Detection of extended spectrum B-lactamase in E. coli from clinical samples

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(Ann. Coll. Med. Mosul 2011; 37 (1 & 2): 122-128).
Received: 17th May 2011; Accepted: 10th Oct 2011.

ABSTRACT

Objectives: 1- To study the frequency of ESBL (extended spectrum beta lactamase) among E. coli clinical isolates. 2- To determine the antibiotic profile for the isolates. 3- To determine the difference between the antimicrobial susceptibility of the ESBL producing E. coli and non producers.
Methods: A 4-months review of patients from three different hospitals who were diagnosed to have genitourinary tract infections with E. coli. These isolates were identified and assessed for their production of B-lactamase, and their antibiotic susceptibility to 21 different antimicrobial agents was determined.
Results: Out of the total 136 E. coli isolates, 58.82% were found to be ESBL producers. The most effective antimicrobial agent against the isolates was amikacin (85%), followed by ciprofloxacin (67.6%), while all the isolates were fully resistant to penicillin, cephradine, cephalothin and carbencillin. Multi-drug resistance (MDR) were found to be more among the ESBL producers. There was a statistical association between the production of B-lactamase and the resistance to Amikacin, nitrofurantoin, levofloxacin, kanamicin, nalidixic acid, gentamicin, piperacillin, cefotaxime and cephalexin.
Conclusions: This study shows that E. coli recovered from clinical specimens produce B-lactamase in high percentage and are resistant to penicillins and most cephalosporins. In addition, the MDR was higher among the B-lactamase producers. Therefore, determination of B-lactamases production, antimicrobial sensitivity of the isolates and strict antibiotic policy should be adopted in hospitals to take steps for reducing the bacterial resistance.

الخلاصة

الأهداف: دراسة وجود أنزيم البيتا لاكتاميز في العزلات السريرية للشريشيات القولونية وتحديد حساسيتها للمضادات الحيوية. بالإضافة إلى دراسة الفرق بين منتجات أنزيم البيتا لاكتاميز وغير المنتجة للإنزيم من ناحية حساسيتها للمضادات الحيوية.
طرق العمل: كانت مدة جمع العينات ٤ أشهر من مرضى من ثلاث مستشفيات مختلفة. تم عزل الشريشيات القولونية عندهم من خمج الجهاز البولي والتناسلي وفحصت العزلات من ناحية إنتاجها لإنزيم البيتا لاكتاميز ومدى حساسيتها لـ ٢١ نوعا مختلفا من المضادات الحيوية.
النتائج: خلال فترة الدراسة وجد أن ٥٨,٨٢% من العزلات مكونة لإنزيم البيتا لاكتاميز وقد أظهر مضاد الاميكاسين أعلى نسبة حساسية (٨٥%) يليه السبروفلوكساسين (٦٧,٦%). كانت كل العزلات مقاومة للبنسلين والسيفرادين والسيفالوثين والكاربنسلين. كما وجد أن العزلات المقاومة لأكثر من نوع من المضادات الجرثومية كانت الأكثر ضمن منتجات إنزيم البيتا لاكتاميز. كانت هناك علاقة إحصائية ايجابية بين منتجات الإنزيم ومضادات الاميكاسين، نايتروفيورانتوين، ليفوفلوكساسين، كانامايسين، ناليدكسيك اسيد، جنتامايسين، بيبراسيللين، سيفوتاكسيم وسيفاليكسين.

الاستنتاج: أظهرت هذه الدراسة أن نسبه عالية من عزلات الشريشيات القولونية كانت منتجة لإنزيم البيتا لاكتاميز وهي مقاومة للبنسلينات ولنسبة كبيرة من السيفالوسبورينات. كما أن العزلات المقاومة لأنواع متعددة من المضادات الجرثومية كانت أكثر شيوعا ضمن منتجات الأنزيم. لذا فان تحديد حساسية العزلات للمضادات الحيوية وإنتاجها لإنزيم البيتا لاكتاميز وتقليص استخدام المضادات الحيوية في المستشفيات يجب أن يتم العمل به لتقليل إنتاج هذا الإنزيم.

