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Research Article



Detection of carbapenemase production in Enterobacteriaceae using the modified Hodge test

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Abstract

The recurrence of carbapenem-resistant Enterobacteriaceae cases is alarming with potential therapeutic dead-ends that may have serious clinical consequences. Thus, this study aims to perform the modified Hodge test as phenotypic screening for carbapenemase production. This study was conducted at the bacteriology unit of the medical biology laboratory at Diamniadio Children's Hospital in the Dakar region of Senegal between June 2022 and June 2023. The 17 isolates came from pus samples, swabs, and urine collected from patients in neonatology and surgical hospitalization departments. Strains showing intermediate or resistant zones of 17 mm to 22 mm for imipenem and meropenem were subjected to the Modified Hodge Test, with comparison to MIC. Of the 17 strains, 15 were positive (88%) for carbapenemase production by modified Hodge test. The carbapenemase-producing organisms were *Enterobacter* spp (40%), *Klebsiella pneumoniae* (33%), *Escherichia coli* (20%), *Citrobacter freundii* (07%). In the absence of advanced technical platforms, the Hodge test is a good tool for confirming carbapenemase production, however some limitations exist and should be complemented by molecular tests.

Keywords: Modified hodge test, Carbapenemase, Antimicrobial resistance

Introduction

Infections with antibiotic-resistant Enterobacteriaceae present as a major challenge in medical facilities. Resistance to antimicrobial agents correlates with alarming outcomes, including increased mortality, prolonged hospital stays, and higher costs. Often, delays in implementing effective treatment, reduced final therapeutic efficacy, and increased virulence of certain resistant strains are responsible for antimicrobial resistance.

Beyond extended-spectrum β -lactamases (ESBLs) (Allali et al., 2015), another enzyme inducing resistance in Enterobacteriaceae is carbapenemase (Bonomo et al., 2018). Its mode of action is the hydrolysis of carbapenem-class antibiotics, which are known to be stable against ESBLs and

are considered last-resort drugs for infections caused by multidrug-resistant strains. However, precise detection of carbapenem resistance in Enterobacteriaceae is not straightforward with classical disk diffusion antibiograms. These enzymes belong to three Ambler classes of β -lactamases: A, B and D (Hammoudi Halat & Ayoub Moubareck, 2020). Although several class C β -lactamases (AmpC) (Jousset et al., 2019) have been described, their producers typically show reduced carbapenem sensitivity due to the enzyme's low catalytic efficiency and permeability defects; therefore, they are not considered true carbapenemases.

Class A carbapenemases are serine β -lactamases, including several enzymes such as KPC types (Bratu et al., 2005; Woodford et al., 2004; Yigit et al., 2001). Class B

carbapenemases are metallo-β-lactamases (MBLs) with enzymes like IMP (Limbago et al., 2011), VIM (Roschanski et al., 2017), and NDM (Hornsey et al., 2011; Nordmann et al., 2012; Tada et al., 2014; Yong et al., 2009), characterized by requiring zinc ions at their active site. Class D carbapenemases are serine β-lactamases including OXA-type enzymes (Poirel et al., 2004).

Carbapenemase detection is complex and can be achieved using phenotypic and genotypic methods. Beyond antibiograms and molecular biology, a relatively simple phenotypic test useful for laboratories is the Modified Hodge Test.

This study aims to screen for carbapenemase-producing Enterobacteriaceae, as these enzymes do not always generate visible resistance zones to carbapenems using standardized susceptibility testing methods. Thus, misinterpretation may lead to therapeutic failure and spread of resistant strains.

Material & Methods

This study was conducted at the bacteriology unit of the medical biology laboratory at Diamniadio Children's Hospital in the Dakar region of Senegal. Isolates came from pus samples, swabs, and urine collected from outpatients and hospitalized patients. After culture and identification, carbapenem susceptibility was assessed by disk diffusion on Petri dishes by measuring inhibition diameters. Resistant strains were confirmed for carbapenemase production using the modified Hodge test.

A 0.5 McFarland dilution of Escherichia coli ATCC 25922 in 5 ml of broth or saline solution was prepared. A 1:10 dilution was inoculated as for antibiograms on Mueller-Hinton agar. A 10 µg meropenem or ertapenem susceptibility disk was placed at the test zone center. The test strain was streaked in a straight line from the disk edge to the plate edge. The plate was incubated overnight at 35±2°C in ambient air for 16-24 hours. The same was done with positive and negative control strains for quality control per CLSI recommendations.

After 24h, a positive test indicates carbapenemase production by the tested microorganism. By producing carbapenemase, the tested microorganism can inactivate the carbapenem diffusing from the disk. This allows the carbapenem-susceptible E. coli ATCC 25922 strain to grow toward the disk. A negative test shows no E. coli 25922 growth along the test strain's growth streak in the disk diffusion zone.

