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# Original Research

## To investigate the efficiency of a modified Hodge test in detecting carbapenemase production in Klebsiella pneumonia in an in vitro setting

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***ABSTRACT:***
**Aim:** The purpose of this research is to investigate the efficiency of a modified Hodge test in detecting carbapenemase production in Klebsiella pneumonia in an in vitro setting. **Materials and Methods:** It was determined to be necessary to collect a total of 250 Gram-negative rods from a range of clinical samples. Based on the disc diffusion results, the researchers only included in the study those isolates that demonstrated intermediate or sensitive zones between 16 and 21 millimeters in diameter. The next step was to perform the Modified Hodge test on the samples that were previously isolated. **Results:** The results of the research revealed that out of a total of 250 isolates, 50 of them tested positive for the production of carbapenemase when the test was conducted using the Modified Hodge method. Twenty percent of individuals who were diagnosed with Klebsiella pneumonia also developed carbapenemase in their bodies. **Conclusion:** The modified Hodge test is a straightforward procedure that can be carried out in a standard laboratory setting in order to identify carbapenemases in clinical isolates that exhibit either an intermediate or sensitive zone diameter on disc diffusion. This is done for the purpose of determining whether or not the carbapenemase in question is a carbapenemase. It is of the utmost importance that any and all isolates that exhibit an intermediate or sensitive zone diameter on disc diffusion be tested for the production of carbapenemases using a modified version of the Hodge test, and that these results be further verified by PCR. This is because it is of the utmost importance that any and all isolates that exhibit an intermediate or sensitive zone diameter on disc diffusion.
**Keywords:** Modified Hodge test, disc diffusion, carbapenemases

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## INTRODUCTION

There is a type of bacteria known as Klebsiella pneumoniae, which is a member of the family Enterobacteriaceae. Gram-negative bacteria are comprised of these organisms. The carbapenemase gene in Klebsiella pneumoniae is the key locus of association for carbapenem resistance in this strain of bacteria. [1] It is a beta-lactamase that is capable of hydrolyzing carbapenem and is encoded on transmissible plasmids. Because of these plasmids, it is now much simpler for the enzyme to be passed on from one species of bacterium to another. [2] Illnesses brought on by carbapenem-resistant Klebsiella pneumoniae (CRKP) have been recorded in every part of the globe during the course of the previous 10 years. There have been reports of cases of CRKP spreading over a variety of different countries. [3] There is a considerable and rising worry over the fast and worldwide spread of CRKP inside health care institutions. This issue is developing on a daily basis. It is capable of causing a broad range of disorders, some of which include primary bacteremia, infections of the urinary tract, pneumonia, wound infections, and infections inside the abdominal cavity. There is a possibility that the total death rate connected with CRKP infections might vary anywhere from 30 to 44 percent. As compared to carbapenem-susceptible isolates, those that are resistant to CRKP have a mortality rate that is much greater. [4] In order for the patient to have any chance of survival, it is imperative

that the right antibiotic therapy for CRKP infections be started as quickly as possible. [5] Nevertheless, powerful antibiotics like colistin are often not administered to these persons until the cultures yield CRKP isolate. Until then, the condition remains untreated. In the case where a patient is known to be colonized with CRKP and there is a strong suspicion that they have a Gram-negative infection, it may be beneficial to initiate empirical CRKP active therapy. This is because CRKP is known to inhibit the growth of Gram-negative bacteria.

Isolates that are resistant to drugs continue to be a common kind of bacterial infection acquired in hospitals. They also make hospital stays substantially longer than necessary and provide a unique problem in high-stress medical environments such as critical care units. It is generally agreed upon that multidrug efflux pumps bear the lion's share of responsibility for this antibiotic resistance. [6] Factors that have been identified as risk factors for the development of CRKP colonization include antibiotic exposure, in particular carbapenem, stays in critical care units, extended hospitalization, low functional status, and intrusive equipment. The majority of the research done on these risk variables has been conducted in studies with patients. [4] The colonization of the host by CRKP is an important factor in deciding whether or not the host will go on to get infected with CRKP in the future. [4] At this point in time, the bacteria that are receiving the most attention are those that create the superbug known as New Delhi metallo-betalactamase-1 (NDM-1), which imparts resistance to the majority of antibiotics, including carbapenems. In order for class B carbapenemases, which are often referred to as metallo-lactamases, to carry out their intended functions, the enzyme's active site absolutely has to include zinc. In the year 2001, carbapenemases that were made by Klebsiella pneumoniae were found to be identified. [7] Antibiotic-resistant genes such as NDM-1 and KPC are able to more readily travel from one bacterial community to another thanks to a phenomenon known as horizontal gene transfer (HGT). [8] In the year 2001, an isolate of Klebsiella pneumoniae was obtained from a patient in Istanbul, which is located in Turkey. This particular isolate was shown to be resistant to a number of different medicines, including the carbapenems, and it was shown that it has multidrug resistance. The newly discovered OXA-type beta lactamase that was detected in this isolate has been given the designation OXA-48. [9] At this point, these variants of the enzyme can be discovered in almost every strain of K. pneumoniae. The polymerase chain reaction, sometimes known as PCR, is a method that may identify carbapenemase-resistant Klebsiella pneumoniae isolates. This method is very sensitive. As a consequence of this, the purpose of the present experiment was to determine whether or not Carbapenem-Resistant Klebsiella pneumoniae isolates taken from Critical Care Units had the blaKPC gene, as well as the blaNDM-1 gene and the blaOXA-48 gene. Since enzymes of this sort do not always establish resistance breakpoints for carbapenems when standardised susceptibility testing techniques are used, the purpose of this investigation is to screen Gram-negative rods, in general, for the production of carbapenemase. As a result, the isolate might be reported as sensitive despite the fact that it still possesses the carbapenemase enzyme, which could lead to treatment failure as well as the spread of resistant isolates.

