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Systematic Review

A SYSTEMATIC REVIEW ON THE DETECTION OF CARBAPENAMASE-PRODUCING ENTEROBACTERIACEAE

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Abstract

Introduction: Carbapenemase-producing Enterobacteriaceae (CPE) represents one of the most pressing and critical public health challenges associated with antibiotic resistance. Challenges persist in accurately and promptly identifying CPE despite the existence of diverse carbapenemases and multiple detection methods.
Aim: This study investigated diagnostic methods used for the detection of CPEs.

Methods: The systematic review and meta-analysis were conducted based on Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines. Electronic databases like Google Scholar, PubMed, Scopus, and Web of Science were used to find relevant articles. In addition, the Joanna Briggs Institute quality appraisal tool was used to assess the quality of the included studies. STATA 14.0 was used for statistical analysis. Heterogeneity was assessed by using Cochran's Q test and 12 statistics. In addition, publication bias was assessed using a funnel plot and Egger's test. A random effect model was used to estimate the pooled prevalence.

Results: The meta-analysis revealed an overall pooled proportion of 40.53% for phenotypic detection of carbapenemase activity across the 11 studies, with substantial heterogeneity observed. Subgroup analysis highlighted variations in detection proportions based on different methods, with mCIM showing the highest proportion at 58,20%, Carba NP at 27.79%, and MHT at 34,62%. Evaluation of publication bias indicated little impact on the results, maintaining the stability of the meta-analysis outcomes.
Conclusion: In conclusion, this systematic review showed a high prevalence of CPE across the studies. This study emphasizes the importance of standardized detection methods, global collaboration, and the integration of advanced techniques for accurate CPE detection.

INTRODUCTION

Numerous studies have focused on developing and evaluating methods for CPE detection. These studies encompass various approaches, including phenotypic and genotypic techniques. Phenotypic methods involve screening for CPE using antimicrobial susceptibility testing, while genotypic methods target the detection of specific carbapenemase genes, such as KPC, NDM, and OXA. Genotypic methods otherwise known as molecular techniques, include Loop-mediated isothermal amplification (LAMP) and the Polymerase Chain Reaction (PCR). (Lutgring et al., 2016) highlighted the challenges associated with detecting CPE, by using both the phenotypic and genotypic methods. The research group emphasized the need for accurate detection methods to guide appropriate antimicrobial therapy and infection control measures. more importantly, they stressed there isn't a single detection method that suits every circumstance perfectly. (Tamma et al., 2017) compared 11 phenotypic assays, their study emphasized the importance of selecting the most suitable method based on local epidemiology and resource availability and stressed the need for early identification of CPE to prevent their dissemination across different healthcare settings. Researchers examined two sets of bacterial isolates, specifically carbapenem-resistant Enterobacteriaceae (CRE). The first collection comprised 191 bacteria of which 122 were (CP-CRE) and 69 non-carbapenemase-producing (non-CP) CRE. The second set included 45 isolates from recent clinical cases at the Johns Hopkins Hospital (JHH) medical microbiology laboratory, containing 15 CP-CRE and 30 non-CP-CRE, gathered over three months. The study aimed to investigate the accuracy of various tests in detecting CRE strains. They determined the specificity and sensitivity for different tests by comparing their results to genotype analysis, considered the reference standard. (Miller et al.,2016) provided an expert review on the clinical laboratory identification of carbapenem-resistant and CPE, highlighting that resistance mechanisms involve the loss of outer membrane porins and the overexpression of efflux pumps. They discussed the advantages and limitations of various detection methods, including phenotypic assays, molecular techniques, and mass spectrometry-based methods. (Dortet et al.,2015) evaluated three biochemical tests, namely CARBA NP, Rapid CARB Screen, and RAPIDEC CARBA NP, for the Identification of CPE. Their findings indicated the potential utility of these tests in routine diagnostic laboratories.

Bogaerts et al. (2016) assessed the electrochemical assay known as the BYG Carba test for its effectiveness in quickly detecting CPE. They reported high sensitivity and specificity of the test, making it a promising tool for early detection. Apart from laboratory-based detection methods, the environmental reservoirs of CPE play a significant role in its transmission and persistence. (Orabueze et al.,2022) emphasized the importance of targeting environmental reservoirs as primary targets for control and prevention strategies against CPE. They contend that existing control measures are insufficient in curtailing their proliferation. To address this, they advocate that more research is needed to understand the ecology and epidemiology of CPE in the environment. They also highlighted the need for comprehensive surveillance and environmental hygiene protocol to mitigate the spread of CPE. There are also relevant studies that address the broader issue of antimicrobial resistance. (O'Neill et al.,2016) presented the final report and recommendations of the global review on tackling drug-resistant infections. This report emphasizes the urgent need for a global response to combat antimicrobial resistance, including strategies for infection prevention, appropriate antibiotic use, and surveillance systems. (Llor et al.,2014) attempted to address the issues of antimicrobial resistance and risk factors linked with antibiotic misuse. Their review emphasized the importance of antibiotic stewardship programs, education campaigns, and the development of new antibiotics to address the challenges of drug-resistant infections. (Nwafia et al.,2019) Investigated extended-spectrum beta-lactamase-producing *Escherichia coli* in a Nigerian tertiary hospital, focusing on molecular detection methods and assessment of antibiotic resistance patterns. Although their study focused on a different resistant mechanism, it highlights the broader issue of antibiotic resistance in healthcare settings. (Zhong et al.,2019) conducted an extensive review and meta-analysis to determine the efficacy and viability of several phenotypic techniques for Enterobacteriaceae carbapenemase detection. Their findings provide a comprehensive overview of various phenotypic assays and their performance characteristics. The works reviewed are by no means an exhaustive account of all available literature that is available on the detection and treatment of CPE. Many other studies have delved into the subject matter, but from a systematic analysis, a vast majority of scholars emphasize early detection as an essential component of treating CPE.

Comparison of Different Detection Methods

Phenotypic techniques are simpler and more economical, although they may have sensitivity and specificity restrictions. On the other hand, genotypic techniques require specialized equipment and expertise. the decision between these approaches frequently depends on the clinical or research context requirements and the resources at hand. A combination of both methods is often used to enhance accuracy, and ongoing advancements continue to improve their capabilities in CPE detection.

