

An Algorithm for Detection of Extended Spectrum, AMP C and Metallo Beta Lactamases in Clinical Isolates: A Proactive Approach

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With the increasing incidence in extended spectrum, AmpC beta lactamases and metallo beta lactamases in gram negative bacilli, diagnostic microbiology laboratories may soon be called upon to actively detect these drug resistance markers to allow both correct and timely treatment. We developed an efficient, simple and cost effective algorithm for phenotypic identification of these drug resistance markers within an acceptable turn around time. Samples received in the Clinical Bacteriology Laboratory were processed for culture and sensitivity. Gram negative bacilli were screened and confirmed for the presence of ESBL by double disk synergy test by using cefoperazone-sulbactam, piperacillin-tazobactam and ceftazidime-clavulanic acid during routine antibiotic susceptibility testing. Imipenem and ceftazidime were utilized for induction of suspected AmpC producers, when needed, by disk approximation assay on the second day. MBL production was confirmed by modified Hodge Test and Imipenem-EDTA double disk synergy test on the third day in strains resistant to imipenem. Among 251 isolates studied, 138 (54.98%) were ESBL producers, 101 (40.23%) were Amp C producers and 52 (20.71%) were MBL producers. Highest rates of detection of ESBL was by cefoperazone-sulbactam (109/138) 78.98% followed by piperacillin-tazobactam (58/138) 42.02% and ceftazidime-clavulanic acid (20/138). The highest ESBL producers were *Acinetobacter* spp. (100%), followed by *Klebsiella* spp (86.7%) and *Proteus* species (85.7%). AmpC production was detected in 72 (71.3%) members of the family Enterobacteriaceae, of which the most common were *Citrobacter* spp. (68%), followed by *Proteus* spp. (54.1%). Of 101 of the Amp C producing strains 20 (19.80%) were inducible and 81 were stably derepressed (80.19%). Imipenem was a better inducer of AmpC than ceftazidime. 52 (20.71%) isolates were identified as MBL producers, the highest number being of *Pseudomonas aeruginosa* followed by *E. coli* and *Klebsiella* spp. Among the methods employed for detection of MBL production, Hodge test (80%) proved better than DDST (55%). Of all the isolates studied, 40 isolates exhibited multiple mechanisms of resistance. High level of antibiotic resistance as observed in this study merits a proactive approach in detection of ESBL, AmpC and MBL in Gram negative bacteria to enable timely management of patient and also reduce period of hospital stay. We have developed an effective and simple approach to identify these resistance markers.

Key words: Extended spectrum beta lactamases, AmpC beta lactamases and metallo beta lactamases.

Extended spectrum β lactamases (ESBLs) were first reported in 1983¹ and plasmid mediated AmpC β lactamases were reported in 1988².

Typically ESBLs are mutant, plasmid-mediated β lactamases derived from older broad spectrum β lactamases which have an extended profile that permits hydrolysis of all

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cephalosporins, penicillins and aztreonam. These enzymes are most commonly produced by *Klebsiella pneumoniae* and *Escherichia coli* but may also occur on other gram negative bacteria. Plasmids responsible for ESBL production carry resistance to many antibiotics like aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol and cotrimoxazole.

AmpC class β lactamases are cephalosporinases that are poorly inhibited by clavulanic acid. They are differentiated from other ESBLs by their ability to hydrolyse cephamycins as well as other extended spectrum cephalosporins. Plasmid mediated AmpC β lactamases have arisen through the transfer of chromosomal genes for the inducible AmpC β lactamases onto plasmids [3]. Till date all plasmid-mediated AmpC beta lactamases have similar substrate profiles to the parental enzymes from which they appear to be derived. Plasmid-mediated AmpCs differ from chromosomal AmpCs in being constitutive. Both ESBLs and plasmid-mediated AmpC β lactamases are typically associated with broad multidrug resistance, usually a consequence of genes for other antibiotic resistance mechanisms residing on the same plasmids as the ESBL and AmpC genes.

