Comparison of Rapid Resa-Imipenem NP Test with Conventional Disc Diffusion Method for Detection of Carbapenem Susceptibility in *Acinetobacter Baumannii*

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ABSTRACT

Objective: To compare rapid Resa-Imipenem NP test with conventional disc-diffusion method for carbapenem susceptibility testing in *Acinetobacter baumannii*.

Study Design: Cross-sectional analytical study.

Place and Duration of the Study: Department of Microbiology, The Armed Forces Institute of Pathology / National University of Medical Sciences, Rawalpindi, Pakistan, from December 2022 to November 2023.

Methodology: A rapid resazurin-based test to assess imipenem susceptibility was performed on the growth of *Acinetobacter baumannii* in 202 clinical samples from different clinical settings, and the results were compared with the conventional disc-diffusion testing method. Bacterial suspensions of the isolates were added to imipenem and resazurin (a viability colourant) containing Mueller-Hinton broth on a 96-well polystyrene microtitre plate and incubated for 2.5 hours. A colour change from blue to pink indicated resistance. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy were calculated. Cohen's kappa assessed agreement between tests.

Results: Out of 202 isolates, 157 were imipenem resistant and 45 were imipenem sensitive. The sensitivity, specificity, PPV, and NPV were 99.4%, 100%, 100%, and 97.8%, respectively. Diagnostic accuracy was 99.5% with one very major error (VME, 0.5%). Cohen's Kappa showed excellent agreement between the two tests (<0.001).

Conclusion: The rapid Resa-Imipenem NP test is simple, reliable, and significantly faster, providing accurate results within 2.5 hours compared to 18-24 hours by conventional disc-diffusion.

Key Words: *Acinetobacter baumannii, Imipenem, Rapid resazurin test, Microbial sensitivity tests, Disk diffusion method, Carbapenems*.

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INTRODUCTION

Acinetobacter baumannii is a gram-negative, catalase-positive, non-fermenting, and oxidase-negative coccobacilli species with twitching motility. Taxonomists recognised it as a separate genus in 1971.[1] *Acinetobacter baumannii* is most notorious for hospital-acquired infections, namely ventilator-associated pneumonia, line-associated sepsis / bacteraemia, skin and soft tissue infections, and meningitis. It has easily emerged as a cause of nosocomial infections because it is resistant to biocides and desiccation and does not have any special nutrient requirements.[2]

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Infections caused by this pathogen are an increasing cause of concern, especially in the ICU settings, particularly because of the emergence of resistance against many antimicrobial groups.[3] Its pathogenic potential is low in immunocompetent patients but it gets enhanced in patients with immune-compromising conditions such as diabetes mellitus, cancers, and pulmonary disorders.[4] carbapenems are considered to be the most widely used medicines for the treatment of infections in ICU settings, but the emergence of carbapenem-resistant *Acinetobacter baumannii* (CRAB) has caused great worldwide concern among clinicians. It was also included in the WHO's List of Global Priority Bacteria published in 2017, which mentioned the immediate requirement of newer antimicrobial agents for these bacteria.[5]

Resistance against carbapenems has been studied in various set-ups across the world, and some set-ups have even reported rates as high as 90%.[6] In a study conducted in Lahore, Pakistan, 156 isolates were collected from different tertiary care hospitals between June and November 2017. Sensitivity patterns of *Acinetobacter baumannii* were analysed, and

resistance against carbapenems was reported to be as high as 89.1%.[7] Despite the emergence of resistance in this bacteria, carbapenems are still first-line medicines for the treatment of infections caused by many Gram-negative bacteria, especially in ICU settings.[8]

Routinely, the method being followed for susceptibility testing for carbapenems in various clinical microbiology laboratories all over the world is in line with CLSI or EUCAST guidelines, which employ disc-diffusion as the prime susceptibility testing method. This method takes at least 18 to 24 hours to provide results of susceptibility testing for various antibiotics.[9] Recently, in 2021, a newer Resazurin-based method was introduced by Nordmann *et al*. for the rapid detection of susceptibility / resistance to carbapenems in *Acinetobacter baumannii*. *i*mipenem susceptibility was assessed in this method, as it is a general observation that isolates resistant to imipenem are also resistant to meropenem and vice versa.[10] The susceptibility results are obtained within 2 hours and 30 minutes through this test as compared to the standard 18-24 hours by the disc-diffusion method. This method operates on the principle of resazurin reduction by metabolically active cells from blue to pink, and hence the resulting colour change is detected as resistance, as it shows growth / survival of bacterial cells in the presence of an antibiotic.[10] Given the increasing prevalence of carbapenem-resistant *A. baumannii* and the urgent need for timely antimicrobial stewardship in critically ill patients, there is a pressing demand for rapid, reliable susceptibility testing methods. The current delay associated with conventional techniques may compromise early and appropriate therapy. Therefore, this study was conducted to compare the performance of the Resa-Imipenem NP test with the standard disc-diffusion method, to evaluate its potential for reducing diagnostic turnaround time in clinical practice.