Phenotypic Detection Methods

Phenotypic techniques rely on the identification of carbapenemase activity or the presence of phenotypic traits associated with carbapenem resistance. (Tsai et al., 2020) reviewed the clinical laboratory detection methods for CR and CPE They discussed various phenotypic assays, such as the Carba NP test, and modified carbapenem inactivation method (mCIM). (Yamada et al.,2016) compared the Modified-Hodge test, and carbapenem inactivation method as a screening method for CPE. The Modified-Hodge test (MHT) is a phenotypic assay used to identify the production of carbapenemase enzymes, this test assesses the ability of bacterial isolates to hydrolyze carbapenem antibiotics, providing a qualitative indication of carbapenemase activity. This test is particularly useful in clinical laboratories for detecting CPE, which is of great concern due to its association with antibiotic resistance. (Tamma et al.,2017) compared the accuracy of CPE detection in different phenotypic assays, including the Rapidec Carba NP, Neo-Rapid Carb screen, Rapid Carb Blue screen, Manual Carba NP CLSI, Manual blue Carba, Modified Carba NP, Boronic acid synergy test, Metallo-lactamase Etestb, MHT, CIM, mMIC, and discovered that the result varied. (Tsai et al.,2020) Their observation indicated that by increasing the vortexing and incubation duration, they could identify strains with weak carbapenemase presence that initially showed negative results. However, they acknowledge that since this approach wasn't applied uniformly across all isolates, it remained uncertain whether this would universally enhance test performance. Furthermore, their study revealed that both the CIM and mCIM serve as effective straightforward, and cost–efficient techniques for phenotypically detecting CPO. Both methods are user-friendly and utilize materials readily accessible in most diagnostic microbiology laboratories. Notably, the CIM test displayed higher sensitivity (97.9%) and specificity (96.5%) compared to the mCIM test recommended by the Clinical and Laboratory Standards Institute (CLSI). Additionally, the CIM test required less time for completion.

For this study, I will be looking at four phenotypic tests, including the modified Hodge test, the Carba NP test, the Meropenem Hydrolysis Assay, and the modified Carba Inactivation method.

Modified Hodge Test (MHT)

The MHT is a phenotypic method widely used for the detection of carbapenemase production in Enterobacteriaceae. It involves the inoculation of a carbapenem-susceptible indicator strain adjacent to the test isolates on an agar plate, followed by incubation. The presence of a carbapenemase-producing strain leads to the growth of the indicator strain toward the carbapenem disk, producing a characteristic cloverleaf-like indentation. The MHT is easy to perform, inexpensive, and provides rapid results. However, it has limitations, such as false-positive results with certain non-carbapenemase producers and reduced sensitivity for certain carbapenemases, particularly those with weak activity.

The MHT is based on the principle that CPE produces carbapenemases, which can inactivate the carbapenem antibiotic in the presence of a carbapenem-susceptible indicator strain. The MHT involves streaking the indicator strain on an agar plate near a test isolate and examining the presence of a clover-leaf-like indentation or enhanced growth at the intersection of the two strains after overnight incubation. Several studies have reported high sensitivity (85-100%) and specificity (85-100%) of the MHT in detecting various carbapenemase types, including KPC, NDM, VIM, and OXA-48-like enzymes (Lutgring et al.,2016). The MHT method is based on the principle that carbapenemase production in Enterobacteriaceae can enhance the growth of carbapenem-susceptible indicator strains. (Lutgring et al.,2016) highlighted the importance of implementing standardized protocols for the MHT to ensure accurate results and reduce false-positive and false-negative rates. The CLSI provides guidelines for performing the MHT, including the preparation of bacterial suspensions, the inoculation of test organisms, and the interpretation of results They emphasized the need for training and quality control to minimize interpretive errors. (Tamma et al.,

2017) reported that the MHT exhibited good sensitivity and specificity but cautioned that the interpretation of results could be subjective, leading to inter-laboratory variability. They suggested combining the MHT with other confirmatory tests to improve accuracy. (Miller et al.,2016) reviewed the clinical laboratory detection of carbapenem-resistant and CPE. They highlighted the MHT as a cost-effective and easily implementable method. However, they acknowledged that the MHT may not detect all carbapenemase types and cautioned against relying solely on this test for CPE detection.

(Bogaerts et al.,2016) the BYG Carba test, an electrochemical assay for rapid laboratory detection of CPE, and compared it with the MHT. They reported that the BYG Carba test showed higher sensitivity and specificity than the MHT and recommended its use as a screening method in conjunction with the MHT for confirmation. (Yamada et al., 2016) compared the MHT with the Carba NP test and the carbapenem inactivation method as screening methods for CPE. They found that the MHT had a lower sensitivity compared to the Carba NP test but higher specificity than the carbapenem inactivation method. Initial reports of CIM were promising for the detection of OXA-48 and NDM-1, However, subsequent studies reported that CIM has lower detection rates (80 %, 50 %, and 91 %) of the OXA-48-like type carbapenemases. They concluded that combining multiple tests could enhance the accuracy of CPE detection. (Kuchibiro et al.,2018) evaluated the modified carbapenem inactivation method (mCIM) for detecting CPE and compared it with the MHT. They reported that the mCIM showed higher sensitivity and specificity than the MHT and recommended its use as a confirmatory test in conjunction with the MHT. (Zhong et al.,2019) assessed the accuracy and applicability of the MHT, for carbapenemase detection in Enterobacteriaceae. They found that the MHT had moderate sensitivity and specificity, emphasizing the need for combining multiple tests to improve detection rates. While the MHT is relatively simple, cost-effective, and easily implementable, its performance can be influenced by inter-laboratory variability and subjective interpretation. Combining the MHT with other confirmatory tests, such as the Carba NP test or modified carbapenem inactivation method, is recommended to improve the accuracy of CPE detection.

