

## Metallo-beta-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae*: A rising threat for hospitalized children

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### Abstract

**Objective:** To determine the frequency of metallo-beta-lactamase (MBL) producing *E. coli* and *Klebsiella pneumoniae*, better phenotypic techniques for MBL detection and choices of treatment available for such cases.

**Methods:** This study was conducted in The Children's Hospital, Lahore during March, 2013 and February, 2014. A total number of 17,651 samples including blood, urine, CSF, pus and catheter tips from suspected cases of bacterial infections were processed and test organisms were identified using standardized microbiological techniques. MBL phenotypic identification was performed by Modified Hodge Test, Double Disc Synergy Test and Combined Disc Test.

**Results:** Carbapenem resistance was observed in 134/1168 (11.47%) strains which comprised of 89 (67.4%) *Klebsiella pneumoniae* and 45 (32.6%) *E. coli*. All of these carbapenem resistant isolates were found to be carbapenemase producers (CP) by MHT test. Among these CP strains, MBL was detected in 131/134 (97.8%) isolates both by CDT and DDST including 87 (66.4%) *Klebsiella pneumoniae* and 44 (33.6%) *E. coli*. Majority of these organisms were resistant to most of the antibiotics used in the study. The isolates showed good susceptibility to colistin (90.1%), chloramphenicol (60.3%) and fosfomycin (31%).

**Conclusion:** Isolation of such a high number of MBL producers is a serious threat for hospitalized paediatric patients.

**Keywords:** Metallo-beta-lactamases, carbapenem resistance, carbapenemases, *E. coli*, *Klebsiella pneumoniae*. (JPMA 66: 1068; 2016)

### Introduction

Multidrug-resistant (MDR) bacterial infections in hospitalized children are an emerging threat globally.<sup>1</sup> Gram negative bacteria including *Escherichia coli* and *Klebsiella pneumoniae* produce resistance threat with the evolution of multiple resistance mechanisms like enzyme production resulting in extended spectrum beta-lactamases, Amp C  $\beta$ -lactamases and now the carbapenemases especially metallo-beta-lactamases (MBLs).<sup>2</sup> These Gram negative bacteria are particularly linked with high morbidity and mortality rate. Treatment of these so-called "superbugs" remains challenging because conventional drugs are now losing their efficacy and few new antibiotics for Gram-negative bacteria are being developed with a very limited range available for paediatric patients.<sup>3</sup>

*E. coli* and *Klebsiella pneumoniae* infections are particularly important in young children especially neonates including infections of the urinary tract, respiratory tract, blood, meningitis, skin and post-

surgical sites.<sup>4</sup> The emergence of resistance due to enzyme production against other drugs has led to the overuse of carbapenems that also belong to beta-lactam antibiotics but have a wide-range activity against Gram-positive and Gram-negative bacteria and are stable to hydrolysis by most of the  $\beta$ -lactamases. This group (imipenem, ertapenem, meropenem and doripenem) has been until recently successfully used even in paediatric patients. Now, a rise in resistance to carbapenems has been reported in different parts of the world.<sup>5</sup> Genes responsible for the production of these enzymes are either present on plasmids or transposons thus making them highly transmissible in nature.<sup>6</sup>

Carbapenemases have been classified by Ambler scheme on molecular basis into three categories.<sup>7</sup> Bush et al. functional scheme has resulted from amino acid and nucleotide sequencing and classifies carbapenemases into four classes with difference in their bases at the active site. MBLs lie into class B in both the schemes.<sup>8</sup> They have emerged as not only the most significant indicator showing carbapenem resistance but also represent an increasingly detected pan-resistant phenotype.<sup>9</sup> MBLs have not only become a source of increased morbidity and mortality but also represent a clinical threat due to their unrivalled

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spectrum of activity and their resistance to therapeutic serine beta-lactamase inhibitors.<sup>10</sup> Although PCR-based genotyping remains as the golden standard for MBL detection and classification, but for rapid detection of MBL activity, diagnostic laboratories still need culture-based phenotypic tests.<sup>11</sup> This study is focused on the determination of better phenotypic techniques for MBL detection in routine diagnostic laboratories. As no data is available about the occurrence of MBLs in Pakistani paediatric patients, so this study was also aimed to identify the rate of infections caused by MBL producing *E.coli* and *Klebsiella pneumoniae* among paediatric patients and the choices of treatment available for such cases.