Our modified Hodge test evaluation was complemented by MIC testing on the same isolates.

Result

Of 17 strains showing intermediate or resistant zones (17-22 mm for imipenem and meropenem), 15 (88%) were positive for carbapenemase production by modified Hodge test. Carbapenemase-producing organisms were Enterobacter spp (40%), Klebsiella pneumoniae (33%), Escherichia coli (20%), Citrobacter freundii (07%).

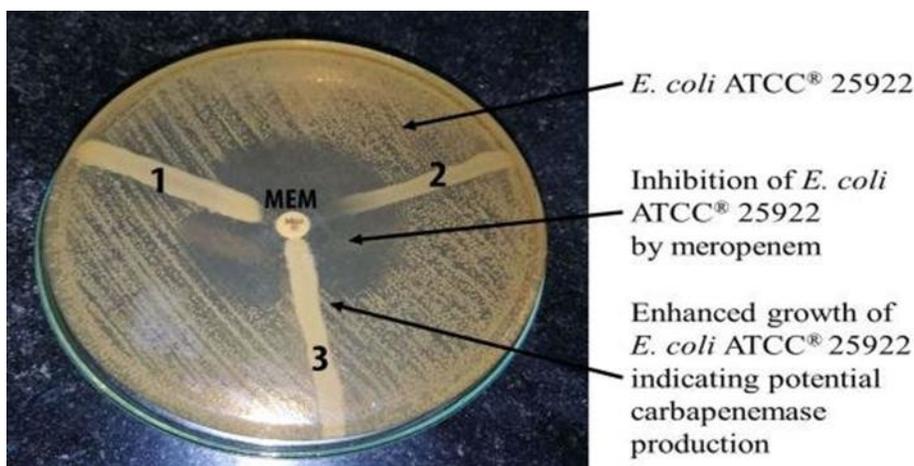


Figure 1. Illustration of a Modified Hodge Test result on Mueller-Hinton medium

Table 1. Distribution of positive Modified Hodge Test results by species

Species	Number of isolates
Enterobacter spp	6 (40%)
K. pneumoniae	5 (33%)
E. coli	3 (20%)
C. freundii	1 (07%)

Table 2. Comparison of modified Hodge test (MHT) and CIM test results for carbapenemase detection

Species (No. of isolates)	MHT Positive (%)	CIM Positive (%)	Concordance MHT/CIM (%)	Concordance Discordances (MHT+/CIM- or MHT-/CIM+)	Discordance Interpretation
Enterobacter spp. (6)	6/6 (100%)	6/6 (100%)	100%	0	-
Klebsiella pneumoniae (5)	5/5 (100%)	5/5 (100%)	100%	0	-
Escherichia coli (3)	3/3 (100%)	2/3 (66.7%)	66.7%	1	OXA 48 ?
Citrobacter freundii (1)	1/1 (100%)	1/1 (100%)	100%	0	-

Discussion

Carbapenem-resistant Enterobacteriaceae remain a global public health threat over thirty years after their initial discovery. Carbapenem resistance in these bacteria can occur through three main mechanisms: enzyme production, efflux pump activation, and porin mutations (Suay-García & Pérez-Gracia, 2019). The modified Hodge test provides phenotypic insight into these resistances (Amjad et al., 2011). In our study, Hodge test results matched antibiogram suspicions in 88% of cases, confirming carbapenemase production. This allows urgent diagnostic confirmation while awaiting PCR.

Though less precise than PCR and genotyping, the modified Hodge test has been studied for its sensitivity, specificity, and limitations. Work adding zinc to culture media showed high sensitivity for detecting class A, B, and D carbapenemases but lacked specificity (Girlich et al., 2012). MHT results in Enterobacteriaceae demonstrated its utility for identifying KPC strains, with negative tests excluding 100% of KPC isolates (Cury et al., 2012), though limitations exist particularly for OXA-48.

The modified Hodge test is an updated version of the original Hodge test. The original evaluated PCR-confirmed isolates producing IMP-1 and VIM-2 metallo- β -lactamases (MBLs). It used 10 μ g imipenem disks, detecting ~67% of MBL-producing *Pseudomonas aeruginosa* and *Acinetobacter* spp. However, some results were ambiguous/negative. Testing with zinc sulfate-containing imipenem disks resolved these cases (Lee et al., 2003).

Conclusion

Discovered nearly three decades ago in Gram-negative bacteria, carbapenemases represent a growing public health threat. Their encoding genes are now widespread in the bacterial world. Despite performance limitations and lacking advanced platforms (VITEK, PCR), the Hodge test remains a valuable carbapenemase detection tool but requires cautious interpretation.

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