Extended spectrum β-lactamases (ESBLs) producing Enterobacteriaceae are a worldwide growing and important problem in hospital practice, which is tied to the extensive use of broad spectrum antibiotics.[1,2]

The most prevalent mechanisms of bacterial resistance among gram negative bacilli are the production of β-lactamases (chromosomal or plasmid mediated), alteration in the penicillin binding proteins, outer membrane permeability, and combination of multiple mechanisms. [3,4]

Beta-lactam antibiotics are among the safest and most frequently prescribed antimicrobial drugs in the world[5]. The most important mechanism of bacterial resistance to B-lactam antibiotics is the production of β-lactamase enzymes which can hydrolyze virtually all β-lactam antibiotics except cephamycins and carbapenems, and are generally inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam, or tazobactam. [6-8]

To date, a wide variety of β-lactamase enzymes continue to be identified, which may be partly attributed to the wide use of β-lactam antibiotics. ESβLs are one of the most common type of β-lactamase enzymes. [2]

ESBL producing strains have emerged among the Enterobacteriaceae, prominently in Escherichia coli and Klebsiella pneumoniae. They were first isolated in Germany in 1983, and a rapid dissemination has been responsible for numerous outbreaks of infections throughout the world[9]. The prevalence of ESBL-producing Enterobacteriaceae specially E. coli and K. pneumonia, among clinical isolates, varies according to the geographical distribution. Moreover, the increase of ESBL mediated resistance amongst E. coli and Klebsiella isolates renders this problem a major public health threat. [10]

The emergence of ESBL has increased the possibility that traditional empiric antimicrobial regimens may be ineffective, resulting in limitation of therapeutic options and making urinary tract infection (UTI), which remains the most common bacterial infection in human populations, and other infections difficult to treat. [1,11]

In genital tract infections E. coli which normally inhabits the rectum can cause infection if spread to the vagina in which the normal balance of bacteria may be disrupted, resulting in the overgrowth of harmful bacteria at the expense of protective bacteria.

Furthermore, antibacterial agents such as trimethoprim-sulphamethoxazole, aminoglygosides, flouroquinolones, tetracyclins and chloramphenicol are often co-transferred on a resistance plasmid resulting in multidrug resistance[7]. However, carbapenems are the treatment of choice for serious infections due to ESBL producing organisms[8].

The National Committee for Clinical Laboratory Standards (NCCLS) recommends ESBL screening methods and confirmatory tests, because delay in the detection and reporting of ESBL production is associated with prolonged hospital stay, increased morbidity, mortality, and health care costs[5].

The aims of this study are to:

1. Study the frequency of β-lactamase and ESBL among E. coli recovered from urinary tract and genital tract infections.
2. Study the antibiotic susceptibility pattern and multiple drug resistant E. coli in these patients.
3. Evaluate the difference between the antimicrobial susceptibility patterns of β-lactamase producing E. coli and non producers.

Materials and methods

This study was conducted in the Microbiology Laboratory, Department of Microbiology, College of Medicine, University of Mosul.

A total of 136 E. coli isolates (112 UTI and 24 genital tract infections) were collected from patients attending Al-Khansa, Al-Batool and Ibn-sena Teaching Hospitals. The period of sample collection was between September 2010 and December 2010. The identification of the isolates was based on morphological features and standard biochemical tests[12].

All the isolates were tested for their susceptibility to 21 selected antibacterial agents using the standard disc diffusion method[12]. A sterile cotton swab soaked in the bacterial suspension in Muller Hinton broth was used to inoculate the organisms onto the surface of Muller Hinton agar plates, then the antimicrobial discs were applied and the plates were incubated at 37C^{o} for 24 hours. The resultant inhibition zone diameter for each disc was measured and compared with the control measure[13]. The used antibacterial discs were: penicillin 10 U, levofloxacin 5 µg, nalidixic acid 30 µg, nitrofurantoin 100 µg, gentamicin 10 µg, ticarcillin 75 µg, cefixime 5 µg, ampicillin 10 µg, amikacin 30 µg, cephalexin 30 µg, cefoxitin 30 µg, cefotaxime 10 µg, kanamycin 30 µg, cloxacillin 10 µg, ciprofloxacin 5 µg, cephradine 30 µg, enrofloxacin 5 µg, cephalothin 30 µg, carbencillin 25 µg, ceftriaxone 10 µg and piperacillin 30 µg (Bioanalyse.UK). The interpretation of the results was as recommended by NCCLS.

