Original Article

Modified Hodge test: A useful and the low-cost phenotypic method for detection of carbapenemase producers in *Enterobacteriaceae* members

K. V. Ramana, Ratna Rao, CH. V Sharada, MA Kareem, L. Rajashekar Reddy, Ratna Mani MS

Department of Microbiology, Apollo Health City, Jubilee Hills, Hyderabad, Andhra Pradesh, India

Address for correspondence:
Dr. K. V. Ramana, Department of Microbiology, Prathima Institute of Medical Sciences, Nagunur, Karimnagar, Andhrapradesh, India Email: ramana_20021@rediffmail.com

Abstract

Background: The global spread of antimicrobial resistance has acquired greater significance in the public health perspective. Drug resistance has posed a threat for the management of various hospital-acquired infections (HAI). For bacteria producing extended spectrum ß lactamase, carbapenems are the drug of choice. However, treatment failures are still a cause of concern due to carbapenemase producers. **Aim:** Various phenotypic and genotypic methods are available for the detection of carbapenemase producers. Studies thus far have mostly concentrated on comparing various methods for detection of carbapenemase producers. We used low-cost and the easily performed modified Hodge test (MHT) for detecting the carbapenemase producers in *Enterobacteriaceae* members isolated from various clinical specimens. **Material and Methods:** The study included 1072 clinical isolates of *Enterobacteriaceae* collected in India between April 2008 and February 2010. MHT was performed on all the isolates in accordance with CDC and CLSI guidelines. **Results:** The carbapenemase activity was detected in 35.9% (385/1072) of the isolates. *Klebsiella* spp. 28.7% (80/278), *Citrobacter* spp. 20.4% (25/122), 11.3% (38/334) in *E. coli*, 20.3% (45/221) in *Enterobacter* spp., and 16.2% (9/117) in *Proteus* spp. revealed variable resistance activities against carbapenems. **Conclusion:** *Enterobacteriaceae* members are among the most common and easily transferable bacterial species responsible for severe HAI. This study revealed a high percentage of *Enterobacteriaceae* clinical isolates producing carbapenemases in India. Detection of such bacteria, formulating hospital antibiogram, and monitoring the usage of antimicrobial drugs is recommended.

Key words: Carbapenemase, *Enterobacteriaceae*, modified Hodge test

INTRODUCTION

In view of the increasing incidence of infections caused by multidrug resistant (MDR) microbes, there are very limited options to treat such infections.[1] Few antimicrobial drugs have, in the past two decades, been introduced in the market, compared to the remarkably growing number of both Gram-positive and Gram-negative MDR bacteria.[2] Several members of the *Enterobacteriaceae* family of bacteria are normally present as harmless human gut flora. However, these bacteria are the leading cause for a wide range of opportunistic infections.[3] Although the carbapenemase activity has mainly been detected in *Pseudomonas* and *Acinetobacter* clinical isolates, recent studies have demonstrated the emergence of carbapenem resistance among *Enterobacteriaceae* members in different geographical regions, which is of great concern as these bacteria are easily transmissible among patients, leading to hospital acquired infections (HAI), but can also spread into the community, resulting in community-acquired cases.[4] Carbapenem resistance in *Enterobacteriaceae* may be due to various reasons that include hyper production of the Amp C beta lactamase, loss of porins, production of metallo-beta-lactamases (MBL) and production of *K pneumonia* carbapenemases. The most important carbapenemase determinants responsible for resistance or reduced susceptibility to carbapenem group

Access this article online	
Quick Response Code:	Website: www.jnsbm.org
	DOI: 10.4103/0976-9668.117009

of antibiotics in *Enterobacteriaceae* members include class A KPC 1-3, Class D (OXA-48, OXA-181), IMP, VIM, NDM, NMC-A, SME1-3, IMI-1, GES-2, SHV, and SFC.[5] Multidrug resistant gram negative bacteria due to production of beta lactamase, metallo-beta-lactamases, and carbapenemases are difficult to treat. In view of the alarming increase in the appearance of Carbapenemase-producing bacteria in the clinical isolates a standard testing method should be followed for detection of carbapenemase producing bacteria. The carbapenemase detection methods include the modified Hodge test (MHT), the double disk test (DDST), blood agar combined disk (BA-CD) assay, PCR amplification, and DNA sequencing.[6] Bacteria susceptible to the carbapenem group of antibiotics also need to be tested as some may still have the enzyme. The study aimed to investigate the carbapenemase activity in *Enterobacteriaceae* members, using the modified Hodge test.

