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Antibiotics susceptibility of *E.cloacae* and *E.sakazakii* that isolated from different clinical specimens

Intidhaar Na'eem Abid
Dep.of Pathological analysis – College of science
Thi-Qar University

Assis.Prof Dr. Mahdi H. Al-Amaar
Dep.of Biology –College of science
Kufa University

Summary

Two species of *Enterobacter* (84 isolates) *E.cloacae* (75 isolates) and *E.sakazakii* (9 isolates) were isolated from different clinical specimens .

They are observed , all isolates (84) were resistant to β– lactam antibiotics (amoxicillin and cefoxitin) but the resistance for cephalothin ,cephalexin and ampicillin , were 98% , 92% and 90% respectively , 75% , 61% and 50% for doxycycline , nitrofurantion and chloramphenicol respectively .The high values of MICs were registered to cefoxitin (46-256) µg / ml , while the lower MICs values of cefepime , imipenem and ciprofloxacin were (0.5 -32 , 0.5 -16 , 0.5 – 4) µg / ml respectively .

From the study 72 (86%) of isolates were produced β– lactamase **,** 63 isolates (84%) of *E.cloacae* can produce β –lactamase , and all isolates (9)of *E.sakazakii* gave positive result for β –lactamase.

Introduction

With few exceptions, the major classes of antibiotics are used to manage of *Enterobacter* infections , include the beta – lactam groups. The fluoroquinolones , the aminoglycosides , and TM – SMZ . Because most *Enterobacter* species are either very resistant to these agents or can develop resistance during antimicrobial therapy, the choice of appropriate antimicrobial agents is complicated (Paterson , 2006) . The emergence of multidrug resistant Enterobacteriaceae is of particular concern because of the potential for widespread dissemination, and difficulties in treating infected patients (Karlowsky *et al* ., 2003 ; Yu *et al* ., 2006) . One of these resistances include :

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β – lactam resistance

β – lactam agents ; penicillins , cephalosporins and other compounds that feature a β– lactam ring in their structure (Greenwood *et al*., 2007) . All of these compounds bind to proteins situated at the cell wall - cell membrane interface (Laurence *et al*., 1997) .The penicillin binding proteins are involved in cell wall construction, including the cross – linking of the peptidoglycan strands that gives the wall its strength.

The β–lactam groups differ from each other by additional rings or side chains (five – membered thiazolidine ring of pencillins, six members dihydrothiazine ring of cephalosporins, carbapenems substitute carbon atom for a sulful atom and add a double bond to the penicillin nucleus , and monobactams are monocyclic compounds). The various antibiotics in each group differ by the nature of one or two side chains (Poole, 2004; Greenwood *et al*., 2007) .

Resistance to β–lactam it is most often caused by the presence of β–lactamase enzymes . But mutations in PBP_S resulting in reduce affinity for β–lactam antibiotics are also commonly observed, and less frequently caused by reduced uptake due to changes in the cell – wall or active efflux. The main drug – resistance mechanism of gram – negative bacteria is producing β–lactamase (Bush *et al*., 1995 ; Brooks *et al*., 2007).

Materials and Methods

. Antibiotics susceptibility tests

It was used two methods for study bacterial susceptibility to antibiotics**:**

1- Disk diffusion method

It was carried out by using Kirby Baur method to show the effect of antibiotics on bacterial isolates (NCCLS, 2003).

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2 - Minimal inhibitory concentration (MIC)

The serial agar dilutions method was used for determination of MICs according to the method suggested by (Piddock , 1990; Stock & Ridgway, 1987)

Detection of β- lactamase production

This test was performed for all bacterial isolates that were resistant to β - lactam antibiotics .It was used the direct capillary tubes method to detecting the bacterial ability to produce β- lactamase (Koneman *et al*., 1992) as follows:

A-It was added 2ml of phenol red reagent (dissolving 2.5 g of phenol red in 40 ml of sodium hydroxide (0.1N) solution, then the volume was completed to 500 ml for result final concentration (0.5%)) to penicillin G solution (dissolving 1 million unit of penicillin powder in 1.16 ml of D.W.).

B-The pH was adjusted by use sodium hydroxide (1N until the color solution was became purple PH =8.5 .