Carba NP Test

The Carba NP test method is based on the detection of carbapenemase activity using a colorimetric indicator. It relies on the addition of a phenol red indicator to the imipenem-containing test tube. The hydrolysis of imipenem by carbapenemases results in a pH change, leading to a colour change in the medium (Rudresh et al., 2017). The Carba NP test is simple, rapid, and has good sensitivity and specificity for detecting carbapenemase production. It can also differentiate between different carbapenemase types. However, the test requires skilled interpretation and false-positive results may occur due to other β-lactamases or porin defects. (Tamma et al., 2017) reported that the Carba NP test exhibited high sensitivity and specificity of 98% and 99% respectively, outperforming other methods such as the modified Hodge test and Carba NP variants. The study demonstrated the effectiveness of the Carba NP test in accurately identifying CPE isolates. Several studies have compared the Carba NP test method with other phenotypic assays. Studies like (Crowe et al., 2018) compared the Carba NP test with the CIM., and mCIM. They concluded that the Carba NP test was a simple and reliable alternative to the CIM and mCIM for detecting CPO. (Zhang et al., 2019) found that the Carba NP test exhibited good concordance with molecular methods, suggesting its potential as a rapid screening tool, to enhance the specificity of the Carba NP test, researchers have developed modified versions of the test. (Kong et al.,2021) proposed that the Carba NP test incorporated different chromogenic substrates to differentiate between different carbapenemases. Their study demonstrated the feasibility of Carba NP test in detecting and differentiating carbapenemase production in carbapenem-resistant Enterobacteriaceae. (Kumaral et al.,2018) also developed Carba NP test specifically for distinguishing between KPC- and MBL-producing Klebsiella species. The rapid detection of CPE is crucial for implementing appropriate infection control measures and selecting effective antibiotic therapy. (Livorsi et al.,2018) conducted a systematic review of the epidemiology of carbapenem-resistant Enterobacteriaceae in the United States. They emphasized the importance of rapid diagnostics, including the Carba NP test, in curbing the spread of CPE and guiding antimicrobial stewardship efforts. (Kunz et al.,2021) investigated the influence of antimicrobial stewardship and molecular rapid diagnostic tests on antimicrobial prescribing for extended-spectrum β-lactamase and carbapenemase-producing bacteria. They highlighted the potential of the Carba NP test to impact clinical decision-making and optimize antibiotic therapy. The Carba NP test method has shown great promise as a rapid and reliable tool for detecting carbapenemase production in Enterobacteriaceae. It has demonstrated high sensitivity and specificity, outperforming other phenotypic assays. Modified versions of the Carba NP test have been developed to

enhance specificity and differentiate between different types of carbapenemases. The clinical impact of the Carba NP test in guiding infection control measures and antimicrobial stewardship efforts is noteworthy. The Carba NP test method holds significant potential for combating the global menace of carbapenem-resistant Enterobacteriaceae.

Meropenem Hydrolysis Assay (MHA)

The MHA is a phenotypic test based on the ability of carbapenemases to hydrolyze meropenem (Bogaerts et al.,2016). It involves the incubation of the test isolate in a meropenem-containing agar plate, followed by the addition of a pH indicator. Carbapenemase production leads to the breakdown of meropenem, resulting in a colour change in the surrounding agar, indicating a positive result (Calderaro et al., 2017). The MHA is relatively easy to perform and provides reliable results. It demonstrates good sensitivity and specificity for several carbapenemase types. However, it may have reduced sensitivity for certain variants, and skilled interpretation is required to differentiate positive and negative results. In their study, partial hydrolysis of meropenem by MHA was observed after both 2 hours and 4 hours of incubation. (Calderaro et al., 2017) To clarify, the MHA method involves placing a carbapenemase-producing isolate onto an agar plate that contains meropenem. As the incubation progresses, CPE strains start producing carbapenemases, which then hydrolyze meropenem. This enzymatic activity leads to a zone of inhibition forming around the strain. The presence of this zone, which typically becomes visible after an incubation period of 18 to 24 hours, indicates carbapenemase production and the potential presence of CPE infection. The MHA method offers several advantages over other traditional methods for CPE detection Such as the Carba NP test, and MHT because it provides rapid results within 4-6 hours, enabling timely implementation of infection control measures. Secondly, it requires minimal specialized equipment and can be performed in routine microbiology laboratories. Moreover, the MHA method exhibits good diagnostic accuracy, which means that the method is effective at correctly identifying whether a sample contains CPE or not with a high degree of precision. comparable to other phenotypic and genotypic assays and has the potential for high-throughput screening due to its simplicity and scalability. Overall, the MHA method demonstrates good sensitivity and specificity for CPE detection. However, it is important to consider the prevalence of CPE in the tested population, as low prevalence can impact the positive predictive value of the assay. While the MHA method shows promise, it is not without limitations. One major concern is the potential for false-positive results due to the presence of other carbapenemase-producing organisms, such as non-Enterobacteriaceae species. Furthermore, the MHA method relies on the hydrolysis of meropenem, and the presence of low-level carbapenem resistance mechanisms may result in false-negative results. Additionally, interpretation of MHA results can be subjective, requiring well-trained personnel to accurately identify and measure the zone of inhibition. The MHA method has been compared with other phenotypic and genotypic assays for CPE detection. These studies demonstrate comparable diagnostic accuracy between the MHA method and reference standards. However, genotypic assays, such as polymerase chain reaction (PCR), offer the advantage of identifying specific carbapenemase genes, aiding in epidemiological investigations and surveillance. The MHA method offers a rapid and cost-effective solution for detecting CPE, enabling the prompt implementation of infection control measures (Zhou et al., 2018). Further research is needed to optimize the MHA method, addressing challenges related to false-positive and false-negative results. Integration of the MHA method with genotypic assays, such as PCR, may enhance the overall accuracy and epidemiological utility of CPE detection. Continued evaluation and validation of the MHA method in diverse clinical settings will contribute to its wider adoption and utility in combating the global challenge of CPE infections (AlTamimi et al.,2017).

Modified Carbapenem Inactivation Method (mCIM)

The mCIM is a phenotypic test that evaluates the ability of carbapenemases to inactivate carbapenem antibiotics (Pierce et al.,2017). It involves the incubation of the test isolate with a meropenem disk, followed by the placement of the disk on a lawn of an indicator strain. If carbapenemase production is present, it inactivates the meropenem, resulting in the growth of the indicator strain around the disk. The mCIM is easy to perform, relatively inexpensive, and provides results within 24 hours (Tamma et al.,2017). It has demonstrated good sensitivity and specificity for detecting diverse carbapenemases. However, the mCIM may produce false-negative results for carbapenemases with weak activity or when porin mutations are present. The mCIM relies on the deactivation of carbapenems through the action of carbapenems enzymes produced by CPE. such as ertapenem or meropenem, to a bacterial culture suspected of harbouring

