PHENOTYPIC DETECTION OF AMPC β-LACTAMASE ENZYME IN GRAM-NEGATIVE BACILLI

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ABSTRACT

Background

Bacterial resistance to antimicrobials are achieved by several mechanisms including production of enzymes such as β-lactamases; according to Ambler classification, β-lactamases are classified into four classes, A to D. AmpC β-lactamase production is related to chromosomal or plasmid genes expression in Gram-negative bacteria.

Objectives

To determine AmpC β-lactamase production among hospital isolates of *Enterobacteriaceae* and non-fermener Gram-negative bacilli in Sulaimani providence and to evaluate different phenotypic methods for detection of AmpC β-lactamase.

Materials and Methods

A total of 108 Gram-negative bacilli bacterial isolates collected from different infections in several governmental hospitals in Sulaimani city were assessed for β-lactamase production. The isolates were identified using biochemical methods. Antimicrobial susceptibility test was performed by Bauer-Kirby disk diffusion method using 14 antimicrobial agents including cefoxitin. Cefoxitin-resistant isolates were tested for AmpC production by four phenotypic tests including disk approximation test, disk antagonism test, hodge test, and AmpC disk test.

Results

Among 108 isolates, 47 (43.5%) were *Escherichia coli* and 35 (32.4%) were *Pseudomonas aeruginosa*. From the isolates, 98.1% were resistant to ampicillin while 71.3% were sensitive to imipenem and 68 isolates (63%) were resistant to cefoxitin. We found that 59 out of 68 to cefoxitin-resistant isolates (86.8%) produce AmpC by hodge test while other tests detected less AmpC production. In regard to hodge test, specificity of the other three tests reached 100% while sensitivity ranged from 55.93 in disk approximation test to 79.66% from both disk antagonism test and AmpC disk test.

Conclusion

AmpC b-lactamase production was common among Gram-negative bacilli from hospital isolates. Cefoxitin screening followed by hodge test for AmpC production was the best test to find b-lactamase producers. Different AmpC b-lactamase production tests can be used according to the tested bacteria.

Keywords: *AmpC β-lactamase detection, Enterobacteriaceae, Gram-negative bacilli, Sulaimani.*

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INTRODUCTION

Bacterial resistance to antimicrobial agents is achieved by several mechanisms including production of enzymes such as β-lactamases that degrade the β-lactam ring of many antibiotics. AmpC β-lactamase enzyme was detected in *Escherichia coli* and believed to be among the first bacterial enzymes reported to destroy penicillin [1]. Currently two classification systems for β-lactamases are available; Ambler classification of β-lactamase classifies the enzymes according to their amino-acid structure into four molecular classes, A through D [2], while the Bush-Jacoby-Medeiros system classifiy the enzymes to several groups according to their substrate profile and susceptibility to β-lactamase inhibitors, such as clavulanic acid [3] .

Class A, C, and D β-lactamases contain penicillin-binding motifs and are penicillin binding proteins (PBPs); they hydrolyze the β-lactam ring through a serine residue at their active site. Class B β-lactamases (metallo-β-lactamases) are structurally unrelated to PBPs, they are zinc-dependent enzymes that use a different series of reactions to open the β-lactam ring [4].

In Gram-negative bacteria, AmpC β-lactamase is linked to chromosome or plasmid genes. Chromosomal AmpC genes are expressed constitutively at a low level. Bacteria such as *Enterobacter* spp., *Citrobacter* spp., and *Serratia* spp., carry an inducible *AmpC* gene in which the gene is strongly induced by β-lactams agents such as cefoxitin and imipenem, with expression mediated by the regulator AmpR [1]. In other organisms, AmpC regulation is achieved by different factors. *Escherichia coli* lacks an *ampR* gene [5], thus AmpC in *E. coli* is non-inducible but is regulated by promoter and attenuator mechanisms [6]. Inducible AmpC overexpression confers resistance to broad-spectrum cephalosporins including cefotaxime, ceftazidime, and ceftriaxone and this make a problem especially in infections due to *E. aerogenes* and *E. cloacae*, where an isolate initially susceptible to these agents may become resistant upon therapy [1].

