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Phenotypic Detection Of Carbapenemase Production Among Klebsiella Spp From Clinical Samples From A Tertiary Care Hospital

Dr Rishi Dhurve[1], Dr Manish Purohit[2*], Dr Anita Mutha[3]

[1]Ex PG Dept of Microbiology, MGMMC, Indore

[2*]Associate Prof Microbiology, MGMMC, Indore

[3]Professor, Microbiology,MGMMC, Indore

***Corresponding Author:** Dr Manish Purohit

*Associate Prof, Microbiology,MGMMC,Indore, Email: mainshpurohit75@yahoo.co.in

Abstract:
Carbapenemase produced by *Klebsiella pneumoniae* can confer resistance to all β-lactam agents including carbapenems. The enzyme may develop low-level carbapenem resistance, leads to failure of routine antimicrobial susceptibility testing methods to identify this mechanism of resistance. Automated and nonautomated methods for carbapenem susceptibility were evaluated by various studies. In our study a total of 160 isolates of *Klebsiella* were isolated from various clinical specimens. Out of 160, 101(63.1%) showed resistance to carbapenem tested in study and was considered screen test positive. On confirmation of Carbapenamase production by Modefied Hodge test, Modified Carbapenem Inactivation(mCIM) method and EDTA –Modified Carbapenem inactivation method(eCIM), 76.2%, 92.1% and 92.1% isolates showed production of carbapenemase respectively .

Keywords: Antibiotic, Carbapenem, Klebsiella

Introduction:

Emergence of resistance to carbapenem antibiotics has been increased recently among clinical isolates of *Klebsiella pneumoniae.* Production of carbapenem-hydrolyzing enzyme is one of the major resistance mechanisms to this group of antibiotics(1).Carbapenemsare one of the most important broad-spectrum β-lactam antibiotics and have greater activity against many resistant bacteria such as those which have the ability to produce AmpC β-lactamase. Across the globe, first reports of Carbapenemases occurred in the 1980s. Its subsequent spread raised a number of concerns, and these have coincided with years in which no new antimicrobial agents against Gram-negatives have been developed. Japan reported the first Carbapenemase from an Aeromonas hydrophila isolate in the 1980s. Sequentially, followed in London (1982) by Seoul imipenemase(SME-1) from Serratia marcesces, imipenemases(IMI-1) in California (1984) and NMC-A in France (1990) ,both from Enterobacter cloacae.Carbapenems members of β-lactam family of antimicrobials that are structurally related to the Penicillins. Mechanism of actionin carbapenems is initiated first by penetrating the bacterial cell wall and binding to enzymes known as penicillin-binding proteins (PBPs). The inhibiting series of PBPs are 1a, 1b, 2and 3; and the resultant lethal effect, the inactivation of an inhibitor of autolytic enzymes with in the cell wall which leads to killing of bacteria.[8-11]In India it has been reported that 65.4% isolates were ESBL producers, 28.5% were AmpC producers, 9.4% were combined ESBL and AmpC producers and 48.6% were Carbapenamases producers in which 25.6% were KPC and 23% MBL producers and 8.2% were KPC and MBL coproducers. The presence of a carbapenemase can be detected by a

different method in clinical laboratories. These include automated systems or disc diffusion, MICs,selective agar, modified Hodge test, synergy tests (e.g., E-tests or double disctests),spectrometrics, genome sequencing and by molecular methods.
Performing antimicrobials susceptibility testing and resistance pattern identification plays an important role in treating patients since these isolates showing multidrug resistance are of great concern for the treating physician and it plays a significant role in the mortality of the patient. Hence,the current study was conducted to determine the most common *Klebsiella* species, antibiotic sensitivity pattern, diverse resistance mechanism among isolates for carbapenem resistance and Detection of MIC for colistin among Carbapenamase producing *Klebsiellaspp.* Further Comparison of different methods for detection of Carbapenamase production was also done.

Aim:
Present study was aimed for Phenotypic detection of Carbapenemase production among *Klebsiella spp*. Isolated from clinical specimen from a tertiary care center of Central India.

MATERIAL&METHODS:

Study Design: Cross-sectional Observational Study.

Study Centre: The study was carried out in the Department of Microbiology, of a tertiary care hospital.

Duration Of the Study: Study duration was 1 year from 11 June 2021 to 10 June 2022 after the approval from the institutional ethics committee.

SampleSize: 500 isolates were taken out of which160 isolates found to be *Klebsiella spp*.

Inclusion criteria: All *Klebsiella spp.*isolated from various specimen received for culture and sensitivity in Department of Microbiology.

Exclusion Criteria: Repeat isolate of *Klebsiella* spp.from same patient.

Methodology

All specimens received for culture and sensitivity were processed as per standard guidelines.Isolation and identification of *Klebsiella spp.* was done using Colony morphology, Gram staining and biochemical tests as per standard guidelines. Antimicrobial sensitivity testing of isolated *Klebsiella spp* was done using Kirby Bauer disc diffusion method as per CLSIM 1002022 guidelines[2].

Screening criteria for carbapenamase production: Organism whichwere found resistant to imipenem and/or Meropenem were considered as probable Carbapenamase producers.

