

DISSEMINATION AND DETECTION OF CARBAPENEMASES PRODUCING GRAM-NEGATIVE RODS

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ABSTRACT

Objective: To evaluate the burden of carbapenemases producers among carbapenem-resistant isolates.

Study Design: Cross-sectional study.

Place and Duration of Study: This cross-sectional study was conducted at microbiology lab, Pathology department, Allama Iqbal Medical College Lahore, from Jul 2016 to Apr 2017.

Material and Methods: A total of 12126 clinical specimens were enrolled through a non-probability/consecutive sampling technique, every sample was processed for bacterial culture, identification was done on the basis of colonial morphology, Gram stain, and biochemical profile. All those isolates that were Gram-negative rods, were processed for carbapenemases detection by Modified Hodge test (MHT) in accordance with CLSI 2016.

Results: Out of total n=12126 samples, culture positive were 35.9% (n=4361) of which 40.5% (n=1770) were Gram-negative rods, among these 9.6% (n=170) were carbapenem-resistant isolates of which 50% (n=85) were carbapenemases producers (MHT positive). Organisms wise carbapenem resistance was detected in *Acinetobacter species* 44%, *Pseudomonas species* 34%, *E.coli* 7%, *Klebsiella species* 8%, *Proteus species* 3%, *Citrobacter species* 1%, *Enterobacter species* 0.7%. Among these isolates MHT positivity was as followed *Acinetobacter species* were 53%, *Pseudomonas species* 51%, *E.coli* 36%, *Klebsiella species* 50%, *Proteus species* 20%, *Citrobacter species* 66%, *Enterobacter species* 50%, department wise distribution of carbapenemases producer showed that among total sample received from surgical units 51.4%, ICU 65.3% medical units 43.5%, pediatric ward 71.4%, orthopedic 11.1% neurosurgery 20%, other wards 55.1% were MHT positive. The almost similar resistant pattern was observed in MHT positive and negative isolates.

Conclusion: Emergence of carbapenem resistance is an alarming situation in clinical settings.

Keywords: Carbapenem, Carbapenemases, Healthcare workers, Modified hodge test.

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INTRODUCTION

Emergence of drug resistance is one of the growing concerns in the field of medical sciences. A simple bacterial infection could potentially become fatal if the bacteria acquire resistance to the routinely used antibiotics. Bacteria can preserve themselves and produce resistance to the antibiotics by destroying the active component of the drugs, drug alternation or inactivation. This occurs by the different enzyme produced by the bacteria against the different antibiotics. In most instances, the bacteria produce protective enzymes by their cell-wall. These protective enzymes add acetyl or phosphate group to a specific site on the antibiotic. This results in

reduced ability of the antibiotic to bind to the bacterial ribosome and disrupt protein synthesis¹.

Carbapenemases producing bacteria are the most common cause of nosocomial infection but this is not limited to the hospital anymore². The wide spread infection of such bacteria due to their ability to transfer resistance, chromosomally and by plasmid-mediated, has made it more difficult to control. In the last 10 years, there has been an increase in carbapenemases reporting in Enterobacteriaceae³. The emergence of immunocompromised patients has been observed in Pakistan and also around the globe during the last decades⁴. *Pseudomonas aeruginosa* and *Klebsiella species* have been associated with varied nosocomial infections like skin and soft tissue infections in immunocompromised adults and paediatric population⁵. Antimicrobial drugs

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are used empirically to reduce the episode of illness and these are based on the susceptibility pattern of the pathogens against these drugs in a specific institute from time to time⁶.

The detection of these carbapenemases producing bacteria has become a necessity. Multiple studies have reported carbapenem resistance from Pakistan^{7,8}. As the carbapenemases producing bacteria show multidrug resistance, there is a very limited option left for the treatment of such bacterial infections⁹. Carbapenem antibiotics include imipenem, Meropenem, Ertapenem, and Doripenem. Organism producing carbapenemases show resistance against all these drugs, except for *Pseudomonas* which has intrinsic resistance to ertapenem.

The detection of β -lactamases can be done by phenotypic and genotypic detection methods, in phenotypic detection, different methods can be used such as MIC by agar dilution, MIC by E-Test, modified hodge test (MHT), EDTA imipenem combine disc synergy test, Vitec MIC detection (automated), Nitrocefin, a chromogenic cephalosporin substrate which changes color from yellow to red upon beta-lactamase mediated hydrolysis¹⁰. Double-Disc Synergy Test (DDST), Carba NP, Carbapenem Inactivation Method (CIM)¹¹. The genotypic method includes PCR and NGS (Next Generation Sequencing) for the specific genes¹².

The study was conducted to evaluate the burden of carbapenemases producers among carbapenem-resistant Gram-negative bacilli.

