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Comparison of Rapidec Carba NP test versus modified Hodge test in Finding Frequency and Resistance Pattern of E. Coli and Klebsiella Species

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ABSTRACT

OBJECTIVE: To compare the investigations that would ultimately benefit the health care professionals to opt for better management against the disease process in a timely manner.
METHODOLOGY: This cross-sectional study was conducted at Lifeline General Hospital, Karachi, from March to November 2023. The CLSI guidelines for isolates showing inhibition zone size of antimicrobial agents were documented as potential carbapenemase producers and short-listed for confirmation of carbapenemases and their respective classes. SPSS 22 was used to analyze data. A chi-square test was used, keeping a p-value of ≤0.05 as significant.
RESULTS: The frequency of positive and negative samples was recorded at 287(75.5%) and 93(24.4%) respectively. The distribution of microorganisms within samples indicated *E. coli* presence as 47(12.3%), 18(4.7%), 21(5.5%) and 4(1%) in urine, pus, respiratory tracts and blood samples respectively. The distribution and identification of microorganisms as per the techniques used reported no statistically different results, with p-values of 0.81 and 0.26 for E.coli and Klebsiella, respectively.
CONCLUSION: This study concludes that Carba NP is cost-effective and provides rapid results within 30 to 120 minutes. The high specificity and sensitivity of the test contribute to better patient management and prevent the spread of healthcare associated infections.

KEYWORDS: Carbapenemase, MHT, Carba NP, *E. coli*, Klebsiella, resistance pattern, frequency

INTRODUCTION

In the past seventy years, the era of antimicrobials has witnessed the enormous discoveries of a diverse array of antibiotics against microorganisms. However, there has been an alarming emergence of antimicrobial resistance. The upward trajectory of the resistance pattern persists, with the pharmaceutical research and manufacturing sectors failing to develop new drugs to substitute existing antimicrobials that have already shown great resistance[1].

Recently, antibiotic resistance has undergone swift changes and poses an imminent challenge to public health across various health sectors that demand coordinated global interventions. In Europe, it has already led to a significant number of fatalities, and the European Center for Disease Prevention and Control (ECDC) anticipates an annual toll of 25,000 lives lost due to infections associated with antimicrobial resistance[2]

Gram-negative bacteria, especially Enterobacteriaceae, *E. coli*, and Klebsiella, are associated with a range of infectious diseases such as urinary tract infections, respiratory infections, gastrointestinal infections and bloodstream infections[3]. The resistance of Klebsiella strains, particularly to third-generation cephalosporins, was initially reported in 1981, Since then, these bacteria have demonstrated a persistent trend of evolving resistance, posing an ongoing challenge in their susceptibility to various antibiotics[4].

Carbapenems are bactericidal β-lactam antibiotics that have demonstrated efficacy against severe infections caused by extended-spectrum beta-lactamase (ESBL) producing bacteria; a few examples include meropenem, Imipenem, ertapenem and panipenem[5]. Global carbapenem resistance in Gram-negative bacteria has become a widespread problem. Evidence indicates that individuals infected with carbapenem-resistant pathogens have higher chances of morbidity and mortality than those infected with susceptible pathogens. The advancement of rapid diagnostic tests for improving the detection of carbapenem resistance and the use of extensive population-based data sets can provide a better understanding of this pressing thread and enable physicians to make more informed decisions in selecting the most appropriate antibiotics[6].

In the modern era, antimicrobial susceptibility testing (AST) plays a vital role in the laboratory processes that assess antimicrobial agents' efficacy against pathogens, especially bacterial and fungal infections. It aids the healthcare professional in identifying the optimal treatment for the patients by evaluating the susceptibility of microorganisms to specific drugs. Moreover, AST works beyond individual patient care, significantly addressing the hazards of antibiotic resistance globally by providing crucial data to antimicrobial stewardship programs. It guides the development of public health strategies to restrain the emergence and spread of resistant strains[7].

The issue of carbapenem resistance in Enterobacteriaceae poses a significant challenge for healthcare providers. The Modified Hodge Test (MHT), endorsed by the CDC, provides sensitivity and specificity up to 90%. Carbapenems serve as crucial antibiotics of last resort for multidrug-resistant Enterobacteriaceae. Unfortunately, there is a concerning global increase in resistance to carbapenems, leading to substantial therapeutic failures and a rise in mortality rates. Consequently, the timely and accurate identification of carbapenemase-producing, carbapenem-resistant Enterobacteriaceae (CRE) is essential to curb the spread of carbapenem resistance in both nosocomial and community-acquired infections[8].