METHODOLOGY

It was a cross-sectional analytical study carried out at the Armed Forces Institute of Pathology/ National University of Medical Sciences, Rawalpindi, Pakistan, from December 2022 to November 2023.

Owing to the publication of a single study in the literature on the accuracy of this test by the developers of the test, which quoted the sensitivity and specificity of this test to be 100%, it was decided to include all clinical isolates of *Acinetobacter baumannii* yielded in the lab from various samples during the entire duration of the study (i.e. one year). A total of 202 isolates, out of which 157 were carbapenem-resistant and 45 were carbapenem-sensitive, were included in the study.[10]

The sampling technique was non-probability consecutive sampling. Growths of *Acinetobacter baumannii* in clinical samples for culture from both genders and all age groups were included in the study. Duplicate samples from the same patient were excluded.

The 202 isolates of *Acinetobacter baumannii* included in the study were identified to species level using API 20 NE®. For imipenem Disc QC, ATCC 25922 *Escherichia coli* was used according to CLSI guidelines.[9] Furthermore, two additional isolates, one imipenem-sensitive and the other imipenem-resistant *Acinetobacter baumannii* (institutional controls), were also used as internal controls for both tests.

The imipenem susceptibility of all bacterial isolates was checked twice using the disc-diffusion method according to CLSI guidelines. Bacterial suspensions of 0.5 McFarland standard were prepared in normal saline and lawned on Mueller Hinton agar. Imipenem discs 10 µg were applied and incubated in ambient air at 35 ± 2˚C for 18-24 hours. A zone diameter of ≥22mm was considered as the susceptible breakpoint for that particular isolate.[9]

For the Resa-Imipenem NP test, a 96-well polystyrene microtitre plate was used. Mueller Hinton (MH) broth was used, and its solution with imipenem (imipenem monohydrate, Sigma-Aldrich®) was also prepared (6.67 µg/ml to obtain a final concentration of 6 µg/ml according to the developer guideline).[10] Bacterial suspension of 1 McFarland standard prepared for each isolate was inoculated in parallel in two wells on the microtiter plate, one containing imipenem solution to test susceptibility and one without the solution, which acted as growth control for that particular isolate. Imipenem-free MH solution (180 µL) was added to wells A1, A2, A3, A4, and 180 µL of imipenem-containing MH solution was added to wells B1, B2, B3, B4, and so on (Figure 1). Sterile saline (20 µL) was added to wells A1 and B1, which was added as sterility control for the media. 20 µL of imipenem-resistant isolate suspension (institutional control) was added to wells A2 and B2. 20 µL of imipenem-sensitive isolate suspension (institutional control) was added to wells A3 and B3. The test isolates were inoculated 20 µL each in the successive wells in a similar way. Resazurin reagent (20 µL) was added to all the wells, mixed by pipetting up and down and incubated at 35 ± 2˚C in ambient air for 2 hours and 30 minutes after covering the tray. All the tests were repeated in duplicate, and the results were considered valid if wells A1 and B1 showed no colour change / remained blue (sterility control), and wells A2 and B2 turned pink (showing imipenem resistance of positive control), and well A3 turned pink (growth control) and B3 remained blue (showing imipenem sensitivity of negative control). The results were observed for a maximum of 2 hours and 30 minutes since inoculation (Figure 1).

The data analysis was done using Statistical Package for the Social Sciences (SPSS) version 23. Sensitivity, Specificity, PPV, NPV, and diagnostic accuracy were calculated to evaluate the performance of the Resa-Imipenem NP test. To estimate the level of agreement between the disc-diffusion test and the rapid Resa-Imipenem NP test, Cohen's Kappa was calculated. In addition, very major error (VME), which was the percentage of resistant isolates identified as susceptible, and major error (ME) which was the percentage of sensitive isolates incorrectly identified as resistant were also calculated.

Table I: Frequency of *Acinetobacter baumannii* isolated from different samples and their susceptibility profiles on disc-diffusion (reference method).