MATERIAL AND METHODS

This cross-sectional study was conducted at microbiology lab, Pathology department Allama Iqbal Medical College, Lahore, Pakistan, during the period of ten months from July 2016 to April 2017. A total of 12126 clinical samples calculated from win-Pepi software ver:11.15 for finding at least 48 sample carbapenem-resistant Gram-negative rods with 95% confidence interval and assumed a rate of 5/1000 samples (urine, blood, pus, pus swabs, tips, respiratory samples, body fluids) were enrolled for study through a non-

probability/consecutive sampling technique presenting to a different department of Jinnah hospital, Lahore (JHL).

Every sample was cultured on blood and MacConkey agar. Selectively CLED (cysteine lactose electrolyte deficient) in urine samples, blood, MacConkey and chocolate agar were used for respiratory samples and fluids. After 24 hours of incubation at 37°C, bacterial identification was done on the basis of colonial morphology, Gram stain, and biochemical profile.

After identification, all those isolates that were Gram-negative rods, were further processed for antimicrobial susceptibility testing by modified Kirby bauer disc diffusion method following CLSI guidelines 2016. Next day all those isolates that were resistant to meropenem (MEM) or imipenem (IPM) were tagged as carbapenem-resistant isolates, furthermore, all these carbapenem-resistant isolates were further processed for MHT (phenotypic method) for the detection of carbapenemases.

MHT is based on the inactivation of a carbapenem by carbapenemases - producing strains (test isolate) that enable a carbapenem-susceptible indicator strain (*E. coli* ATCC® 25922) to extend growth towards a carbapenem-containing disc along the streak of inoculum of the test strain. Positive test result gives cloverleaf-like indentation.

This test was done in accordance with CLSI 2016. Briefly, A 0.5 McFarland standard suspension (using either direct colony suspension or growth method) of *E. coli* ATCC® 25922 (the indicator organism) in broth or saline, and dilute 1:10 in saline or broth was prepared. MHA plate as for the routine disk diffusion procedure was inoculated and allowed to dry for 3 to 10 minutes. A 10 ug imipenem,/meropenem disk was placed in the center of the test area on the plate. Using a 10-ul loop or swab, 3 to 5 colonies of test organism grown overnight on a blood agar plate were inoculate in a straight line out from the edge of the disk. And were incubated overnight at 35°C \pm 2°C in ambient air for 16-24 hours. After

18-24 hours of incubation, the plates were examined for a cloverleaf-type indentation at the intersection of the test organism and the *E. coli* 25922, within the zone of inhibition of the carbapenem susceptibility disk. MHT Positive test had a cloverleaf-like indentation of the *E. coli* 25922 growing along the test organism growth streak within the disk diffusion zone. While MHT

RESULTS

During the study period, of total specimens n=12126, bacterial growth was obtained in 35.9% (n=4361) samples of which 40.5% (n=1770) were Gram-negative rods (GNR). Of these 40.5% (n=1770) GNRs, Carbapenem-resistant isolates were 9.6% (n=170) isolates, of which equal freq-

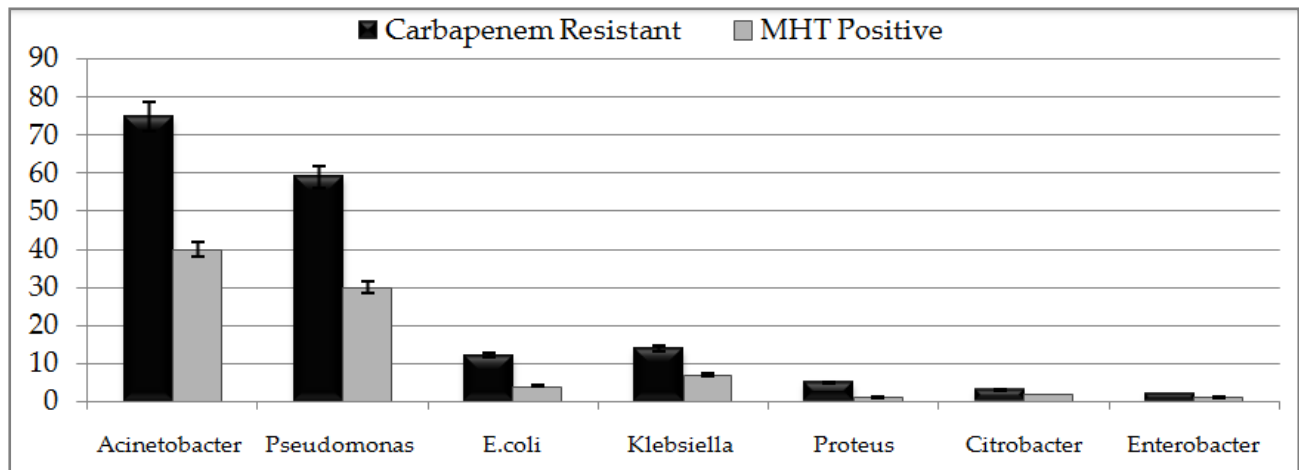


Figure-1: Organisms wise breakup of MHT positivity among carbapenem-resistant isolates.