The Carba NP test, which is a swift iteration of the Carba NP test, is a rapid commercial phenotypic test which operates on the principle of hydrolyzing the β-lactam ring of Imipenem by carbapenemase-producing bacteria, leading to a noticeable color change in a pH indicator (phenol red) from red to yellow/orange. This test utilizes specially designed strips for single-patient studies, featuring strips with pre-made reagents that streamline processes and reduce the potential for errors. With a rapid turnaround time of 2 hours, the Carba NP test exhibits

high specificity and sensitivity for detecting class A and B carbapenemases. However, its sensitivity is comparatively lower for OXA carbapenemases. Notably, class A and B carbapenemase producers obtain results faster than class D carbapenemase producers[9].

The adapted CNP test demonstrates favourable outcomes when compared to MHT. This test is cost-effective, straightforward, and reproducible, making it easy to execute and interpret, with results available in under 5 minutes. It exhibits a high level of sensitivity and specificity comparable to molecular tests. In contrast, MHT is a complex procedure with challenging result interpretation and a lengthy 24-hour incubation period[10].

The MHT and Carba NP tests are the most frequently used tests within healthcare institutes; comparing these two techniques will enable healthcare providers to estimate efficacy and ease of use within centres. There is insufficient data available to demonstrate the effectiveness of the Carba NP and Modified Hodge tests in assessing the frequency and susceptibility pattern of *E. Coli* and *Klebsiella* Pneumonia. The aim is to compare the investigations above, which ultimately would benefit healthcare professionals in opting for better management of the disease process promptly.

METHODOLOGY

This cross-sectional study was conducted at Lifeline General Hospital, Karachi, from March to November 2023. After getting ethical approval from the head of the department, the data was collected from the microbiology department from patients with systematic or local infections admitted in wards and ICU. The sample size was calculated with the help of the Raosoft sample size calculator, keeping total samples from the previous year as the numerator (n=800), a confidence interval as 95% and a margin of error as 5%; the minimum required sample was **380**. Probability, a consecutive sampling technique was used, and the samples were divided into four groups; group A had urine samples (n=100) collected from diagnosed patients with urinary tract infections, preferably first urine in the morning. Group B had pus samples (n=100) collected from patients' wounds (any site) with the help of a sterilized cotton swab. While group C had respiratory tract, tracheal aspirates and sputum (n=90) collected with the help of suction, and group D had blood samples collected from patients suspected of septicemia (n=90), after collecting samples through a venous site, blood was injected into brain heart infusion broth in 1:5 ratio of 1 part blood and 5 parts broth.

The CLSI guidelines for isolates showing inhibition zone size of antimicrobial agents were documented as potential carbapenemase producers and short-listed for confirmation of carbapenemases and their respective classes. The carried-out procedures were as follows:

The Modified Hodge Test, sometimes called the cloverleaf approach, is a phenotypic method for measuring carbapenemase activity. The mechanism involves carbapenem inactivation by bacteria that produce carbapenemase, allowing an indicator strain sensitive to carbapenem to grow farther toward a disc that contains carbapenem along the tested strain's inoculum streak. With minor adjustments, MHT was carried out by the body of existing research. A positive screening test for the production of carbapenemases is defined as a clover leaf-shaped indentation of the indicator strain's zone of inhibition along the test or QC strain's inoculum streak. Negative findings were interpreted when there was no indentation.

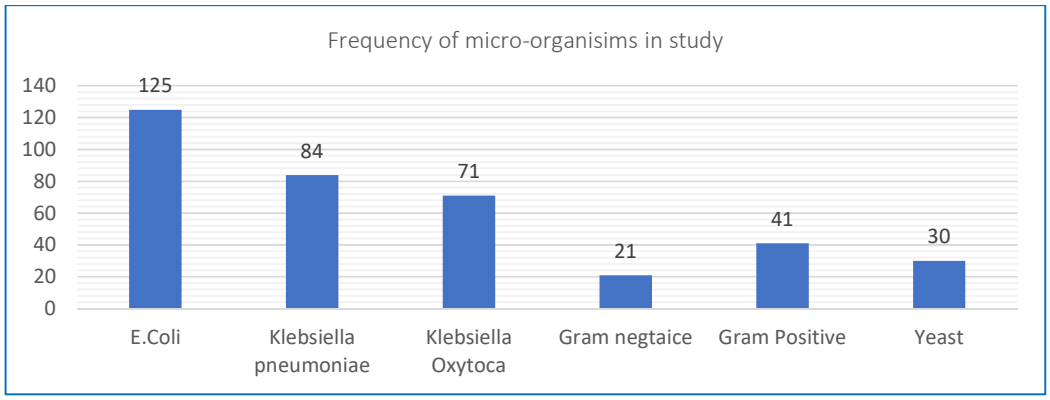
The detention of carbapenem hydrolysis by bacteria that produce carbapenemases is the basis for the RAPID CARBA NP test. The pH indicator changes color due to hydrolysis due to the medium becoming more acidic. Reading is done visually by contrasting a response well containing Imipenem with a control well that does not contain Imipenem after incubation for a maximum of two hours. The findings were interpreted following the literature at the time of publication. An increase in the width of the zone surrounding the Imipenem and meropenem discs that included EDTA was compared to that of Imipenem, and the discs without EDTA were thought to be positive for MβL.

