

Research Article



Detection of carbapenem resistant gram-negative bacteria in clinical isolates from a tertiary care hospital

Abstract

Carbapenem resistance is one of the major threats faced in antimicrobial treatment of infections caused by Gram negative organisms. In India and throughout the world the use of carbapenems has increased resulting in an increased resistance of pathogens to this class of antibiotics. Bacteria are capable enough to become resistant to antibiotics by a number of mechanisms both intrinsic and acquired, most common of which include enzymatic degradation of antibiotics. Carbapenem resistant Gram-negative bacteria usually spreads in the hospital settings to other patients and caregivers or relatives by unwashed hands or from contact with soiled equipment and surfaces such as bedrails, tables, chairs, countertops and door handles. These carriers are the ultimate sources of dissemination in the community. Detection of carbapenems is a crucial infection control issue because they are often associated with extensive antibiotic resistance, treatment failures and infection-associated mortality. The present study was undertaken to determine Carbapenem resistant Gram-negative bacteria in clinical isolates from a tertiary care hospital.

Keywords: carbapenemases, gram negative bacteria, modified hodge test, EDTA disk synergy test

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Abbreviations: MHT, modified hodge test; ESBLs, extended-spectrum b-lactamases; NFGNB, non-lactose fermenting gram negative bacilli; CLSI, clinical laboratory standard institute; MHA, mueller hinton agar; MIC, minimum inhibitory concentration

Introduction

Multidrug-resistant Gram-negative bacteria are a major public health threat. However, intense efforts to limit their spread, the number of multidrug resistant Gram-negative bacteria continues to increase globally. Carbapenems, the most broad-spectrum beta lactam antibiotics active against Gram-negative organisms, are very slowly hydrolyzed by most beta-lactamases. Because of their broad-spectrum activity, carbapenems have served as the last line of defense against multidrug-resistant Gram-negative organisms since their introduction in the early 1980s.¹

Carbapenemases-betalactamases hydrolyze carbapenem class of antibiotics efficiently. Such Carbapenemases, for e.g. the serine carbapenemases SME and the metallo-beta-lactamase IMP-were detected in *Enterobacteriaceae* in the 1980s.¹ The use of Carbapenems for the treatment of infections caused by Gram-negative organisms which already harbour extended-spectrum b-lactamases (ESBLs) and AmpC b-lactamases has led to selection of clinical isolates with carbapenem resistance.² Carbapenemases are enzymes produced by organisms which hydrolyse almost all b-lactams and are thus not inhibited by b-lactamase inhibitors. These Carbapenemase enzymes have been identified in *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* and these organisms are often found to be multidrug resistant.³

Bacteria are capable enough to become resistant to antibiotics by a number of mechanisms both intrinsic and acquired, most common of which include enzymatic degradation of antibiotics.⁴

The carbapenemases producing Gram negative bacteria are easily transmitted among humans. These Carbapenemase producing bacteria acquire genetic material from each other through horizontal gene transfer by the means of plasmids and by transposons.⁵ Increasing resistance to carbapenems is now frequently being observed in many hospital acquired and several community-acquired infections.⁶

The present research was done for the detection of patients and carriers with Carbapenemase producing Gram negative bacilli. It is necessary for prevention of their nosocomial outbreak as patients colonized with Carbapenemase producers can be an important source of transmission in a healthcare setting. Hence, identifying patients colonized with Carbapenemase producing Gram negative bacilli and placing those patients in isolation may be an important step in preventing transmission of these nuisance pathogens. These detection tests may help practitioner identify Carbapenemase producing Gram negative bacilli early and thus preventing the wastage of antibiotics. Some other alternatives may be thought of on the basis of the antibiotic resistance pattern. This research is novel in the way that all the Carbapenemase producer detection tests are informative about the epidemiological issues and this study is novel in our area in India.

Materials and methods

This clinical study was carried out at Gian Sagar Medical College and Hospital, Punjab (India) from October 2014 to May 2015. From different clinical samples like endotracheal secretions, blood, urine, pus, and bronchial aspirates, a total non-duplicative, 300 isolates of Gram negative bacteria which includes 180 isolates of *Escherichia coli*, 50 isolates of *Klebsiella* species, 50 isolates of *Acinetobacter* species and 20 isolates of *P. aeruginosa* were included in this study.

