Emergence of Extended spectrum β - Lactamases (ESβL) in clinical Isolates of *Klebsiella Pneumoniae* in Baghdad.

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Received 8 April 2012 ; Accepted 13 May 2012

Abstract

The spread of Gram- negative bacteria with plasmid-born Extendend- Spectrum β -Lactamases (ESβLs) had become aworldwide problem, that enzymes confer resistance to penicillin,cephalosporin the first, second and third generations and aztreonam via hydrolysis of the antibiotic ,that sample collected from urine and sputum sample .five isolates from forty-four of Klebsiella pneumoniae was production ESβL (11.36%).
Tested for susceptibility using Disk diffusion method ,and determination of (MIC)by standard agar dilution method ,and phenotypic detection of ESβL by using disc approximation method.

Keyword: *klebsiella pneumoniae*, Extended spectrum β -Lactamases, disc approximation method، minimum inhibitory concentration.

إنتشار إنزيمات البيتالاكتاميز الواسعة الطيف في عزلات بكتريا *Klebsiella Pneuomoniae* السريرية في بغداد.

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الخلاصة

ان انتشار انزيمات البيتالاكتاميز الواسعة الطيف المشفرة بلازميديا في البكتريا السالبة لصبغة غرام اصبحت مشكلة عالمية. هذه الانزيمات تكون مقاومة للبنسلينات وسيفالوسبورينات الجيل الاول والثاني والثالث والازتريونام عن طريق تحليلها لهذه المضادات الحيوية.العينات جمعت من الادرار والقشع ، خمس عينات موجبة من بكتريا *Klebsiella Pneuomoniae* كانت منتجة لانزيمات ESβL (11.36%) من مجموع العينات البالغة اربعة واربعين عينة. تم اجراء فحص الحساسية وتحديد التركيز المثبط الادنى وتشخيص انتاجها لانزيمات ESβL مظهريا باستخدام طريقة الاقراص المتاخمة.

الكلمات الدالة:- الكلبسيلا الرئوية، انزيمات البيتالاكتاميز الواسعة الطيف، طريقة الاقراص المتاخمة، التركيز المثبط الادنى.

Introduction

The spread of antibiotic resistant bacteria has become alarg problem in most countries including Iraq. This bacteria is usually due to the production of extended spectrum β - Lactamase enzyme (1).

The excessive use of the oxyimino- cephalosporins in clinical practice had Resulted in *Klebsiella* species and other member of the Enterobacteriaceae showed diaminished susceptibility (2). *Klebsiella pneumonia*e is anormalflora bacteria, become opportunistic when transfer to urinary tract. its conceder the second cause of Nosocomial infection, (3). it showing increase of resistance when it found with another bacteria it showed co-resistance to (Aminoglycoside, Quinolones). That resistance increas with produce (ESβL) enzyme (4).

This study performed to investigate by phenotypic detection of ESβL in *Klebsiella pneumonia* isolated from patients in hospitals in Baghdad.

Material and method

Bacterial isolates:

One thousand and five hundred (1500) sample of urin and one hundred (100) sample from sputum were collected from Baghdad hospitals .

Diagnosis Isolates:

Forty- four isolates of *Klebsiella pneumoniae* were collected, That isolates diagnosis by morphological diagnosis, Biochemical tests and using Api 20Esystem.

The positive sample was culture in blood Agar, MacConkey Agar, Eosin methylen blue Agar and were stored in medium containing 15% glycerol in brain heart infusion broth and stored in deep freezing at -20C°.

Antimicrobial Susceptibility Testing

Organisms were tested by disc diffusion method using Muller-Hinton agar as described by(5).

Phenotypic Detection of ESβL

Detection of ESβL in positive sample was ascertained using disc approximation method (6). Using Mulller- Hinton agar and three disc containing 30 µg for cefotaxime , ceftriaxone were Placed 30 mm (center to center) around adisc containing amoxicillin plus clavulanic acid (Augmentin) an increase in the zone diameter of Augmentin compared with other antibiotic considered positive for ESβLs.

Results

All sample collcted from urin and sputum (1600) were analysed for *K..pneumoniae* by identication according to morphological and biochemical tests .which showed (44) positive sample of *K.pneumoniae* Distribution according to clinical source in Table (l). This sample showed high resistance to antibiotic in this study, and was showed high MIC Value to antibiotic , the result showed five positive sample (11.36%) from 44 samples of *K.pneumoniae* have ability to producing ESβL enzyme.

Table 1 :distribution of *K.pneumoniae* according to clinical source.

source	Total sample	Isolate	
		NO	%
Urine	1500	25	1.6
sputum	100	19	19

Discussion

The phenotypic Identification of producing ESβL enzyme can do by tow stebs. The first is antimicrobial susceptibility test and the second stebs is synergy between oxyimino cephalosporine and clavulanate (7). Disk approximation Method used to phenotypic method, the result showed 5 isolat (11. 36)% from 44 isolat of *K.pneumoniae* producing ESβL . is show in figure (1).