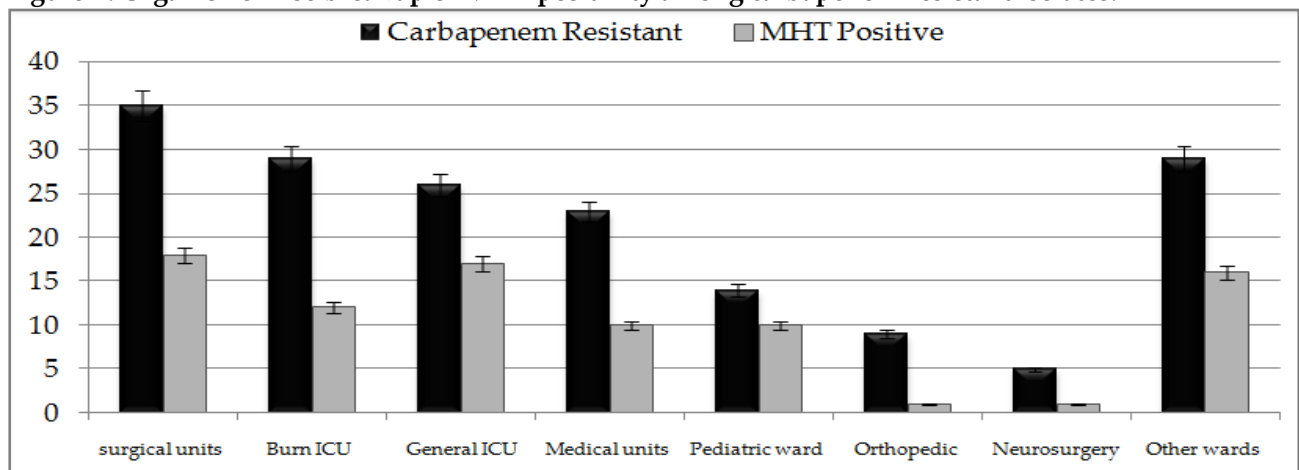


Figure-2: Department wise breakup of MHT positivity among carbapenem-resistant isolates.

Negative test had no growth of the *E. coli* 25922 along the test organism growth streak within the disc diffusion. For quality control purpose *Klebsiella pneumoniae* ATCC® BAA-1705 were used as positive control and *Klebsiella pneumoniae* ATCC® BAA-1706 were used as negative control.

SPSS version 21.0 was used for data analysis, frequencies and percentages were calculated.

ency of MHT positive and negative isolates observed.

Out of total 170 carbapenem-resistant isolates, *Acinetobacter* species were 44%, *Pseudomonas* species 34%, *E.coli* 7%, *Klebsiella* species 8%, *Proteus* species 3%, *Citrobacter* species 1%, *Enterobacter* species 0.7% Among these isolates MHT positivity was as followed *Acinetobacter* species were 53%, *Pseudomonas* species 51%, *E. coli*

36%, *Klebsiella species* 50%, *Proteus species* 20%, *Citrobacter* 66%, *Enterobacter* 50% (fig-1).

In case of ward wise distribution 35 specimens received from surgical units and 18 (51.4%) showed MHT positive. 29 specimens were received from burn ICU and 12 (41.3%) were MHT positive. 26 samples were received from ICU and 17(65.3%) were MHT positive. A total of 23 specimens were received from medical units and 10 (43.5%) were MHT positive. From pediatric ward, 14 specimens were received and 10 (71.4%) were MHT positive. From orthopedic 9 samples were received and 1 (11.1%) was MHT

carbapenem-resistant isolates, 138 (69%) MHT positive by MHT test. Among MHT positive isolates frequency of *E. coli* was 38%, *Pseudomonas aeruginosa* 30% *Klebsiella pneumoniae* 17%, *Acinetobacter baumannii* 12%, *Citrobacter diversus* 2%, *Enterobacter agglomerans* 1.4%¹³.

Ramana *et al* from India reported that among 1072 clinical isolates of Enterobacteriaceae carbapenemases production was detected in 385 (35.9%)¹⁴. MHT positivity for *Klebsiella spp* was 28.7%, *Citrobacter spp* 20.4%, *E. coli* 11.3%, *Enterobacter spp* 20.3%, *Proteus spp* 16.2%¹⁴. Rangnekar *et al* from India reported, among

Table-I: Gender wise distribution of MHT positive isolates.