The isolates were identified as lactose fermenting or non-lactose fermenting Gram negative bacilli (NFGNB) on the basis of colony characteristics, Gram staining and a panel of biochemical reactions. The antimicrobial susceptibility of clinical isolates was performed by the Kirby Bauer's disc diffusion technique on Mueller-Hinton agar, as per Clinical Laboratory Standard Institute (CLSI) guidelines. Isolates with reduced susceptibility to meropenem and imipenem (diameter of zones of inhibition ≤ 13 mm) by disc diffusion method were screened for the production of carbapenemase.

Phenotypic detection of carbapenemases

A phenotypic detection of carbapenemases was done by Modified Hodge test and EDTA disk synergy test.

Modified hodge test (MHT): In Modified Hodge Test the growth was suspended in Normal saline and matched to Mcfarland standard (0.5). It was diluted 1:10 by adding 0.5ml of the Mcfarland to 4.5ml of saline. Standard strain of *E. coli* (ATCC 25922) was first inoculated on the Mueller Hinton Agar (MHA) plate as lawn culture. A 10 μ g Imipenem disk was placed at the centre of the plate and each clinical isolate was streaked from the disk to the edge of the plate and the later was incubated at 37°C for 12hours.⁷

After incubation period, the plates were examined for a clover leaf type of pattern of indentation at the intersection of growth of the test organism and the standard strain *E. coli* ATCC 25922, within the zone of inhibition of the Imipenem disc.¹² Interpretation of Modified Hodge Test: A positive test shows a clover leaf like pattern of indentation of *E. coli* ATCC 25922 which grows along the growth of test isolate within the zone of disc diffusion. A negative test shows no growth of *E. coli* ATCC 25922 along the growth of test organism within the zone of disc diffusion.⁷

EDTA disk synergy test: EDTA Disk synergy test was used for the detection of metallo- β - lactamases in the imipenem and meropenem resistant clinical isolates. First of all, an EDTA solution with 0.5M strength was prepared by dissolving 186.1g of Disodium EDTA in 1000ml of distilled water. The pH was adjusted to 8.0 and the solution was sterilized by autoclaving. An overnight culture broth of the test isolate was adjusted to a turbidity of 0.5 McFarland standard and was spread on the surface of a Mueller Hinton Agar plate. A 10 μ g imipenem disk (HI - MEDIA) was placed on the agar surface. A blank disk (6mm diameter) was then kept on the inner surface of the lid of the Muller Hinton Agar plate and 10 μ l of 0.5 M EDTA was poured onto it with the help of an auto pipette. This EDTA disk was then placed on the surface of agar and was kept about 10mm edge-to-edge apart from the imipenem disk. After overnight incubation at 37°C, the presence of an expanded growth inhibition zone between the two disks was interpreted as positive for MBL production.⁸

Detection of minimum inhibitory concentration (MIC) by different methods

MIC by Agar dilution method: Agar Dilution Method used to determine the minimal inhibitory concentration of antimicrobial substances. Minimum inhibitory concentration (MIC) of each bacterial isolate against imipenem was determined by agar dilution method according to CLSI guidelines. In Agar dilution method a varying concentrations of antibiotic were incorporated in series of agar plates onto which a standardized suspension of the test isolate was inoculated. The drug dilution was 10times the required concentration. Mix 2ml of each dilution with 18ml of molten agar at 50°C per plate. After incubation, the lowest concentration of the agent which shows no growth of test organism was the MIC.⁹

Results

A total no of 300 isolates of Gram negative bacteria were included in this study. These 300 isolates included 180 *E.coli* isolates, 50 *Klebsiella* species isolates, 50 *Acinetobacter* species isolates, 20 of *P. aeruginosa* isolates. Out of these 300 isolates, 44 isolates of Gram negative bacteria were found resistant to imipenem by Kirby Bauer's disc diffusion method. The 44 isolates that were found resistant to imipenem included 7 *E.coli* isolates, 7 *Klebsiella* spp. isolates, 28 *Acinetobacter* species, 2 *P. aeruginosa* isolates. All these 44 isolates showed MIC values for imipenem ranging from 0.5 to 64 μ g/ml. Among 44 isolates tested, 44 isolates were found to be Metallo-Beta Lactamases producers by EDTA disk synergy method. All these 44 isolates were found to be carbapenemase producer by Modified Hodge Test.

