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Research Article

Comparison of Phenotypic Tests-Carba NP and Modified Carba NP Test for Rapid Detection of Carbapenem Resistance in *Escherichia coli*

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A B S T R A C T

Background: To determine the antibiotic resistance pattern, the prevalence of extended-spectrum beta-lactamases (ESBLs) and carbapenem resistance in blood culture isolates of *E. coli*. Further, we evaluated and compared Carba NP, Modified Carba NP

Methods: Initial screening was performed, following which 21 carbapenem-resistant strains and four carbapenem-susceptible strains were selected for further analysis. Two phenotypic methods—the Carba NP test and the Modified Carba NP test—were evaluated according to Clinical Laboratory Standards Institute (CLSI) guidelines. These assays rely on the biochemical detection of carbapenem hydrolysis, specifically the breakdown of the imipenem beta-lactam ring, which induces a colour change in a pH indicator. Results: The Carba NP test yielded positive results in 18 of the 21 resistant isolates, while the Modified Carba NP test was positive in 20 out of 21 isolates.

Conclusion: The Modified Carba NP test is easier and cheaper than the Carba NP test, making it possible to find carbapenemase activity directly from *Escherichia coli* bacterial cultures.

The test could be used in low-income countries with large reservoirs for carbapenemase producers and can be implemented in any laboratory worldwide.

Keywords: Carba NP, carbapenem resistance, Modified Carba NP

Introduction

Bloodstream infections (BSIs) are among the most common community and hospital-acquired infections worldwide and are severe public health problems. BSIs are common in intensive care units (ICUs) and have been shown to predict severe sepsis. Community-acquired BSIs, which are usually caused by susceptible bacteria, should be distinguished from hospital-acquired BSIs, which are commonly caused by resistant hospital strains.[1] Gram-negative bacteria are responsible for approximately 25% of all occurrences of healthcare-associated bacteraemia and almost 50% of all cases of community-acquired bacteraemia. They enter the bloodstream most commonly through infections in the respiratory tract, genitourinary tract, gastrointestinal tract or hepatobiliary system.[2] Gram-negative bacteria associated with bacteraemia commonly were *Escherichia coli*,[3] *E. coli* is frequently isolated in adult patients with bacteraemia.[4] Antibiotics are commonly prescribed everywhere as a part of both empirical and regular therapy for BSIs. Multiple resistance mechanisms have been reported increasingly in *E. coli* isolates, one of which is the phenotypic expression of plasmid Ambler class A or D β-lactamases that confer resistance to most beta-lactam antibiotics, including 3rd and 4th generation cephalosporins and monobactams.[5] Carbapenems are recommended as the last-resort antibiotics for treating infections *E. coli*.[6] Hence, the wide use of carbapenems has led to the emergence of carbapenem-resistant strains referred to as carbapenem-resistant *Enterobacteriaceae* (CRE).[7] A variety of carbapenem-hydrolysing beta-lactamases (carbapenemases) have been reported in *Enterobacteriaceae*, such as KPC (Ambler class A), metallo beta-lactamases of VIM-, IMP- and NDM-type (Ambler class B) and OXA-48-types (Ambler class D). These carbapenemase genes are more diverse and laboratory detection is more challenging.[7, 8]

As India is one of the largest consumers of antibiotics globally, the efficacy of several antibiotics is compromised due to the emergence of resistant bacterial strains. Antimicrobial resistance threatens healthcare at every level and has become a major international concern for public health. Bacterial pathogens can evolve to transmit, cause disease and resist antibiotics. As bacteria evolve with an increased risk to human health, we need systematic approaches for collecting and identifying these at a local level and integrated systems to collate data to provide an international overview. In this study, we aimed to determine the antibiotic resistance pattern, and carbapenem resistance in pus and urine isolates of *E. coli*. We further evaluated and compared the results of Carba NP, Modified Carba NP for rapid detection of carbapenem resistance.

Methods

Study design: the retrospective study reviews the specimen records of patients at Muzaffarnagar medical college, Muzaffarnagar, which is a tertiary care hospital, including rural and semi - urban areas, from January 2024 to December 2024. Due to the retrospective study,patient consent was not obtained; therefore, the patient data was kept confidentially to protect the patient's privacy.