## Materials and Methods

This descriptive study was conducted between March, 2013 and February, 2014 in the department of Microbiology, The Children's Hospital and The Institute of Child Health, Lahore after the approval by institutional ethical committee. A total number of 17,651 consecutive paediatric samples were processed using standardized microbiological techniques. These specimens comprised of 9,418 (53.4%) blood, 5,023 (28.5%) urine, 2,605 (14.8%) cerebrospinal fluid (CSF), 660 (3.7%) pus/wound swabs and 145(0.8%) indwelling catheter tips. The samples were inoculated on Blood, Chocolate and MacConkey agar plates and organisms were identified using the routine microbiological methods including Gram's staining, colony morphology, biochemical tests and API 20E.<sup>12</sup> Antimicrobial susceptibility testing was performed by Kirby Bauer disc diffusion method and interpreted according to Clinical and Laboratory Standards Institute guidelines. Carbapenem resistant (CR) organisms were further processed for carbapenemase (CP) and MBL detection by different phenotypic methods.<sup>13</sup> Data analysis was completed using SPSS version 17.0.

Phenotypic screening test of modified Hodge test was used for carbapenemase detection and is recommended by CLSI.<sup>13</sup> The test was performed by preparing 0.5McFarland dilutions of the *E.coli* ATCC 25922 in 5 ml of broth or saline. The suspension of this strain was diluted 1:10 and was streaked as lawn on to a Mueller Hinton agar plate. A 10µg imipenem susceptibility disc was placed in the center of the test area. Test organism was streaked in a straight line from the edge of the disc to the edge of the plate. The plates were incubated overnight at 35±2°C in ambient air. MHT positive ATCC strain no. 1705 of *Klebsiella pneumoniae* and MHT negative *Klebsiella pneumoniae* ATCC1706 were

streaked on each plate as quality control strains.<sup>13</sup> Clover leaf like indentation shown by MHT positive ATCC strain was used as a standard for screening of CP strains.

Combined Disc Test and Double disc synergy test were performed on all MHT positive strains for phenotypic detection of MBLs.<sup>14</sup> In combined disc test, MBL positive strains were identified by the addition of 0.5 M EDTA to the imipenem disc and a disc of imipenem alone was also placed as a control. Synergistic effect of EDTA on imipenem enhanced the inhibition zone by 8-15 mm in case of MBL positive strains while the increase for MBL negative isolates was only 1-5 mm.<sup>15</sup> In DDST, an imipenem (10µg) disc was placed 20mm apart (centre to centre) from a blank disc containing 10µl of 0.1 M EDTA solution. Enhancement in zone of inhibition in the area between the two discs was considered positive for MBL.<sup>16</sup>

## Results

Carbapenem resistance was observed in 139/1168 (11.9%) strains which comprised of 93 (66.9%) *Klebsiella pneumoniae* and 46 (33.1%) *E.coli*. Out of the carbapenem resistant isolates, 138 were found to be carbapenemase producers (CP) by MHT test. Among the CP strains, MBL was detected in 134/138 (97.1%) isolates both by CDT and DDST including 90 (67.16%) *Klebsiella pneumoniae* and 44 (32.84%) *E.coli*.