MATERIALS AND METHODS

The study, performed at Apollo Health City, Hyderabad, India, included 1072 consecutive clinical isolates of *Enterobacteriaceae*, collected between April 2008 and Feb 2010. The MHT was performed on all the isolates irrespective of their susceptibility pattern to carbapenems (Imipenem, Meropenem, and Etrapenem), in accordance with guidelines of the Centers for Disease Control (CDC) and Clinical and Laboratory Standards Institute (CLSI) (http://www.cdc.gov/; http://www.clsi.org/).[7] *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 1706 and *Pseudomonas aeriginosa* ATCC 27853 were used as controls.

A lawn culture was prepared on Mueller Hinton agar (MHA) using an overnight culture suspension of *E. coli* (ATCC 25922) adjusted to 0.5 McFarland's standards. The plate was left for 15 min for drying and then a disc of 10 μg meropenem was applied at the centre of plate. The isolates under study were streaked from the edge of the disk to the periphery of the plate. Four isolates were tested in each plate. After an overnight incubation at 37° C, the clover leaf like appearance between the test streaks near the disk was taken as positive for carbapenemase production.

RESULTS

The isolates were identified as *E. coli* (n = 334), *Klebsiella* spp. (n = 278), *Enterobacter* spp. (n = 221), *Proteus* spp. (n = 117), and *Citrobacter* spp. (n = 122). Overall, the carbapenemase activity was detected in 35.9% (385/1072) of the isolates. The activity varied between 28.7% (80/278) in *Klebsiella* spp., 20.4% (25/122) in *Citrobacter* spp., 11.3% (38/334) in *E. coli*, 20.3% (45/221) in *Enterobacter* spp., and 16.2% (9/117) in *Proteus* spp. MHT positive and negative is shown in Figures 1 and 2.

DISCUSSION

The increased occurrence of MDR and resistance to carbapenems in *Enterobacteriaceae* has been a cause of concern to public health. This study detected a high percentage 35.9% (385/1072) of the potential carbapenemase activity among a collection of *Enterobacteriaceae* isolates obtained from various clinical specimens in India. Among the different *Enterobacteriaceae* members tested in the present study, *Klebsiella spp.* showed the highest percentage of carbapenem resistance (~30%), whereas *Proteus spp.* and *Citrobacter* spp revealed comparatively low carbapenem resistance of (~17%) and (~12%), respectively. A recent study from South India done as a part of antimicrobial surveillance program (SENTRY) that tested 39 *Enterobacteriaceae* isolates collected between 2006 and 2007 that showed reduced susceptibility to carbapenem antibiotics revealed 26 (66.6%) were found MHT positive.[8] Deshpande *et al.* in their recent study tested 24 carbapenem resistant *Enterobacteriaceae* members and found 22 (91.6%) were MHT positive and later confirmed by PCR.[9] A study from Greece included ~117 ESBL negative *Enterobacteriaceae* members that revealed a MHT positivity of 41.8%.[10] The high carbapenemase positivity in this study can be attributed