Carbapenems are often used as antibiotics of last resort for treating infections due to multidrug resistant gram negative bacilli because they are stable even in response to extended spectrum and AmpC β lactamases. However gram negative bacilli producing the acquired metallo- β lactamases (MBLs) are on the rise⁴⁻⁸. Given that MBLs will hydrolyse virtually all classes of β lactams and that we are several years away from the development of a safe therapeutic inhibitor; their continued spread would be a clinical disaster.

CLSI recommendations exist for *E.coli*, *Klebsiella* spp. and *Proteus mirabilis* alone. No guidelines exist for ESBL detection in other microorganisms or for detection of AmpC β lactamases⁹.

The aims of our study were to find out the prevalence of ESBL, AmpC and MBL in gram negative bacteria isolated from clinical specimens; to evaluate various phenotypic methods for identification of ESBL, AmpC and MBL production (we concentrated on phenotypic methods as molecular methods are not cost effective, especially

in developing countries), and to essentially provide a holistic antibiotic susceptibility report which aids in timely and appropriate patient management.

MATERIAL AND METHODS

The study was carried out in the Department of Microbiology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh over a 9-month period from August 2007 to April 2008.

Patient Evaluation

Samples received in the Clinical Bacteriology Laboratory were processed for microbiological confirmation of clinically suspected infection in patients based on clinical signs and symptoms. Patients in whom gram negative bacteria were isolated during routine diagnostic testing were included in the study. Relevant clinical information was also collected for each patient. These isolates were obtained from surgical site infections (84), orthopaedic infections (65), urinary tract infections (28), cerebrospinal fluid (11), sputum (23), and ear and eye samples²⁵. Samples were collected from patients as per standard procedures. *Antimicrobial Susceptibility Testing*

Antibiotic susceptibility testing was performed by disc diffusion method by the Kirby Bauer technique according to CLSI guidelines on Mueller Hinton agar⁹. The isolates were tested against amikacin (30 g), gentamicin (10 g), gatifloxacin (5 g), ofloxacin (5 g), ceftriaxone (30 g), cefoperazone (75 g), cefoperazone-sulbactam (75 g, 1:1) cefixime (5 g), netilmicin (30 g). All discs were obtained from Himedia, India.

Identification of β lactamases

ESBL

On the first day, the initial screening was done according to CLSI (2005) [10] guidelines. Phenotypic detection of ESBL production was attempted by β lactam and β lactamase inhibitor combination. The combinations used were Cefoperazone-sulbactam, Piperacillin-tazobactam and Ceftazidime-clavulanic acid.

Three criteria which had to be fulfilled for phenotypic identification of an ESBL producing isolate were:

1. Zone diameters of various third generation cephalosporins should be: Ceftriaxone (30 μ g) \leq 25mm; Cefotaxime (30 μ g) \leq 27mm;

Ceftazidime (30µg) ≤22mm.

2. Susceptibility to Cefoxitin (30µg)
3. Increase in zone diameter with addition of inhibitor by ≤5mm with special reference to Cefoperazone (75µg) and Cefoperazone-sulbactam (75/30µg) Piperacillin-tazobactam and Ceftazidime-clavulanic acid combination.

Ceftriaxone and cefoperazone were used as screening agents while cefoperazone sulbactam was used for confirmation of ESBL. This combination is not routinely used to confirm the production of an ESBL but we standardized this combination against amoxicillin-clavulanic acid. The latter combination was more sensitive than the former in identifying ESBL producers (unpublished data). The cut-off zone of cefoperazone for screening of possible ESBL was kept at 22 mm and that of ceftriaxone was as recommended by CLSI (< 25 mm). Confirmation was done on the same day by noting the potentiation of the activity of cefoperazone (CP) in the presence of cefoperazone-sulbactam (CPS)⁹. An increase in diameter of 5 mm was considered positive for ESBL detection. As there is a mounting problem of AmpC beta-lactamases in our region, clavulanic acid is being reported as a suboptimal inhibitor of ESBLs. Sulbactam is unlikely to cause this problem and could be a better alternative to clavulanic acid for detection of ESBLs⁶. This was our rationale for using cefoperazone-sulbactam.

