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Detection ESBL enzymes in gram-negative bacteria

Muna Jabbar Tuama Hardany[1] , Amani Abd Al-Ridha Al-Abdullah[2] , Saad S. Mahdi Al-Amara[3] , Hasanain Mohammed Ali Makki[4]

[1],3 Basrah University /College of Sciences/Biology Department/Basrah, Iraq
[2] Basrah University /College of Sciences/pathological analysis /Basrah, Iraq
[4] Basrah General Hospital /Basrah, Iraq
*Corresponding Author: Email: Dewpond80@gmail.com , saadshaker2003@yahoo.com

ABSTRACT

The prevalence of Extended-spectrum ß-lactamase (ESBLs) enzymes are considered a large problem all over the world. The misuse of broad-spectrum-antibiotics especially in immunocoprmized and hospitalized patients has lead to emergence of antibiotic-resistant strains of Enterobacteriaceae and several Gram-negative bacteria (GNB), for that reason, there's an urgent need to investigate these enzymes by a continuously examination and blood culture of these patients. Blood samples n=147 were collected from Hemodialysis patients that treatment in Basrah General Teaching Hospital and Al-Sader Hospital in Basrah governorate south of Iraq, conventional microbiological methods (Biochemical tests) and also molecular methods by implified 16s rDNA and genetic sequence analysis, were used to dignosis the common types of Gram-negative bacteria and two methods were used, double disc synergy test (DDST) and double disc approximation method (DAM) to detect the present of ESBLs-enzymes in these samples. Twenty seven of Gram-negative bacteria were obtained out of 147 blood samples, and out of 27 GNB, 16(59.3 %) was positive produced ESBLs-enzymes. Cefotaxime (CTX), was found to be the more sensitive to detect the ESBLs-enzymes.

Keywords: *ESBLs-enzymes, DDST, DAM.*

1. INTRODUCTION

Antibiotics are one of the most critical discoveries which have affected human and animal health in the records of mankind. β-lactam antibiotics can be divided into six unique groups, penicillins, cephalosporins, monobactams, cephamycins, carbapenems, and β-lactamase inhibitors[1]. The β-lactamases are the collective name of enzymes that hydrolyze the β-lactam ring (addition H2O molecule to the common ß-lactam bond) of penicillins, cephalosporins, and diverse β-lactam antibiotics[2]. And due to the hydrolyzing process by β-lactamases, the β-lactam antibiotic undergoing change in their structure. This inactivates the drug, giving it a slightly extraordinary structure.

This structure change makes it not able to bind to the penicillin binding protein (PBP) energetic spot. β-lactamases are categorized into narrow-spectrum (penicillinases), broad-spectrum (ampicillinases), extended-spectrum β-lactamases (ESBLs), and carbapenemases[3,4] based totally on range of activity. ESBLs-enzymes can break done the antibiotic wich contain β -lactam ring such as, third generation cephalosporins (3rd GC) (ceftriaxone, cefotaxime, ceftazidime, cefepime and aztreonam (monobactam) but retain sensitivity to the cephamycins (i.e. cefotetan and cefoxitin) or carbapenems (i.e. ertapenem, meropenem, and imipenem)[5,6] and can inhabited in vitro by clavulanic acid. The first ESBLs-enzymes was detected in 1980s in France. One of the most common species that carry this type of enzymes was the *E. coli* and *Klebsiella* spp, and other members of the *Enterobacteriaceae*, and in *P. aeruginosa*[7]. ESBLs are found in the Gram-negative bacteria, and are plasmid-mediated enzymes, In addition, they have been derived from mutations that occurred to the original β-lactamases[8,9]. The plasmid that encoding to the ESBLs frequently also encodes a resistance to other antimicrobial classes, includes, aminoglycosides, trimethoprim-sulfamethoxazole, aminoglycosides, fluoroquinolones, leaving very narrow a therapeutic options that available for treatment[10].