Statistical Package of Social Sciences (SPSS) version 22 was used to enter, sort and analyze data. The normality of data was assessed with the help of the Shipro Wilk test for continuous variables, and frequency, percentages, mean, and standard deviation were used. A paired sample t-test was used to compare the results of both devices. The chi-square test assessed the difference between two mean values, keeping a p-value of ≤ 0.05 as significant.

RESULTS

A total of 380 samples were added to the study from admitted patients. The gender distribution of enrolled patients indicated male dominance, with 241(63.4%) and 139(36.5%) females. The mean age was 41.8±9.81 years. The samples were collected from three different departments of institutes; the maximum number of samples were collected from the surgical department with 164(43.5%), followed by the medical department with 121(31.8%), and intensive care unit samples were least in numbers with 95(25%). The frequency of positive and negative samples was documented as 287(75.5%) and 93(24.4%) respectively.
The positive samples were distributed as 179(47.1%) from Urine samples, 112(29.4%) from pus samples, 77(20.2%) from respiratory tract samples, and 12(3.1%). Frequency of different microorganisms in collected samples: the highest reported microorganism was *E. coli* with 125(32.8%), followed by *Klebsiella* pneumonia with 84(22.1%), *Klebsiella* oxytoca with 71(18.6%), Gram-negative with 21(5.5%), gram-positive 41(10.7%) and yeast 30(7.8%). (**Figure I**)

Figure I: Frequency of microorganism distribution within study samples.



The distribution of microorganisms within samples indicated *E. coli* presence as 47(12.3%), 18(4.7%), 21(5.5%) and 4(1%) in urine, pus, respiratory tracts and blood samples respectively. Similarly, K.Pneumoniae and K. Oxytoca were identified in all samples with different frequencies. (**Table I**)

Table I: Frequency and distribution of reported microorganisms within collected samples of urine, pus, respiratory System and blood

Organism	Urine (n=179)	Pus (n=112)	Respiratory System (n=77)	Blood (n=12)
E. coli	47 (12.3%)	18 (4.7%)	21 (5.5%)	4 (1%)
K.Pneumoniae	41 (10.7%)	20 (5.2%)	11 (2.8%)	1 (0.2%)
K.Oxytoca	19 (5%)	11 (2.8%)	7 (1.8%)	0

The distribution and identification of microorganisms as per the techniques used reported the highest sensitivity of MHT compared to Carba NP, with p-values of 0.81 and 0.26 for *E. coli* and Klebsiella, respectively (**Table II**).

Table II: Difference between MHT and Carba NP test frequencies in study samples

Organism	MHT	Carba NP	P-Value
E. Coli	84 (22.1%)	41 (10.7%)	0.81
Klebsilla	95 (25%)	60 (15.7%)	0.26

Antibiotic resistance was assessed, and resistance from multiple antibiotics was reported from each sample; a comprehensive explanation is provided in (**Table III**).

Table III: Reported distribution of antibiotic resistance in study samples

Antibiotics	Abbreviation	*E. coli*	*Klebsiella*
Ampicillin	AMP	97	104
Amoxicillin Clavulanic acid	AMC	60	83
Piperacillin/Tazobactem	TZP	20	35
Aztreonam	ATM	76	83
Cephalethin	CI	93	95
Cefuroxime	CXM	83	88
Cefotaxime	CTX	74	81
Ceftazidime	CAZ	73	83
Ceftriaxone	CRO	74	81
Cefepime	FEP	70	79
Ofloxacin	OFX	74	47
Ciprofloxacin	CIP	53	45
Gentamicin	CN	11	59
Amikacin	AK	83	28
Trimethoprim - sulphamethoxazole	SXT	7	83
Imipenem	IPM	7	9
Meropenem	MEM	0	9
Tigecyclin	TGC	3	4
Polymyxin	PB	0	0

DISCUSSION

Nosocomial infection refers to infection acquired after the 48 hours of hospital admission and after the 3 days of hospital discharge. These infections affect one out of ten patients in the hospital, imposing a significant financial burden on the patients and the healthcare system. Most infections are associated with invasive devices like endotracheal tubes, central venous catheters, and urinary catheters. Roughly one-third of them could be potentially avoided. Each infection contributes to the patient's extended hospital stays, morbidity and mortality[11].