C-Capillary tubes in diameter (0.7 -1) mm was immersed in phenol red – penicillin G solution until the high was reached to (1-2) cm.

D-The end of capillary tube was embittered on several colonies of 24 hr.-old bacterial cultures on nutrient agar for make microbial blocking .for touched the colonies with solutions inside the tubes. With checking the air bubbles were not found between the solution and bacterial mass.

C-The tube were incubated in vertical at 37˚ C, and the result was reading during 5-15 min. , change of color above the colonies to yellow was indicated to positive result .

Results

Effect of different antibiotics on *Enterobacter* isolates

A- Disk diffusion methods

The susceptibility patterns of *Enterobacter* isolates to antibiotics was tested using disk diffusion method , the result revealed that all bacterial isolates

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(*E.cloacae* ,*E.sakazakii*) were resistant to amoxicillin , all isolates of *E.sakazakii* were resistant to ampicillin , but 89% of *E.cloacae* were resistant to ampicillin .

The results showed that all isolates of *E.cloacae* were founded to be resistant to cephalothin , but 78% of *E.sakazakii* were resistant to this antibiotic, while 95% , 66.7% isolates of *E.cloacae* and *E.sakazakii* were resistant to cephalexin respectively .

The finding revealed that all isolates 100% were recorded to be resistant to cefoxitin .

The resistance of isolates to cefotaxime , ceftriaxone and ceftazidime were variant , 56% of *Enterobacter* isolates have intermedium resistance to cefotaxime , and most isolates of *Enterobcter* were high resistance to ceftriaxone , it was reached to 84% for *E.cloacae* and 100% for *E.sakazakii* , but they were more sensitive to ceftazidime (51% , 66.7%) respectively .

Enterobacter spp. were resistant to aztreonam , which reached to 72% in *E. cloacae* isolates and 56% in *E.sakazakii* isolates. It was demonstrated that, the resistance of *Enterobacter* isolates to cefepime were low, the sensitivity of *E. cloacae* and *E.sakazakii* were registered 67% and 78% respectively.

Bacterial isolates were found to be sensitive to imipenem , the sensitive rate was reached to 51% in *E. cloacae* and 66.7% in *E.sakazakii*.

The bacterial susceptibility to other non β –lactam antibiotics were also revealed 56% of *E. cloacae* and 66.7% of *E.sakazakii* were sensitive to TM – SMX .

The resistance of *E. cloacae* to aminoglycosides (tobramycin , gentamicin and amikacin) was recorded 44% , 28% , 39% respectively and it found in (33.3% , 33.3% , 66.7%) of *E.sakazakii* isolates respectively . The susceptibility of quinolones (ciprofloxacin, nalidixic acid) was found 61%, 77.8% for ciprofloxacin respectively, but 82% and 11% for nalidixic acid respectively.

High resistance was appeared for doxycycline (77% and 56%) and (60%, 89%) for tetracycline respectively.