Genders	Carbapenem resistant		MHT positive	
	Frequency	Percentages	Frequency	Percentages
Male	98	57.6	57	67
Female	72	42.3	28	32.9
Total	170	100	85	100

Table-II: Antimicrobial resistant pattern among MHT positive and negative isolates.

Antimicrobials drugs	Carbapenem Resistant MHT negative	Carbapenem Resistant MHT positive
Piperacillin/tazobactam	99	99
Co-amoxycylav	99	100
Doxycycline	78	80
Ciprofloxacin	98	99
Nitrofurantoin	58	55
Gentamicin	94	92
Amikacin	94	91

positive, 5 samples were received from neuro-surgery and 1 (20%) was MHT positive, 29 specimens were received from other wards and 16 (55.1%) were MHT positive (fig-2).

Almost similar resistant pattern was observed in both cases table-II.

DISCUSSION

Infection rate by drug-resistant Gram-negative rods is on the rise, therefore present study was planned to evaluate the frequency of carbapenemases producer isolates among carbapenem-resistant isolates, via MHT test.

A similarly study from Armed Forces Institute of Pathology (AFIP) Rawalpindi, conducted in 2011. In that study, among 200

153 Extended-Spectrum Beta-Lactamases (ESBL) producing *Klebsiella pneumoniae* isolates, 54 were resistant to one of the carbapenems. Among these, 13 were positive for MHT on MHA, while 23 were positive by MHT on MCA¹⁵. Sultan *et al* from Pakistan reported that among 100 Gram-negative rods, MHT positivity among *Klebsiella pneumoniae* was 63 (63%), *Escherichia coli* 32 (32%), others 5 (5%)¹⁶. The present study reported a high rate of carbapenem resistance as well as MHT positivity among surgical units, followed by Burn ICU and General ICU fig-2 higher infection rate enhancing transmission, is observed in this department, this might be credited to exposure to a large number of antibiotics and saturation of vulnerable patients¹⁷. The five key mechanisms

of antimicrobial resistance are i) enzymatic degradation of anti-bacterial drugs, ii) modification of bacterial proteins that are antimicrobial targets, iii) changes in membrane permeability to antibiotics, iv) export of drug from bacteria and v) Biofilm formation¹⁸.

A bacterium that is resistant to a wide range of antibiotics is termed as "SUPERBUG". These strains of bacteria show multidrug resistance. They carry genes for several antibiotic resistance. These resistant strains are the cause of very serious disease¹⁹. Bacteria can preserve themselves and produce resistance to the antibiotics by destroying the active component of the drugs, drug alternation or inactivation. This occurs by the different enzyme produced by the bacteria against the different antibiotics. In most instances, the bacteria produce protective enzymes by their cell-wall. These protective enzymes add acetyl or phosphate group to a specific site on the antibiotic. This results in reduced ability of the antibiotic to bind to the bacterial ribosome and disrupt protein synthesis¹. There are several different types of enzymes produced by the bacteria i) Aminoglycoside-modifying enzymes, ii) Chloramphenicol acetyl-transferases, iii) Beta-lactamases²⁰. Beta-lactamases is a wide group of enzymes, produced by bacteria against β -lactam antibiotics. They provide multidrug resistance against these antibiotics²¹ B-lactam antibiotic all have a common structure in them, they have a four-ringed structure in them. The beta-lactamase enzyme hydrolyzes this ring and renders the antibiotic ineffective. The β -lactam drugs include Aminopenicillins, Cephalosporins, Carbapenems, and Monobactams. There are different enzymes produced correspondingly against these antibiotics such as penicillinase, cephalosporinases, and carbapenemases²². The ability of an organism to produce a β lactamase may be chromosomal and constitutive or a plasmid-associated acquired property.

Carbapenemases are a diverse group of β -lactamases. They have a wide range of hydrolytic capacities. They have the ability to hydrolyze penicillin, cephalosporin, monobactam, and car-

bapenems. Carbapenems have high-level production of chromosomal AmpC with decreased outer membrane permeability (porins). The carbapenemases have been organized based on amino acid homology in the Ambler molecular classification system. Class A, and D beta-lactamases all share a serine residue in the active site, while class B enzymes require the presence of zinc for activity (and hence are referred to as metallo-beta-lactamases). Currently, there are five major classes of carbapenemases.

- i. KPC (Klebsiella pneumoniae carbapenemases)
- ii. IMP (imipenemase metallo-beta-lactamase)
- iii. VIM (Verona integron-encoded metallo-beta-lactamase)
- iv. OXA (Oxacillin carbapenemases)
- v. NDM (New Delhi metallo-beta-lactamase)

CONCLUSION

The carbapenem resistance is increasing among Gram-negative rods. This is an alarming situation in clinical settings for treating patients with Gram-negative infections. Also in nosocomial infections, this is a critical scenario.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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