Scholars Academic Journal of Biosciences (SAJB)
Abbreviated Key Title: Sch. Acad. J. Biosci.
ISSN 2347-9515 (Print)
ISSN 2321-6883 (Online)

A Unit of Scholars Academic and Scientific Society, India
www.saspublishers.com

Microbiology

Comparison of Blue-Carba and Modified Hodge Test for Carbapenamase Production in Blood Culture Isolates of *Klebsiella pneumoniae*

Deepinder Singh*, Ashima Katyal, Madhu Sharma, Uma Chaudhary
Department of Microbiology, Pt. B. D. Sharma PGIMS, Rohtak, Haryana, India

Original Research Article

***Corresponding author**
Deepinder Singh

Article History
Received: 25.01.2018
Accepted: 08.02.2018
Published:15.02.2018

DOI:
10.36347/sajb.2018.v06i02.006

Abstract: Multidrug resistant (MDR) Gram negative infections have resulted in high rates of morbidity and mortality in patients with diverse clinical conditions. *Klebsiella pneumoniae* carbapenamase (KPC)-producing bacteria is one of the emerging MDR pathogens causing bacteremia with limited therapeutic options such as colistin and tigecycline. An investigational laboratory based study was conducted (over 1 year) to know the prevalence of *Klebsiella pneumoniae* carbapenemase (KPC)-producing bacteria from blood culture isolates. Blood culture samples were processed and *Klebsiella pneumoniae* was identified by colony morphology, Gram staining and biochemical tests, followed by antibiotic susceptibility testing as per CLSI (clinical and laboratory standard institute) guidelines. The isolates were confirmed by automated identification system (BD phoenix). Hundred & ten such isolates were enrolled in the study. The production of carbapenamase was detected by blue carba test and modified Hodge test. A comparative evaluation of the two tests was also done. The majority of the 110 pts were newborn (62%). A total of 16 (14.54%) isolates were positive for carbapenamase production by blue carba test and 12 (11%) by Modified Hodge test. The most sensitive drug for all carbapenamase producing isolates were colistin and polymyxin B (100% each). MDR KPC was detected to be important organism in NICU and post-surgical patients for enhanced morbidity & mortality. The clinicians are faced with the dilemma as to how to control nosocomial spread of this organism in ICU as there are limited therapeutic options available. Therefore, there is a need to evaluate new detection methods for carbapenamase detection & blue carba test is a simple, rapid and sensitive test for their detection.
Keywords: Blue carba test, Modified hodge test, carbapenemase production.

INTRODUCTION

Extensive use of broad-spectrum antibiotics in hospitalized patients has led to both increased carriage rate and development of multi-drug resistant strains. During last decade, dissemination of *K. pneumoniae* carbapenemase (KPC) has led to an increase in the prevalence of carbapenem-resistant or pan resistant *Enterobacteriaceae*. These enzymes are also found on mobile genetic elements having the capacity to transfer these genes to other members of the family and frequently encode for resistance to other class of antimicrobials, thereby limiting the choice of antimicrobials left for treatment of such infections. Infection with such strains often lead to complications, increased hospital stay, treatment cost, morbidity and mortality [1].

Blue-Carba Test (BCT) is a modified version of Carba NP. The test is based on the principle of direct detection of carbapenem hydrolysis by carbapenemase-producing bacteria. It detects different classes of carbapenemases that is KPC & MBL (Metallo-β-lactamases) in first 30 mins and OXA types in 2 hours by performing test directly from colonies [2]. The detection of carbapenemase production can be done with various tests namely Modified Hodge test, Blue-Carba test, Carba NP, Phenotypic Inhibitor Test but each test has its own advantages and disadvantages. Therefore, this study was designed to compare the two methods of detection of carbapenemase production in blood culture isolates of *K. pneumoniae.*

MATERIALS AND METHODS

An investigational laboratory based study was conducted in the department of Microbiology, Pt. B.D. Sharma, PGIMS, and Rohtak over a period of one year (October, 15 to September, 16). A total of 15141 samples were received in the department. Out of these, 2301(15.2%) samples were culture positive. Two hundred thirty three (10.12%) *K. pneumoniae* isolates were recovered on processing of cultures by the conventional methods i.e. by studying the colony morphology on the culture plates, gram staining, catalase test and various biochemical reactions as well as automated system. One hundred ten *Klebsiella pneumoniae* isolates were processed further for the

purpose of this study and confirmed by automated identification and antibiotic susceptibility testing system (BD phoenix). All the *K. pneumoniae* isolates were tested for carbapenemase production by Blue-Carba test [3] and MHT [4].