2. MATERIALS AND METHODS

Twenty seven of Gram-negative bacteria were collected from clinical blood samples of hemodialysis patients identified by conventional methods (biochemical tests) and by molecular methods implifide 16s rDNA and genetic sequence analysis. All these bacteria tested to detection the ESBL-enzymes. Two phenotypic methods were used to investigate the enzymes, double disc approximation methods (DAM) and double disc synergy test(DDST). These two tests were done by using these antibiotics Ceftriaxone (30 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Aztreonam (30 µg) and Augmentin (amoxicillin-clavulanate(20/10µg)). The DAM was used to screen the presence of ESBL in all isolates by placing a disc of Augmentin (AMC) in the center of the Muller Hinton agar surface, then discs of Ceftazidime (CAZ), Cefotaxime (CTX), Aztreonam(ATM) and Ceftriaxone (CRO) were distributed around it at a distance of about from (20-30 mm center to center). After an overnight incubation[11,12]. DDST performed by using the method according to the CLSI recommendation. The organism to be scanned was spread on to Mueller-Hinton agar plates, then the (30µg) disc of Ceftriaxone (and /or Cefotaxim and /or Aztreonam and /or Ceftazidime) and a disc of Augmentin (20 µg amoxicillin / 10 µg clavulanic acid) located at a distance of (30mm) (centre to centre). In both methods, the organisms were considered to be producing ESBLs when the inhibition zone around any of these antibiotic discs presented a clear-cut increase headed for the Augmentin disc[13].

3. RESULTS

During this study, 27 gram negative bacteria were diagnosed by biochemical tsets and by implified 16s rDNA and genetic sequence analysis, table 1. Out of these, 17(62.9%) female and 10(37.0%) male of age group (9-78) years.

Table 1: The percentage of Gram-negative bacteria

Bacterial isolates	The percentage
Acintobacter haemolyticus	4(14.8%)
Acintobacter baumanii complex	3(11.1%)
Acintobacter lowffii	1(3.7%)
Pseudomonas aeruginosa	5(18.5%)
Pseudomonas luteola	2(7.4%)
Klebsiella pneumoniae	4(14.8%)
Burkholderia cepacia	4(14.8%)
Sphingomonas paucimobilis	3(11.1%)
Ralstonia mannitoilyfica	1(3.7%)

While the percentage of ESBL-Producing gram negative bacteria was 59.3% (16 out of 27) investigated by using (DAM) and (DDST), table (2). In present study, *Acinetobacter* spp. recorded highest percentage 37.5%(6 out of 16) that harboring ESBLs including *(Acintobacter haemolyticus*, *Acintobacter baumanii complex* and *Acintobacter lowffii)*, followed by *Pseudomonas aeruginosa* 18.8% (3 out of 16), while the percentage of *Klebsiella pneumonia* and *Pseudomonas luteola* was 12.5% (2 out of 16) for each other, and percentage of *Burkholderia cepacia* group, *Ralstonia mannitoilytica* and *Sphingomonas paucimobilis* was 6.3% (1 out of 16).

Table 2: The percentage of ESBL phenotypes by using (DAM) and (DDST).

Bacterial isolates	No. of isolates	ESBL positive
Burkholderia cepacia group	4	1
Ralstonia mannitoilytica	1	1
Pseudomonas luteola	2	2
Sphingomonas paucimobilis	3	1
Acinetobacter baumanii	3	2
Klebsiella pneumonia	4	2
Pseudomonas aeruginosa	5	3
Acinetobacter haemolyticus	4	3
Acinetobacter lowffii	1	1
Total	27	16

The percentage of detection of ESBL enzyme was 59.3% (16 from 27) by using Cefotaxime (CTX) against Augmentin disc (AMC) and this percentage was the higher among the other antibiotics disc, the second antibiotics also show high detection percentage for ESBL enzymes was Ceftriaxone (CRO) with 52.0% (14 out of 27), while the other percentage were 48.1%(13 out of 27) and 44.4%(12 out of 27) for Aztreonam (ATM) and Ceftazidime (CAZ) respectively against Augmentin (AMC) as shown in table (3). The positive result was considered by increasing the inhibition zone between 3rd GC and\or ATM headed for AMC, as shown in figure (1,2 and 3).

Figure 1: Positive double disk approximation method (DAM) for ***K. pneumoniae*** ,showing the typical and specific synergy between AMC and ATM .