Plasmid-mediated AmpC β-lactamases represent a new threat. They confer resistance to cephalosporins such as cefoxitin or cefotetan, are not affected by commercially available β-lactamase inhibitors, and can, in strains with loss of outer membrane porins, provide resistance to carbapenems [7]. Transmissible plasmids have acquired genes for AmpC enzymes, which consequently can now appear in bacteria lacking or poorly expressing a chromosomal *blaAmpC* gene, such as *E. coli*, *K. pneumoniae*, and *P. mirabilis*. Resistance due to plasmid-mediated AmpC enzymes is less common than extended-spectrum-beta-lactamase (ESBL) production in most parts of the world but it may be both harder to detect and broader in spectrum [1].

Coproduction of both ESBL and AmpC are becoming more common, therefore it is suggested that laboratories should be able to detect both AmpC β-lactamase and ESBL because they have been associated with false cephalosporin susceptibility and also to recognize isolates for which there is the potential to falsely report as ESBL-negative isolates [8]. AmpC producing strains have been reported worldwide. Plasmid-mediated AmpC-type enzymes were found to be responsible for an appreciable fraction of resistance in clinical isolates of *Klebsiella* spp. and *E. coli*, and are disseminated around the United States (9).

Detection of AmpC production is important to limit their rapid spread. There are no guidelines recommended by Clinical and Laboratory Standards Institute (CLSI) for detection of Amp C b-lactamases but several detection methods were suggested [10]. AmpC β-lactamase are rather cephalosporinases that can confer resistance to a wide range of β-lactam drugs including cephalosporins [1], thus tests using cephalosporin are reported to identify the of AmpC β-lactamase production.

Different tests are suggested to detect AmpC production [11]. AmpC production by phenotypic tests cannot distinguish various families of plasmid-mediated AmpC enzymes and may also overlook chromosomally determined AmpC b-lactamases with an extended spectrum effect [5]. PCR detection of AmpC genes can be used and multiplex PCR has been developed for plasmid-mediated AmpC -lactamase by using six primer pairs [12].

Objectives

The aim of this study is to determine the occurrence of AmpC β-lactamase production among hospital isolates of *Enterobacteriaceae* and non-fermenter Gram-negative bacilli in Sulaimani providence and to evaluate different phenotypic methods for detection of AmpC β-lactamase.

MATERIALS AND METHODS

All Media and reagents were prepared as recommend by the manufacturer's instructions. Patient samples were cultured on MacConkey agar, blood agar media and incubated aerobically at 37 °C for 24 h. Growth was identified by colony morphology and Gram staining features.

We confirmed the collected isolates by API 32 ® system and Vitek® 2 system (bioMérieux, France). We performed antimicrobial susceptibility test according to Bauer-Kirby disk diffusion method [13] on Mueller-Hinton agar using the following antimicrobial disks (MASTDISCS ™ Mast Diagnostics, UK); ampicillin (AM, 10 μg), amoxicillin-clavulanic acid (AMC, 20/10 μg), imipenem (IPM, 10 μg), meropenem (MEM, 10 μg), piperacillin/tazobactam (PTZ, 100/10 μg), cefepime (CPM, 30 μg), ceftazidime (CAZ, 30 μg), cefotaxime (CTX, 30 μg), cefixime (CFM, 5 μg), aztreonam (AMT, 30 μg), ciprofloxacin (CIP, 10 μg), gentamicin (GM, 10 μg) and trimethoprim (TM, 5 μg) and cefoxitin (FOX, 30 μg) [14].

For screening of AmpC production, we used cefoxitin disk (FOX, 30 μg, MASTDISCS ™ Mast Diagnostics, UK). The disk was placed on a Mueller-Hinton agar plate already inoculated with the test organisms. The inhibition zone around the cefoxitin disk was observed after overnight incubation at 37 °C. Isolates that yielded a diameter less than 18 mm were labeled as AmpC β-lactamases positive [15].

In disk approximation test, an antibiotic was used to induce for AmpC production (imipenem or cefoxitin) whereas, others antibiotics were used as substrates (ceftazidime, cefotaxime, piperacillin/tazobactam). A bacterial suspension (0.5 McFarland) was inoculated on a Mueller-Hinton agar and then an imipenem disk was added. The substrate disks were added near the imipenem disk and the plate was incubated at 37°C for 18-24 h. Occurrence of blunting around the substrate disks indicated AmpC production [16].