Blue-Carba test (BCT): [3]

Procedure

Imipenem powder (Hi Media) was used as the substrate for carbapenemases. The test solution consists of an aqueous solution of bromothymol blue at 0.04% adjusted to pH 6.0,0.1mmol/literZnSO4, and 3mg/ml of imipenem, with a final pH of 7.0. A negative-control solution (0.04% bromothymol blue solution, pH 7.0) was prepared to control the influence of bacterial components or products in the pH of the solution. A loop (approximately 5 µl) of a pure bacterial culture recovered from Mueller-Hinton agar was directly suspended in 100 µl of both test and negative-control solutions in a 96-well microtiter plate and incubated at 37°C with agitation (150 rpm) for 2 hrs.

Interpretation

Carbapenemase activity was revealed when the test and negative-control solutions, respectively, were

- yellow versus blue,
- yellow versus green, or
- green versus blue.

Non carbapenemase producers remained blue or green in both solutions.

Modified Hodge Test (MHT)[5]

Procedure

0.5 McFarland standard suspension of *E. coli* ATCC 25922 in broth was prepared and it was diluted to 1:10 in broth. Then it was inoculated on MHA plates for the routine disk diffusion procedure. Plate was allowed to dry for 3 to 10 minutes. Then one disk of meropenem was placed in the center of the plate. Using a swab, 3 to 5 colonies of test or QC organism were picked from blood culture plate grown overnight at 37ºC and inoculate in a straight line out from the edge of the disk. Test the number of isolates per plate.

Interpretation

Following incubation, MHA plate was examined for enhanced growth around the test or QC organism at the intersection of the streak and the zone of inhibition.

Enhanced growth = positive for carbapenamase production

No enhanced growth= negative for carbapenamase production

Some test isolates produced substances that inhibit growth of *E. coli* ATCC 25992. When this occurred, a clear area was seen around the streak and the MHT was considered uninterpretable for these isolates.

STASTICAL ANALYSIS

The data was collected using Microsoft Excel spread sheet and doubly checked for errors. Qualitative data was presented as mean and standard deviation and quantitative data as proportions. Association was tested using Chi-square and Z-test for proportions. Evaluation of Blue Carba test was done by calculating sensitivity, specificity, positive predictive value and negative predictive value. Statistical significance was considered when $p<0.05$. SPSS 20.0 software was used for analysis.

RESULTS

The age range in the study population in case of females was from newborn to 84 years with mean age 6.62±1.02. The age range in case of males was newborn to 62 with mean age 7.13±2.07. The maximum number of patients i.e. 62 (56.36%) belonged to <1 years age group followed by age group 21-30 years i.e. 13 (11.8%). Only 2 (1.8%) patients found in 1-10 and >60 years age group each. Blood stream *Klebsiella* infections were found to be more common in new born (54.54%).

The study reveals that these isolates had varying degree of susceptibility to the antimicrobials tested (Table 1). All the strains i.e. 100% were sensitive to colistin and polymyxin B. Most of these showed high susceptibility to carbapenems group of antibiotics in-vitro viz. imipenem (84%), meropenem (82%) and ertapenem (77%). Least susceptibility of the isolates was seen against followed by gentamicin (11%) and amoxicillin-clavulanic acid (15%). This shows *K. pneumoniae* have developed resistance to most commonly employed antibiotics namely fluoroquinolones, penicillin group, cephalosporins in this tertiary care study. It was observed that out of 110 isolates, 88.1% were resistant to ≥1 agent in ≥ 3 antimicrobial categories i.e. multidrug resistant.