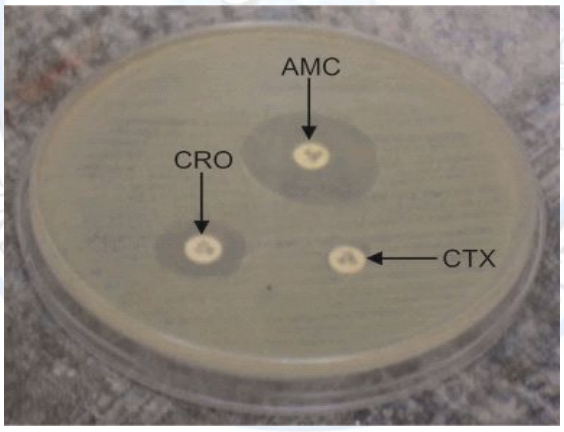

Figure (1):ESBL detection by using disc approximation method.

CTX:Augmentin CRO:Ceftriaxone CTX:Cefotaxime

Our result agree with Yan (8) , and disagree with Lal et al (2007) (9).our study showed high resistance to antibiotic, 100% to (ceftazidime, cefotaxime , ceftriaxone Aztreonam, carbencillin, piperacillin, Ampicillin), 80% to cefepime and ciprofloxacin 40% to Augmantin and streptomycin, 60% to Kanamycin. 20% to Amikacin, 0% to meropenem and Imipenem as show in figure (2)

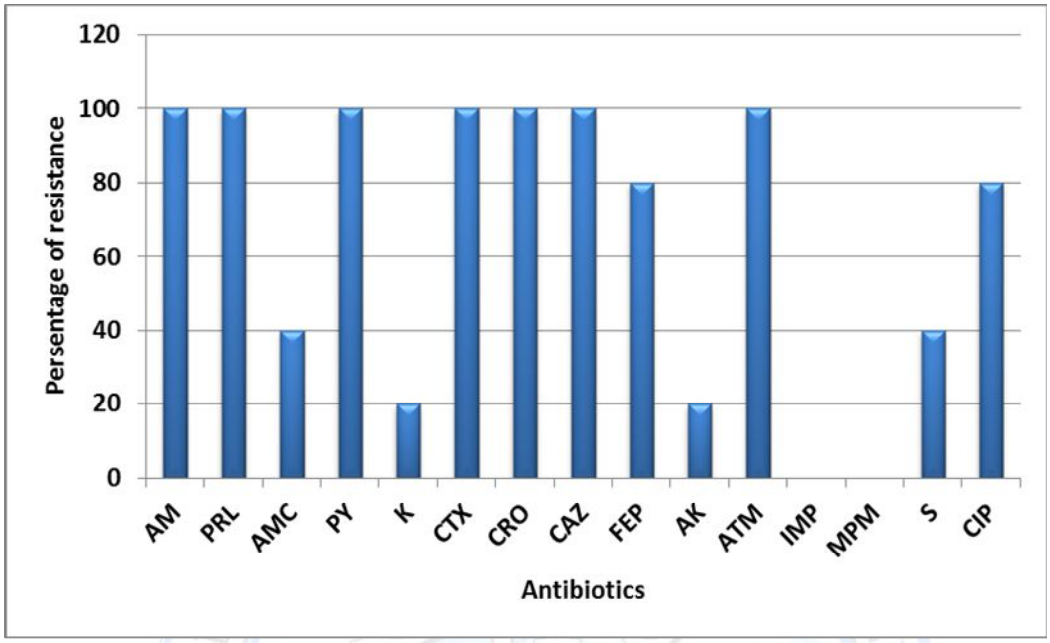

Figure (2):Antibiotic resistance of ESβL produce *K.pneumoniae*

CTX: Cefotaxime	AM:Ampicillin	ATM: Azetreonam
PRL: pipracillin	CRO: ceftriaxone	IMP: Imipenem
AMC: Amoxicillin /clavulanic acid	CAZ: ceftazidime	MPM: Meropenem
Py: Carbenicillin	FEP: Cefepime	S: Streptomycim
K:kanamycin	AK: Amikacin	CIP: Ciprofloxacin

The Resistance to carbencillin, pipracillin may be prduce by bacteria enzyme broking that antibiotic(10).

The resistance to ceftriaxone, ceftazideme, cefotaxime by producing ESβL enzyme (11) resistance to cefotaxime, ceftriaxone, ceftazidime. Agree with Amin *et al* (2009)(12). While resistance to ceftriaxone was disagree with Nasehi *et al* (2010)(13). The resistance to Imipenem & meropenem was agree with Lim *et al*. (2009)(14)

Our study determination the minimum inhibitory concentration for four β-lactam antibiotic (Imipenem, ceftazidime, Ceftriaxone, cefotaxim.) . as show in table:2.

Table 2: Minimum Inhibitor concentration of ESBL –Producing *K .Pneumoniae*

Antibiotic / NO. of Isolates	Imipenem	Ceftazidime	Ceftriaxone	cefotaxime
Break point µg/ml	≥ 16	≥ 32	≥ 64	≥ 64
K 22	0.05	128	512	512
K 26	0.05	32	256	128
K28	0.05	32	512	256
K 29	0.05	64	512	512
K36	0.1	265	1024	1024

The Imipenem MIC Value agree with nasehi *et al* (2010)(13).while ceftazidime MIC value Was disagree with GrÖber *et al* (2009)(15).and our study showed MIC to cefotaxime and cefriaxone disagree with Jones et al (2009) (16).

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