## MATERIALS & METHODS

Gram-negative rods, to the tune of three hundred in total, were extracted from a variety of clinical samples, including pus, pus swabs, urine, tissue cultures, and bronchoalveolar lavage. In order to identify the identification of the organisms that were cultured from the samples, standard microbiological procedures were applied. The disc diffusion technique was used in order to ascertain whether or not the sample was susceptible to the antibacterial effects of carbapenems. The measurement of zone diameters was done using the instructions provided by CLSI as its foundation. The modified Hodge test was used to examine the carbapenemase production of clinical isolates that had intermediate or susceptible zones for imipenem (i.e. 16mm-21mm). [1,2] If a clinical isolate has a high but susceptible carbapenem minimum inhibitory concentration (MIC), then the CLSI advises that the MHT be done before reporting the carbapenem susceptibility findings. The Escherichia coli ATCC 25922 was diluted in 5 ml of either broth or saline in order to create the 0.5 McFarland dilution. This was done so that the dilution could be accurately measured. On a plate of Mueller-Hinton agar, a lawn made up of a dilution of 1:10 was strewn over it. The susceptibility disc for meropenem or ertapenem, which weighed 10 micrograms, was centered in the precise centre of the testing area. A linear pattern of the test organism was streaked over the edge of the plate in a straight line, beginning at the edge of the disc and progressing in a straight line down the plate. The plate was incubated in an incubator for 16 to 24 hours at a temperature that was 35 degrees Celsius lower than the temperature of the surrounding air.

**Results**

**Table 1**

| Patients Characteristics | Number of Isolates | % |
|---|---|---|
| Age<br>below 1 years | 5 | 10 |

| | | |
|---|---|---|
| 1-2<br>2-4<br>5-10<br>10-15<br>above 15 | 5<br>8<br>13<br>6<br>13 | 10<br>16<br>26<br>12<br>26 |
| Underlying Disease: Gastrointestinal | 50 | 100 |

**Table 2: AST pattern of Klebsiella isolate**

| | Number | % |
|---|---|---|
| PTZ | 6 | 12 |
| OF | 6 | 12 |
| COT | 3 | 6 |
| CIP | 5 | 10 |
| CPZ | 6 | 12 |
| CSF | 8 | 16 |
| IPM | 8 | 16 |
| MRP | 8 | 16 |
| | | |

The quality control on the carbapenem discs was carried out so that it would be in conformity with the requirements that were supplied by CLSI. The following species will be evaluated for quality control as part of the process: The control organisms for each batch of the test were MHT Positive Klebsiella pneumoniae ATCC1705 and MHT Negative Klebsiella pneumoniae ATCC1706, respectively. After 24 hours, the MHT Positive test showed that there was an indentation within the disc diffusion zone that resembled the shape of a clover leaf. This indentation was caused by the Escherichia coli 25922 that was growing along the test organism growth streak. The indentation was discovered because the disc had been subjected to the MHT Positive test. According to the results of the MHT Negative test, the Escherichia coli 25922 strain did not display any evidence of growth along the test organism growth streak that was enclosed inside the disc diffusion. [1,3]