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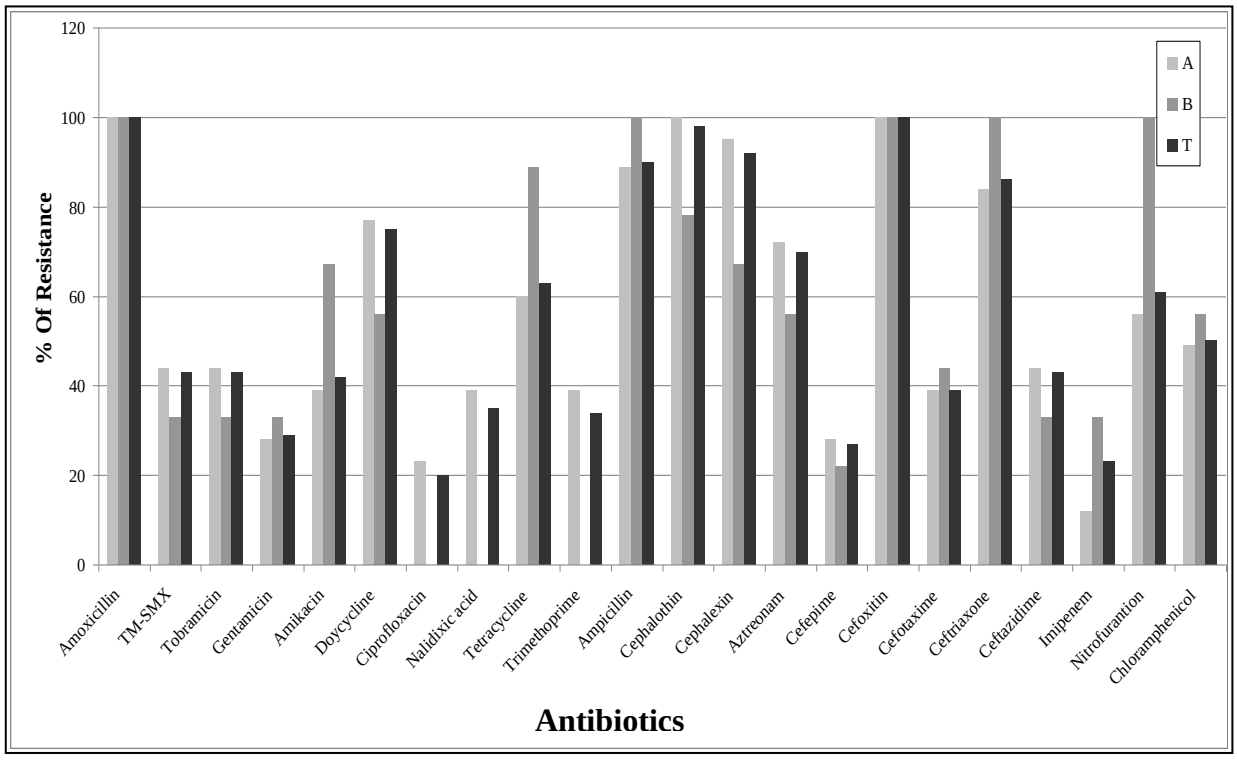
All isolates of *E. sakazakii* 100% were appeared sensitive to trimethoprime , versus 39% of *E.cloacae* were resistant to this antibiotic .Resistance of isolates to nitrofurantion registered 56% and 100% in *E.cloacae* and *E.sakazakii* isolates respectively . At the same time, it was also demonstrated that , 49% of *E.cloacae* were resistant to chloramphenicol and resistance of *E.sakazakii* was registered in 56% of isolates (Fig.1).



A: *E.cloacae* , B: *E.sakazakii* , T: Total of isolates

Figure (1): Antibiotics resistance of *Enterobacter* isolates.

B - Determination of Minimum Inhibitory Concentration (MIC)

The study include the determined of MIC_S for ten of antibiotics from that were used in disk diffusion method , these antibiotics include : gentamicin ,ciprofloxacin ,tetracycline , ampicillin , cefotaxime , cefepime , cefoxitin , ceftazidime , aztreonam and imipenem .The values were compared with the break point recommended by CLSI (2007) . The results were shown in table (1) indicated that all isolates of *Enterobacter* (*E.cloacae* and *E.sakazakii*) were

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highly resistant to cefoxitin (100%) with concentrations reached above the break point values (≥64 µg /ml) .The table also revealed that the MIC_S values of ampicillin were less than that of cefoxitin (8-32) µg /ml for *E.cloacae* isolates and ≥32 µg /ml for *E.sakazakii* . The results showed the MIC_S values of imipenem were (0.5-16) µg /ml, which were a very effective drug on two species of *Enterobacter* , followed by cefepime which its MICs values were (0.5-32) µg /ml , and ciprofloxacin (0.5-4) µg /ml for *E.cloacae* isolates and (0.5-1) µg /ml for *E.sakazakii* .