carbapenemases. If the organism produces carbapenemases, the antibiotic is inactivated, resulting in growth within the inhibition zone around the antibiotic disk. whereas MHT assesses the ability of the organism to hydrolyze carbapenem antibiotics, leading to distinct observable changes in the test result. The mCIM method has shown excellent sensitivity and specificity for detecting CPE, making it a reliable diagnostic tool (Bogaerts et al.,2016). Studies have reported high sensitivity values ranging from 96% to 100% and specificity values ranging from 98% to 100% when compared to molecular methods. The mCIM method is relatively simple to perform, requiring minimal resources and equipment. It does not necessitate specialized molecular techniques, making it more accessible in resource-limited settings (Tamma et al., 2017). This simplicity contributes to its cost-effectiveness compared to molecular methods. It applies to various Enterobacteriaceae species, including *K. pneumoniae*, *E. coli*, and *E. cloacae*. It can detect different types of carbapenemases, including KPC, OXA, NDM, and VIM, making it versatile for epidemiological surveillance and infection control purposes. The mCIM method relies on the phenotypic expression of carbapenemase activity, which can vary among different strains and carbapenemases. (Bogaerts et al.,2016). Some CPE isolates may exhibit weak carbapenemase activity, leading to false-negative results. Combining the mCIM method with molecular tests can help overcome this limitation (Pierce et al., 2017). It requires overnight incubation, which may delay the detection of CPE compared to rapid molecular methods. However, the simplicity and cost-effectiveness of the mCIM method still make it a valuable tool in settings where rapid molecular methods are not available. Variations in the interpretation of mCIM results and the absence of standardized guidelines can lead to inconsistencies in reporting. Standardization efforts, such as establishing clear breakpoints and interpretive criteria, are essential to ensure uniformity and comparability of results across laboratories. In a meta-analysis by (Tamma et al., 2017) the mCIM method demonstrated a pooled sensitivity of 97.8% (95% CI: 96.6% to 98.7%) and specificity of 99.5% (95% CI: 98.8% to 99.8%) compared to molecular reference methods. Other studies have reported similar high sensitivity and specificity values, supporting the reliability of the mCIM method for CPE detection.

mCIM offers a valuable phenotypic approach for detecting CPE (Miller et al., 2016). Its simplicity, cost-effectiveness, and wide applicability make it a valuable tool, especially in resource-limited settings. Despite its limitations, the mCIM method demonstrates excellent diagnostic accuracy when compared to molecular methods. Standardization efforts and further research are needed to address its limitations and enhance its performance as a reliable diagnostic tool for CPE detection (Miller et al., 2016). Phenotypic methods play a crucial role in the detection of CPE. Each method has its advantages as well as limitations, and the decision of method should consider the specific requirements of the laboratory. A combination of multiple phenotypic tests like MHT and the Carba NP test may be necessary for the accurate detection and characterization of CPE strains.

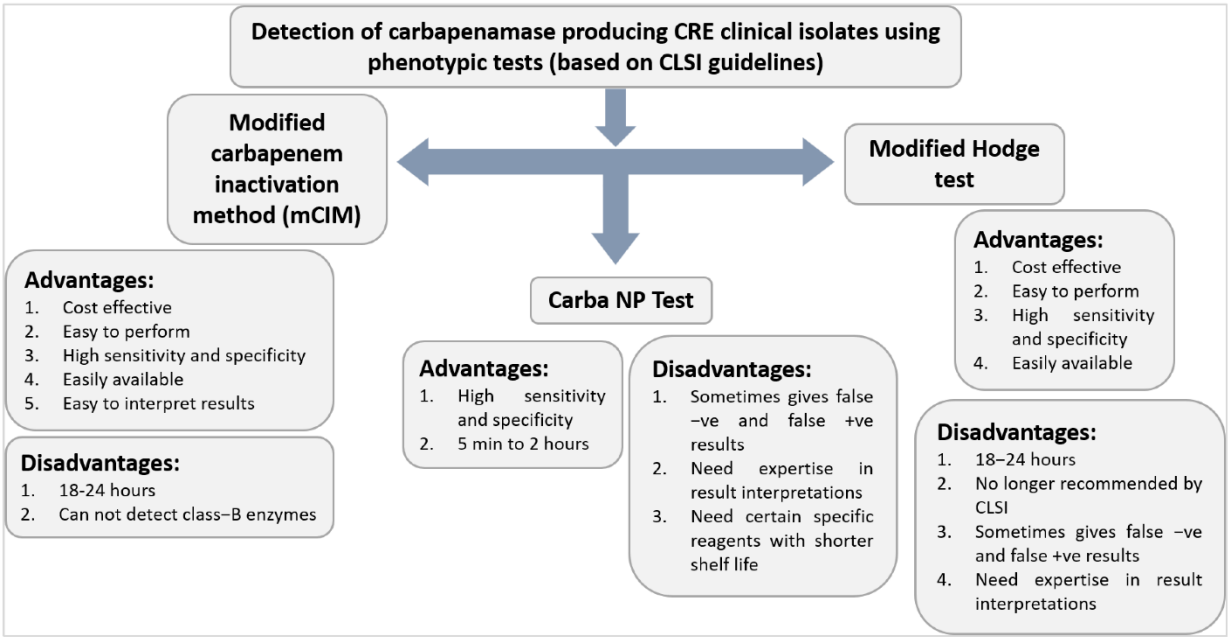

Figure 3: Characteristics of specific phenotypic tests, as outlined by CLSI guidelines, for identifying clinical isolates of carbapenemase-producing (Rabaan et al., 2022).

