ORIGINAL RESEARCH PAPER
Volume - 12 | Issue - 04 | April - 2023 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

MODIFIED CARBA NP TEST: SIMPLE AND RAPID METHOD TO DIFFERENTIATE MBL PRODUCING E.COLI SPECIES

Microbiology

Dr. Shama Tomar Professor and Head Department of Microbiology NIMS Jaipur, Rajasthan.

Dr. Siva Prasad Reddy B Professor Department of Microbiology NIMS Jaipur ,Rajasthan

Dr. Sapna Chauhan Professor and Head Department of Microbiology Microbiology Muzaffarnagar Medical college, Muzaffaranagar U.P

Mr. Ravi Vashistha Assistant Professor Department of Microbiology Muzaffarnagar Medical College, Muzaffarnagar.c-47 Faculty Residence , Muzaffarnagar.

ABSTRACT

Introduction: Carbapenemases are β-lactamase enzymes that render bacteria resistant to nearly all classes of antibiotics including carbapenem, a last-resort antibiotic, posing an immense threat to public health. Carbapenemases have been classified as, metallo-β-lactamase (Ambler class B), and OXA carbapenemase (Ambler class D). **Method and Material-**l A total of 115 clinical samples (sputum, pus, urine, blood, and fluids) were processed and E.coli spp. were identified as per standard operating procedures including microscopy, culture and biochemical tests, which were further subjected for carbapenemase detection **Result-**A total of 250 bacterial isolates were obtained,. including 90 (15.6%) carbapenemase producers, comprising 66 (88.6%) MBL producers. E.coli spp. were predominately isolated from sputum samples followed by pus and blood samples ie. Modified Carba NP test shows low performance after 30 minutes, when we increases to 2 hours. Sensitivity, specificity, PPV, and NPV 95.7%, 100%, 100%, and 99.8% for MBL detection, Modified Carba NP test detected (90.1%) and combined disc test detected 60.0%, whereas both equally detected MBL producers. **Discussion-**This study evaluates the performance of modified Carba NP test for the characterization of carbapenemase-producing E.coli spp. phenotypically. In this study, 13.9% (90 out of 250) E.coli isolates were carbapenemase producers. Similarly Chakkarapani et al10 reported 12% and 14.4% carbapenemase producing E.coli spp, respectively. Carbapenemase-producing E.coli spp. were more predominately isolated from pus samples.

KEYWORDS

Carbapenemase, E coli species, MBL Modified carba NP.

INTRODUCTION

Carbapenemases are β-lactamase enzymes that render bacteria resistant to nearly all classes of antibiotics including carbapenem, a last-resort antibiotic, posing an immense threat to public health[1]. Carbapenemases have been classified as, metallo-β-lactamase (Ambler class B), and OXA carbapenemase (Ambler class D). Classes A & D enzymes have a serine based hydrolytic activity (hence also called serine carbapenemases), while class B enzymes require the presence of metal, i.e, zinc for their activity (thus known as metallo-β-lactamases)[2,3].In 2013, the US Center for Disease Control and Prevention (CDC) put three drug-resistant superbugs in urgent category, namely carbapenem-resistant Enterobacteriaceae (CRE), drug-resistant Neisseria gonorrhoeae, and Clostridium difficile[4] .Conventional methods for carbapenemase detection utilize either enzyme inhibitors (combined disc test)[5,6] or chromogenic substance (chrome agar culture media), which are time-consuming and expensive, respectively. Some rapid and cheap methods (Carba NP) have been developed[7,8]. However, they lack the ability to characterize carbapenemases. MBL producers may pose greater resistance compared with E.coli producers because their plasmid may harbor a high number of other resistance genes like plasmid-mediated cephalosporin's genes, extended spectrum β-lactamase (ESBL) genes, aminoglycoside resistance genes (16S RNA methylases), macrolide resistance genes (esterase), rifampin genes (rifampin-modifying enzymes) and sulfamethoxazole resistance genes as a source of multidrug resistance[9]. Thus, accurate MBL carbapenemases is one of the utmost importance for the betterment of treatment modality and reduction in the clinical mortality rate.

MATERIALS AND METHODS

A total of 115 clinical samples (sputum, pus, urine, blood, and fluids) were processed and E.coli spp. were identified as per standard operating procedures including microscopy, culture and biochemical tests, which were further subjected for carbapenemase detection.

Carbapenemase Detection

Screening Test

In all, 250 E.coli isolates were tested for meropenem susceptibility (10 µg) by Kirby-Bauer method as per Clinical Laboratory Standard Institute (CLSI) guidelines.8 Strains, showing the zone diameter less than 22mm, considered as suspected carbapenemase producers and further processed for confirmation by CDT (combined disc test) and modified Carba NP test.

Combined Disc Test (CDT)

Strain was inoculated on (MHA) Mueller-Hinton agar plate, one disc temocillin (30 µg) and 4 discs meropenem (10 µg): one disc without inhibitor, second disc with phenylboronic acid (PBA) (400 µg), third disc with EDTA (292 µg), and fourth disc with both PBA and EDTA, were placed. Then, plate was incubated at 37°C for overnight 18-24 hours. Test strain, showing more than 5-mm zone diameter around meropenem with PBA and meropenem with both PBA and EDTA than zone diameter around meropenem disc alone, was considered as serine carbapenemase producer. Test strain, showing more than 5-mm zone diameter around meropenem with EDTA and meropenem with both PBA and EDTA than zone diameter around meropenem disc alone, was considered as MBL producer. However, test strain, showing no increase zone of inhibition around meropenem plus inhibitor.