For the detection of β -lactamase production, both the rapid iodometric tube method and ESBL activity were tested. In the latter method (ESBL), the double disc synergy test was performed using ceftriaxone and a combination disc of amoxicillin 20 µg and clavulanic acid 10 µg[11].

Statistical analysis was performed using chi square test where appropriate, and P value < 0.05 was considered significant.

Results

In the current study the β-lactamase enzyme production was detected in the isolated E. coli from urinary tract and genital tract (Figure1). Out of the total 136 tested E. coli, 80 (58.8%) were found to be ESβL producers (Figure 2).

There was no statistical association between the production of β-lactamase enzyme and the source of isolation (UTI and genital tract infection) P>0.05.

The antibiogram profile of the E. coli isolates was determined against a panel of 21 antimicrobial agents. The highest sensitivity percentage was noted in case of amikacin (85.3%) followed by ciprofloxacin, enrofloxacin, nitrofurantoin and levofloxacin (67.6%, 66.2%, 64.7% and 58.8% respectively). In addition, all the isolates were fully resistant to penicillin, cephalothin, cephradine and carbenicillin (Table 1).

The sensitivity to certain antibiotics was statistically decreased (P<0.05) with the production of β-lactamase enzyme particularly in case of amikacin, nitrofurantoin, levofloxacin, kanamicin, nalidixic acid, gentamicin, ticarcillin, piperacillin, cefotaxime and cephalexin (Table 2).

Broad spectrum resistance, which is defined as the resistance to ampicillin or cephalothin was present in the current work for all the isolates, apart from two (98.5%) [5].

Extended spectrum beta lactam resistant E. coli, is defined as resistance of bacteria to ceftriaxone, which was observed in 114 isolates (83.8 %)[5].

The MDR ESBL was considered as resistance to 3 of the following 4 antibiotic groups: trimethoprim- sulphamethxazole, aminoglycosides (amikacin or gentamicin), fluoroquinolones (ciprofloxacin, norfloxacin, or nalidixic acid), and nitrofurantoin. This MDR was detected in 34 isolates (25%) and were all β-lactamase producers, hence, a co-resistance to non β -lactam antibiotics was observed more with ESBL producing E. coli[5].

Table (1): The antimicrobial sensitivity of E. coli isolates from UTI and genital tract infections.

Antimicrobial Agents	UTI No.(%)	GTI No.(%)	Total No.(%)
Amikacin	94(83.9)	22(91.7)	116(85.3)
Ciprofloxacin	72(64.3)	20(83.3)	92(67.6)
Enrofloxacin	68(60.7)	22(91.7)	90(66.2)
Nitrofurantoin	76(67.8)	12(50)	88(64.7)
Levofloxacin	66(58.9)	14(58.3)	80(58.8)
Kanamicin	46(40)	14(58.3)	60(44.1)
Nalidixic acid	40(35.7)	8(33.3)	48(35.3)
Gentamicin	36(32.1)	8(33.3)	44(32.4)
Ticarcillin	22(19.6)	0	22(16.2)
Ceftriaxone	16(14.3)	6(25)	22(16.2)
Cefixime	10(8.9)	6(25)	16(11.8)
Piperacillin	14(12.7)	0	14(10.3)
Cefotaxime	8(7.1)	0	8(5.9)
Cephalexin	8(7.1)	0	8(5.9)
Cefoxitin	4(3.6)	0	4(2.9)
Cloxacillin	2(1.8)	0	2(1.5)
Ampicillin	2(1.8)	0	2(1.5)
Penicillin	0	0	0
Cephradine	0	0	0
Cephalothin	0	0	0
Carbenicillin	0	0	0

Table (2): The antimicrobial susceptibility percentages of ESBL producing and non-producing E. coli.