Disk antagonism test was performed for detection of inducible AmpC β-lactamases. A test isolate (0.5 McFarland standards) was spread over a Mueller-Hinton agar plate. Cefotaxime (CTX, 30 μg) and cefoxitin (FOX, 30 μg) disks were placed 20 mm apart from center to center. Isolates showing blunting of the cefotaxime zone of inhibition adjacent to the cefoxitin disk were screened as positive for AmpC β-lactamase [11].

In Hodge test, MacConkey agar plates were inoculated with a lawn of the indicator strain (*E. coli* ATCC 25922). After the agar surface was dried, a test strain was heavily streaked from the center of the plate to the periphery and a cefoxitin disk (cephamycin) was placed at the center. The plates were incubated overnight at 35°C. The presence of definite growth of the indicator organism in the inhibition zone along with the test strain was interpreted as positive test result [17].

For AmpC disk test, a lawn culture of *E. coli* ATCC 25922 was prepared on Mueller-Hinton agar plate. A sterile disk of 6 mm moistened with 20 μl of sterile saline was kept and several colonies of test organism were inoculated on this disk. A cefoxitin disk was placed next to this disk (almost touching) on the inoculated plate. The plates were incubated overnight at 37°C. A flattening or indentation of the cefoxitin inhibition zone in the vicinity of the disk was considered a positive test for AmpC production [18]. Statistics was done with Microsoft Excel.

RESULTS

We have collected 108 bacterial isolates of Gram-negative bacilli from several governmental hospitals in Sulaimani city during a period from September 2015 to December 2016. The bacteria were isolated from patients with surgical infections, burn and cancer patients. The isolates, both Enterobacteria and non-fermenter Gram-negative bacilli, were from specimen such as urine, blood, wounds and burn wounds, sputum. The predominant isolates among the *Enterobacteriaceae* species was *Escherichia coli* followed by *Pseudomonas aeruginosa*, Table 1.

Table 2 shows the antimicrobial susceptibility of the isolates and overall response to each agent. Resistance response was a predominate feature. Regardless the isolates, resistance was detected mostly to ampicillin in 98.1% of the isolates followed by amoxicillin-clavulanate 90.7%, ceftazidime 88%. The most effective agents were imipenem (71.3%), meropenem (66.7%) and piperacillin-tazobactam (62.6%).

All *Escherichia coli* were shown to be resistant to amoxicillin, 91% resistant to amoxicillin-clavulanate and ceftazidime, while 85.1% of them were susceptible to imipenem and 80.8% to meropenem. Among the *Pseudomonas aeruginosa*, 97% were resistant to amoxicillin and, amoxicillin-clavulanate, 94.2% for trimethoprim, 91.4% to ceftazidime, while 48.5% of

them were susceptible to meropenem and 62.8% gave intermediate response to imipenem, Table 2.

Most of the isolates were multi-drug resistant strains. We found that *E. coli* were resistant to an average of 9.25 of 14 tested antimicrobial agents, *Pseudomonas aeruginosa* to 10.5 agents, *Klebsiella pneumoniae* to 8 agents and *Proteus mirabilis* to 8.1 agents, Table 3.

For detection of AmpC production, we used four detection methods on 68 cefoxitin-resistant isolates. Table 4 shows the results of these tests; Hodge test was the method which mostly detected AmpC production (59 out of 68 isolate, 86.76 %) followed by both disk antagonism and AmpC disk test (47 out of 68, 69.11%) and then disk approximation method. Depending on the results of hodge test, sensitivity and specificity of these tested was calculated. The sensitivity ranged from 55.93% for disk approximation method to 79.66% for both AmpC disk and disk antagonism. Specificity was 100 % for the all three tests in regard to hodge test, Table 5.

We made a comparison between cefoxitin-resistant two most frequent isolates (*Escherichia coli* [*n*=24] and *Pseudomonas aeruginosa* [*n*=28]) regarding the true, false results of disk approximation test, antagonism test, and AmpC disk test and the calculated sensitivity and specificity of these tests in regard to hodge test. While specificity for all the tests in both organisms reached 100%, but sensitivity for all tests was 75.86% in *Escherichia coli* and it ranged from to 37.5% in disk approximation test to 95.83% in AmpC disk test and reaching 100% for disk antagonism test in *Pseudomonas aeruginosa*, Table 6.

Table 1. The bacterial isolates and their sample sources.