Ethical Approval

All permission is taken from their parents to fill in the information required. They were assured of the confidentiality of their responses. The aim of this study was explained, and only those who agreed to participate were included in this study. The study was approved by the department of microbiology. at Muzaffarnagar Medical College, Muzaffarnagar, U.P.

This prospective study was conducted in the Department of Microbiology of a tertiary care hospital in North India on the *E. coli* strains isolated from pus and urine collected from patients. In total, 21 carbapenem-resistant clinical isolates of *E. coli* were included in the study. The bacterial isolates were identified to species level as per standard microbiological procedures. Based on Clinical Laboratory Standards Institute (CLSI) 2021 guidelines, the antimicrobial susceptibility of the following drugs was determined by the Kirby-Bauer method: amikacin (AMK, 30 µg), ciprofloxacin (CIP, 5 µg), piperacillin-tazobactam (PTZ, 100/10 µg), cefotaxime (CTZ, 30 µg), ceftazidime (CAZ, 30 µg), cefepime (CEP, 30 µg), imipenem (IMP, 10 µg)/meropenem (MEM, 30 µg). Carbapenem-resistant strains were detected by using meropenem (10 µg) discs and the resistant strains were confirmed by minimum inhibitory concentration (MIC) detetection. Carbapenem resistance was reported if MIC to meropenem was ≥ 4 µg/mL. Standard strains of *E. coli* (ATCC 25922) were used as a control. The detected carbapenem-resistant strains were then tested by the Carba NP, Modified Carba NP, and the results were evaluated. These tests are based on biochemical detection of the hydrolysis of the beta-lactam ring of a carbapenem-imipenem, followed by the color change of a pH indicator.[11, 12] Carba NP test uses reference standard imipenem powder, while Modified Carba NP uses a therapeutic IV imipenem/cilastatin. These tests were performed on strains grown on Mueller-Hinton agar plates in triplicates for each isolate and results were interpreted by more than one independent reader.[13, 14]

Loopfull of colonies was used to pick up a bacterial colony from overnight-incubated MHA plates and then mixed into suspension medium. The bacterial suspension was then transferred to wells in the test strip and incubation at 37 °C. The test strip was read visually after 30 min and 2 h. A

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colour change from red to yellow-orange was considered positive, whereas red was interpreted as a negative result.[15]

Quality control strains used were *K. pneumoniae* ATCC BAA- 1705-MHT positive and *K. pneumoniae* ATCC BAA-1706-MHT negative.

Statistical Analysis

In this study, Statistical Package of Social Science (SPSS) version 22.0 software was used for calculating the indices like sensitivity and specificity of Carba NP and Modified Carba NP

Results

Out of the total 68 strains of *E. coli*, which were isolated from samples of patients, 21 isolates were carbapenem-resistant and were therefore selected for the study. The antibiotic susceptibility profiles of 68 isolates were analysed (Figure 1). The most effective antibiotics for these isolates were fluroqunilones and piperacilin-tazobactam with susceptibilities being 60% (39/68) and 51% (33/68), respectively as shown in Table 1. Piperacilin belongs to penicilin class of antibiotics and tazobactam is a beta-lactamase inhibitor. The prevalence of carbapenem resistance was observed in 21 isolates out of the 68 strains (30% resistance).

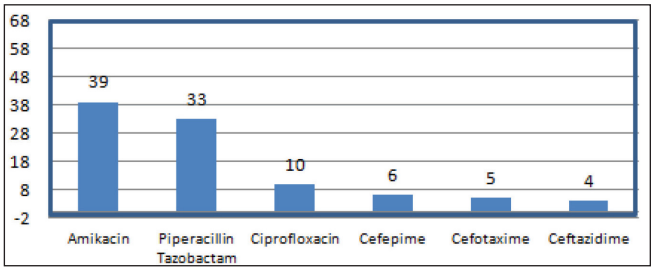

Figure 1.Antibiotic graph shown resistance

Amongst the 21 carbapenem-resistant strains of *E. coli*, These strains were resistant to imepenem, ciprofloxacin, cefepime, cefotaxime and ceftazidime. Based on carbapenemase detection conducted on these carbapenem-resistant isolates,. Carba NP test was positive on 18/21, Modified Carba NP and the kit based Rapidec Carba NP test were positive for all the isolates (20/21). The sensitivity varied with Modified Carba NP slightly better than the Carba NP testThese carbapenem susceptible strains were tested to rule out or minimise false positivity and evaluate the test methodology's specificity. The specificities of all three tests were excellent.