Specimen	No. of isolates (n = 202)	Imipenem susceptible (n = 45)	Imipenem resistant (n =157)
Bronchioalveolar lavage (BAL)	49 (24.3%)	14	35
Non-directed bronchial lavage (NBL)	29 (14.4%)	4	25
Endobronchial washings (EBW)	30 (14.9%)	5	25
Sputum	25 (12.4%)	6	19
Blood	18 (8.9%)	7	11
Tissue	12 (5.9%)	0	12
Cerebrospinal fluid (CSF)	11 (5.4%)	5	6
Pus	10 (5%)	1	9
Wound swab	6 (3%)	0	6
CVP tip	6 (3%)	2	4
Pleural fluid	6 (3%)	1	5

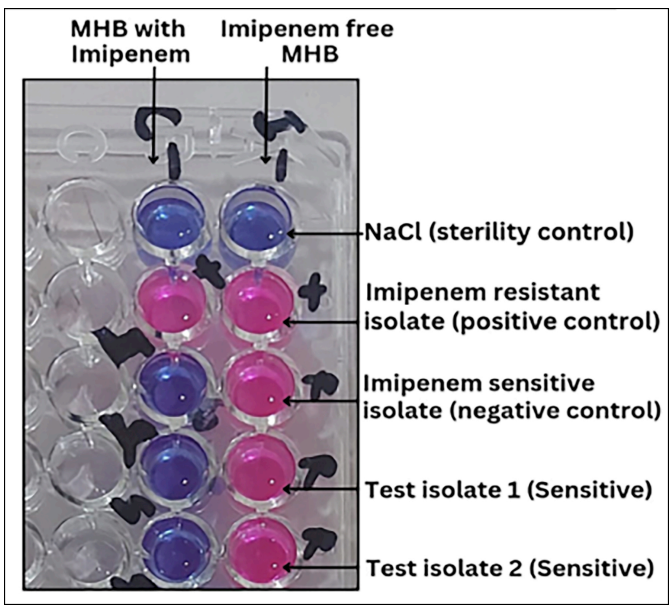

Figure 1: Representative results of rapid Resa-Imipenem NP test for imipenem susceptibility testing.

RESULTS

The results of the rapid Resa-Imipenem NP test were compared with the standard disc-diffusion method. Out of 202 isolates tested, 157 isolates were found to be resistant to imipenem by the disc-diffusion method repeated in duplicate for all isolates (zone diameter ≤18 mm). Forty-five out of 202 were sensitive to imipenem by disc-diffusion method (zone diameter ≥22mm, Table I). On rapid Resa-Imipenem NP test, 156 out of 157 imipenem-resistant isolates showed correct change in colour (from blue to pink) within 2 hours and 30 minutes, indicating resistance. One isolate, however, failed to show colour change until after 3 hours and 30 minutes. The tests were repeated in triplicate for this isolate, but the result remained the same. The results for this isolate were counter-checked *via* the MIC method which, similar to the disc-diffusion method, also declared the isolate to be resistant to imipenem (MIC ≥8). All 45 isolates declared sensitive to imipenem by disc-diffusion method showed no colour change at the end of 2 hours and 30 minutes, showing correct results, i.e. imipenem sensitivity *via* Resa-Imipenem NP test too. The results of colour change were interpreted by three independent observers to rule out observer bias. All tests were independently repeated in duplicate by different team members, yielding consistent results.

The sensitivity of the test (number of resistant isolates correctly identified as resistant) was calculated to be 99.4%. The specificity of the test (number of sensitive isolates correctly identified as sensitive) was calculated to be 100%. The PPV of the Resa-Imipenem / Acinetobacter NP test was calculated to be 100%, and the NPV was 97.8%. The diagnostic accuracy of the test was 99.5%. Overall, no ME and only one VME (0.5%) were observed in the whole study, where the resistance or the correct result was observed at 3 hours and 30 minutes instead of the cut-off time of 2 hours and 30 minutes. This could be due to the poor metabolism of the individual isolate and not the procedural fault. The value of Cohen's Kappa came out to be 0.986, showing excellent agreement between the two tests with a p-value of <0.001.