Moreover cefoperazone-sulbactam is cheaper, and also routinely used in our setup thus, making this exercise cost effective as well.

It is noteworthy that we attempted screening and confirmation on the same day, thereby saving a day. Also, an isolate resistant to Cefoxitin or showing no increase in zone diameter with addition of inhibitor was suspected to be an AmpC producer; tests for which were put up on the second day.

K. pneumoniae ATCC 700603 (positive control) and *E. coli* ATCC 25922 (negative control) were used for quality control of ESBL test.

AmpC

The three criteria followed for inducible Amp C on Day 2 of which at least two had to be fulfilled were:

1. Blunting of zone towards inducer namely Imipenem (10µg) by 2mm.
2. No increase in zone size with addition of

inhibitor specifically sulbactam, clavulanic acid and tazobactam.

3. Susceptibility to Cefepime (30µg).

Induction of AmpC synthesis was by the disc approximation assay according to the CLSI guidelines [9]. Inducer/substrate discs were placed on the surface of Mueller Hinton agar plates (HiMedia) at a distance of 25 mm from center to center using the template. Strains were considered stably derepressed if resistance was observed to all substrate drugs. Quality control of the performance of the assay was accomplished with positive control (*Pseudomonas aeruginosa* ATCC 27853) and negative control (*Escherichia coli* ATCC 25922). Strains were considered inducible if a positive test was obtained with any of the inducer/substrate combinations. A test was considered positive if the zone of inhibition was reduced by 2 mm on the induced side of the substrate disc. Imipenem (10 g) and cefoxitin (30 g) were used as inducers of AmpC. Discs of 100/10 g piperacillin-tazobactam (PIP-TZP), 30 g cefotaxime (CTX), 100 g piperacillin, (PIP) 30 g ceftazidime (CAZ), 30/10 g ceftazidime-clavulanic acid (CAZ-CLAV), 75 g (1:1) cefoperazone-sulbactam (CPS), (30 g) cefepime, and 30 g cefoxitin (FOX) were substrates to Imipenem (IMI).

Criteria followed for derepressed AmpC mutant were

1. Resistance to all cephalosporins
2. No increase in zone size with addition of an inhibitor.

Strains were considered stably derepressed if resistance was observed to all substrate drugs⁹.

MBL

MBL was suspected when an isolate exhibited:

1. Resistance to Imipenem (10µg) (zone size <16mm)
2. Heaping and zone size >16mm and <20mm.

The suspected isolate was then subjected to the modified Hodge Test¹¹ and Imipenem-EDTA double disk synergy test¹¹.

Modified Hodge test

Escherichia coli ATCC 25922 at a turbidity of 0.5 McFarland standard was used to swab the surface of a Mueller Hinton Agar plate, and the test strain was heavily streaked from the centre to plate periphery. After the plate was

allowed to stand for 10 minutes at room temperature, a 10µg imipenem disk was placed in the centre and the plate was incubated overnight. The presence of a distorted inhibition zone was interpreted as a positive result for carbapenem hydrolysis screening. . Quality control was run with

▮ MHT Positive *Klebsiella pneumoniae* ATCC BAA-1705

▮ MHT Negative *Klebsiella pneumoniae* ATCC BAA-1706

Imipenem-EDTA Double Disk Synergy Test

The suspected isolate was swabbed onto a plate of Mueller Hinton agar (turbidity 0.5 McFarland standard). A 10µg imipenem disk and a disk onto which 10µl 0.5 M EDTA solution was added were placed at a distance of 10mm edge to edge. After overnight incubation the presence of even a small zone of synergistic inhibition was interpreted as positive.

Multiple Mechanisms

To identify isolates with multiple mechanisms of resistance, the criteria followed were:

1. Resistance to Cefoxitin (30µg) (AmpC)
2. Blunting of zone towards inducer (Inducible AmpC)
3. Increase in zone size with addition of an inhibitor by >5mm (ESBL)

4. Decrease in zone diameter around imipenem, confirmed by the modified Hodge test or DDST (MBL).

RESULTS

This study aimed to assess the prevalence rates of β lactamases among various gram negative bacteria isolated in our clinical laboratory, and to evaluate the sensitivity of various phenotypic detection methods, which could subsequently be incorporated into mainstream antibiotic susceptibility testing.