Conflict of Interest: This study was contribution by Dr Siva Prasad Reddy B. and Dr. Sapna Chauhan. All method and material, anaylsis were covered by Dr. Siva Prasad Reddy B. all manuscript was prepared by both Dr. Siva Prasad Reddy B and Dr. Sapna Chauhan. Review and editing were conducted by Dr. Sapna Chauhan and overall supervision were provided by Dr. Siva Prasad Reddy B.

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Discussion

Regarding the antibiotic susceptibility, *E. coli* was found to be vulnerable to a bet-lactamase/beta-lactamase inhibitor (BL/BLI) combination such as piperacillin-tazobactam, aminoglycosides and carbapenems. However, all of these are parenteral antibiotics with limited usage for indoor patients. Carbapenems should be reserved for severe cases and a carbapenem sparing policy should be implemented to stop emergence of resistance. In outpatient setting, oral antibiotics are always preferred although very limited options are left for sepsis treatment. The oral drugs such ciprofloxacin and amoxicillin-clavulanate can be used, but only after susceptibility reports are available as empirical therapy which may not be helpful in the present scenario. Moreover, the choice of antibiotic must be based on several aspects like clinical condition, renal function tests and whether the patient is indoors or outside.

The present study also highlights the supreme importance of detection of carbapenem resistance for effective therapy. The Carba NP test is the most important and recent development for the accurate identification of carbapenemase- producing *Enterobacteriaceae*.[22] The Carba NP test is a novel phenotypic method for carbapenemase detection. It is based on the in vitro hydrolysis of imipenem by a bacterial lysate, which is detected by changes in pH values by using the indicator phenol red (red to yellow/orange).[13]

The Modified Carba NP test is another variant that uses 0.02% cetyltrimethylammonium bromide lysis buffer and a starting pH of 7.5 instead of 7.8, allowing for better carbapenemase producer identification. It also detects OXA-48carbapenemases to some extent.[14]

Hydrolysis of imipenem is detected by a change in the pH value of the indicator (from red to yellow/orange). These tests are rapid (2 h), easy to use and do not require any specific equipment. Various studies have reported this test to be 100% sensitive and specific, according to the molecular techniques.[11, 20, 21] It detects not only all known carbapenemases (belonging to Ambler A, B and D classes) in *Enterobacteriaceae*, but also identifiesvirtually any new emerging carbapenemase in contrast to molecular techniques. while Carba NP showed results. However, found a lower sensitivity as it identified 18/21 isolates

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(86%) correctly on multiple occasions with no false positives (100% specificity). Due to the high cost of reference standard imipenem powder and its instability in solution form, this test is also costly, labour-intensive and inconvenient. To identify a cheaper or more convenient test of similar accuracy, a modified Carba NP test that used intravenous imipenem/cilastatinwhich is cheaper and more stable than the reference standard imipenem powder.[23, 24, 25] Our Carba NP test was modified and provided positive findings with 100% sensitivity and specificity. Isolates were retested by using a more concentrated extract. When the inoculum was higher, the Carba NP test showed clearer colour changes that were positive. Similar findings have been documented in a study by Tijet et al. (26), suggesting OXA 48 could be the reason. These results improved the overall sensitivity of the Carba NP test in our study. The limitation of our study was that we were unable to perform molecular based identification of the various carbapenemases and confirm the reason for the discrepant result.

Conclusion

The Modified Carba NP is an easier and cheaper alternative to the Carba NP test, allowing carbapenemase activity to be deleted directly from bacterial cultures of *Enterobacteriaceae*. The test can be used in low-income countries that have large reservoirs for carbapenemase producers and can be implemented in any laboratory worldwide. These tests have the potential to contribute to a better stewardship of carbapenems by changing the paradigm of controlling carbapenemase producers worldwide, especially in ICU patients. Further, the best choice of empirical therapy option should be determined. The aetiology agents of BSI should be monitored periodically and their resistance patterns as well. This study would help to explore the possibilities for revising the antimicrobial stewardship programme which would reduce morbidity and hospitalisation costs.

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