Table (1): MIC_S of selected antibiotics to *Enterobacter* isolates

Antibiotics	***E.cloacae*** **N= 75**			***E.sakazakii*** **n=9**			**Total n=84**	
	MIC_S range µg /ml	**No.& (%) S**	**No.& (%) R**	**MIC_S range µg /ml**	**No.& (%) S**	**No.& (%) R**	**No.& (%) S**	**No.& (%) R**
Gentamicin	0.5-16	50 (66.7)	16 (21%)	0.5-8	7 (78%)	2 (22%)	57 (68%)	18 (21%)
Ciprofloxacin	0.5-4	53 (71%)	12 (16%)	0.5-1	9 (100%)	0 (0%)	62 (74%)	12 (14%)
Teyracycline	1-256	12 (16%)	40 (53%)	1-128	1 (11%)	8 (89%)	13 (15%)	48 (57%)
Ampicillin	8-32	4 (5%)	66 (88%)	32-256	0 (0%)	9 (100%)	4 (4.8%)	75 (89%)
Cefotaxime	0.5-512	5 (6.7%)	28 (37%)	0.5-128	4 (44%)	4 (44%)	9 (11%)	32 (38%)
Cefepime	0.5-32	57 (76%)	18 (24%)	0.5-32	8 (89%)	1 (11%)	65 (77%)	19 (23%)
Cefoxitin	64-256	0 (0%)	75 (100%)	64-128	0 (0%)	9 (100%)	0 (0%)	84 (100%)

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Ceftazidime	0.5-256	43 (57%)	32 (43%)	0.5-128	7 (78%)	2 (22%)	50 (60%)	34 (40%)
Aztreonam	0.5-256	20(27%)	53 (71%)	0.5-128	4 (44%)	5 (56%)	24 (29%)	58 (69%)
Imipenem	0.5-16	65(87%)	10 (13%)	0.5-16	7 (78%)	2 (22%)	72 (86%)	12 (14%)

β –lactamase detection

The β –lactamase –producing isolates of *Enterobacter* species will be illustrated in table (2) . It was found that, 63 / 75 isolates (84%) of *E.cloacae* can produce β –lactamase , and all isolates of *E.sakazakii* gave positive result for β –lactamase 9/9.

Table (2) : β –lactamase –producing of *Enterobacter* isolates

***Enterobacter* species**	**No. of Isolates**	**No.&(%)of β –lactamase –producers**	**No.&(%)of β –lactamase –Non-producers**
E.cloacae	75	63 (84%)	12(16%)
E.sakazakii	9	9(100%)	0(0%)
Total	84	72(86%)	12(14%)

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Discussion

The antibiotics susceptibility patterns

One of the most curious and puzzling aspects of infections due to *Enterobacter* species is the predilection of these organisms to develop antimicrobial resistance during therapy (Chow *et al*., 1991).

The study, used two methods (disk diffusion method and MICs)to determined antibiotic susceptibility of *Enterobacter* isolates.

The results were revealed, that all isolates of *Enterobacter* (*E.cloacae* and *E.sakazaki*i) have higher resistance to penicillins (amoxicillin , ampicillin , this may be due to the production of β – lactamases or failed antibiotics in reaches to the target (PBPs) (Harwood *et al*., 2000) . In relation to cephalosporins , it was also most isolates of two species of *Enterobacter* are resistant to cephalothin and cephaloxin (first generation cephalosporins) and the resistance to these antibiotics can be attributed to poor permeation of bacteria by the drug or lack of BPBs or degradation of drug by β-lactamases (Brooks *et al*., 2007) . As well as all isolates of this study were resistant to cefoxitin (second generation of cephalosporins) , these results are agreement with Lngram *et al*.(2011) they found that no isolates were susceptibile to cefoxitin among the isolates of Enterobacteriaceae , this may be due to have production of AmpC β –lactamase enzymes that hydrolysis of cephamycin or porin deficiency and carbapenemases (Livermore , 1995).

Regarded to third generation of cephalosporins (cefotaxime , ceftriaxone and ceftazidime) , it was appeared that all isolates (*E.cloacae* and *E.sakazaki*i) are possess high resistant to cefotaxime and ceftriaxone , its an important indicator for the presence of ESBLs (CLSI, 2006) . Most of isolates of *Enterobacter* that included with this study were sensitive to ceftazidime , these results are identical with those obtained by Tzouvelekis *et al*.(2000) they found CTX –M β-lactamases play a significant role in resistance to third generation cephalosporin ,and the activity of these enzymes against cefotaxime is markedly higher than that against ceftazidime .

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Resistance of *Enterobacter* isolates to aztreonam also, may be results from production of ESBLs (CLSI, 2006).

At the same time, it was also demonstrated from the results, that, the overall bacterial isolates were sensitive to cefepime (fourth generatin cephalosporins).

Cefepime and cefpirome rapidly penetrate into gram –negative bacteria, have a high affinity for essential PBPs and are stable to attack from chromosome – encoded β -lactamase (Kesseler *et al*., 1985) .

