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ORIGINAL RESEARCH

DETECTION OF CARBAPENEM RESISTANCE IN GRAM NEGATIVE BACTERIA

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ABSTRACT

Background: The development of antibiotic resistance in bacterial isolates is the primary contributor to higher rates of death and morbidity across the globe. Carbapenems, which were originally thought to be useful but are now primarily rendered ineffective due to the appearance of carbapenemase, were once thought to be effective. The purpose of this study was to assess the in vitro efficacy of the modified Hodge test for the detection of carbapenemase synthesis in Gram-negative rods. (klebsiella pneumonia).

Materials and Methods: Between the months of September 2019 and December 2021, the research was carried out at the Department of Microbiology located at Govt General Hospital, Nalgonda. A total of 200 Gram-negative rods from a variety of clinical samples were collected. On the basis of disc diffusion, the researchers only included those isolates for the study that displayed intermediate or sensitive zones between 16 and 21 millimetres. After that, the Modified Hodge test was carried out on these isolated samples.

Results: The outcome of the study showed that out of a total of 300 isolates, 29 were positive for the synthesis of carbapenemase using the Modified Hodge test. The percentage of patients who had Klebsiella pneumonia that produced carbapenemase was 16 %.

Conclusion: The modified Hodge test is a straightforward procedure that can be carried out in a standard laboratory setting for the purpose of identifying carbapenemases in clinical isolates exhibiting either an intermediate or sensitive zone diameter on disc diffusion.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 these results be further verified by PCR.
Keywords: Modified Hodge test, disc diffusion, carbapenemases.

INTRODUCTION

Klebsiella pneumoniae is a kind of bacteria that belongs to the family Enterobacteriaceae. These bacteria are gramme negative. The Klebsiella pneumoniae carbapenemase gene is the primary locus of association for carbapenem resistance in Klebsiella pneumoniae.[1] It is a carbapenem hydrolyzing beta-lactamase that is encoded by transmissible plasmids. These plasmids make it easier for the enzyme to spread between different bacterial species.[2] Over the course of the past ten years, illnesses brought on by carbapenem-resistant Klebsiella pneumoniae (CRKP) have been reported in every region of the world. There have been reports of CRKP outbreaks in a number of different nations.[3] At health care facilities, there is a significant and growing concern regarding the rapid and global spread of CRKP. It is capable of causing a wide variety of diseases, such as primary bacteremia, infections of the urinary tract, pneumonia, wound infections, and infections within the abdominal cavity. The overall mortality rate associated with CRKP infections might range anywhere from 30 to 44 percent. The mortality rate is significantly higher for CRKP strains when compared to carbapenem-susceptible strains.[4] Starting the appropriate antibiotic treatment for CRKP infections as soon as possible is absolutely necessary for the patient's life.[5] Nevertheless, effective antibiotics such as colistin are not usually treated to these individuals until the cultures produce CRKP isolate. If a patient is known to be colonised with CRKP, it may be useful to begin empirical CRKP active treatment in the event that there is a strong suspicion that they have a Gram-negative infection.

Drug-resistant isolates continue to be a prominent hospital-acquired bacterial pathogen. They also add significantly to the length of hospital stays and provide a particular challenge in high-impact medical settings such as intensive care units. It is believed that multidrug efflux pumps are mostly to blame for this antibiotic resistance.[6] Antibiotic exposure, particularly carbapenem, intensive care unit stays, prolonged hospitalisation, poor functional status, and invasive devices have been identified as risk factors for the acquisition of CRKP colonisation. These risk factors have primarily been evaluated in studies involving patients.[4] CRKP colonisation of the host is a crucial element in determining whether or not the host will go on to acquire later CRKP infection.[4] At this time, the bacteria that are attracting the most attention are those that produce the superbug known as New Delhi metallo-betalactamase-1 (NDM-1), which confers resistance to the majority of antibiotics, including carbapenems. The active site of class B carbapenemases, also known as metallo -lactamases, must include zinc in order for the enzyme to function properly. Carbapenemases produced by Klebsiella pneumoniae were discovered in the year 2001.[7] Through a process known as horizontal gene transfer (HGT), antibiotic-resistant genes like NDM- 1 and KPC are able to more easily migrate from one bacterial population to another.[8] In the year 2001, a Klebsiella pneumoniae isolate was received from a patient in Istanbul, Turkey. This isolate was proven to be resistant to multiple drugs, including the carbapenems, and it was discovered that it was multidrug resistant. OXA-48 is the name given to the newly discovered OXA-type beta lactamase that was found in this isolate.[9] These variations of the enzyme can now be found

in almost all strains of K. pneumoniae. The polymerase chain reaction (PCR) is a sensitive technology that has become the gold standard for detecting carbapenemase-resistant Klebsiella pneumoniae isolates. As a result, the current investigation aimed to identify the presence of the blaKPC gene, as well as the blaNDM-1 gene and the blaOXA-48 gene, in Carbapenem-Resistant Klebsiella pneumoniae isolates obtained from Intensive Care Units.This study is being carried out with the intention of screening Gram-negative rods, in general, for the synthesis of carbapenemase because enzymes of this kind do not always create resistance breakpoints for carbapenems when standardised susceptibility testing methods are utilised. Therefore, the isolate can be reported as sensitive despite the fact that it still carries the carbapenemase enzyme, which could lead to treatment failure and the spread of resistant isolates.