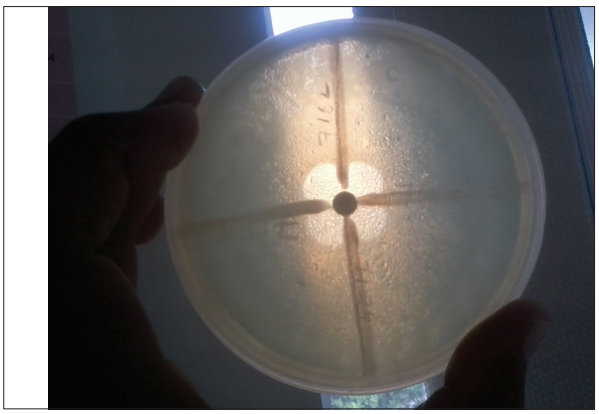

Figure 1: Clover leaf appearance of carbapenemase positive strains

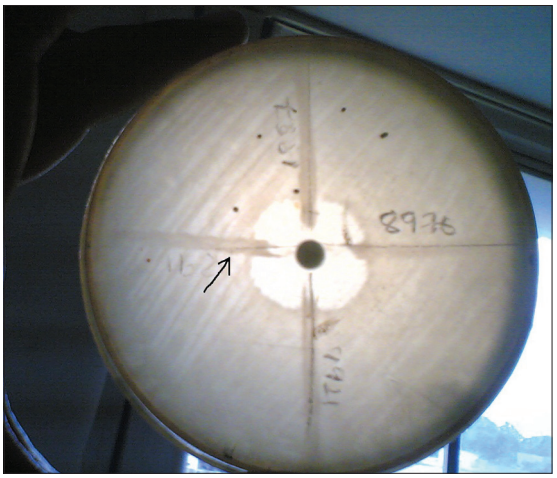

Figure 2: Arrow depicting carbapenemase negative strain

to the low-level hydrolysis of carbapenems by ESBLs of the CTX-m type.[11] The limitation of this study is not comparing the results with a molecular method as the main aim of the study was to present data on the carbapenemase producing *Enterobacteriaceae* members that can be useful in developing strategies to control the spread of such bacteria as we still have no guidelines to treat infections caused by carbapenemase producing bacteria.[12] Previous reports have confirmed that carbapenem susceptible isolates showed the presence of carbapenemase gene by PCR indicating that clinical resistance may not be detected in *Enterobacteriaceae* members and that laboratories should routinely check for carbapenemase production among clinical isolates by possible phenotypic or genotypic methods.[11] Several phenotypic methods for carbapenemase detection are available, of which the MHT is recommended both by CDC and the CLSI.[13,14] Other tests including the combination disc tests have been in use by many laboratories.[15] Studies have compared MHT and combination disc tests and found variable results. Amongst them few studies have raised concern over the false positivity in the MHT test.[16] Few other works have proved combined disk synergy tests as more effective in detecting the carbapenemase activity. The carbapenemases of the OXA, KPC, IMP, and VIM types are clinically important enzymes. They are all encoded on mobile genetic elements, located on plasmids or chromosomes, and are frequently isolated from patients suffering from antibiotic resistant infections.[5] Studies have come to a contrasting conclusions about use of MHT, one finding it as inadequate in detecting the metallo-beta-lactamases and others proving that MHT produces false positive carbapenemases.[16] An Indian study showed MHT is not preferred for carbapenemases in nonfermenting gram negative bacteria and recommended the use of both EDTA-meropenem and the EDTA-ceftazidime combination test.[17] This study results clearly demonstrate the presence of the carbapenemase activity in high percentage of *Enterobacteriaceae* members detected by the MHT that has proven to be easily done in any tertiary care setting with minimal infrastructure and is cost effective. Routine testing of all clinical isolates for possible carbapenemase activity may result in availability of data on such isolates as only few studies have been done in this regard. Confirmation of the resistance mechanism is not required in the public health perspective. Studies must be encouraged to assess the risk factors for infections with carbapenemase producing bacteria. Effective antibiotic policy, infection control programs combined with good medical practices can help in confronting the menace of antibiotic resistance.