Bacterial species	No.	Blood	Burn and wound	Pus	Sputum	Urine
Acinetobacter baumannii	**2**	2				
Aeromonas hydrophila	**1**		1			
Burkholderia cepacia	**1**		1			
Escherichia coli	**47**	4	12	2	1	28
Enterobacter spp.	**4**		2			2
Klebsiella pneumoniae	**9**		4		2	3
Klebsiella oxytoca	**1**		0			1
Morganella morganii	**1**		1			
Proteus mirabilis	**7**		0	1		6
Pseudomonas aeruginosa	**35**	5	16	2	4	8
Total	**108**	**11**	**37**	**5**	**7**	**48**

Table 2. Antimicrobial susceptibility of the isolates using Bauer-Kirby disk diffusion method*.

Bacterial species and number of tested isolates	Response	AM	AMC	CAZ	CPM	FOX	CTX	ATM	CFM	IMI	MEM	CN	TM	PTZ	CIP
Acinetobacter baumannii 2	S					1									
	I														
	R	2	2	2	2	1	2	2	2	2	2	2	2	2	2
Aeromonas hydrophilia 2	S							1		1	1	1		1	1
	I														
	R	1	1	1	1	1	1		1				1		
Burkholderia cepacia 1	S									1					
	I							1						1	
	R	1	1	1	1	1	1		1		1	1	1		1
Escherichia coli 47	S		3	3	10	22	6	11	7	40	38	16	8	20	17
	I		1	1	1	1			1	1	3	2		7	1
	R	47	43	43	36	24	41	36	39	6	6	29	39	20	29
Enterobacter spp. 4	S		1	2	2		2	2	2	3	3	1	3	2	3
	I											1		1	1
	R	4	3	2	2	4	2	2	2	1	4	2	1	1	
Klebsiella pneumoniae 9	S		2	1	3	6	3	3	2	6	6	5	3	4	4
	I			1	1					1	1	1		1	2
	R	9	7	7	5	3	6	6	7	2	2	3	6	4	4
Klebsiella oxytoca 1	S	1	1	1	1		1	1	1	1	1	1	1	1	1
	I														
	R					1									
Morganella morganii 1	S										1			1	
	I														
	R	1	1	1	1	1	1	1	1	1		1	1		1
Proteus mirabilis 7	S		1	1	2	3	2	3	2	3	5	2	3	3	6
	I									2		1		2	
	R	7	6	6	5	4	5	4	5	2	2	4	4	2	1
Pseudomonas aeruginosa 35	S	1	1	1	4	7	3	3	4	1	17	5	2	13	14
	I			2	1			9		22	2	2		7	2
	R	34	34	32	30	28	32	23	31	12	17	28	33	12	19
Total responses and percentage regardless to the bacterial species	S	2 (1.9)	9 (8.3)	9 (8.3)	22 (20.4)	39 (36.1)	17 (15.7)	30 (27.8)	18 (16.7)	77 (71.3)	72 (66.7)	31 (28.7)	20 (18.5)	46 (42.6)	45 (41.7)
	I	0	1 (0.9)	4 (3.7)	3 (2.8)	1 (0.9)	0	4 (3.7)	1 (0.9)	5 (4.6)	5 (4.6)	7 (6.5)	0	19 (17.6)	6 (5.6)
	R	106 (98.1)	98 (90.7)	95 (88)	83 (76.9)	68 (63)	91 (84.3)	74 (68.5)	89 (82.4)	26 (24.1)	31 (28.7)	70 (64.8)	88 (81.5)	43 (39.8)	57 (52.8)

* S: susceptible, I: intermediate response, R: resistance, AM: ampicillin, AMC: amoxicillin-clavulanate, CAZ: ceftazidime, CPM: cefepime, FOX: cefoxitin, CTX: cefotaxime, ATM: Aztreonam, CFM: cefixime, IPM: imipenem, MEM: meropenem CN: gentamicin, TM: trimethoprim, PTZ: piperacillin /tazobactam, CIP: ciprofloxacin.

Table 3. The bacterial isolates and their minimum, maximum and average resistance response to 14 tested antimicrobial agents using Bauer-Kirby disk diffusion method.

Bacterial species	No.	Minimum resistance response	Maximum resistance response	Average resistance response
Acinetobacter baumannii	2	12	14	13
Aeromonas hydrophila	1	8	8	8
Burkholderia cepacia	1	11	11	11
Escherichia coli	47	3	14	9.25
Enterobacter spp.	4	2	12	6.5
Klebsiella pneumoniae	9	1	14	8
Klebsiella oxytoca	1	1	1	1
Morganella morganii	1	12	12	12
Proteus mirabilis	7	1	14	8.1
Pseudomonas aeruginosa	35	3	14	10.5

Table 4. AmpC production detected by four different methods among 68 cefoxitin-resistant isolates.