Regarding to susceptibility to imipenem (carbapenems) , it was found , that some isolates of *Enterobacter* were resistant to imipenem , that are agreement with those obtained by Tumbarello *et al*.(2006) ; Yigit *et al*.(2002) they found resistance to carbapenems among *Enterobacter* isolates are of great concern . In general, carbapenem resistance may be mediated by three major mechanisms: (i) they hyperproduction of a β -lactamase with weak carbapenem – hydrolyzing activity (such as AmpC β-lactamase or ESBLs) combined with decreased drug permeability through the outer membrane , (ii) a decreased affinity of the PBPs that constitute target proteins for carbapenems . and (iii) carbapenem-hydrolyzing β-lactamase production (Nordmann & Poirel, 2002; Tumbarello *et al*., 2006) .

The results were shown, that most isolates of *Enterobacter* were sensitive to TM-SMX. These results were disagreed with those obtained by Salman (2006) who found high resistance of *E.cloacae* to this antibiotic. This sensitivity of isolates to TM –SMX , may be due to have sulfa compund combinat with trimethoprime effects on different segments of bacterial folate synthesis , which has synergistic effects (Wang , 2002) .

Aminoglycosides such as tobramycin , gentamicin and amikacin also used in antibiotics susceptibility test of all isolates of *Enterobacter* . Most isolates of *E.cloacae* are high sensitive to these antibiotics. the results are correlated with results of Kolar *et al*.(2010) who found ,that all isolates were susceptible to aminoglycosides , while all isolates of *E.sakazakii* were sensitive to tobramycin and gentamicin , but were appeared resist to amikacin , these results are agreement with Park *et al*. (2003), had stated that the resistance rate

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of *Enterobacter* to gentamicin was (33.3%) , and amikacin (54%) . The mechanism of *Enterobacter* spp. resistance to aminoglycosides is mediated by the production of more than one type of aminoglycosidases located on the R plasmid (Maes&Vanhoof , 1992).

Results showed that the quinolones (ciprofloxacin) had a good activity against *Enterobacter* isolates, therefore, ciprofloxacin can be used to treat infections caused by these bacterial species , but it is not recommended for patients blew 18 years of age , because of the possibility of the erosion to the growing cartilage (Orthropathy) (Harvey & Champe , 2006) .These results are agreement with that of Kunapuli *et al*.(2003)who found that the susceptibility rate for ciprofloxacin was more than 85% for *E.cloacae*.

In contract the sensitivity of *Enterobacter* isolates to nalidixic acid was lower than that in ciprofloxacin .Nalidixic acid one of earlier quinolones and the resistance to this antibiotic involves one of two mechanism – either an alteration in the A subunit of the target enzyme , DNA gyrase , or a change in outer membrane permeability (Brooks *et al* ., 2007) .Regarding susceptibility to doxycyclin and tetracycline , *Enterobacter* isolates were appeared more resistance to these antibiotics , these results are agreement with those obtained by Zhihui *et al*.,(2003) they found that all isolates of *Enterobacter* have high resistance to both antibiotics . This resistance is under the control of transmissible plasmids (Brooks *et al*., 2007).Furthermore, all isolates of *E.sakazakii* were resist to trimethoprime , but resistance of *E.cloacae* was lower than that in *E. sakazakii* .Mayer *et al*.(1985) reported that TMP resistance in multiple species of Enterobacteriaceae was found to be spread in one hospital by a single , stable conjugative plasmids that has wide host range and encodes the type II DHFR gene .

Resistance rates of *Enterobacter* isolates to nitrofurantion were high. These results are disagreement with other results obtained by Astal (2005) who reported that the *Enterobacter* sp., were more susceptible to this antibiotic.

The relatively high resistance of isolates to chloramphenicol was observed in results of the study , these results resemble those obtained by Abid

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(2006) who showed that the *Enterobacter* spp. that isolated from clinical specimens were highly resistant to chloramphenicol . This resistant may be due to production chloramphenicol acetyl transferase that coden by genes are lies on plasmids (Murray & Show, 1997).

The values of MICs were based on break point recommended by CLSI (2007) for estimation of the response .