Among the 251 isolates studied, 173 (68.9%) were members of the family Enterobacteriaceae, of which 109 (63%) isolates were *Escherichia coli*, 15 (8.7%) were *Klebsiella* spp., 25 (14.5%) were *Citrobacter* spp., 17 (9.8%) were *Serratia* spp. and 7 (4.1%) were identified as *Proteus* spp. Seventy four (29.5%) were *Pseudomonas aeruginosa* and 3 (1.2%) were *Acinetobacter* spp.

Out of all the isolates, ESBLs were produced by 91/173 (52.6%) members of Enterobacteriaceae, 44/74 (59.5%) *Pseudomonas aeruginosa*, 3/3 *Acinetobacter* spp. and 0/1 (none) of *Burkholderia* spp.

Among the family Enterobacteriaceae, ESBLs were produced by 49 out of 109 (44.95%)

Table 1. Distribution of ESBLs, AmpC and Metallo- β - Lactamases in various Gram negative bacilli

Total	ESBL						AmpC	
	Total	Cfs	Pt	Cac	Inducible	Derepressed	Total	
<i>E.coli</i>	109 (43.42%)	49 (44.95%)	38 (77.55%)	24 (48.97%)	10 (20.4%)	8 (19.04%)	34 (80.95%)	42 (38.53%)
<i>Pseudomonas</i>	74 (29.42%)	44 (59.45%)	39 (88.63%)	15 (34.09%)	7 (15.9%)	10 (34.48%)	19 (73.07%)	29 (39.18%)
<i>spp</i>	15 (5.97%)	13 (86.66%)	10 (76.92%)	6 (46.15%)			7 (100%)	7 (46.66%)
<i>Klebsiella</i>	15 (5.97%)	13 (86.66%)	10 (76.92%)	6 (46.15%)			7 (100%)	7 (46.66%)
<i>pneumonia</i>	15 (5.97%)	13 (86.66%)	10 (76.92%)	6 (46.15%)			7 (100%)	7 (46.66%)
<i>Proteus</i> spp	7 (2.78%)	6 (85.71%)	5 (83.33%)	3 (50%)		1 (25%)	3 (75%)	4 (54.14%)
<i>Serratia</i> spp	17 (6.77%)	7 (41.17%)	4 (54.14%)	3 (42.85%)		1 (50%)	1 (50%)	2 (11.76%)
<i>Acinetobacter</i>	3 (1.19%)	3 (100%)	3 (100%)	1 (33.33%)				
<i>spp</i>	25 (9.96%)	16 (64%)	10 (62.5%)	6 (37.5%)	3 (18.75%)		17 (100%)	17 (68%)
<i>Citrobacter</i>	25 (9.96%)	16 (64%)	10 (62.5%)	6 (37.5%)	3 (18.75%)		17 (100%)	17 (68%)
<i>spp</i>	1 (0.39%)							
<i>Burkholderia</i> spp	1 (0.39%)							

Escherichia coli, 13 out of 15 (86.7%) *Klebsiella* spp., 6 out of 7 (85.7%) *Proteus* spp., 7 out of 17 (41.2%) *Serratia* spp., and 16 out of 25 (64%) *Citrobacter* spp.

On analysing each species individually, the organisms that exhibited extensive ESBL production were *Acinetobacter* spp. (100%), *Klebsiella* spp. (86.7%), *Proteus* spp. (85.7%) and *Pseudomonas aeruginosa* (59.5%). These figures reinforce the fact that ESBL production should be looked for routinely in gram negative bacteria, and not just in *E. coli* and *Klebsiella*.