Discussion

Carbapenem resistance in Gram-negative bacteria is increasingly encountered in healthcare-associated infections in India. Bacteremic episodes due to these organisms carry a high mortality as shown by previous studies from other countries.¹⁰ From India various studies have found different rates of carbapenem resistance. In August 2004 and July 2005 a study was conducted in Aligarh. In this study overall Imipenem resistance was 12% for *Klebsiella* species.¹¹ In July 2011 to January 2013 a study was conducted in Meerut which showed 5-6% carbapenem resistant in *Enterobacteriaceae*.¹² Aswani et al.,¹³ found 7% carbapenem resistance in *E.coli* and 5% carbapenem resistant in *Klebsiella* species.¹³ In other developing countries from African continent, the prevalence of carbapenemase producing bacteria ranged from 2.3% to 6.7% in North Africa and from 9% to 60% in Sub - Saharan Africa.¹⁴

In the present study, the overall resistance to carbapenems was 14.6% which is in comparison with the study of Manoharan and Premalatha et al.,¹⁵ who reported 17% resistance to carbapenems in *Enterobacteriaceae*¹⁵ Also, Priya dutta et al.,¹⁶ Wattal C et al.,¹⁷ and Gupta E et al.,¹⁸ showed 7.87%, 13-57% and 17-22% resistance to carbapenems respectively. The MHT screening test for carbapenemases is currently proposed by the Clinical and Laboratory Standards Institute (CLSI) for phenotypic screening of Carbapenemase producers (Table 1). The MHT method is easy to perform, but diverse specificity values have been reported by authors, so should be aware of false-positive results (Figures 1-3).¹⁹

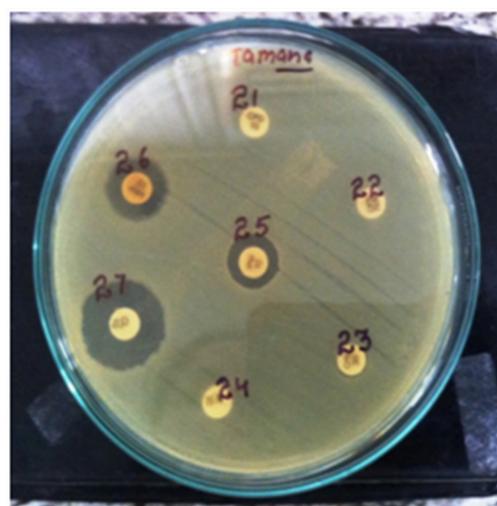
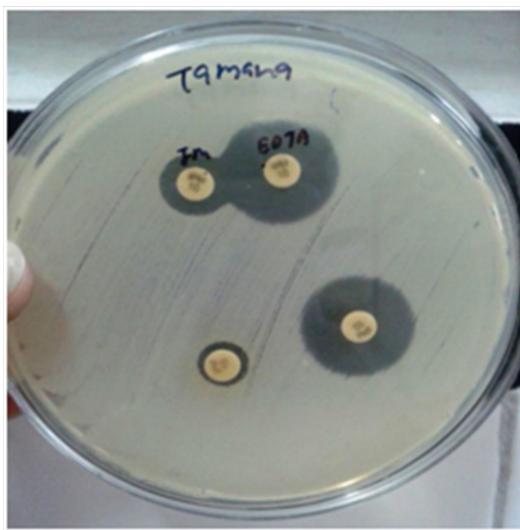


Figure 1 Kirby Bauer's method.

**Figure 2** EDTA disk synergy method.**Figure 3** Modified hodge method.**Table 1** Phenotypic characterization and distribution of Carbapenemase producer from clinical isolates

S. no	Bacterial strains isolated	Total no of isolates	Carbapenemase production by:			Percentage of carbapenemase producers (%)
			Disc diffusion method	Modified hodge method	EDTA disk synergy method	
1	<i>E.coli</i>	180	7	7	7	3.8
2	<i>Klebsiella</i> species	50	7	7	7	14
3	<i>Acinetobacter</i> species	50	28	28	28	56
4	<i>Pseudomonas aeruginosa</i>	20	2	2	2	10
Total		300	44	44	44	

Conclusion

Carbapenemase production in Gram negative bacteria is not the cause of specific types of clinical infections. The role of these bacteria is related to the difficult-to-treat infections rather than to expression of specific virulence traits due to non availability of higher drugs. Carbapenem resistance in Gram-negative bacteria is increasingly encountered in healthcare-associated infections in India. But, active surveillance, hand hygiene, contact precautions, and appropriate antibiotic usage form an effective approach in reducing the incidence of infections caused by these life threatening microorganisms. Non molecular tests for detection of carbapenemases have variable results for Modified Hodge Test, EDTA disk synergy test, MIC by Agar Dilution Test. Out of these three tests the Modified Hodge Test often lacks specificity (false positive results for high - level Ampicillin C producers) and sensitivity (weak screening of NDM producers). But this test works well for the detection of KPC and OXA - 48 producing Gram negative bacteria.

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Conflict of interest

The author declares no conflict of interest.

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