Genotypic Detection Methods

Genotypic methods detect the presence of specific carbapenemase genes using molecular techniques, such as polymerase chain reaction (PCR) and DNA sequencing. (Lutgring et al.,2016) discussed the challenges associated with the genotypic detection of CPE. The genotypic methods primarily target the detection of specific carbapenemase genes, such as *blaKPC, blaNDM, blaOXA*, and *blaIMP*. Genotypic assays allow for the detection of specific resistance genes and can provide valuable information regarding the types of carbapenemases present (Tomokazu et al., 2018). However, genotypic methods require specialized laboratory equipment, trained personnel, and additional time compared to phenotypic methods. PCR is a molecular biology technique that allows the amplification of specific DNA sequences from a complex mixture of genetic materials (Tomokazu et al., 2018). The method relies on the use of DNA primers that bind to the target DNA region of interest and a thermostable DNA polymerase enzyme to synthesize complementary DNA strands (Saliba et al.,2019). The PCR method for the detection of CPE primarily focuses on the amplification and identification of genes encoding carbapenemases (Mariappan et al., 2017). The most common carbapenemase genes include *blaKPC, blaNDM, blaVIM, blaIMP*, and *blaOXA*-48-like. These genes are often located on mobile genetic elements, such as plasmids, facilitating their transfer between bacteria and contributing to the spread of resistance. To detect carbapenemase genes, specific primers are designed to target conserved regions of the genes. The PCR assay involves the use of purified DNA sourced from either bacterial cultures or clinical samples, such as blood or swabs. This technique amplifies specific DNA segments, allowing for the identification of genetic material or pathogens in the samples. The DNA template is mixed with the PCR reaction mixture containing primers, nucleotides, buffer, and DNA polymerase (Mariappan et al., 2017). The PCR reaction is then subjected to a series of temperature cycles, typically involving denaturation at a high temperature, annealing at a lower temperature, and extension at an intermediate temperature. After PCR amplification, the presence of carbapenemase genes can be detected using various methods. Agarose gel electrophoresis allows the visualization of the amplified DNA fragments based on their size, confirming the presence of the target genes. Alternatively, real-time PCR can be employed, which enables the quantification of DNA amplification in real-time during the PCR reaction. This method utilizes fluorescent probes that emit fluorescence when bound to the target DNA sequences, allowing the monitoring of the amplification process. The PCR method offers several advantages for the detection of CPE Firstly, it provides high sensitivity, capable of detecting even low amounts of target DNA in complex samples. This sensitivity is critical for early detection and accurate diagnosis. Secondly, PCR assays are highly specific, as the primers are designed to specifically amplify the carbapenemase genes of interest, reducing the chances of false-positive results. Thirdly, PCR assays can be performed rapidly, typically within a few hours, enabling timely interventions and appropriate patient management.

The PCR also has certain limitations. The method requires specialized equipment, including a thermocycler and gel electrophoresis apparatus, which may limit its accessibility in resource-limited settings. PCR assays require skilled laboratory personnel to perform and interpret the results accurately. Furthermore, the method is susceptible to contamination, which can lead to false-positive results (Mariappan et al.,2017). Appropriate measures, such as the use of negative controls and adherence to good laboratory practices, must be taken to mitigate this risk. Lastly, PCR detects the presence of genes encoding carbapenemases but does not provide information about their expression or the phenotypic resistance of the bacteria. PCR method has revolutionized the detection of CPE Its high sensitivity, specificity, and rapid turnaround time make it an invaluable tool in the fight against antibiotic resistance. By targeting specific carbapenemase genes, PCR allows for the accurate identification of resistant bacteria, facilitating timely interventions and infection control measures. However, PCR should be complemented with phenotypic methods to confirm the expression of carbapenemase genes and the antibiotic resistance phenotype. Future advancements in PCR technology, such as the development of multiplex assays and point-of-care devices, hold promise for further enhancing the detection and management of CPE infections.

Loop-mediated Isothermal Amplification (LAMP) molecular method emerges as a potent tool for identifying CRE with speed and precision. This innovative technique harnesses the principle of isothermal amplification, sidestepping the need for elaborate equipment and offering a rapid and efficient approach to

CRE detection (Notomi et al.,2015). In the LAMP method, target DNA sequences within CRE are amplified under constant temperature conditions, bypassing the thermal cycling of traditional PCR methods. This not only expedites the process but also reduces the complexity of the equipment required, making it more accessible for various laboratory settings (Wong et al., 2018). The LAMP assay boasts high sensitivity and specificity, enabling the accurate identification of CRE strains. It exhibits minimal cross-reactivity with non-CRE organisms, thereby minimizing false positives. The simplicity of result visualization, often through colour changes or fluorescent signals, further enhances the usability of the LAMP method. With its quick turnaround time of about 40 minutes and user-friendly nature, the LAMP molecular method holds significant promise for combating CRE outbreaks. Its potential to revolutionize CRE screening and surveillance, particularly in resource-limited settings, underscores its importance in enhancing clinical decision-making and infection control measures. As an innovative diagnostic approach, the LAMP method equips healthcare providers with a potent tool to swiftly and accurately identify CRE, thus contributing to more effective patient management and public health responses (Zhang et al., 2019). Genotypic methods, such as PCR and LAMP, are highly specific and can detect a wide range of carbapenemase genes simultaneously. These techniques provide rapid results and enable the identification of specific carbapenemase types.

Identification of Gaps in the Existing Literature

Despite the extensive research on CPE detection, several gaps exist in the current literature. Firstly, there is a need for more adoption of standardized methods and guidelines for CPE detection like the European Committee on Antimicrobial Susceptibility Test (EUCAST) standards to ensure consistency and comparability of results across different laboratories. The lack of standardized protocols hinders the accurate estimation of CPE prevalence and limits the comparability of studies. Although molecular methods, particularly PCR and LAMP, are highly sensitive and specific, their feasibility and cost-effectiveness for routine diagnostic use in resource-limited settings require further evaluation. Simplified and cost-effective molecular assays that can be easily implemented in settings with limited resources would greatly enhance CPE detection and surveillance. (Zhang et al.,2014) compared the performance of different detection methods in various sample types, such as rectal swabs, urine, blood, and respiratory specimens. They found that LAMP was the most sensitive and specific method for detecting the mcr-1 gene in rectal swabs, urine, and blood samples, while PCR was the most sensitive and specific method for detecting the mcr-1 gene in respiratory samples. Such studies are essential to determine the optimal detection method for different clinical scenarios and improve our understanding of the diagnostic accuracy of these methods. The identification of CPE is crucial for the timely implementation of infection control measures and appropriate antibiotic therapy.

RESEARCH METHODOLOGY

This study seeks to perform a systematic review on the identification of CPE, which presents a significant public health threat owing to its resistance to carbapenem antibiotics. The systematic review will provide a comprehensive analysis of the various methods and techniques employed for the detection of CPE, including phenotypic and genotypic assay. This section presents an overview of the research methodology employed for conducting the systematic review.

Research Design

the preferred reporting items for systematic review and meta-analysis (PRISMA) guidelines, among others provided the basis for the research methodology .an already established process was followed during this review to guarantee objectivity, reliability, and minimal bias.

Search Strategy

An extensive search strategy was developed to identify relevant studies from electronic databases, including Google Scholar, PubMed, Scopus, and Web of Science. The search terms were carefully selected to cover the topic of interest, such as CPE, "detection methods," and related variations of carbapenemase resistant, Enterobacteriaceae, and carbapenem. The search was conducted without any language or time restrictions to include as many relevant studies as possible.

Study Selection

The selection process consists of two stages: screening titles and abstracts and full-text assessment. the titles and abstracts of the retrieved articles were screened to identify potentially relevant studies. Full-text articles were assessed against predefined inclusion and exclusion criteria.