Bacterial species	No. tested	Disk approximation test, %	Disk antagonism test, %	Hodge test,%	AmpC Disk test,%
Acinetobacter baumannii	**1**	1 (100)	0 (0)	1 (100)	1 (100)
Aeromonas hydrophila	**1**	0 (0)	0 (0)	1 (100)	0 (0)
Burkholederia cepacia	**1**	0 (0)	0 (0)	1 (100)	0 (0)
Escherichia coli	**24**	15 (62.5)	15 (62.5)	22 (91.6)	15 (62.5)
Enterobacter spp.	**4**	3 (75)	3 (75)	4 (100)	4 (100)
Klebsiella pneumoniae	**3**	2 (66.6)	2 (66.6)	3 (100)	2 (66.6)
Klebsiella oxytoca	**1**	1 (100)	1 (100)	1 (100)	0 (0)
Morganella morganii	**1**	0 (0)	0 (0)	1 (100)	1 (100)
Proteus mirabilis	**4**	2 (50)	2 (50)	1 (25)	1 (25)
Pseudomonas aeruginosa	**28**	9 (32.1)	24 (85.7)	24 (85.7)	23 (82.1)
Total	**68**	**33 (48.5)**	**47 (69.1)**	**59 (86.8)**	**47 (69.1)**

Table 5. The true, false results of disk approximation test, antagonism test, and AmpC disk test and the calculated sensitivity and specificity of these tests in regard to hodge test among 68 cefoxitin-resistant isolates.

Test	Hodge test	Disk approximation test	Disk antagonism test	AmpC disk test
Test result	59/68	33/68	47/68	47/68
True Positive	59	33	47	47
False Positive	0	0	0	0
True Negative	9	9	9	9
False Negative	0	26	12	12
Sensitivity%		55.93	79.66	79.66
Specificity%		100	100	100

Table 6. Comparison between cefoxitin resistant *Escherichia coli* (*n*=24) and *Pseudomonas aeruginosa* (*n*=28) regarding the true, false results of disk approximation test, antagonism test, and AmpC disk test and the calculated sensitivity and specificity of these tests in regard to hodge test.

Test	Hodge test		Disk approximation test		Disk antagonism test		AmpC disk test	
	E. coli	***Pseud. aeru.***	***E. coli***	***Pseud. aeru.***	***E. coli***	***Pseud. aeru.***	***E. coli***	***Pseud. aeru.***
Positive test	22/24	24/28	15	9	15	24	15	23
True Positive	22	24	15	9	15	24	15	23
False Positive	0	0	0	0	0	0	0	0
True Negative	2	4	2	4	2	4	2	4
False Negative	2	0	7	15	7	0	7	1
Sensitivity%			**75.86**	**37.5**	**75.86**	**100**	**75.86**	**95.83**
Specificity%			**100**	**100**	**100**	**100**	**100**	**100**

DISCUSSION

In this study, we detected and compared AmpC production among 108 Gram-negative hospital isolates; *Escherichia coli* was the most predominate isolates followed by *Pseudomonas aeruginosa*. Urine was the main specimen source (44%) followed by burn and wound pus (34%). Among the 47 *E. coli* isolates, 26 (59.97) were from urinary tract infection 12 (26%) were from burn and surgical infection pus while among 35 *Pseudomonas aeruginosa* isolates 16 (45%) was from burn and surgical infection and 8 (22%) were from urinary tract infection. These Gram-negative infections are reported to be the common nosocomial infections although there are differences in infection types and frequency of causative agents [19, 20].

Testing the antimicrobial susceptibility of these isolates, we found that most of the isolates were multi-drug resistant strains and they were averagely resistance to eight antimicrobial agents out to the tested fourteen antimicrobials. These high resistance

figures were reported in our hospitals and community and it is alarming and must be taken in consideration [21, 22]. Production of AmpC β-lactamase is frequently accompanied by multi-drug resistance, conjugative dissemination of these AmpC β-lactamase encoding plasmids is thought to facilitate the spread of resistance against a wide range of antibiotics among different members of *Enterobacteriaceae* [23].