Antimicrobial Agent	B-lactamase producer		B-lactamase non -producer		Total sensitive	P-value
	Sensitive	Resistant	Sensitive	Resistant		
Amikacin	75	25	100	0	85.3	<0.01
Ciprofloxacin	65	35	71.4	28.6	67.6	>0.5
Enrofloxacin	65	35	67.9	32.1	66.2	>0.5
Nitrofurantoin	42.5	57.5	96.4	3.6	64.7	<0.01
Levofloxacin	47.5	52.5	75	25	58.8	<0.01
Kanamicin	15	85	85.7	14.3	44.1	<0.01
Nalidixic acid	17.5	82.5	60.7	39.3	35.3	<0.01
Gentamicin	5	95	71.4	28.6	32.4	<0.01
Ticarcillin	0	100	39.3	60.7	16.2	<0.01
Ceftriaxone	12.5	87.5	21.4	78.6	16.2	0.5>p>0.1
Cefixime	7.5	92.5	17.9	82.1	11.8	>0.1
Piperacillin	0	100	25	78	10.3	<0.01
Cefotaxime	0	100	14.3	85.7	5.9	<0.01
Cephalexin	0	100	14.3	85.7	5.9	<0.01
Cefoxitin	2.5	97.5	3.6	96.4	2.9	>0.5
Cloxacillin	0	100	3.6	96.4	1.5	0.5>p>0.1
Ampicillin	0	100	3.6	96.4	1.5	0.5>p>0.1
Penicillin	0	100	0	100	0	---
Cephradine	0	100	0	100	0	---
Cephalothin	0	100	0	100	0	---
Carbencillin	0	100	0	100	0	---

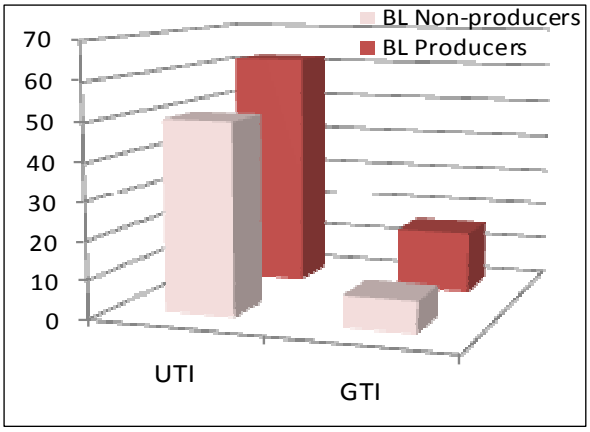

Figure (1): Beta lactamase enzyme distribution in urinary and genital tract isolates.

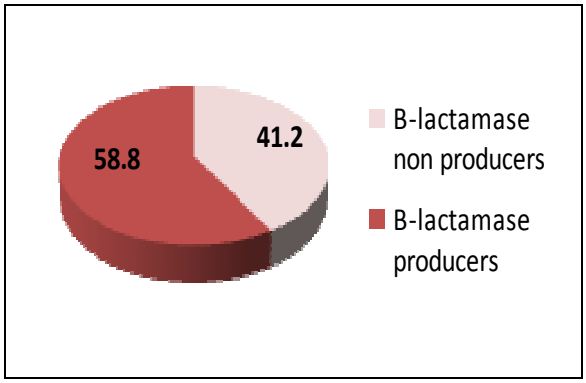

Figure (2): B-lactamase production in Ecoli isolates.

Discussion

ESBLs have been detected worldwide, and they are forming a major contributor of drug resistance in many Enterobacteriaceae[14].

In the present work the ESBL E. coli was detected in 58.8% of our isolates which is in accordance with results of other studies that ranged between 41%-68% [3,10,15-18]. Another study done in a Turkish Hospital has reported a higher percentage (79.3%) [19], while other researchers reported a lower percentages of such isolates ranging between (11.4-38.2%) [1,4,7,11,20].

The prevalence of ESBLs among clinical isolates varies greatly in different geographical areas and are rapidly changing over time[14]. This variation may be attributed to the difference in the use of antibiotics between different localities particularly B-lactam antibiotics.

Broad spectrum resistance, in the current study, was detected in 98.5% of E. coli isolates, which was somewhat relative to the result of study[5] done in Iran where 87.9% of the isolated E. coli were found to have a broad spectrum resistance.