A total of 86 (61.9%) CP strains were isolated from male

**Table-1:** Results of CDT& DDST among CP strains (n=138).

DDST & CDT Results (%)	Frequency (n)	Percentage
Positive	134	97.1
Negative	4	2.9

**Table-2:** General characteristics of the enrolled patients (n=139).

General characteristics (%)	Number
<b>Gender</b>	<b>n (%)</b>
Male	86 (61.9)
Female	53 (38.1)
<b>Age Groups</b>	
Neonates	47 (33.8)
Between 1 month to 5 years	60 (43.2)
Between 5 to 15 years	32 (23.0)
<b>Specimens</b>	
Urine	62 (44.60)
Blood	50 (35.97)
Tips and secretions	15 (10.79)
Pus & wound swabs	8 (5.76)

**Table-3:** Antimicrobial sensitivity pattern of MBL producing *Klebsiella pneumoniae* and *E.coli* (n=134).

Antibiotics	Resistant n (%)	Intermediate sensitive n (%)	Sensitive n (%)
Amikacin	123 (90.2)	7 (5.2)	4 (2.9)
Aztreonam	131 (97.8)	0	3 (2.2)
Co-amoxiclav	134 (100)	0	0
Cefotaxime	134 (100)	0	0
Ceftriaxone	134 (100)	0	0
Ceftazidime	134 (100)	0	0
Cefuroxime	134 (100)	0	0
Cefixime	134 (100)	0	0
Ciprofloxacin	115(85.8)	17 (12.7)	2 (1.5)
Chloramphenicol	53 (39.6)	0	81(60.4)
Cefoperazone-Sulbactam	118 (88.1)	3 (2.23)	13 (9.7)
ColistinSulphate	13 (9.7)	0	121 (90.3)
Fosfomycin	27 (20.15)	64(47.76)	43 (32.09)
Gentamycin	133 (99.2)	0	1 (0.8)
Rifampicin	133 (99.2)	0	1 (0.8)
Piperacillin-Tazobactam	101 (75.4)	22 (16.4)	11 (8.2)

patients and 53 (38.1%) from female patients. There were 60 (43.2%) cases isolated in age group of 1 month to 5 years, 47 (33.8%) in neonates and 32 (23%) of the patients were between the age of 5-15 years. There were 62 (44.6%) strains isolated from urine and 50 (35.97%) from blood samples.

In antimicrobial susceptibility testing, colistin sulphate was found to be the most effective antibiotic as 121 (90.3%) organisms were sensitive to this antibiotic followed by 82 (61.2%) to chloramphenicol and 43 (32.09%) to fosfomycin. All of the organisms were resistant to co-amoxiclav, cefotaxime, ceftriaxone, ceftazidime, cefuroxime and cefixime. Only 13 (9.7%) MBL strains were sensitive to cefoperazone-sulbactam and 11 (8.2%) were sensitive to piperacillin-tazobactam.

## Discussion

Resistance due to carbapenemase enzymes especially MBL is emerging as a serious threat for hospitalized paediatric patients. In our study, carbapenemase producing (CP) strains were 138/1168 (11.81%) while 134/138 (97.1%) of these strains proved to be MBL producing strains by both CDT and DDST. Out of these 134 MBL producers there were 89(67.16%) *Klebsiella pneumoniae* and 45(32.84%) *E.coli*. Our results are in accordance with the study conducted in Islamabad where the frequency of CP was 9%.<sup>17</sup> CP frequency in *E.coli* has been reported up to 4.0% and 10.8% in *Klebsiella pneumoniae* isolates in a USA based study.<sup>18</sup> Another study conducted in the Military Hospital

Rawalpindi, Pakistan reported 3.5% prevalence rate of CP strains.<sup>19</sup> This rate is much lower as compared to our study which may be due to the rapidly disseminating nature of MBL genes at interspecies and intra-species level which is aggravated by overcrowded environment.