The tested strains were resistant to ampicillin (98.1%), amoxicillin-clavulanate (90.7%), and 68 out of the tested 108 isolates (63%) were resistant to cefoxitin. Resistance to cefoxitin in our isolates was higher than previously reported elsewhere. It was reported that cefoxitin-resistance increased from 7.4% in 2000 to 15% in 2006 among *Escherichia coli* from humans and food animals in United States [24]. Cefoxitin resistance in fecal *E. coli* isolates in healthy persons and patients with diarrhea in Korea was less than 10% [25], and resistance reaching in 19.57% (from 81 isolates) of *E. coli* from tertiary care hospital of Islamabad, Pakistan [26]. Insusceptibility of *Pseudomonas aeruginosa* to cefoxitin however is more prevalent and may reach more than 90% as we also showed [27].

We also compared the number of antimicrobials which an isolate is resistant among cefoxitin-resistant versus cefoxitin-susceptible isolates of both *E. coli* and *Pseudomonas aeruginosa* and found that cefoxitin-resistance strains were resistance to more antimicrobial than cefoxitin-susceptible stains in which was statistically significant ($p < 0.1$) in both *E. coli* and *Pseudomonas aeruginosa*. This shows that when bacteria become resistant to more agents they become more selected and acquired more resistant.

We subjected cefoxitin-resistant strains for AmpC production detection tests, as cefoxitin resistance is a reliable method for screening AmpC production [28, 29]. AmpC production ranged 48.5% by disk approximation test to 86.8% for hodge test indicating variability in AmpC expression related to the AmpC detection. Among cefoxitin-resistant *Escherichia coli* isolates, AmpC production ranged from 62.5% to 91.6% respectively by different tests. The same results are applied to cefoxitin-resistant *Pseudomonas aeruginosa* isolates which showed AmpC production in range of 32.1% to 85.7%. From our results individual bacteria may shows different abilities for AmpC production and this is affected by the detection method or induction of resistance property.

Comparing AmpC production among the isolates of this study to other studies, we have higher AmpC β-lactamase production. In the Netherlands, the pAmpC in the community was found to be low. However, since pAmpC-producing isolates were not identified as ESBL producers by routine algorithms, there may be risk that further increase of their prevalence might go undetected [30]. In other countries, such as in India, Egypt and Iran, it was found that the prevalence of AmpC β-lactamases is increasing [23, 31, 32]. In a study in Nigeria, the prevalence of AmpC β-lactamase among Gram-negative bacteria recovered from clinical specimens was found to be 15.23 % and it was found that *Pseudomonas aeruginosa* was the most prevalent producer of AmpC enzymes [33], while in India 32.7% of *Pseudomonas aeruginosa* isolated from burn injury were AmpC producer which cause big treatment failure and therefore demand early detection of these AmpC producers [11]. A study from Erbil city reported AmpC producers among Gram-negative bacteria; from the screened 34 strains, 11 (32.4%) were shown to be AmpC producers and this was detected among *Sphingomonas paucimobilis, Enterobacter hormaechei* and *Pseudomonas aeruginosa* [34].

In comparing the performances of these four tests, we found that hodge test is one that reveals most of AmpC production in the cefoxitin-resistant isolates; this was followed by disk antagonism test. Others reported that methods, like Bauer- Kirby disk potentiation with some β-lactamase inhibitors, or the cefoxitin-hodge test, AmpC disk test combined disk diffusion test and AmpC E-test methods are giving good results, but some of these methods are labor-intensive, technically complex, expensive and may be unsuitable for routine screening in clinical microbiology laboratory [35]. Based on hodge test, specificity of the tests reached 100% so excluding any false positive tests, so with any test we can detect true AmpC producer while sensitivity of the tests were different with some tests there is chance to miss true producers and the AmpC production can be induced with a method and not another. This was shown when we compared *Escherichia coli* to *Pseudomonas aeruginosa*, so different tests can be selected according to the tested bacteria.

We concluded that AmpC β-lactamase production was common among Gram-negative bacilli from hospital isolates. Cefoxitin screening followed by hodge test for AmpC production was the best test to find β-lactamase producers. Different AmpC β-lactamase production tests can be used according to the tested bacteria.