Highest rates of ESBL detection were observed to be by cefoperazone-sulbactam (109/138) 78.98%, followed by piperacillin-tazobactam (58/138) 42.02% and ceftazidime-clavulanic acid (20/138) 14.5%. Even in individual bacteria, cefoperazone-sulbactam combination proved to be the most sensitive for detection of ESBL production. Cefoperazone-sulbactam detected 77.55% ESBL-producing isolates in *E. coli*, 88.63% in *Pseudomonas aeruginosa*, 76.92% in *Klebsiella* spp., 83.33% in *Proteus* spp., 57.14% in *Serratia* spp., 100% in *Acinetobacter* spp., and 62.5% in *Citrobacter* spp.

Table 2. Etiology of metallo-beta lactamases

MBL Producers	Number	Percentage
<i>E. coli</i>	9	20
<i>Pseudomonas</i> spp	19	42
<i>Klebsiella</i> spp	5	11.1
<i>Proteus</i> spp	2	4.5
<i>Serratia</i> spp	9	20
<i>Acinetobacter</i> spp	1	2
<i>Citrobacter</i> spp	5	11.1
Total	45/251	17.9

AmpC β lactamase was suspected in 101 isolates, of which 72 (71.3%) were Enterobacteriaceae [42 (41.6%) *E. coli*, 7 (6.9%) *Klebsiella* spp., 17 (16.8%) *Citrobacter* spp., 4 (3.97%) *Proteus* spp. and 2 (1.98%) *Serratia* spp.]. Twenty nine (28.7%) isolates of *Pseudomonas aeruginosa* were AmpC producers, whereas none of the *Acinetobacter* spp. isolated produced AmpC. Of all the AmpC producing strains 20 (19.8%) were inducible, and 81 (80.2%) were stably derepressed. Such a high percentage of derepressed mutants is noteworthy and a cause for alarm.

Among individual bacteria, the most common AmpC producer was *Citrobacter* spp. (68%), followed by *Proteus* spp. (54.1%) and *Klebsiella* spp. (46.7%). In *E. coli*, ESBL (44.95%) and AmpC (38.5%) production was comparable. AmpC production was higher in *Citrobacter* spp. as compared to all the members of the Enterobacteriaceae put together (58.4%). The production of both ESBL and AmpC in *Citrobacter* spp. was also comparable (64% and 68% respectively). Imipenem was found to be a more sensitive inducer for detection of AmpC than cefoxitin.

Out of 251 strains studied fifty two (20.7%) were phenotypically identified as MBL producers. The highest incidence was seen in *Pseudomonas aeruginosa* with 24/74 (35.1%) isolates producing MBLs. Among the other Gram negative bacteria, MBL production was observed in 5 of 15 *Klebsiella* isolates and 1 of 3 *Acinetobacter* isolates (33.3% each), followed by 2 of 7 (28.6%) *Proteus* spp., 19 of 74 (25.7%) *Pseudomonas aeruginosa*, 4 of 17 (23.5%) *Serratia* spp., 5 of 25 (20%) *Citrobacter* spp., and 9 of 109 *E. coli* (8.3%).

Table 3. Distribution of multiple mechanisms of drug resistance

	AmpC+MBL	AmpC+ESBL	MBL+ESBL	AmpC+MBL+ ESBL	TOTAL
<i>E. coli</i>	5	3	3	5	16
<i>Klebsiella</i> spp	2	1	1		4
<i>Pseudomonas</i> spp	3	1	4	1	9
<i>Proteus</i> spp					
<i>Serratia</i> spp	5		2		7
<i>Acinetobacter</i> spp					
<i>Citrobacter</i> spp	2			1	3
<i>Burkholderia</i> spp			1		1
TOTAL	16	5	10	6	40

Among the methods employed for detection of MBL production, Hodge test (80%) proved better than DDST (55%). In 14 (35%) isolates, both the DDST and Hodge tests were positive. We observed that although heaping may be used as a screening tool, not all isolates that exhibited heaping were MBL producers.

Amongst a total of 251 isolates, 40 (15.94%) were identified as exhibiting multiple mechanisms of resistance. 10 (25%) elaborated ESBL along with AmpC, 11 (27.5%) isolates produced ESBL and MBL together, 19 (47.5%) produced MBL along with AmpC, and 1 (2.5%) exhibited all the three mechanisms of resistance.