The MICs values of selected β – lactam antibiotics were high for all isolates. MICs of cefoxitin were with concentrations reached beyond the break point values, this may be due to production of AmpC- β – lactamase or to the lack of permeation porin (Manchanda &Singh ,2003) . It was also found, high resistant to ampicillin with MIC values (8-32) µg /ml for *E.cloacae* and exceeding 32 µg / ml for *E.sakaakii* . Bradford *et al*. (1996) showed that the β - lactamase provided selective resistance to penicillins and cephamycins .

According to results of MIC determination of imipenem which was a very effective drug against *Enterobacter* isolates were more sensitive to this antibiotic with MIC values ≤16 µg / ml. These results were in accordance with results being reported by Vatopoulos *et al*.(1990) who showed that , the MICs of imipenem for some strains of gram negative which included *Enterobacter* spp. remained low . MIC values of cefepime were equal for two species of *Enterobacter* and below the break point value ≤ 32 µg / ml**.** These results are identical with those obtained by Sanders *et al*. (1996) who found, that the MICs of cefepime in derepresed strains of *Enterobacter* spp. remain within the susceptible rang (MICs ≤4 µg / ml). These antibiotics may be suitable for treating infections caused by inducible and derepressed AmpC producing Enterobacteria .

Ciprofloxacin was another antibiotic that effect on *Enterobacter* isolates which had MIC values , extremely below the break point value (0.5-1) µg / ml for *E.sakazakii* and above these values for *E. cloacae* , this may be due to the distribution of *E.sakazakii* in hospitals was less than that with *E. cloacae* . The sensitivity of isolates to this antibiotic may be due to that limited use of this antibiotic in treatment of infections (Martinez *et al*., 1998; Abid ,2006) .

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The production of β - lactamases

In gram – negative bacteria, one of the important mechanisms of β-lactam resistance, is the production of beta – lactamases which inhibit protein transpeptidases participating in bacterial cell wall synthesis (Bradford , 2001 ; Paterson & Bonomo ,2005).

It was used direct capillary tubes method to detection of β - lacamase , the principle of this method depends on the detection of penicilloic or cephalospoic acid that resulted from breakdown of amide bond in the β - lactam ring of each penicillins or cephalosporins (Livermore , 1995) . It was found from the results , that two species of *Enterobacter* were able to produce β – lactamase, these results were agreement with those proposed by Pitout *et al* .(1997) who indicated , that the species of *Enterobacter* can possess a variety of β – lactamases that are responsible for beta –lactam resistance.

Increase and the prevalence of *Enterobacter* spp. producing β – lactamase may be due to prevalence of this genus as a nosocomial pathogen.

الحساسية الدوائية لبكتريا *E.sakaakii* and *E.cloacae* المعزولة من عينات مرضية مختلفة

انتظار نعيم عبد
قسم التحليلات المرضية - كلية العلوم
جامعة ذي قار

ا. م. د. مهدي حسين العمار
قسم علوم الحياة – كلية العلوم
جامعة الكوفة

الخلاصة

تم عزل نوعين من بكتريا .*Enterobacter* spp (84) ، تضمنت بكتريا *E. cloacae* 75 عزلة (89.3%) و *E. sakazakii* إذ عزل بواقع 9 عزلات (10.7%) من عينات مرضية مختلفة.

لوحظ أن جميع العزلات (84) كانت مقاومة لمضادات البيتا – لاكتام Amoxicillin ، Cefoxitin أما نسبة المقاومة لكل من Ampicillin،Cephalexin و Cephaothin فكانت 98% ،92% و90% على التوالي ،كذلك أظهرت مقاومة بنسبة 75%، 61% و 50% لكل من مضادات Doxycycline، Nitrofurantion و Chloramphenicol على التوالي.سجل مضاد Cefoxitin

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أعلى قيم للتراكيز المثبطة الدنيا (MICs) ، اذ تراوحت بين (64 –256) مايكروغرام / مل ، بينما اقل قيم لـ MICs كانت لمضادات Cefpime، Imipenem، Ciprifloxacin اذ تراوحت بين((0.5–32) ، (0.5–16) ، (0.5–4)) مايكروغرام / مل على التوالي .

أظهرت نتائج الدراسة الحالية أن 72(86%) من العزلات كانت لها القابلية على إنتاج انزيمات البيتا –لاكتاميز.

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