DISCUSSION

In this study, all growths of *Acinetobacter baumannii* yielded on clinical samples in one year, received from all sorts of clinical setups and not just the ICUs were included. The percentage of carbapenem resistance detected in these isolates was 77.7%. A study was conducted in three tertiary care hospitals in Pakistan, where the incidence of carbapenem resistance (imipenem resistance) was calculated in 681 isolates of *Acinetobacter baumannii* isolated from ICU samples. They found imipenem to be resistant in 85.5% of the isolates.[11] The difference observed could be because the medicine resistance is generally higher in bacteria isolated from ICU samples, and this study included isolates from all clinical settings. In a meta-analysis conducted by Tavasol *et al.*, which included 62,779 cases of infections caused by *Acinetobacter baumannii*, they found the highest rate (67%) of resistance against imipenem in Asia.[12] Globally, infections caused by CRAB are considered the fourth leading cause of death due to antimicrobial resistance.[13] Published data also show that patients with CRAB infections are at a higher risk of adverse outcomes than patients with infections caused by other carbapenem-resistant pathogens.[14] The emergence and spread of CRAB have been directly linked to carbapenem overuse in the treatment of Gram-positive and Gram-negative infections in hospital settings.[15]

Rapid molecular tests and other techniques that can initiate early treatment in CRAB infections are not routinely used in various laboratory setups mainly due to cost limitations, which leads to delays in the start of treatment and resultantly poor patient outcomes, especially in cases of pneumonia caused by CRAB.[16] Owing to this, efforts to develop cost-effective and rapid tests for susceptibility testing in *Acinetobacter baumannii* have been on the rise. Apart from carbapenems, the medicines with increased activity and a better sensitivity profile against *Acinetobacter baumannii* include polymyxins, tetracyclines (minocycline, tigecycline), and newer agents such as cefiderocol. Moreover, combination therapy using two *in vitro* active agents has been considered to be superior to monotherapy in both IDSA (Infectious Diseases Society of America) and ESCMID (European Society of Clinical Microbiology and Infectious Diseases) guidelines.[14,17,18] Rapid testing methods are also being developed for these other treatment options.

A similar rapid resazurin-based test to detect Colistin susceptibility in *Acinetobacter baumannii* was developed by Lescat *et al*., which had a sensitivity of 100% and specificity of 97%. This test is now available as commercial kits as well, and this also reduces the time span from 18-24 hours to 4 hours for obtaining colistin susceptibility results.[19] This resazurin-based technique has also been applied in a study to obtain early results for cefiderocol susceptibility testing in *Acinetobacter baumannii*, which showed a sensitivity of 95.5%, specificity of 100%, and overall accuracy of 98.9%. The turnaround time for susceptibility results was reduced in this test to 4 hours and 45 minutes, which is still very less as compared to that by the standard methods.[20] In the study published by the developers of the test, the sensitivity and specificity of the rapid Resa-Imipenem / Acinetobacter NP test were calculated to be 100%, which is quite close to the results of this study.[10] This shows promising results if this technique is inculcated in routine susceptibility testing in labs by providing earlier results to the physicians and prompt change of treatment when required.

A small limitation of the Resa-Imipenem NP test and this study is that there is no clear-cut guideline about the isolates that fall in an intermediate susceptibility zone (19-21mm) to carbapenems according to CLSI guidelines for disc-diffusion testing and more studies with the inclusion of such isolates are required.[9] Another limitation of this study is that disc-diffusion, which is a CLSI-approved method, was used as the reference method for calculating diagnostic accuracy metrics and MIC method which is the absolute gold standard, was not used for all isolates due to resource constraints. However, for isolate with discordant results between the Resa-Imipenem NP test and disc diffusion, MIC testing was performed, and MIC results were consistent with the disc-diffusion interpretation.

CONCLUSION

This test is a simple, easy-to-perform, reliable, and significantly faster alternative to routine lab methods. It can be performed with routine lab materials, and no advanced equipment is required. If incorporated into routine sensitivity testing, it can facilitate the early initiation of appropriate antimicrobial therapy, potentially improving clinical outcomes. Moreover, by enabling timely therapeutic decisions, this rapid method supports effective antimicrobial stewardship and may play a crucial role in combating multidrug resistance.

ETHICAL APPROVAL:

Ethical approval was taken by the Institutional Review Board and Ethical Committee (Letter No: FC-MIC21-16/READ-IR-B/23/1672; Dated: 29-03-2023).

PATIENTS' CONSENT:

Consent was taken from all the patients prior to their inclusion in the study.

COMPETING INTEREST:

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

SM: Conception, interpretation, and drafting.
IAM, SHN: Analysis and revision of the manuscript.
AZ: Contribution to the interpretation and revision of the manuscript.
AHG: Conception, drafting, and accountability.
RS: Contribution to interpretation, revision, and accountability.
All authors approved the final version of the manuscript to be published.

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