DISCUSSION

β lactamase producing gram negative bacteria are fast emerging pathogens worldwide. Plasmid-mediated ESBLs have emerged as an important mechanism of resistance to β lactam antibiotics among members of the family Enterobacteriaceae. In addition, the presence of AmpC and MBL in a pathogen can certainly result in therapeutic failure, especially with the production of MBLs where carbapenems, most often used as drugs of last resort, are rendered inactive. Thus there is an imperative need to develop a simple, inexpensive and sensitive approach to detect ESBLs, AmpCs and MBLs on a routine basis.

CLSI recommends detection of ESBLs in *E. coli* and *Klebsiella* spp. However, in our region, bacteria other than *E. coli* and *Klebsiella* spp. are increasingly being isolated from both community and hospital-acquired infections. Therefore, we evaluated this mechanism of resistance in other bacteria as well and found a significant incidence of resistance in bacteria not commonly tested for ESBL production, like *Citrobacter* spp., *Serratia* spp., *Pseudomonas aeruginosa* and *Acinetobacter* spp. Based on our findings, we recommend testing all bacterial isolates for this resistance mechanism.

In the present study, *E. coli* (43.4%) was the predominant pathogen, followed by *Pseudomonas aeruginosa* (29.4%) and *Citrobacter* spp. (9.96%). *Klebsiella* spp. (5.97%) was unusually low in its presence in this region.

In our study, the highest ESBL producers were *Acinetobacter* spp. (100%), followed by

Klebsiella spp. (86.7%) and *Proteus* spp. (85.7%). The overall incidence of ESBL production amongst all our isolates was 54.98%. These ESBL detection rates were in the range reported in other studies from India¹³⁻¹⁴. Although in absolute numbers, *Klebsiella* isolates were fewer than many other bacterial pathogens, it is important to note that 86.6% of those identified produced ESBLs. Studies which have investigated carbapenem resistance in *Klebsiella pneumoniae* have found a combination of porin loss and β lactamase production to induce resistance¹⁵. Almost 45% of our *E. coli* isolates produced ESBLs. This finding is similar to that of Ananthakrishnan et al (2000)^[16] who reported a high prevalence of ESBL among *E. coli*. This high incidence of ESBLs in *E. coli* may be peculiar to the Indian subcontinent. In our study, *Citrobacter* spp. and *Serratia* spp. also exhibited notable resistance.

In our study, Cefoperazone-sulbactam was the most sensitive combination for the detection of ESBL compared to Piperacillin-tazobactam or Cefazidime-clavulanic acid. The increased efficacy of Cefoperazone-sulbactam in phenotypic detection of ESBL production may be attributed to its increased stability as compared to a penicillin inhibitor combination. This may be the case especially in those isolates co-producing an ESBL and penicillinase at high levels since concentration of inhibitor in their periplasmic space may be insufficient to protect a penicillin¹⁷. Moreover, sulbactam is also not easily hydrolysed by AmpC, and so is a better screening agent for ESBL than clavulanic acid in combination with a third-generation cephalosporin. Although on weight basis clavulanic acid is more potent than sulbactam, its ability to induce AmpC production may interfere with ESBL production¹⁸.

We observed a high incidence (80.2%) of derepressed AmpC mutants among our isolates. This is quite high as compared to 46% derepressed mutants reported by Rodrigues *et al*, 2004¹². If this trend continues it may pose a serious threat to effective antimicrobial therapy in the near future. On analysing each bacterial species individually, *Citrobacter* spp. (68%) was the leading AmpC producer followed by *Proteus* spp. (57.1%), *Klebsiella* spp (46.7%), and *Pseudomonas aeruginosa* (39.2%). AmpC production by *E. coli* was lower than these Gram negative bacteria. These

figures also alert us to the emergence of *Citrobacter* spp. as drug resistant bacteria. This is also the first study with *Citrobacter* spp. exhibiting so much of resistance. Imipenem was found to be a better inducer than cefoxitin for the detection of inducible AmpC.

