بنسلين ج، أمبسلين، أموكسيسيلين، ببراسيلين، سيفالوثين، سيفاكلور وسيفوتاكسيم، مقاومة متوسطة لمضادات: سيفاليكسين، سيفتازديم وسيفيكيم ومقاومة قليلة لمضادات: أوكمنتين، سيفوكسيتين وسيفتيزوكسيم.

التراكيز المثبطة الدنيا لمضادات (MICs) : أموكسيسلين، بيراسيلين، سيفاليكسين وسيفالوثين µg/ml (1024-512) أعلى من المضادات الأخرى (512-32) µg/ml وأن 10 عزلات (90.9%) أنتجت أنزيم β-lactamase.

أيونات المغنيسيوم والكالسيوم أكثر تأثيرا في تقليل التركيز المثبط الأدنى (MIC) للمضاد ceftizoxime مقارنة ببقية الأيونات. لم يظهر تأثير واضح لايونات الحديد والزنك على التركيز المثبط الأدنى في جميع التراكيز المستخدمة (10, 5, 2.5, 1) µg/ml بينما أيون الصوديوم سبب زيادة في التركيز المثبط الأدنى عند التراكيز (1, 0.5)%.