MATERIALS & METHODS

This investigation was carried out at the Govt General Hospital, Nalgonda in the Department of Microbiology from September 2019 all the way through December 2021. A total of three hundred Gram-negative rods were isolated from various clinical samples, such as pus, pus swabs, urine, tissue cultures, and bronchoalveolar lavage. Standard microbiological methods were utilized in order to determine the identities of the organisms that were cultivated from the samples. The disc diffusion method was used to determine the antibacterial susceptibility of the sample to carbapenems. CLSI recommendations served as the basis for the measurement of zone sizes. The carbapenemase production of the clinical isolates that showed intermediate or susceptible zones for imipenem, i.e. 16mm-21mm, was evaluated using the modified Hodge test.[1,2] The CLSI recommends that the MHT be performed before reporting carbapenem susceptibility results if a clinical isolate has an elevated but susceptible carbapenem MIC. In order to prepare the 0.5 McFarland dilution, the Escherichia coli ATCC 25922 was diluted in 5 ml of either broth or saline. On a Mueller-Hinton agar plate, a lawn consisting of a 1:10 dilution was streaked across it. The meropenem or ertapenem susceptibility disc, weighing 10 micrograms, was positioned in the exact middle of the testing area. The test organism was streaked in a linear pattern down the edge of the plate, moving from the edge of the disc in a straight line. The plate was kept in an incubator at a temperature of 35 degrees Celsius below ambient air for 16 to 24 hours.

RESULTS

Table 1: Depicts Patient Characteristics and the Number of Isolates

Patients Characteristics	Number of Isolates	%
Age		
< 1 year	3	9 %
1 year	3	9 %
2-4 years	5	15 %
5-11 Years	8	24.8 %
12-18 Years	4	12.4 %
>18 Years	8	24.8 %
Underlying Disease:	17	51 %

Gastrointestinal		

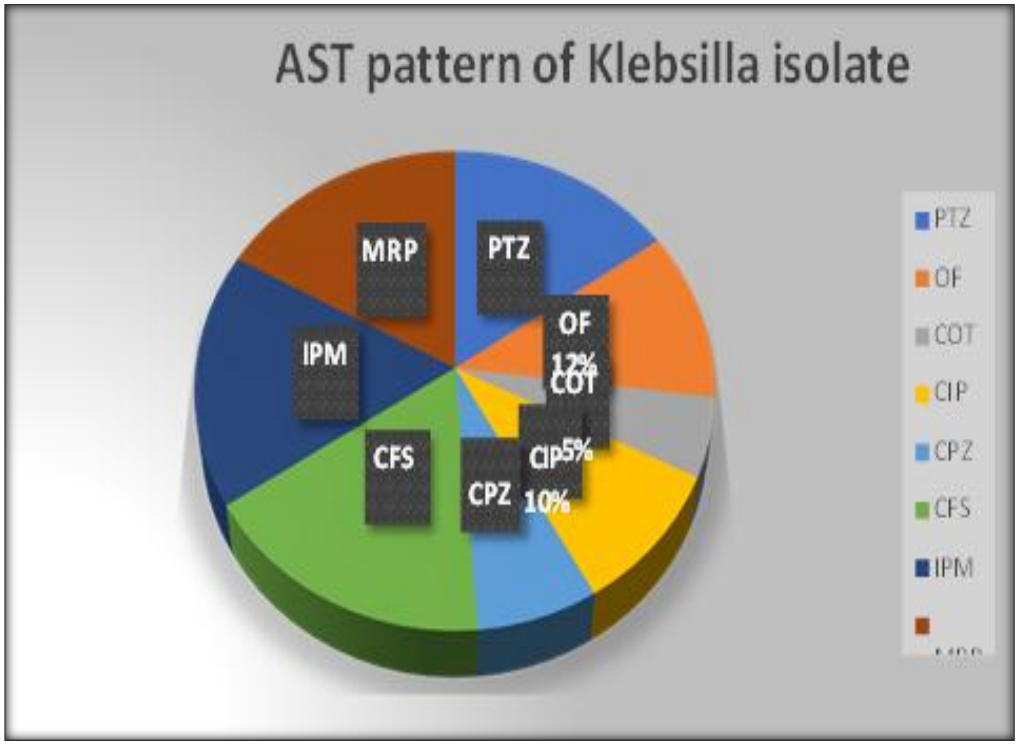

Figure 1: AST pattern of Klebsiella isolate

In accordance with the guidelines provided by CLSI, quality control on the carbapenem discs was carried out. The following organisms will be subject to quality control: Each batch of the test was done using MHT Positive Klebsiella pneumoniae ATCC1705 and MHT Negative Klebsiella pneumoniae ATCC1706 as the control organisms. After 24 hours, the MHT Positive test revealed that there was an indentation within the disc diffusion zone that resembled the shape of a clover leaf caused by the Escherichia coli 25922 that was growing along the test organism growth streak. The MHT Negative test revealed that the Escherichia coli 25922 did not show any signs of growth along the test organism growth streak contained within the disc diffusion.[1,3]