Inclusion and Exclusion Criteria

Studies meeting the following criteria were included on the detection of CPE, describing the use of phenotypic or genotypic methods for detection, reporting the performance metrics (e.g., sensitivity, specificity) of the detection method, and being published in peer-reviewed journals. Studies that do not meet these criteria, such as review articles, case reports, or conference abstracts, will be excluded.

Data Analysis

A narrative synthesis of the included studies was conducted, summarizing the characteristics, findings, and limitations of each study. Key themes and patterns were identified to address the research objectives, If feasible and appropriate. a meta-analysis has been performed to estimate pooled sensitivity, specificity, and other relevant outcomes. The research evaluated the heterogeneity among the studies and conducted subgroup analysis to identify the presence of significant heterogeneity.

Risk of Bias and Publication Bias

Bias risk assessments were conducted, both within individual studies and across the included studies, encompassing aspects such as selection bias, performance bias, and reporting bias. To explore the potential for publication bias, funnel plots, and statistical tests were employed.

Ethical Considerations

No ethical authorisation was necessary for this systematic review, every piece of the document was gathered from publicly accessible sources, and the evaluation steps were conducted with full adherence to ethical standards and copyright.

Limitations

This systematic review may have several limitations. Firstly, the inclusion of only published studies may introduce publication bias. Secondly, the heterogeneity among the included studies, including differences in study designs, settings, and methodologies, may have limited the ability to perform a meta-analysis. Additionally, the exclusion of non-English language articles may introduce a language bias. The systematic review will involve a thorough search strategy, study selection based on predefined criteria, data extraction, quality assessment, and data synthesis. The outcome of this review will contribute to the understanding of various detection methods for CPE, identify knowledge gaps, and guide future research in this critical area of antimicrobial resistance.

RESULTS

In the initial exploration across scientific databases, including PubMed, Medline, the Cochrane Library, Web of Science, and Google Scholar, a comprehensive set of 31 articles was procured. After meticulous scrutiny, seven articles were excluded due to duplications, leaving 24 full-text articles for in-depth quality assessment. Remarkably, all 18 of these articles garnered assessments exceeding the 50% threshold on the Joanna Briggs Institute (JBI) quality assessment scale, attesting to their robustness. Subsequently, two articles were omitted from consideration due to their lack of pertinent outcome data, while five additional articles were excluded due to an absence of primary data. Consequently, this review culminated in the inclusion of 11 scientifically eligible studies. The detailed process of article selection is presented in Figure 4, while Table 1 provides a concise overview of the primary characteristics of the encompassed studies.

Characteristics of Study

The particulars of the 13 studies analysed, as outlined in Table 1 (Belouad et al., 2023; Beresford & Maley, 2019; Davari et al., 2022; Eltahlawi et al., 2023; Enterobacterales, 2021; Ghaith et al., 2019; Lefebvre et al., 2015; Li et al., 2019; Mulla et al., 2016; Olowo-Okere et al., 2020; Shrief et al., 2022; Too et al., 2023; Velasco et al., 2017), provide a comprehensive overview of research activity in this field. Importantly, all the reviewed articles were composed in English, ensuring that their findings are accessible and can be disseminated globally**.**

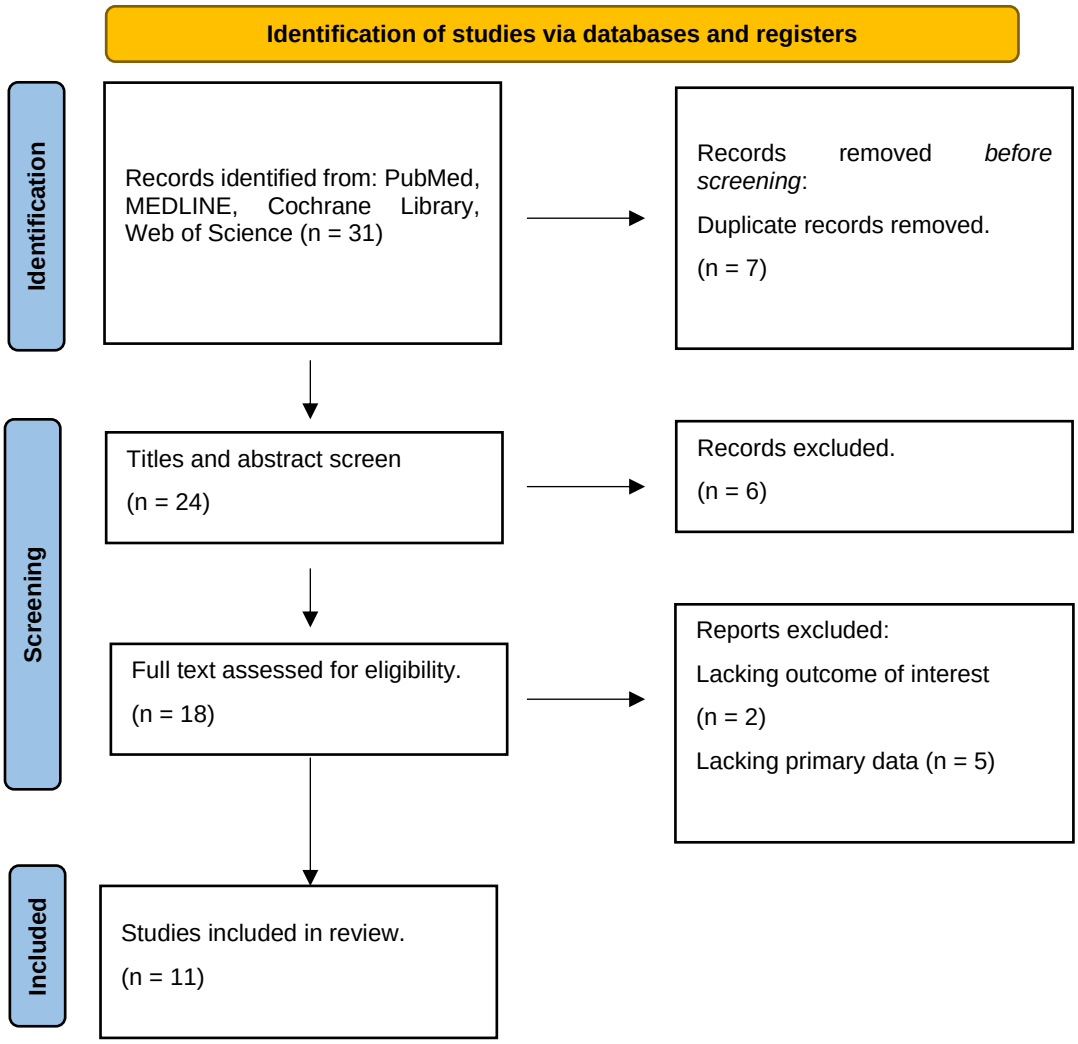

Figure 4: Flow chart for study eligibility following PRISMA criterion. (Formatted as per Page et al., 2020)

