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Phenotypic Methods for the Detection of Various Betalactamases in Carbapenem Resistant Isolates of Acinetobacter baumanii at a Tertiary Care Hospital

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Abstract

Aim of the Study: *Carbapenem resistance in Acinetobacter baumannii has become a major concern for treating physicians. The reason for this study was to identify the predominance of different beta-lactamases among multi-drug resistant Acinetobacter baumannii from clinical specimens.*

Methods: *October 2024 to May 2024 marked the conduct of a cross-sectional survey. Thirty-three non-repetitive A. baumannii confines resistant to carbapenem were gathered. Testing for AST (Antimicrobial Susceptibility Testing) was done using the Kirby-Bauer disk diffusion technique. The E-test method demonstrated MICs. The MHT (Modified Hodge Test) was used to determine whether carbapenemase production was detected in the tests. Using the EDTA-disk synergy test, the MBL (Metallo-B-Lactamases) in the carbapenem-resistant segregates of A. baumannii were examined.*

Results: *All isolates exhibited resistance to multiple drugs and antimicrobial agents, such as carbapenems, but they were susceptible to colistin. The Modified Hodge test confirmed carbapenemase production in 31 (93.93%) of these confines. The phenotypic strategy revealed that 18 (or 54%) of the separates generated MBL.*

Conclusion: *According to the results of our investigation, one of the main issues is the high prevalence of MBL-generating Acinetobacter baumannii isolates that are resistant to carbapenem. Strict measures for infection control are required in this situation. Hence, it is significant to limit the emergence of pathogens that cause resistance by using the proper anti-microbial approach and limiting the indiscriminate use of carbapenems.*

Keywords

Acinetobacter baumannii, Carbapenem resistance, Metallo-beta-lactamases, Modified hodge test.

INTRODUCTION

Pleomorphic and aerobic, Gram-negative coccobacillus is A. baumannii. It is encapsulated, nonmotile, and does not ferment lactose. It is a member of the Acinetobacter class, which has become “one of the global multidrug-resistant (MDR) nosocomial pathogens.[1] A. baumannii is the cause of many infections, particularly meningitis, septicemia, wound infections, urinary tract infections, and ventilator-associated pneumonia. This” is particularly prevalent in hospitalized as well as immunocompromised patients. A. baumannii clinical strains that “are resistant to third-generation cephalosporins, ureidopenicillins, aminoglycosides, and” fluoroquinolones are typically multidrug resistant.[2] When “extended spectrum β-lactamases and AmpC enzymes produce resistance to beta-lactams, carbapenems are frequently employed as higher anti-microbial to treat serious nosocomial infections caused by multidrug-resistant strains.[3] Enzymes that hydrolyze carbapenems are classified as carbapenemases and are found in classes A, B, & D of the molecular Ambler classification.[4] Either way, over the past few years, carbapenem-hydrolyzing β-lactamases of molecular classes B & D have” increased. However, class B carbapenemases are referred to as MBL because they need one or two zinc ions to function fully catalytically.[5] Because MBLs have the capability to hydrolyze all β -lactam antibiotics, they are regarded as more significant resistance mechanisms than other types. Since there aren't any clinically approved MBL inhibitors at the moment, these enzymes actually pose a risk to human health.[6] [7] Plasmid-mediated horizontal gene transfer facilitates the simple transfer of MBL-encoding genes from one bacterium to another.[8]

The present investigation aimed to differentiate between the incidence of distinct β -lactamases in MDR A. baumannii segregates from clinical specimens.

MATERIALS AND METHODS

October to May of 2024 marked the completion of a cross-sectional study. Thirty-three non-repetitive A. baumannii separates resistant to carbapenem were gathered. Testing for AST “was performed employing the Kirby-Bauer disk diffusion technique. The E-test” method demonstrated MICs. The MHT was used to determine

whether carbapenemase generation had been detected in the tests. A. baumannii's carbapenem-resistant confines had been tested for MBL employing the "EDTA-disk synergy test as well as the double disc synergy test.

Kirby-Bauer Disk Diffusion Method:

The Kirby-Bauer Disk Diffusion" approach had been employed to conduct antibiotic sensitivity testing and determine the sensitivity design of test life forms. A variety of Himedia antimicrobials were tested, including amoxicillin-clavulanic acid (AMC), gentamicin (CN), piperacillin-tazobactam (PTZ), aztreonam (ATM)cefuroxime (CXM), colistin (CL), amikacin (AK), cefixime (CFM), cefotaxime (CTX), ceftazidime (CAZ), , meropenem (MEM), imipenem (Imp), and ciprofloxacin (CIP). Resistance to three or more classes of anti-microbials (quinolones, cephalosporins, and carbapenems, for example) used as treatments for Acinetobacter infections is known as multidrug resistance (MDR).