A study conducted in Mumbai, India reported 100% MBL positivity among 22 CP strains which is similar to the findings of our study.<sup>20</sup> Different results were reported from a study conducted in Greece, where 32.4% CP strains were of MBL type.<sup>21</sup> CDT and DDST have shown good sensitivity in MBL detection. Similar results have been reported by Yalda et al., (2012) where they have also reported 100% sensitivity with these methods.<sup>11</sup> Arunava et al., (2013) has reported 100% positivity by combined disc test but 72.7% by DDST.<sup>22</sup> Such cases should be confirmed with genotyping as CP strains especially MBL strains are more prevalent in developing countries due to noncompliance to antibiotic protocols, insufficient infection control surveillance in hospitals, lack of good healthcare facilities, poor socioeconomic status, self-medication practices, off the counter availability of antibiotics and lack of education.

In our study, majority of the MBL producing isolates were isolated from urine samples. Most of our patients were males and belonged to the age group of 1 month to 5 years and neonates. A study conducted in Karachi reported highest number of MBL cases from urine samples.<sup>23</sup> Carbapenemase producing *Klebsiella pneumoniae* associated septicemia has been reported more in male patients.<sup>24</sup> In contrast, many international studies reported more cases of MBLs in female patients.<sup>25</sup> Only limited data is available to compare the prevalence of CP in paediatric patients.

Antimicrobial susceptibility of MBL producing *Klebsiella pneumoniae* and *E. coli* was tested against 16 different antibiotics. Colistin sulphate, chloramphenicol and fosfomycin showed better sensitivity results in our study than all other groups in which less than 10% sensitivity was seen against other antibiotics. A study reported high resistance in MBL producing *Klebsiella pneumoniae* to extended spectrum antibiotics like cephalosporins, aztreonam and meropenem which are in accordance with our results.<sup>26</sup> Another study conducted in America revealed high antimicrobial resistance in CP strains against piperacillin, ciprofloxacin, cefotaxime, tazobactam and rifampicin.<sup>27</sup> A study from India is also in accordance with our work which reported high resistance to CP strain to beta-lactams,

aminoglycosides, fluoroquinolones, nitrofurantoin and sulphonamides.<sup>28</sup>

Pan-resistance in MBL producing strains and high resistance to chloramphenicol and ciprofloxacin was also reported in a study conducted in USA.<sup>29</sup> Colistin sulphate proved to be the best alternate antibiotic for the treatment of MBL producing strains in our study. These results are in accordance to the results of an American study which reported 92.6% strains sensitive to colistin.<sup>20</sup> MBL isolates in a study conducted at two military hospitals Rawalpindi, Pakistan were 97% sensitive to colistin.<sup>24</sup> A study conducted in Rome, Italy has reported decrease in colistin sensitivity in MBL producing strains.<sup>30</sup> Colistin is being used against MBL producing strains in European countries and eventually they are facing the problem of increase in resistance. Colistin has not yet been registered in Pakistan and is not being used regularly. The isolates showed 60.3% sensitivity to chloramphenicol which has come up as an alternative antibiotic.

## Conclusion

Routine screening of Carbapenemases especially MBL type can be easily done with these inexpensive phenotypic methods in diagnostic laboratories. Our study has unveiled high frequency of MBL producing *Klebsiella pneumoniae* and *E. coli* in a paediatric set up leaving the paediatricians with very limited treatment options. This problem can only be solved with effective infection control measures, control of overcrowding, educating general public to adopt clean habits, avoid self-medication and proper use of antibiotics. A dedicated hospital management team can play an utmost important role in eradication of such resistant strains. The role of continuous microbiological surveillance is pivotal in prompt identification and reporting of these multidrug resistant bugs.

## Limitations

The class of carbapenemase cannot be determined by the results of the MHT. Some isolates show a slight indentation but do not produce carbapenemase. For this purpose, Carba NP test is now recommended in current CLSI. Although, CDT and DDST are good phenotypic tests, genotyping is the gold standard which could not be performed due to non-availability in our setup.

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**Conflicting Interest:** None declared.

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