A comprehensive overview of the included studies related to the detection of CPE. The table presents key details for each study, including the authors and publication year, the study's duration in months, the geographical location where the study was conducted, the total number of bacterial isolates examined, the number of isolates that tested positive for CPE, the phenotypic detection method employed, and the genotype detection method used. Notably, these studies were conducted in diverse global locations, such as Canada, Nigeria, Australia, Morocco, Iran, India, the USA, China, Egypt, and Thailand. The phenotypic detection methods include MHT, Carba NP, and mCIM, while genotype detection methods primarily involve Polymerase Chain Reaction (PCR). The timeframe for these studies ranged from 2015 to 2023, with an

average study duration of 22 months. In this table, we can observe that a total of 5228 carbapenemase isolates were collected, and out of these, 1121 were found to be positive.

Table 1: Description of studies included in phenotypic and genotypic detection method.

SN	Author	Year of Publication	Study Duration (Months)	Location	Total isolates	CPE positive isolates	Phenotypic Detection Method	Genotype Detection Method
1	Lefebvre et al	2015	29	Canada	742	169	MHT	PCR
2	Olowo-Okere et al	2020	6	Nigeria	292	19	Carba NP	RT-PCR
3	Beresford and Maley	2019	116	Australia	137	135	mCIM	PCR
4	Belouad et al	2023	4	Morocco	2875	330	Carba NP	PCR
5	Davari et al	2022	5	Iran	173	20	mCIM	PCR
6	Mulla et al	2016	3	India	95	40	MHT	PCR
7	Hosoda et al	2021	7	USA	70	51	Carba NP	RT-PCR
8	Li et al	2019	24	China	101	79	mCIM	PCR
9	Ghaith et al	2019	6	Egypt	413	169	MHT	PCR
10	Shrief et al	2022	7	Egypt	200	64	mCIM	PCR
11	Velasco et al	2017	36	Thailand	130	45	Carba NP	PCR

Pooled proportion based on phenotypic detection method.

Figure 5 shows the meta-analysis adopting a random-effect model to assess studies that investigated the phenotypic detection of carbapenemase activity associated with carbapenemase-producing Enterobacteriaceae. The overall pooled proportion of positive results in these studies, which used methods such as the MHT, Carba NP, and mCIM method, was found to be 40.53% (95% confidence interval: 24.79% - 57.44%). The analysis included data from 11 different studies, indicating a broad range of sources. However, it's important to note that there was substantial heterogeneity between these studies, as indicated by an I^2 value of 99.11%. This high heterogeneity suggests that the results from the different studies were not consistent with each other, and the variation in findings was statistically significant (as indicated by a p-value of less than 0.001). In other words, the methods used, or the populations studied in these 11 studies might have differed significantly, leading to the observed variation in outcomes.

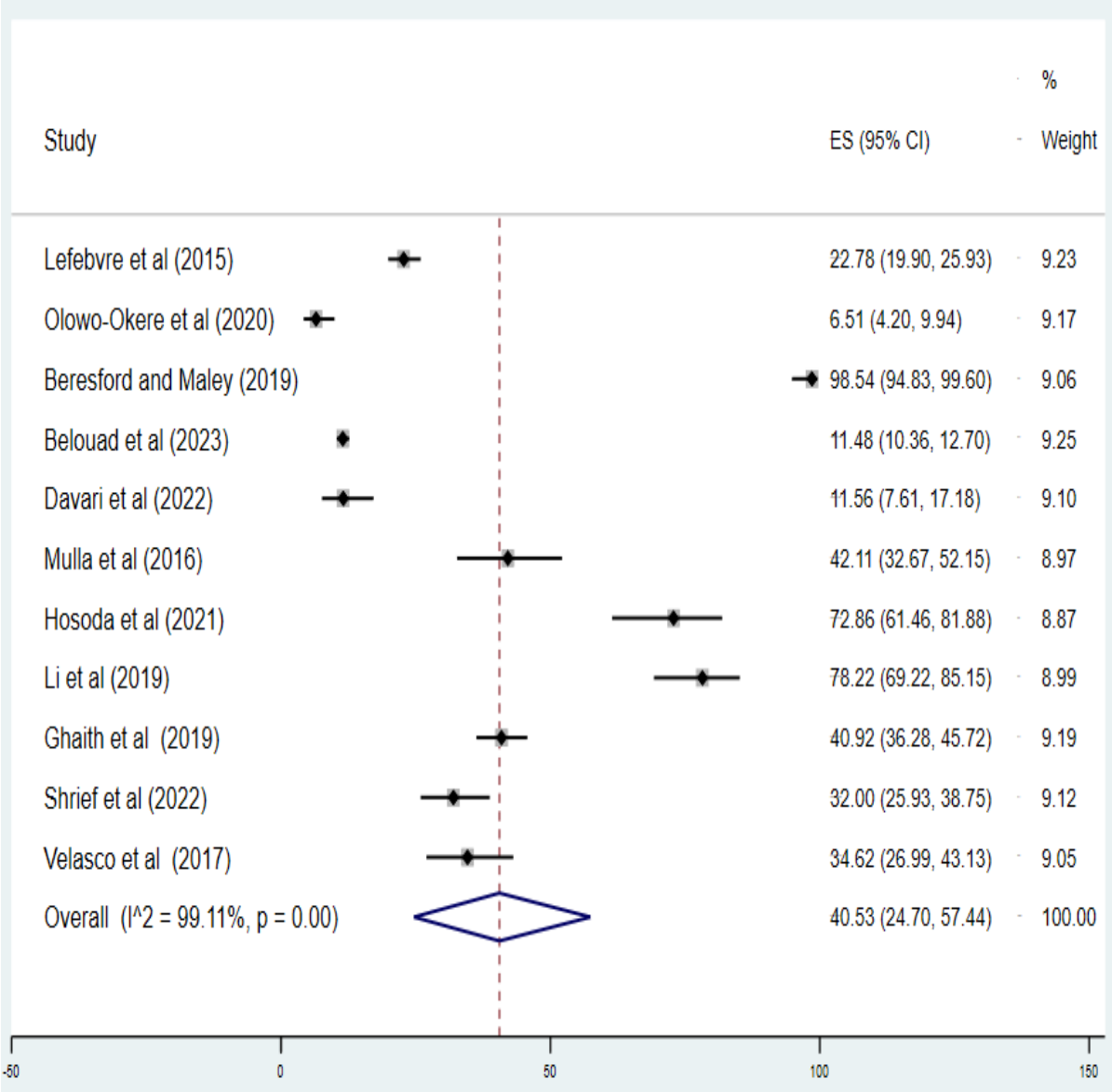

Figure 5: Forest plot of the pooled proportion of phenotypic detection of carbapenemase activity.