Modified Hodge Test (MHT):

To find carbapenemases, MHT was applied to all of "the A. baumannii strains that were resistant to carbapenem. Using the direct colony suspension method, the ATCC E. coli 25922 suspension was made with 0.5 McFarland standard in 5mL of peptone water. A sterile tube was pipetted with 4.5mL of peptone water. Next, a 1: 10 dilution was created by adding 0.5mL of the ATCC E. coli 25922 suspension to 4.5mL of peptone water. Zn sulfate was added to Mueller Hinton agar (MHA) plates, which were lawn cultured with" diluted ATCC E. Coli 25922. The center of the MHA plate was covered with a 10-gram Ertapenem susceptibility disk. After that, the test organism, positive control, and negative control "were streaked in a straight line from the Ertapenem disk's edge to the plate's edge. The organisms were then incubated for the entire night at 37°C at room temperature. The appearance of a clover leaf-like indentation by the ATCC E. coli 25922 development along the test" organism, which indicated a positive test, was employed to identify carbapenemase generation.

Double Disk Synergy Test (DDST):

The EDTA disk test as well as imipenem were employed to identify the generation of metallo β-lactamases. A 10µg imipenem disk, as well as an Imipenem-EDTA (IE) disk, had been placed 20 mm apart on the plate. Ipenem and imipenem's hindrance zones, along with EDTA disks, were compared after being incubated at 37˚C for the entire night. The imipenem disk was identified as the MBL producer if the hindrance zone increased by ≥ 7 mm when combined with the EDTA disk.

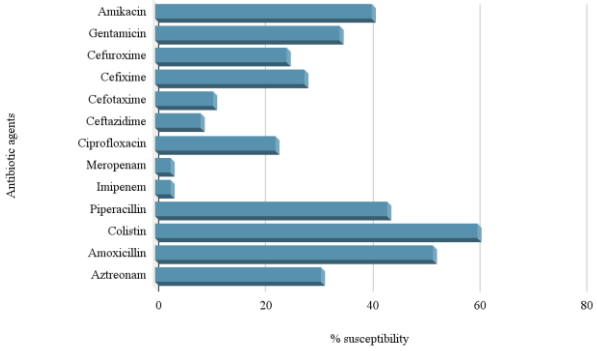
RESULTS

Thirty-three non-repetitive A. baumannii confines resistant to carbapenem were gathered. All of the segregates were resistant to colistin but not to any other anti-microbials, including carbapenems. They were also resistant to multiple drugs. The Modified Hodge test confirmed carbapenemase generation in 31 (93.93%) of these separates. The phenotypic strategy showed the generation of MBL in 18(54%) separates.

Maximum sensitivity (52%) was seen with Amoxicillin clavulanic acid, followed by moderate action with piperacillin/tazobactam (43.5%), amikacin (40.5%), and gentamicin (34.5%), and poor vulnerability patterns with the remainder of the drugs.

Table 1. Total number of carbapenemase producers.

Total Number of isolates	Modified Hodge test	Double Disk Synergy Test (DDST)
33	31	18



Graph 1. Antibiotic susceptibility pattern of Acinetobacter baumannii

DISCUSSION

In research by Hussein et al. (2013), the majority of confines showed carbapenem resistance, with 58.26% of them resistant to both imipenem and meropenem.[8] Consistent with the 93.93% found in the current study, 83.3% of the carbapenem-resistant strains that underwent MHT screening for carbapenemase generation were found to be producers of the enzyme.[9] [10] [11] [12] As per Kumar et al. (2011), the MHT produced carbapenemase in seventy-one percent of the separates.[13] [14] This was also in accordance with the outcomes from Korea noted by Lee et al. (2003), where the MHT showed that seventy-three percent of the segregates were positive for carbapenemase.[15] For MBL generation, the meropenem-resistant segregates underwent additional screening. By DDST, 95.4% were positive.[16] [17] A comparable study "by Pandya et al. (2011) revealed that 81.4% of the subjects tested positive" for DDST.[14] [6] [7]

CONCLUSION

Our analysis comes to the conclusion that one of the main issues is the high prevalence of Acinetobacter baumannii which segregates with MBL generation and is resistant to carbapenem. When comparing various phenotypic measures for Acinetobacter baumannii MBL detection, it is evident that the MHT and DDST yield high percentages of positive

results. This circumstance needs strict disease control measures. Hence legitimate anti-microbial policy and measures to limit the unpredictable utilization of carbapenems ought to be taken to limit the rise of resistance-producing pathogens.

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