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REFERENCES

1. Jacoby GA. AmpC beta-lactamases. Clinical microbiology reviews. 2009;22(1):161-82

2. Ambler RP. The structure of beta-lactamases. Philosophical transactions of the Royal Society of London Series B, Biological sciences. 1980;289(1036):321-31

3. Bush K, Jacoby GA. Updated functional classification of beta-lactamases. Antimicrobial agents and chemotherapy. 2010;54(3):969-76

4. Yohei Doi, Henry F. Chambers. Penicillins and β-Lactamase Inhibitors. In: John E. Bennett, Raphael Dolin, Martin J. Blaser, editors. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. Eighth edition. 8th Edition ed: Saunders, Elsevier; 2105. p. 362-278.

5. Honoré N, Nicolas MH, Cole ST. Inducible cephalosporinase production in clinical isolates of *Enterobacter cloacae* is controlled by a regulatory gene that has been deleted from *Escherichia coli*. The EMBO Journal. 1986;5(13):3709-14

6. Jaurin B, Grundstrom T, Edlund T, Normark S. The *E. coli* beta-lactamase attenuator mediates growth rate-dependent regulation. Nature. 1981;290(5803):221-5

7. Philippon A, Arlet G, Jacoby GA. Plasmid-Determined AmpC-Type β-Lactamases. Antimicrobial agents and chemotherapy. 2002;46(1):1-11

8. Thomson KS. Extended-spectrum-beta-lactamase, AmpC, and Carbapenemase issues. J Clin Microbiol. 2010;48(4):1019-25

9. Alvarez M, Tran JH, Chow N, Jacoby GA. Epidemiology of Conjugative Plasmid-Mediated AmpC β-Lactamases in the United States. Antimicrobial agents and chemotherapy. 2004;48(2):533-7

10. Hassan A, Usman J, Kaleem F, Gill MM, Khalid A, Iqbal M, et al. Evaluation of different phenotypic methods for detection of amp C Beta-lactamase producing bacteria in clinical isolates. Journal of the College of Physicians and Surgeons--Pakistan : JCPSP. 2013;23(9):629-32

11.Kumar V, Sen MR, Nigam C, Gahlot R, Kumari S. Burden of different beta-lactamase classes among clinical isolates of AmpC-producing *Pseudomonas aeruginosa* in burn patients: A prospective study. Indian Journal of Critical Care Medicine: Peer-reviewed, Official Publication of Indian Society of Critical Care Medicine. 2012;16(3):136-40

12. Perez-Perez FJ, Hanson ND. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol. 2002;40(6):2153-62

13. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. American journal of clinical pathology. 1966;45(4):493-6

14. CLSI. Performance Standards for Antimicrobial Disk Susceptibility Tests: Twenty Third Informational Supplement. Wayne, PA2013.

15. Hemalatha V, Padma M, Sekar U, Vinodh TM, Arunkumar AS. Detection of Amp C beta lactamases production in *Escherichia coli* & *Klebsiella* by an inhibitor based method. The Indian journal of medical research. 2007;126(3):220-3

16. Dunne WM, Jr., Hardin DJ. Use of several inducer and substrate antibiotic combinations in a disk approximation assay format to screen for AmpC induction in patient isolates of *Pseudomonas aeruginosa, Enterobacter spp., Citrobacter spp., and Serratia spp.* J Clin Microbiol. 2005;43(12):5945-9

17. Lee W, Jung B, Hong SG, Song W, Jeong SH, Lee K, et al. Comparison of 3 Phenotypic-detection Methods for Identifying Plasmid-mediated AmpC β-lactamase-producing *Escherichia coli, Klebsiella pneumoniae,* and *Proteus mirabilis* Strains. The Korean journal of laboratory medicine. 2009;29(5):448-54

18. Tanushree Barua, Malini Shariff, S.S. Thukral. Detection and Characterization of AmpC B-Lactamases in Indian Clinical Isolates of *Escherichia coli, Klebsiella pneumoniae and Klebsiella oxytoca*. Universal Journal of Microbiology Research. 2013;1 (2):15 - 21

19. Peleg AY, Hooper DC. Hospital-Acquired Infections Due to Gram-Negative Bacteria. The New England journal of medicine. 2010;362(19):1804-13

20. Farzianpour F, Bakhtiari A, Mosavee SH, Akbarzadeh A. The Most Important Species of Nosocomial Infection and the Most Important Wards Susceptible to Nosocomial Infection. Iranian Journal of Public Health. 2014;43(7):1016-7