Table-1: Antibiotic susceptibility pattern of the *K. pneumoniae* isolates

Antibiotics	Sensitive isolates (%)	Resistant isolates (%)
Gentamicin	12(11)	98(89)
Amoxicillin-clavulanic acid	16(15)	94(85)
Co-trimoxazole	23(21)	87(79)
Piperacillin-tazobactam	26(24)	84(76)
Cefazolin	26(24)	84(76)
Cefoperazone	28(25)	82(75)
Ciprofloxacin	28(25)	82(75)
Ceftazidime	29(26)	81(74)
Cefuroxime	31(28)	79(72)
Levofloxacin	31(28)	79(72)
Cefepime	45(41)	65(59)
Amikacin	55(50)	55(50)
Ertapenem	85(77)	25(23)
Meropenem	89(81)	21(19)
Imipenem	92(84)	18(16)
Colistin	110(100)	0(0)
Polymyxin B	110(100)	0(0)

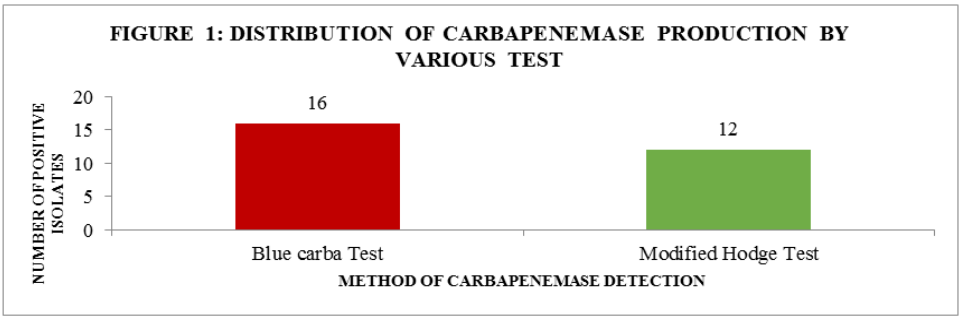
Table 2 shows the carbapenemase production among blood culture isolates of *K. pneumoniae*. It was observed that out of 110 *K. pneumoniae* isolates 16 (14.54%) were carbapenemase producers**.** All the carbapenemase producers were multidrug resistant. The carbapenemase production in the study isolates were detected by Blue-Carba test and Modified Hodge test as shown in Table 3. It shows that number of isolates producing carbapenemases are more i.e. 16 (14.54%) by Blue-Carba test than 12(10.9%) by Modified Hodge test**.** Blue-Carba test showed sensitivity 100%, specificity 95.91%, positive predictive value 75% and negative predictive value 100 %. Similar findings are depicted in figure 1.

Table-2: Carbapenemase production among *K. pneumoniae* isolates

Total number of *K. pneumoniae* isolates	Number of isolates producing carbapenemase	Percentage (%)
110	16	14.54

Table-3: Detection of carbapenemase production by Blue-Carba test and Modified Hodge test

Method of detection of carbapenemase production	Number of isolates positive for carbapenemase production	Percentage (%)
Blue-Carba test	16	14.54
Modified Hodge test	12	10.9



DISCUSSION

In the present study, it was observed that out of 110 *K. pneumoniae* isolates 16 (14.54%) were carbapenemase producers. In a cross-sectional study conducted in Egypt by Moemen *et al.* [6] included all the patients admitted in ICU'S over one year period. One hundred and twenty five (125) *K. pneumoniae* isolates were enrolled for the purpose of study and 42 out of 125 (33.6%) patients had CRKP infection in various samples. CRKP infection among blood isolates was 9.5%. Similarly, Jeniffer *et al.* [7] who studied epidemiology of carbapenem-resistant *K. pneumoniae* in a network of long-term acute care hospitals found that out of 3846 *Klebsiella pneumonia* samples from various sites of infection. CRKP was found in 9.4 % of blood culture isolates. CRKP

prevalence in the above 2 studies is comparable with present study.

The antimicrobial profile of the *K. pneumoniae* isolated from blood culture reveals that these isolates had varying degree of susceptibility to the antimicrobials tested. All the strains i.e. 100% were sensitive to colistin and polymyxin B. Most of them showed high susceptibility to carbapenem group of antibiotics *in vitro* viz. imipenem (84%), meropenem (82%) and ertapenem (77%). Least susceptibility of the isolates was seen against cephalosporin group (20-30%), ciprofloxacin (25%), amoxicillin-clavulanic acid (15%) and gentamicin (11%). The fact that cephalosporins are one of the most common antibiotics used for inpatients as well as for outpatients could be the reason for such high resistance being observed in the developing countries.