Modified Carba NP Test

Modified Carba NP test was performed as described with modification; 4-5 loops (10 µL) of a pure bacterial culture was taken from blood agar plate, suspended into 400 µL TrisHCl bacterial lysis buffer, and then vortexed it for 1 minute. One hundred microliters of this bacterial suspension was added in four microcentrifuge tubes (0.5 mL capacity) each. Tube 1 (control tube) contained 100 µL of diluted phenol red and 0.1 mmol/L ZnSO4; tube 2 contained 100 µL of diluted phenol red, 0.1 mmol/L ZnSO4, and 6 mg/mL imipenem-cilastatin powder (equivalent to 3 mg/mL of imipenem); tube 3 contained 100 µL of diluted phenol red, 0.1 mmol/L ZnSO4, 6 mg/mL imipenem-cilastatin, and 4 mg/mL of tazobactam sodium salt; and tube 4 contained 100 µL of diluted phenol red, 0.1 mmol/L ZnSO4, 6 mg/mL imipenem-cilastatin, and 0.003 mol/L EDTA. Test strain turning yellow color from original red color in tubes 2 and 3 it was considered as MBL producer.

RESULTS

A total of 250 bacterial isolates were obtained,. including 90 (15.6%) carbapenemase producers, comprising 66 (88.6%) MBL producers. E.coli spp. were predominately isolated from sputum samples followed by pus and blood samples ie. Modified Carba NP test shows low performance after 30 minutes, when we increases to 2 hours. Sensitivity, specificity, PPV, and NPV 95.7%, 100%, 100%, and 99.8% for MBL detection, Modified Carba NP test detected (90.1%) and

combined disc test detected 60.0%, whereas both equally detected MBL producers.

DISCUSSION

This study evaluates the performance of modified Carba NP test for the characterization of carbapenemase-producing E.coli spp. phenotypically. In this study, 13.9% (90 out of 250) E.coli isolates were carbapenemase producers. Similarly Chakkarapani et al[10] reported 12% and 14.4% carbapenemase producing E.coli spp, respectively. Carbapenemase-producing E.coli spp. were more predominately isolated from pus samples .Similar study by Radhika et al[11] revealed that highest 45% carbapenemase-producing E.coli spp. were isolated from pus sample, Several studies have been conducted with the modification of Carba NP test using imipenem-cilastatin for the detection of carbapenemase but with the limitation to MBL producers. A study by Hamid El Abd et al[12] concluded the sensitivity and specificity to be 100% for the detection of MBL producers. Another study carried out by Campana et al[13] reported 73.1% sensitivity and 100% specificity. These studies support the current study, concluding 91.7% sensitivity and 96.7% specificity.. High rate of MBL producers may be because MBLs have been documented throughout the world, while carbapenemases have been reported from New York City area, recently in Europe, but seldom reported from India. The reason behind the regional variation is not clear[14].

CONCLUSION

Antimicrobial resistance among gram-negative bacteria is rapidly increasing and leaving behind very limited treatment options. Rapid and accurate identification of resistant strains results in better treatment modality and reduction in the mortality rate. Modified Carba NP test is a rapid, cheap, highly sensitive, and specific method for MBL carbapenemase-producing E.coli spp.

REFERENCES

1. Meletis G. carbapenem resistance: overview of the problem and future prespectives. Ther adv infect Dis. 2016,3:15-21.
2. Bush K, jacoby G. updated functional classification of bets lactamase. Antimicrob agents chemother.2010: 54:969-976.
3. Kumar N,singh V, potttathil S. metallo beta lactamase and serine carbapenemase producing klebseilla spp: a global challenge. J glob antimicrob resist.2018:12:185-186.
4. Centers for disease control and prevention. Antibiotic resistance threats in the united states,2013. http://www.cdc.gov/drugresistance/threats-report-2013/index.html. Accessed september25,2017.
5. Pournaras S, Zarkotou O, poulou A,et al. a combined disc test for direct differentiation of carbapenemase producing Enteriobacteriaceae in surveillance rectal swabs. J clin microbial. 2013:51:2986-2990.
6. Birgy A, bidet P, genel N, et al. phenotypic screening of carbapenemase and associated beta lactamase in cabapenem resistant enteriobacteriaceae.J clin microbial.2012: 50:1295-1302.
7. Clinical and laboratory standard institute (CLSI). Performance standards for antimicrobial susceptibility testing. Approved standard eleventh edition. CLSI document Mo2-A12:WaynePA: clinical and laboratory standards institute:2015.
8. BernabeuS. Dortet L, nase T. Evaluation of the beta CARBA test, a colorimetric test for the rapid detection of carbapenemase: activity in gram negative bacilli. J Antimicrob chemother.2017:72:1646-1658.
9. Nordmann P, nass T, Poriel L. Global spread of cabapenemase producing enteribacteriaceae. Emerg infect dis. 2011:17:1791-1796.
10. Chakkarapani AA, AmboiramP, Balakrishnan U, Ninan B, Sekar U pattern and antimicrobial susceptibility of carbapenem resistance organism in tertiary care neonatal intensive care unit, India. J clin neonatal 2014:3:200-2004.
11. Radhika B, Padmaja J. detection of serine carbapenemase and metallo carbapenemase enzymesin klebseilla pneumonia in a tertiary care hospital.Am Jsci Med Res. 2015:1:136-147.
12. Hamid El Abd D, Msry, et al. Carba NP test as a rapid diagnostic tool for carbapenemase producers.JMSR. 2017:5:19133-19143.
13. Campana E, chuster S et al. Modified Carba NP test for the detection of carbapenemase production in gram negative rods:optimized handling of multiple samplew, Braz J microbial,2017:48:242-245.
14. Nordmann P, nass T, poriel I. global spread of carbapenemase producing enteriobacteriaceae. EMERG INFECT DIS.2011:17:1719-1796.