Extended spectrum B-lactam resistant E. coli was recorded in 16.2% of the isolates. Such finding is in contrast with the results of another work[5], where 45.2% of their isolates found to be ESBL resistant. However in Pakistan a lower result (8%) was reported[3].

Multi drug resistance (MDR) is a major problem in the management of uropathogens. It has been noticed that the clinical isolates of E. coli that are ESBL producers are more likely to be resistant to other non β-lactam antimicrobial agents. This MDR may be due to plasmid carrying several genes coding multi-resistance which are transferred from one bacterium to another. The future treatment of MDR ESBL producing E. coli may become more complex because of further limitations of the available drugs.

In the present work, MDR ESBL formed 25% of the isolates, where all are β-lactamase producers. Aminzadeh, et al[5] in Iran also reported 25% to be MDR E. coli[5]. Other studies reported much higher percentages of MDR ESBL-E. coli (69.6% and 90.5%) [4,11]. Actually determination of the resistance pattern can help in great deal to select the best antibiotic in such a situation.

Actually, a statistical significant difference ($p<0.05$) was found in the susceptibility profile between ESBL producers and non ESBL producing E. coli for amikacin, nitrofurantoin, levofloxacin, kanamicin, nalidixic acid, gentamicin, ticarcilin, piperacillin, and cefotaxime. These findings support the hypothesis that extended spectrum ESBL producing strains of E. coli are more likely to have diminished susceptibility to non beta-lactam antibiotics compared to non beta-lactamase producing E. coli[21]. Hence, the antimicrobial susceptibility profile of the individual isolates should be used to guide treatment.

Penicillins are bactericidal agents that inhibit the bacterial cell wall synthesis. In this study

the resistance of the isolated E. coli to penicillin and ampicillin was 100%. Similar results were reported too by other studies[5] particularly among the β-lactamase producers. This low susceptibility to penicillins may be due to the continuous use of these drugs for many years. Moreover, earlier other studies[22] reported that ampicillin has no more effect on urinary tract pathogens.

The cephalosporins particularly second and third generations are generally used for the treatment of E.coli infections. Sensitivity to ceftriaxone in this study was detected only in 16.2% which was lower than that reported in other studies (28.1%, 50% and 24%) [4,5,17]. Furthermore the resistance to cefotaxime and cephalexin was 94.1% in non β-lctamase producers, while in B-lactamase producers it was 100%, which is in agreement with the result of another work by Jirachai, et al[23]. This high resistance to cephalosporins could be explained by the fact that in our locality these drugs are easily available from pharmacy without doctor prescription and are relatively inexpensive antibiotics. Also, inadequate doses of these agents are sometimes used for treatment of different types of infections which may result in the development of high degree of resistance.

Flouroquinolones are particularly useful for the treatment of UTI because a high concentration of the drug in the urine can be achieved. The sensitivity to ciprofloxacin in the present work was observed in 67.6 % of the isolated E. coli which was in agreement with other studies[4,5,17,23]. The sensitivity to levofloxacin among B-lactamase non-producers was 75% which is similar to that reported by Jirachai and his co-workers (73%) [23].

Concerning aminoglycosides, they are generally prescribed against infections caused by gram negative bacilli. Amikacin really showed a high sensitivity percentage (85%), which is in agreement with the findings of other researchers[1,5,11,18,20], while other studies[10,17,24] revealed a lower sensitivity which may be due to the extensive use of this drug in those localities. Also, the sensitivity to gentamicin was 61.7% which is similar to that reported by other studies[1,23,25].

In conclusion, E. coli isolates recovered from clinical specimens in this region produced B-lactamase in high percentage, they are resistant to penicillins and most cephalosporins and the MDR was higher among the B-lactamase producers. Therefore strict antibiotic policy should be adopted in hospitals to estimate the impact of higher resistance in bacteria and to take steps for reducing this resistance. Knowledge of the resistance pattern in a geographical area will help to guide appropriate antibiotic use, and screening for ESBL production as a routine procedure in clinical laboratories which may give a valuable information to the clinician in appropriate selection of antibiotics.

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