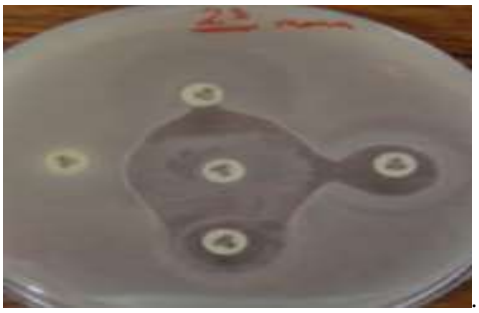

Figure 2: Positive double disk approximation method (DAM) for ***Burkholderia cepacia*** **group** for detection of ESBLs , showing the synergy between AMC against CRO,CTX and ATM (the inhibition zone increased between 3rd GC and ATM headed for AMC .

Figure 3 :Positive double disk synergy test to detect ESBL for ***Pseudomonas luteola*** showing the synergy between AMC and CTX (The inhibition zone increased between 3rd GC headed for AMC).

Table (3): The ESBL phenotypes by using DDST and DAM

Bacterial isolates	No.	Isolate no.	Antibiotic types that used to confirm ESBL				ESBL positive	ESBL negative
			CTX + AMC	CAZ + AMC	CRO + AMC	ATM + AMC		
Burkholderia cepacia group	**4**	**17**	-	-	-	-		**N**
		42	-	-	-	-		**N**
		21	-	-	-	-		**N**
		23	+	+	-	+	P	
Ralstonia mannitoilytica	**1**	**74**	+	+	+	+	P	
Pseudomonas luteola	**2**	**28**	+	+	+	+	P	
		160	+	+	+	+	P	
Sphingomonas aucimobilis	**3**	**76**	-	-	-	-		**N**
		76R	-	-	-	-		**N**
		106	+	-	+	-	P	
Acinetobacter baumanii	**4**	**20**	+	+	+	+	P	
		30	+	+	-	+	P	
		124	-	-	-	-		**N**
Klebsiella pneumonia	**4**	**32**	-	-	-	-		**N**
		37a	-	-	-	-		**N**
		18a	+	+	+	+	P	
		18b	+	+	+	+	P	
Pseudomonas aeruginosa	**5**	**37b**	-	-	-	-		**N**
		25	+	+	+	+	P	
		132	-	-	-	-		**N**
		71	+	+	+	+	P	
		16	+	-	+	+	P	
Acinetobacter haemolyticus	**4**	**69**	-	-	-	-		**N**
		64	+	+	+	+	P	
		6	+	-	+	-	P	
		78	+	-	+	-	P	
Acinetobacter lowffii	**1**	**118**	+	+	+	+	P	
Total		**27**	**59.3% (16)**	**44.4% (12)**	**52.0% (14)**	**48.1% (13)**	**59.3% (16)**	40.7% (11)

P=positive, N=negative (+ indicated by increased inhibition zone around 3rd GC and/or ATM headed for AMC disc).

DISCUSSION

The excessive andd misuse of antibiotics in both the hospital and community has led to an increase in health care costs, morbidity, and mortality,the emergence of bacteria that resistant of antibiotic is a matter of great concern, mainly in hospitals. Development of bacterial resistance towards more recent antibiotics makes the principle focus of the studies. ESBL-Producer bacteria are important not only for inffected person, but also for suitable infection control measures to prevent the prevalence of these bacteria. Bacteria harboring ESBLs have been isolated from various infections, like urinary-tract-infection, septicemia, hospital acquired-pneumoniae, intra-abdominal-abscesses, brain abscesses[14]. The antibiotics sensitivity test by using DDST and DAM to detect the production of ESBL enzyme revealed that the percentage of ESBL production was 59.3 % (16 out of 27) in all Gram-negative bacteria isolates from blood the positive result was determined by the synergy between augmantin disc and aztreonam or any other disc of third generation cephalosporins, scince the ESBL enzymes are resistant to 3rd GC, the use of AMC with 3rd GC in the test to increase and enhancement the inhibition effectiveness. The purpose of using more than one types of cephalosporin in DDST and DAM is to