DISCUSSION

Nosocomial infections caused by carbapenem-resistant K. pneumoniae are a serious public health concern because they are associated with high mortality rates, particularly in immunocompromised patients who are treated in intensive care units (Patel etal., 2008; Ulu etal., 2015).[10,11] Band etal., 2018 studied that 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.[12] The CDC tracking tool indicates that KPC and OXA-48 type CRE have rapidly spread around the world. Along with the growth of resistance to carbapenem medicines, the possibility of Klebsiella pneumoniae Carbapenemase (KPC) synthesis among gram-negative bacteria has created a challenging and crucial problem in the treatment of infection. A decreased sensitivity to carbapenems is an indirect sign that can be used to speed up the detection of KPC.[1,12] Since

the introduction of carbapenem resistance among GNB a few years ago, they have become one of the primary causes of death among hospital-acquired infections. According to Tamma etal. 2016 and Illés etal. 2019, these organisms are likewise considered to be a concern to public health all over the world. Because of this, there has been an increased focus on the research and development of precise and speedy methods for the identification of carbapenemases via phenotypic or genotypic methods, which are the primary factor in the propagation of carbapenem resistance.[13,14] In this investigation, we tested and compared three distinct phenotypic approaches for identifying carbapenemases in carbapenem- resistant strains of K. pneumoniae, A. baumannii, and E. coli. In addition, a conventional PCR was carried out in order to identify five genes that code for carbapenemases as a reference approach (Tijet et al. 2016;).[16] The findings of this investigation indicated that the frequencies of antibiotic resistance are quite high among the clinical strains that were investigated. In keeping with the findings of prior research carried out in Iran, practically all of the isolates tested positive for resistance to three or more antibiotics, with the exception of colistin, which showed the highest antibacterial activity. In the current research, high MIC values for carbapenems were found, which indicated a significantly diminished efficacy of these drugs. This greatly reduced efficacy could be the result of their unregulated availability or of their misuse. In addition, our recent research has indicated 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 (Bialvaei et al. 2017).[15] An further likely reason for such a growth in the incidence of resistance is the trade between countries, such as Iran, Iraq, and Turkey, which report the largest number of MDR cases.

The remaining 13 cases, all of which had previously shown equivocal or negative results, were retested with imipenem discs containing zinc sulphate and were found to be positive after the testing. In a different study that was carried out at the Centers for Disease Control and Prevention in Atlanta, Georgia in the year 2007, 45 isolates were evaluated by 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.[17] These isolates included 26 strains of K. pneumoniae, 9 strains of

K. oxytoca, and 10 strains of E. coli (25). This demonstrates that the modified Hodge test is an extremely sensitive and trustworthy method for the detection of carbapenemases. In 2007, researchers in Greece evaluated many different laboratory tests for the detection of MBLs in Enterobacteriaceae as part of a study that was carried out there. According to Table 1, there were 3 individuals who belonged to the age group of less than 1 year old, 3 individuals who belonged to the age group of 1 year old, 5 individuals who belonged to the age group of 2-4 years old, 8 individuals who belonged to the age group of 5-11 years old, 4 individuals who belonged to the age group of 12-18 years old, and 8 individuals who belonged to the age group of over 18 years old. Recently, a new variant of carbapenemases called New Delhi metallo— lactamase-1 (NDM- 1) has been generating a lot of buzz in media outlets all over the world.[1] Even though the PCR test is the only one that can definitively diagnose NDM-1, the modified Hodge test can be an extremely helpful screening test when looking for possible cases of the disease for epidemiological research. Bacterial infections that express diverse resistance mechanisms are becoming increasingly prevalent in many healthcare

institutions throughout the world. This makes treatment more difficult and contributes to an increase in human morbidity as well as the associated financial expenses.[12,15] Because of this, it is imperative that the resistant bacteria be identified in order to prevent the needless application of antimicrobials with a broad spectrum of activity. The fact that quite a large percentage, or 71 percent of our isolates, which showed intermediate or susceptible zone sizes on disc diffusion were detected positive by MHT indicates the huge importance of this simple test.[1] This was the most significant finding of our research, and it shows how important it is to perform this test. As a result, 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.

CONCLUSION

The Modified Hodge test is an uncomplicated and uncomplicated test that may be carried out in order to identify bacteria that produce carbapenemases.In our culture, carbapenemases that produce Gram-negative rods make up an extremely high proportion of the population. It is absolutely necessary to check the generation of carbapenemase in any and all isolates that show an intermediate or sensitive zone diameter on disc diffusion.

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