21. Adnan M Hamawandi, Sherko A Omer, Tara Hussain Tayib, Mahammd Karim Mustafa. Antibacterial susceptibility in urinary tract infection among children in Sulaimani. Journal of Sulaimani Medical College. 2015;5(1):1-6

22. Karwan Raoof Hassan, Ismaeel Hama Ameen, Ramzi ZS. Types of organisms causing urinary tract infection and their antibiotic sensitivity pattern in Sulaimani Teaching Hospital. Journal of Sulaimani Medical College 2014;4(1):11-17

23. Mohamudha Parveen R, Harish BN, Parija SC. Ampc Beta lactamases among gram negative clinical isolates from a tertiary hospital, South India. Braz J Microbiol. 2010;41(3):596-602

24. Daniel AT, Shaohua Z, Emily T, Sherry A, Aparna S, Mary JB, et al. Antimicrobial Drug Resistance in *Escherichia coli* from Humans and Food Animals, United States, 1950–2002. Emerging Infectious Disease journal. 2012;18(5):741

25. Cho S-H, Lim Y-S, Park M-S, Kim S-H, Kang Y-H. Prevalence of Antibiotic Resistance in *Escherichia coli* Fecal Isolates From Healthy Persons and Patients With Diarrhea. Osong Public Health and Research Perspectives. 2011;2(1):41-5

26. Shafiq M, Rahman H, Qasim M, Ayub N, Hussain S, Khan J, et al. Prevalence of plasmid-mediated AmpC β-lactamases in *Escherichia coli* and *Klebsiella pneumoniae* at tertiary care hospital of Islamabad, Pakistan. European Journal of Microbiology & Immunology. 2013;3(4):267-71

27. Loureiro M, Moraes Bd, Mendonça V, Quadra M, Pinheiro G, Asensi M. *Pseudomonas aeruginosa*: study of Antibiotic Resistance and Molecular Typing in Hospital Infection Cases in a Neonatal Intensive Care Unit from Rio de Janeiro City, Brazil. Memórias do Instituto Oswaldo Cruz. 2002;97:387-94

28. Polsfuss S, Bloemberg GV, Giger J, Meyer V, Bottger EC, Hombach M. Practical approach for reliable detection of AmpC beta-lactamase-producing *Enterobacteriaceae*. J Clin Microbiol. 2011;49(8):2798-803

29.Black JA, Moland ES, Thomson KS. AmpC disk test for detection of plasmid-mediated AmpC beta-lactamases in *Enterobacteriaceae* lacking chromosomal AmpC beta-lactamases. J Clin Microbiol. 2005;43(7):3110-3

30. Reuland EA, Halaby T, Hays JP, de Jongh DMC, Snetselaar HDR, van Keulen M, et al. Plasmid-Mediated AmpC: Prevalence in Community-Acquired Isolates in Amsterdam, the Netherlands, and Risk Factors for Carriage. PLoS ONE. 2015;10(1):e0113033

31. El-Hady SA, Adel LA. Occurrence and detection of AmpC β-lactamases among *Enterobacteriaceae* isolates from patients at Ain Shams University Hospital. Egyptian Journal of Medical Human Genetics. 2015;16(3):239-44

32. Maleki A, Khosravi A, Ghafourian S, Pakzad I, Hosseini S, Ramazanzadeh R, et al. High Prevalence of AmpC β-Lactamases in Clinical Isolates of *Escherichia coli* in Ilam, Iran. Osong Public Health and Research Perspectives. 2015;6(3):201-4

33. Helen O. Ogefere, James G. Osikobia, Omoregie R. Prevalence of AmpC β-lactamase among Gram-negative bacteria recovered from clinical specimens in Benin City, Nigeria. Tropical Journal of Pharmaceutical Research. 2016;15 (9):1947-53

34. Sevan H. Bakir, Fattma A. Ali. Evalution of Multi-drug Resistance and ESBL, AmpC, Metallo β-Lactamase Production in Gram Negative Bacteria Causing Pharyngotonsillitis. International Journal of Research in Pharmacy and Biosciences 2015;2(7):8-17

35. Saad N, Munir T, Ansari M, Gilani M, Latif M, Haroon A. Evaluation of phenotypic tests for detection of Amp C beta-lactamases in clinical isolates from a tertiary care hospital of Rawalpindi, Pakistan. JPMA The Journal of the Pakistan Medical Association. 2016;66(6):658-61