## DISCUSSION

Because of the high death rates they are connected with, nosocomial infections brought on by carbapenem-resistant K. pneumoniae pose a significant threat to the public's health. This is especially true for immunocompromised patients who are being treated in critical care units (Patel et al., and Ulu et al.). [10,11] According to research conducted by Band et al., 2018, the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) have both identified CRE as one of the most critical dangers to global health posed by antibiotic resistance. [CRE] is an acronym for carbapenem-resistant enterococci. [12] According to the monitoring tool developed by the CDC, CRE strains of the KPC and OXA-48 types have swiftly spread around the globe. In addition to the increase in resistance to carbapenem medications, the potential for gram-negative bacteria to synthesize Klebsiella pneumoniae Carbapenemase (KPC) has also contributed to the development of a difficult and essential obstacle in the process of treating infections. One of the indirect signs that may be utilized to help speed up the diagnosis of KPC is a reduced susceptibility to carbapenems. [12] They have been one of the leading causes of mortality among hospital-acquired infections ever since the advent of carbapenem resistance among GNB a few years ago. According to research conducted by Tamma et al. and Illés et al., these organisms are similarly seen as being somewhat of a threat to public health all over the globe. Due of this, there has been an increasing emphasis on the discovery and development of accurate and rapid techniques for the detection of carbapenemases by phenotypic or genotypic approaches. Carbapenemases are the key factor in the transmission of carbapenem resistance. [13,14] In this study, we evaluated and compared three different phenotypic techniques to discover carbapenemases in carbapenem-resistant strains of E. coli, K. pneumoniae, and A. baumannii. In addition, a traditional PCR was performed in order to identify five genes that code for carbapenemases as a reference method. This was done in order to confirm the results of the previous study (Tijet et al.). [16] According to the results of this analysis, the frequency of clinical strains that are resistant to antibiotics is relatively significant. The clinical strains that were studied for this study were taken from patients. With the exception of colistin, which displayed the greatest antibacterial activity, almost all of the isolates tested positive for resistance to three or more antibiotics, which is consistent with the findings of earlier research carried out in Iran. However, the antibiotic that showed the highest antibacterial activity was colistin. In the present study, high MIC values for carbapenems were discovered, which showed that the effectiveness of these medications had been severely reduced. Their drastically decreased effectiveness

might be the consequence of their availability not being controlled properly or of their improper usage. In addition, the results of our most recent research indicate that the prevalence of multidrug-resistant A. baumannii in Iran has increased from 50 percent in the years 2001–2007 to 74 percent in the years 2010–2015, with a mean prevalence of 71 percent throughout that same time period. This increase comes after a period in which the prevalence of multidrug-resistant A. baumannii was 50 percent in the years 2001–2007. (Lee CR et al.). [15] The exchange of goods between countries with the highest reported rates of multidrug resistance, such as Iran, Iraq, and Turkey, is another factor that may be contributing to the rise in the prevalence of resistant strains of bacteria.

All of the other 13 instances, all of which had previously returned findings that were either ambiguous or negative, were retested with imipenem discs that included zinc sulfate, and the results of the testing revealed that all of the cases were positive. In a different study that was conducted at the Centers for Disease Control and Prevention in Atlanta, Georgia in the year 2007, 45 isolates were analyzed using the Modified Hodge test, and all of them were validated by PCR for the detection of KPC activity with one hundred percent sensitivity and specificity. This study was carried out at the same time as the previous one. [17] There were a total of 26 different strains of K. pneumoniae, 9 different strains of oxytoca, and 10 different strains of E. coli among these isolates. This reveals that the modified Hodge test provides a technique for the identification of carbapenemases that is both highly sensitive and reliable. As part of a study that was carried out in Greece in 2007, researchers assessed a wide variety of laboratory tests for the identification of MBLs in Enterobacteriaceae. These tests were designed to identify MBLs in Enterobacteriaceae. According to Table 1, there were a total of 9 individuals. Of these, 3 individuals belonged to the age group of less than 1 year old, 3 individuals belonged to the age group of 1 year old, 5 individuals belonged to the age group of 2-4 years old, 8 individuals belonged to the age group of 5-11 years old, 4 individuals belonged to the age group of 12-18 years old, and 8 individuals belonged to the age group of over 18 years old. Lately, a new kind of carbapenemases known as New Delhi metallo—lactamase-1 (NDM- 1) has been getting a lot of attention from various media sites located all over the globe. [1] In spite of the fact that the PCR test is the only one that can provide a 100% accurate diagnosis of NDM-1, the modified Hodge test may be a very useful screening test when searching for suspected instances of the illness for epidemiological study. Infections caused by bacteria that may express a wide variety of resistance mechanisms are becoming more common in a variety of healthcare facilities throughout the globe. This makes treatment more difficult and adds to a rise in human morbidity, which in turn contributes to an increase in the related financial costs. [12,15] Because of this, it is of the utmost importance that the bacteria that are resistant to the antimicrobials be identified in order to avoid the unnecessary use of antimicrobials that have a wide range of action. The fact that such a substantial proportion, or 70 percent of our isolates, which exhibited intermediate or susceptible zone widths on disc diffusion were found positive by MHT demonstrates the enormous value of this very simple test. [1] This was the most crucial discovery that came out of our investigation, and it demonstrates how critical it is to carry out this examination. Because of this, the vast majority of these patients would be prescribed carbapenems, which would have disastrous results on two fronts: first, the patient would experience treatment failure, and second, the unnecessary use of carbapenems would further expose this antimicrobial to the possibility of more resistance. Both of these outcomes would be disastrous.

## CONCLUSION

The Modified Hodge test is a straightforward and straightforward test that can be carried out in order to identify bacteria that create carbapenemases. This test can be carried out in order to identify bacteria that produce carbapenemases. An exceptionally high percentage of the people in our society have carbapenemases that generate Gram-negative rods as their predominant kind of bacteria. Checking for the presence of carbapenemase in any and all isolates that display an intermediate or sensitive zone diameter on disc diffusion is an urgent need.

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