REFERENCES

1. Nordmann P, Cuzon G, Naas T. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. Lancet Infect Dis 2009;9:228-36.
2. Ramana KV, Mohanty SK, Kumar A. *In vitro* activities of current antimicrobial agents against isolates of pyoderma. Indian J Dermatol Venereol Leprol 2008;74:430.
3. Galani I, Souli M, Koratzanis E, Koratzanis G, Chryssouli Z, Giamarellou H. Emerging bacterial pathogens: *Escherichia coli*, Enterobacter aerogenes and *Proteus* mirabilis clinical isolates harbouring the same transferable plasmid coding for metallo-beta-lactamase VIM-1 in Greece. J Antimicrob Chemother 2007;59:578-9.
4. Villegas MV, Lolans K, Correa A, Suarez CJ, Lopez JA, Vallejo M, *et al*. First Detection of the Plasmid-Mediated Class A Carbapenemase KPC-2 in Clinical Isolates of *Klebsiella pneumoniae* from South America. Antimicrob Agents Chemother 2006;50:2880-2.
5. Nordmann P, Dortet L, Poirel L. Carbapenem resistance in *Enterobacteriaceae*: Here is storm!. Trends Mol Med 2012;18:263-72.
6. Carrër A, Fortineau N, Nordmann P. Use of ChromID Extended-Spectrum β-Lactamase Medium for Detecting Carbapenemase-Producing *Enterobacteriaceae*. J Clin Microbiol 2010;48:1913-4.
7. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. Twentieth Informational Supplement M100-S20 June Update. Wayne, Pennsylvania: CLSI; 2010. Available from: http://www.clsi.org/source/orders/free/m100-s20-u.pdf [Last accessed on August 10th 2012].
8. Castanheira M, Deshpande LM, Mathai D, Bell JM, Jones RN, Mendes RE. Early dissemination of NDM-1- and OXA-181-producing *enterobacteriaceae* in indian hospitals: Report from the SENTRY Antimicrobial Surveillance Program, 2006-2007. Antimicrob Agents Chemother 2011;55:1274.
9. Deshpande P, Rodrigues C, Shetty A, Kapadia F, Hedge A, Soman R. New Delhi Metallo-b lactamase (NDM-1) in *Enterobacteriaceae*: Treatment options with carbapenems compromised. J Assoc Physicians India 2010;58:147-9.
10. Petropoulou D, Tzanetou K, Syriopoulou VP, Daikos GL, Ganteris G, Malamou-Lada E. Evaluation of imipenem/imipenem+EDTA disk method for detection of metallo- beta-lactamase-producing *Klebsiella pneumoniae* isolated from blood cultures. Microb Drug Resist 2006;12:39-43.
11. Girlich D, Poirel L, Nordmann P. Do CTX-M b-lactamases hydrolyse ertapenem? J Antimicrob Chemother 2008;62:1155-6.
12. Centers for Disease Control and Prevention (CDC) Guidance for control of infections with carbapenem-resistant or carbapenemase-producing *Enterobacteriaceae* in acute care facilities. MMWR Morb Mortal Wkly Rep 2009;58:256-60.
13. Galani I, Rekatsina PD, Hatzaki D, Plachouras D,Souli M, Giamarellou H. Evaluation of different laboratory tests for the detection of metallo-b-lactamase production in *Enterobacteriaceae*. J Antimicrob Chemother 2008;61:548-53.
14. Rai S, Manchanda V, Singh NP, Kaur IR. Zinc-dependant carbapenemases in clinical isolates of family *Enetrobacteriaceae*. Indian J Med Microbiol 2011;29:275-9.
15. Anderson KF, Lonsway DR, Rasheed JK, Biddle J, Jensen B, McDougal LK, *et al*. Evaluation of Methods To Identify the *Klebsiella pneumonia* Carbapenemase in Enterobacteriaceae. J Clin Microbiol 2007;45:2723-5.
16. Carvalhaes CG, Pica˜ o RC, Nicoletti AG, Xavier DE, Gales AC. Cloverleaf test (modified Hodge test) for detecting carbapenemase production in *Klebsiella pneumoniae*: Be aware of false positive results. J Antimicrob Chemother 2010;65:249-51.
17. Noyal MJ, Menezes GA, Harish BN, Sujatha S, Parija SC. Simple screening tests for detection of carbapenemases in clinical isolates of nonfermentative Gram negative bacteria. Indian J Med Res 2009;129:707-12.

How to cite this article: Ramana KV, Rao R, Sharada C, Kareem MA, Reddy LR, Ratna Mani MS. Modified Hodge test: A useful and the low-cost phenotypic method for detection of carbapenemase producers in *Enterobacteriaceae* members. J Nat Sc Biol Med 2013;4:346-8.

Source of Support: Apollo Hospitals, Hyderabad. **Conflict of Interest:** None declared.