The carbapenem resistance in the present study accounted to 16% and all these strains were multidrug resistant. The greatest threat with MDR and carbapenem-resistant strains is that the infections caused by such strains are usually difficult to treat with limited antibiotic options available. The carbapenemase production in the study isolates were detected by Blue-Carba test and Modified Hodge test. It was seen that number of isolates producing carbapenemases are more i.e. 16 (14.54%) by Blue Carba test than 12(10.9%) by Modified Hodge test**.** Pires *et al.* [3] evaluated Blue-Carba test on 101 previously characterized *Enterobacteriaceae* (n=44), *Acinetobacter* (n=43) and *Pseudomonas* (n=14) species. The strains producing Ambler's class A, B and D carbapenemases (KPC, IMP, NDM, VIM, SPM, and OXA) and 49 non-carbapenemase producers were enrolled. They concluded that the BCT detected all carbapenemase producers with 100 % sensitivity and specificity. All non carbapenemase producers with or without alterations in outer membrane permeability gave negative results. They also concluded that BCT is an quick, simple, and reliable method which demonstrated sensitivity and specificity similar to those of Carba NP test and better than those of MHT with additional advantages [8]. In a similar study by Pasteran *et al.* [9] 300 clinical isolates were included (188 carrying known carbapenemase encoding gene and 112 without these genes). They found that BCT detected all class A and class B carbapenemases. They reported overall sensitivity of 97% and specificity of 100%. In the present study, the sensitivity and specificity of BCT came out to be 100% and 95.91% respectively. Hence, BCT is a simple quick and sensitive method as compared to MHT.

CONCLUSION

Therefore, BCT is an accurate and inexpensive method for detection of carbapenemase in laboratory which can help in early detection of carbapenemase producing strains. This will lead to proper management of these patients and thereby reducing carriage rates and spread of these strains.

REFERENCES

1. Gupta N, Limbago BM, Patel JB, Kallen AJ. Carbapenem-resistant Enterobacteriaceae: epidemiology and prevention.Clin Infect Dis.2011 Mar;53(1):60-7.
2. Rao N, Indumathi VA. Detection of carbapenem resistant Enterobacteriacae from clinical isolates. Int J of CurrMicrobiol and ApplScie. 2016; 5(1):864-69
3. Pires J, Novais A, Peixe L. Blue-Carba, an easy biochemical test for detection of diverse carbapenemase producers directly from bacterial cultures. J Clin Microbiol.2013 Dec 51(12):4281–83.
4. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; Twenty-Fifth informational supplement. CLSI document M100-S25. Wayne, PA: CLSI;2015:50-6.
5. Eshetie S, Unakal C, Gelaw A, Ayelign B, Endris M, Moges F. Multidrug resistant and carbapenemase producing Enterobacteriaceae among patients with urinary tract infection at referral Hospital, Northwest Ethiopia. Antimicrobial resistance and infection control. 2015 Dec;4(1):12.
6. Moemen D, Masallat D. Prevalence and characterization of carbapenem-resistant *Klebsiellapneumoniae* isolated from intensive care units of Mansoura University hospitals Egypt J Bas Appl Sci. 2017 Jan ;37–41
7. Han JH, Goldstein EJ, Wise J, Bilker WB, Tolomeo P, LautenbachE. Epidemiology of carbapenem-resistant *Klebsiellapneumoniae* in a network of long-term acute care hospitals. Clin Infect Dis. 2017 Apr;64(7):839-44
8. Wang Q, Li B, Tsang AK, Yi Y, Woo PC, Liu CH. Genotypic analysis of Klebsiella pneumoniae isolates in a Beijing Hospital reveals high genetic diversity and clonal population structure of drug-resistant isolates. PloS one. 2013 Feb 21;8(2):e57091.
9. Pasteran F, Veliz O, Ceriana P, Lucero C, Rapoport M, Albornoz E, Gomez S, Corso A, ReL AVRA Network Group. 2015. Evaluation of the Blue-Carba test for rapid detection of carbapenemases in Gram-negative bacilli. J Clin Microbiol.2015Jun.;53(6):1996 –98.