Gram negative bacilli producing acquired MBLs have been increasingly reported in Asia and Europe^{4,6,7,19}. Infections with carbapenem-resistant *Enterobacteriaceae* (CRE) or carbapenemase-producing *Enterobacteriaceae* are emerging as an important challenge in health-care settings^[20]. Currently, carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is the species of CRE most commonly encountered in the United States. In our study, the detection of 20.7% of our isolates overall as MBL producers on phenotypic identification is alarming. Although all our isolates showed significant MBL production, the highest MBL producers were *Pseudomonas aeruginosa* (35.1%), *Klebsiella* spp. and *Acinetobacter* spp. (33.3% each). Other authors have also reported a high incidence of MBLs in *Pseudomonas aeruginosa* isolates²¹. In another study by Sharma et al (2010)^[22], 69.5% of their imipenem-resistant *Pseudomonas aeruginosa* isolates were MBL producers. Thus, a high level of antibiotic resistance pattern exists in various clinical isolates. Among detection methods employed for MBL detection, we found the Hodge test (80%) better than DDST (55%). The Hodge test and DDST together detected 35% of the isolates.

The modified Hodge test (MHT) is a phenotypic test used to detect carbapenemases in isolates demonstrating elevated but susceptible carbapenem MICs and has demonstrated sensitivity and specificity exceeding 90% in identifying carbapenemase-producing *Enterobacteriaceae* (CLSI 2009). Carbapenemase-producing pathogens produce infections that are difficult to treat and have high mortality rates²³. Detecting their presence at the entry level into the hospital environment is the first crucial step that the microbiologist can take to address this problem, so that measures can be instituted to prevent the spread of these pathogens. Care in detection is important because high carbapenem MICs are not always evident. Therefore we suggest that the initial complete screening of the isolate should include the Hodge test for the detection of MBL

as well as for multiple resistance mechanisms²⁴.

Thus, a holistic and timely antibiotic susceptibility report can be made available in the following manner: Screen and confirm ESBL, also screen for AmpC on the first day; confirm presence of AmpC (inducible/derepressed), and also screen for MBL on the second day; and confirm the presence of MBL on the third day.

REFERENCES

1. Knothe H, Shah P, Kremery V, Antal M, Mitsuhashi S. Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. *Infection* 1983; **11**: 315-7.
2. Bauernfeind A, Chong Y, Lee K. Plasmid-encoded AmpC beta-lactamases: how far have we go 10 years after the discovery. *Yonsei Med J* 1998; **39**: 520-5.
3. Bauernfeind A, Chong Y, Schweighart S. Extended broad-spectrum β -lactamase in *Klebsiella pneumoniae* including resistance to cephamycin. *Infection* 1989; **17**: 316-21.
4. Chu YW, Shah MA, Houang TS, Palepou MFI, Lyon DJ, Woodford N, Livermore DM. IMP-4, a novel metallo- β -lactamase from nosocomial *Acinetobacter* spp. Collected in Hong Kong between 1994 and 1998. *Antimicrob Agents Chemother* 2001; **45**: 710-14.
5. Iyobe, Kusadokoro SH, Ozaki J, Matsumura N, Minami S, Haruta S, Sawai T, O'Hara K. Amino acid substitution in a variant of IMP metallo- β -lactamase. *Antimicrob Agents Chemother* 2000; **44**: 2023-7.
6. Lee K, Lim JB, Yum JH, Yong D, Chong Y, Kim JM, Livermore DM. bla_{VIM-2} cassette-containing novel integron in metallo- β -lactamase producing in *Pseudomonas aeruginosa* and *Pseudomonas putida* isolated disseminated in a Korean Hospital. *Antimicrobial Agents Chemother* 2002; **46**: 1053-8.
7. Livermore DM, Woodford N. Carbapenemases: a problem in waiting? *Curr Opin Microbiol* 2003; **3**: 489-95.
8. Watanabe M., Iyobe S, Inoue M, Mitsuhashi S. Transferable imipenem resistance in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1991; **35**: 147-51.
9. Thomson KS. Controversies about extended spectrum and AmpC beta lactamases. *Emerg Infect Dis* 2007; **7**: 333-36.
10. Clinical and Laboratory Standards Institute.

- Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically; Approved Standard—8th Ed. *Clinical and Laboratory Standards Institute document*-(M07-A8), Wayne, 2009
11. Lee K, Lim YS, Yong D, Yum JH and Chong Y. Evaluation of Hodge Test and Imipenem EDTA Double Disc Synergy for differentiating metallo-β lactamase producing isolates of *Pseudomonas* spp. & *Acinetobacter* spp. *J Clin Microbiol* 2003; **41**: 4623-9.
 12. Rodrigues C, Joshi P, Jani SH, Alphonse M, Radhakrishnan R, Mehta A. Detection of β lactamases in nosocomial gram negative clinical isolates. *Indian J Med Microbiol* 2004; **22**: 247-50.
 13. Mathur P, Kapil A, Das B, Dhawan B. Prevalence of extended spectrum β lactamase producing Gram negative bacteria in a tertiary care hospital. *Indian J Med Res* 2002; **115**: 153-7.
 14. Mohanty S, Kapil A, Dhawan B, Das BK. Bacteriological and antimicrobial susceptibility profile of soft tissue infections from northern India. *Indian J Med Science* 2004; **58**: 10-15.
 15. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: a clinical update. *Clin Microbiol Rev* 2005; **18**: 657-86.
 16. Ananthakrishna AN, Kanungo R, Kumar A, Badrinath S. Detection of extended spectrum β lactamase producers among surgical wound infections and burn patients in JIPMER. *Indian J Med Microbiol* 2004; **18**: 160-5.
 17. Stratchounski L, Edelstein I, Narezkina A, Edelstein M, Pimkin M. *In vitro* activity of cefoperazone/sulbactam vs amoxicillin/clavulanic acid and piperacillin/tazobactam against extended spectrum β-lactamase (ESBL)-producing strains of *Escherichia coli* and *Klebsiella pneumoniae*. 12th European Congress of Clinical Microbiology and Infectious diseases 2000; 24-27 April,; Milan, Italy: Poster # 1413.
 18. Strutenberg E, Mack D. Extended spectrum β lactamases: implications for the clinical microbiology laboratory, therapy and infection control. *J Infect* 2003; **47**: 273-95.
 19. Migliavancea R, Docquier JD, Mugnaioli C, Amicosante G, Daturi R, Lee K, Russolini GM, Pagani L. Simple microdilution test for detection of metallo-β-lactamase production in *Pseudomonas aeruginosa*. *J Clin. Microbiol* 2002; **40**: 4388-90.
 20. Schwaber MJ, Carmeli Y. Carbapenem-resistant *Enterobacteriaceae*: a potential threat. *JAMA* 2008; **300**: 2911-3.
 21. Varaiya A, Kulkarni M, Bhalekar P, Dogra J. Incidence of metallo-beta-lactamase producing *Pseudomonas aeruginosa* in diabetes and cancer patients. *Indian J Pathol Microbiol* 2008; **51**: 200-3.
 22. Sharma M, Yadav S, Chaudhary U. Metallo-beta-lactamase producing *Pseudomonas aeruginosa* in neonatal septicaemia. *J Lab Physicians* 2010; **2**: 14-16.
 23. Queenam AM, Bush K. Carbapenemases: The versatile beta-lactamases. *Clin Microbiol Rev* 2007; **20**: 440-58.
 24. Fontana C, Favaro M, Sarmati L, Natoli S, Altieri A, Bossa MC, Minelli S, Leonardi F, Favalli C. Emergence of KPC-producing *Klebsiella pneumoniae* in Italy. *BMC Research Notes* 2010; **3**: 40.
 25. Lee K, Chong Y, Shin HB, Kim YA, Yong D, Yum JH. Modified Hodge and EDTA-disk synergy tests to screen metallo-β-lactamase-producing strains of *Pseudomonas* and *Acinetobacter* species. *Clin Microbiol Infect.* 2001; **7**: 88-91.