There is a plethora of studies that explain the risk factors for various types of nosocomial infections. Still, four significant factors cause these infections, including the patient's underlying health status, acute disease process, invasive devices, and factors related to the treatment[12]. The most important of these factors are the antibiotics susceptibility pattern and resistance to the organisms that significantly burden the doctors and healthcare professionals to tackle the pathogens effectively.

Infections that were previously easily manageable become intricate health issues, complicating medical interventions and escalating hospital stays of the patients. Bacteria that resist conventional antibiotics lead to prolonged illnesses and increased mortality rates. Common infection sites include the Urinary tract, respiratory tract, wound infections (pressure sores at the ankles, back and hip joint) and bloodstream infections[13].

Pathogens containing Carbapenemase activities are increasingly observed in hospitals and community settings. The rapid and accurate laboratory testing of carbapenemase-producing isolates is vital in preventing the spread of infections. It enables the healthcare provider to find the best way to manage[14].

A fundamental mechanism underlying carbapenem resistance involves the hydrolysis of carbapenems by carbapenemase enzymes, primarily encoded on plasmids and possessing high transmissibility. Other mechanisms are the non-enzymatic mechanism, which includes loss of expression of porin-encoding genes, mutations in chromosomally encoded porin genes (such as OprD), and overexpression of genes encoding efflux pumps (such as MexAB-OprM, MexXY-OprM, or MexCD-OprJ), particularly in P. Aeruginos. Porins serve as non-specific channels located in the outer membrane of gram-negative bacteria. They facilitate the passive transport of hydrophilic small molecules, nutrients, and certain antibiotics across an otherwise impermeable membrane. The reduction of porins and the increased expression of efflux pump characteristics are linked to carbapenem resistance[15].

CRE (carbapenem-resistant Enterobacteriaceae) isolates exhibit resistance to beta-lactam antibiotics and demonstrate significant cross-resistance across various antibiotic classes. This is attributed to plasmids carrying carbapenem resistance genes that harbor multidrug-resistant (MDR) determinant genes. Infections caused by CRE are challenging to treat, as they resist most available antibiotics, resulting in therapeutic failures. Additionally, the rapid transmission of carbapenem resistance due to carbapenemase production occurs swiftly among different Enterobacteriaceae[16,17].

The present study found carbapenem resistance in many organisms, creating the acronym CRE, which now defines Carbapenem-Resistant Enterobacterales. The organisms were more prevalent in infected urine and tracheal aspirates of the patients. Among the organisms, *E. Coli*, followed by Klebsiella pneumonia and Klebsiella Oxytoca, were the most common pathogens.

While alternative tests like aminophenyl boronic acid and dipicolinic acid tests could be viable for phenotypic carbapenemase screening, the required facilities for these tests are not commonly accessible in most laboratories. The Modified Hodge test is a straightforward investigation that can be conducted in a routine laboratory to identify carbapenemases in isolates exhibiting intermediate or sensitive zone peripheries on disc diffusion testing. For

epidemiological purposes, the Modified Hodge test is a genuinely valuable screening test for identifying suspected cases[18,19]. This study confirms that the Modified Hodge Test (MHT) was a practical and effective method for confirming carbapenemase production. Consistent with the results of other studies[20,21].

Following the other study, E. Coli and Klebsiella pneumonia were found to be MDRO[9-11]. MHT detected *E. coli* resistant to multiple antibiotics, including Ampicillin, Piperacillin/Tazobactam, Aztreonam, Amikacin, and Cefuroxime. Similarly, Klebsiella pneumonia was resistant to Amoxicillin Clavulanic acid, Ampicillin, Cephaletin, Ofloxacin, and Trimethopim-Sulfamethoxazole.

In our study, no organism was found to be resistant to polymyxin. Colistin (or polymyxin E) is one of the limited choices for addressing life-threatening infections induced by multidrug-resistant (MDR) bacteria, especially CRE. Initially isolated in 1947 by Koyama and colleagues in Japan, colistin originated from the spore-forming soil bacterium Bacillus polymyxa subsp. colistinus[22,25].

Most phenotype-based methods face specificity and sensitivity challenges, are time-consuming (taking at least 12 to 24 hours), and lack specificity regarding the type of carbapenemase produced. Consequently, they are inadequately suited to the clinical imperative of promptly identifying cases[26].

CONCLUSION

This study concludes that MHP is cost-effective and provides accurate results. The high specificity and sensitivity of the test contribute to better patient management and prevent the spread of healthcare associated infections.

Ethical permission: Lyari General Hospital, Karachi, REC letter No. LGH/REC/163.
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AUTHOR CONTRIBUTION

Faisal H: Write-up, data collection
Idris A: Objecitve, data collection
Siddiqui HZ: Data analysis, results interpretation
Razzak S: Data entry, results interpretation
Yaseen M: Ethical consideration
Faisal A: Participant enrollment, consent forms, laboratory work

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