Confirmation of Carbapenamase
Modified Hodge Test, Modified carbapenem inactivation method (mCIM), EDTA-modified carbapenem inactivation method(eCIM) was use to confirm carbapenemase production among screen positive *Klebsiella spp.*

Result and Discussion:

A total of 500 isolates from various clinical samples were taken for thestudy, out of which 160

clinically consecutive, nonduplicate, significant isolatesof *Klebsiella spp*were isolated and included in the study. Out of 160
Klebsiella spp .isolates 129 were obtained from the IPD and remaining 31 were from OPD.Number of Klebsiella *spp* isolated from various clinical samples were infollowingorder:urine(61/38.1%),pus(53/33.1%),sputum(13/8.1%),bronchoalveolar (11/6.9%), ear swab (9/6.9%), throat swab (8/5%) and CSF(5/3/1%). In the current study majority of *Klebsiella spp* were isolated from urine cultures (38.1%). This observation is in accordance to that of PS Patil et al.[3](29.7%), Leavitt et al.(2010), [4] Parveen et al. (2010) [5] Bashir et al. (2014) [6] andVivan et al. (2017)[7] who reported maximum isolation of *K. Pneumoniae* from urine samples. Amongst the 160 isolates, 101 (63.1%) were found resistant to Imipenem and/or meropenemand considered to be screen positive(IPD: 82; OPD: 19) whereas 59 (36/9%) were screen negative (IPD: 47; OPD:12). This was in concurrence with results obtained by Vamsi SK et al.[8]whoreported that out of the 220 isolates, 207 (94.0%) had screen test positivity. Further,the results were also comparable to that reported by Diwakaret al. [9]
For confirmation of carbapenemase production among screen positive*Klebsiella spp.*, Modified Hodge test, MCIM and ECIM was done. ECIM and MCIM test showed similar sensitivity with 92(91.1%) positives and 9 (8.9%) negatives. However, Modified Hodge test (MHT) showed 77(76.2%) positives and 24 (23.8%) negatives. For ECIM positive outcome, i.e., 92 isolates; 100% concurrence was observed when cross checked by MHT test. However, for MTH positive outcomes i.e., 77 isolates; 98.7% (76) positive outcomes were observed when cross checked by mCIM test.
In the study of Amjad et al. (2011),[10] 17% of K. *pneumoniae* showed positive modified Hodge test. Anderson and colleagues at CDC evaluated 45 isolated by modified Hodge test and further validated by PCR for detection of Carbapenamase activity.[103]Modified Hodge test was found to be 100% sensitive and specific for detection of carbapenem resistance in *K.pneumoniae*. Bashir H et al. reported modified Hodge test as a reliable andsensitive laboratory method for detection of carbapenem.The treatment of *Klebsiella* infections remain a great challenge because resistant to aminoglycosides, cephalosporins and quinolones has substantially increased world wide.
Carbapenems are the drug of choice for MDR *Klebsiella* infections, for ESBL and AmpC producing isolates, but resistance to carbapenems by the production of carbapenamases and various other mechanisms has limited the therapeutic options.
Amongtotal160*Klebsiella*isolates,92showedcarbapenamaseproductionitisabout57.5%whereasCarbapenamase production among screen test positive isolates was found to be 91.1% indicating need for continuous screening of all carbapenem resistant isolates.MCIM and ECIM test showed similar sensitivity with 92 (91.1%) positives and 9 (8.9%) negatives. However, Modified Hodge test (MHT) showed lower sensitivity with 77(76.2%) positives and 24 (23.8%) negatives.Therefore, we can do MCIM testing among all MHT negative isolates to increase sensitivity of test.

Conclusion

The treatment of *Klebsiella* infections remain a great challenge because of multidrug resistant strains. Routine monitoring of Carbapenamaseis need of hours so that appropriate and timely right treatment can be given.Simple and inexpensive method for detection of Carbapenamase will help in rapid and correct identification . So, updating ourselves, exploring new diagnostic options and implementing new testing strategies can help to detect these carbapenemase for early and proper patient management. Emergence of antimicrobial resistance in bacteria has serious effects like high morbidity and mortality rates, increased length of hospital stays and additional financial burden on patients.

Table1.Sample Distribution on Basis of Location

Lab	Frequency	Percent
IPD	129	80.6
OPD	31	19.4
Total	160	100.0

Table2.Distribution on Basis of Screening for carbapenamase production

Screening Outcome	Frequency	Percent
Positive	101	63.1
Negative	59	36.9
Total	160	100.0

Chart-1.DistributiononBasisof Specimen

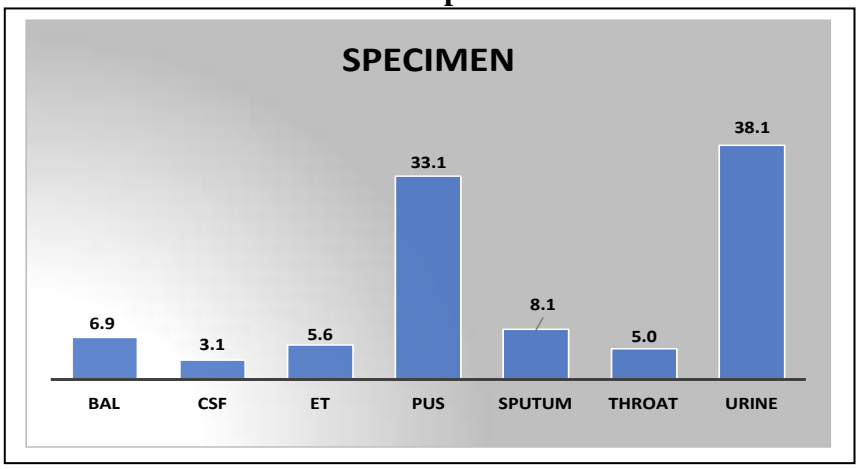

Image-1 Modified Hodge test

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