increase the sensitivity of the test[13]. The detection of ESBL enzymes by antibiotic sensitivity method is useful and costefective, but there is a possibility of technical failure and problems of storage. Studies from different regions show different percentage. In a study by Yasmin *et al* .,2012[15] and a study by Vijayvergia and his Co-workers[16] from Bangladesh 2013, found the percentage of ESBLs that was detected using DDST was 71.3% and 46.0% respectively, from various clinical specimens (urine, stool, blood and body fluids). Two studies by Shivaprakasha *et al*.,2007[17] and Sasirekha *et al*., 2010[18] from India reported as ESBL producers were 51.4% and 53.4% respectively. In a study included deferent clinical samples (Blood, Urine, , Ear swab ,Wound swab, and Miscellaneous body fluids) in Sudan Teaching hospital the rate of ESBL-Producing bacteria that detected by DDST was (47.5%)[19]. In the present study the percentage of ESBL-Producing bacteria was 59.3% that agreement with a study by Delela *et al*., 2012 and Al-Agamy *et al*.,2006 that reported 61.6% in India and 60.9% in Egypt respectively[20,21]. Difference and variation in percentage of ESBL-Producing bacteria that detected by phenotypic methods in studies dealt with different samples such as urine ,wounds, stool ,body fluids and others, the fact that deferent studies have deferent design[22] and maybe due to different level of hygiene in different regions. The incidence of the ESBL-producing bacteria in clinical infection can cause a real and noticeable problem in the treatment, because the presence of these ESBL-producing bacteria eventually leads to failure of the treatment if the group of cephalosporin (e.g. cefotaxime, cefttriaxone and ceftazidime) or aztreonam, were used[21]

CONCLUSION

The present study revealed the high incident of *Acintobacter* spp. followed by *Pseudomonas* spp. among GNB isolated from the blood. The percentage of detection ESBL enzyme was higher by using cefotaxime disc.

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الكشف عن انزيمات ESBL في البكتريا السالبه لصبغة كرام

[1]منى جبار طعمة حرداني و اماني عبد الرضا العبدالله[2] و سعد شاكر الامارة[3] و حسنين محمد علي مكي[4]

[1و3]كلية العلوم / قسم علوم الحياة

[2]كلية العلوم / قسم تحليلات مرضية

[4] مستشفى البصرة العام / البصرة– العراق

*Corresponding Author: Email: Dewpond80@gmail.com , saadshaker2003@yahoo.com

الخلاصة

ان انتشار الانزيمات الواسعة الطيف (ESBLs) يعد مشكلة كبيرة في جميع انحاء العالم. ان سوء استخدام المضادات الحيوية واسعة الطيف ، وخاصة في المرضى الموهنين مناعيا والراقدين في المستشفى، قد ادى الى ظهور سلالات مقاومة للمضادات الحيوية من العائلة المعوية والعديد من البكتريا السالبه لصبغه كرام، ولهذا السبب ، هناك حاجة ماسة للتحري عن هذه الانزيمات بواسطة الاختبارات المستمرة وزرع الدم لهؤلاء المرضى. تم جمع عينات دم (n=147) من مرضى الغسيل الكلوي الذين يتلقون المعالجة في مستشفى البصرة العام التعليمي ومستشفى الصدر في محافظة البصرة جنوب العراق، تم استخدام الطرق التقليدية (الاختبارات الكيميائية الحيوية) وكذلك الطرق الجزيئية تضخيم جين 16s rDNA وتحليل التتابع الجيني، لتشخيص الانواع الشائعة من البكتريا السالبة لصبغة كرام و استخدمت طريقتين، اختبار تأزر القرص المزدوج (DDST) وطريقة تقريب القرص المزدوج (DAM) للكشف عن تواجد انزيمات ESBL في هذه العينات. تم الحصول على سبعة و عشرون من البكتريا السالبة لصبغة كرام من اصل 147 عينة دم، ومن اصل 27 من البكتريا السالبة لصبغة كرام، 16(59.3%) كانت موجبة لإنتاج انزيمات ESBL . كما وجد ان Cefotaxime (CTX) هوالمضاد الحيوي الاكثر حساسية للكشف عن انزيمات ESBL.

الكلمات المفتاحية: ESBLs–enzymes(الانزيمات الواسعة الطيف)، DDST (double disc synergy test) اختبار التآزر القرص المزدوج ، DAM(double disc approximation method) طريقة تقريب القرص المزدوج .