Subgroup analysis of Phenotypic detection method (PDM)

Figure 6 shows the subgroup analysis was performed to estimate the difference in phenotypic detection method and to determine the existence of heterogeneity. This analysis aimed at assessing variations in Carba-NP test, MHT and mCIM. The results unveiled noteworthy differences in the efficacy of these methods. Notably, the mCIM method emerged as the most effective, with a pooled proportion of 58.20% (95% CI: 14.23–95.26%), signifying its potential sensitivity in detecting carbapenemase activity. Conversely, the Carba-NP test displayed the lowest pooled proportion at 27.79% (95% CI: 10.72–49.07%), suggesting comparatively reduced sensitivity. The MHT method fell in between, with a pooled proportion of 34.62% (95% CI: 20.92–49.74%). These findings underscore the importance of selecting the most appropriate phenotypic detection method based on specific clinical or research contexts, as their effectiveness in identifying carbapenemase activity can substantially differ.

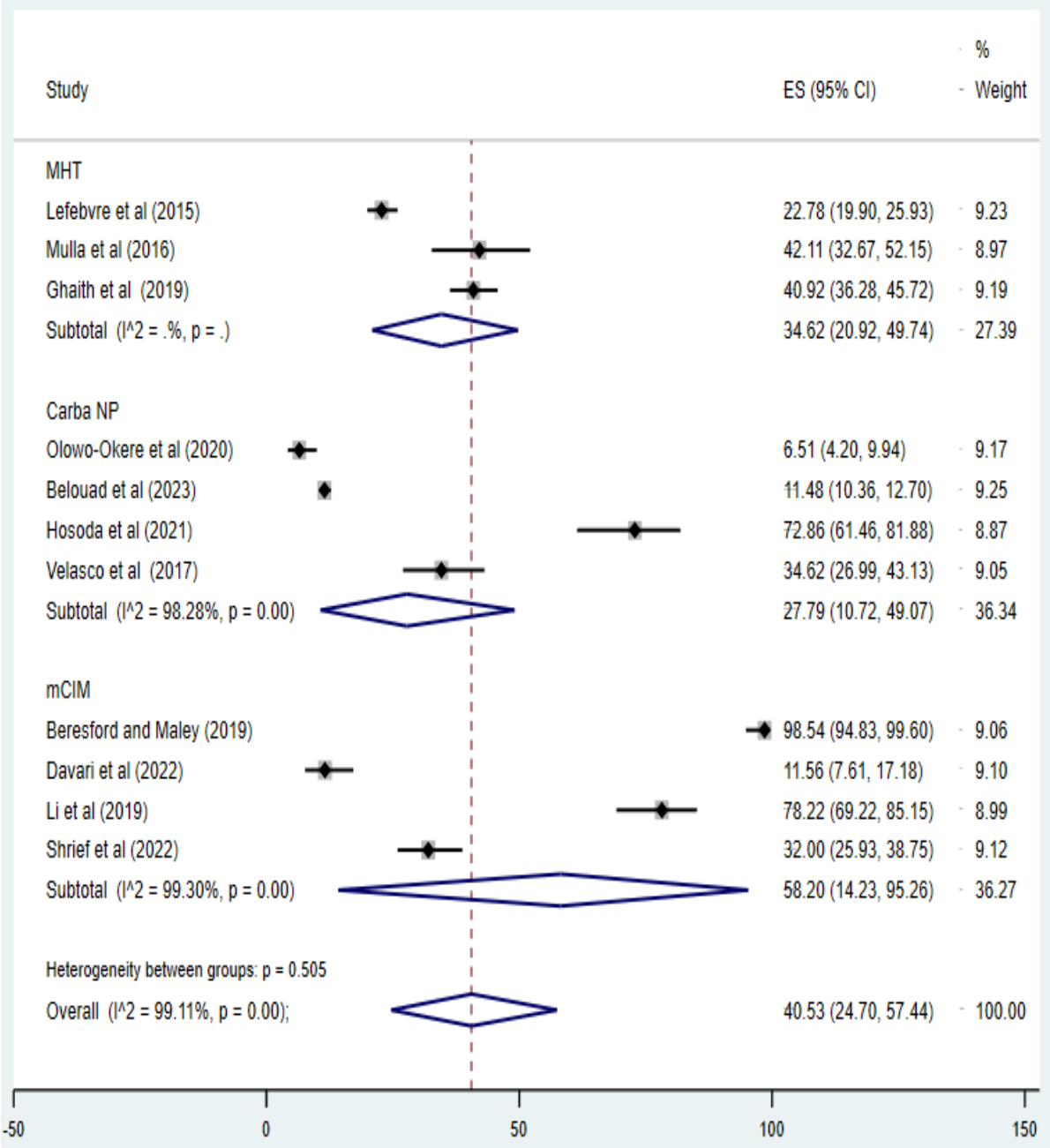

Figure 6: Forest plot displayed the combined proportion in a subgroup analysis for the phenotypic detection of carbapenemase activity.

Publication bias

Figure 7 shows the subgroup analysis was conducted to estimate the difference in phenotypic detection method to determine the presence of heterogeneity. This analysis aimed at assessing variations in Carba-NP test, MHT, and mCIM. The results unveiled noteworthy differences in the efficacy of these methods. Notably, the mCIM method emerged as the most effective, with a pooled proportion of 58.20% (95% CI: 14.23–95.26%), signifying its potential sensitivity in detecting carbapenemase activity. Conversely, the Carba-NP test displayed the lowest pooled proportion at 27.79% (95% CI: 10.72–49.07%), suggesting comparatively reduced sensitivity. The MHT method fell in between, with a pooled proportion of 34.62% (95% CI: 20.92–49.74%). These findings underscore the importance of selecting the most appropriate

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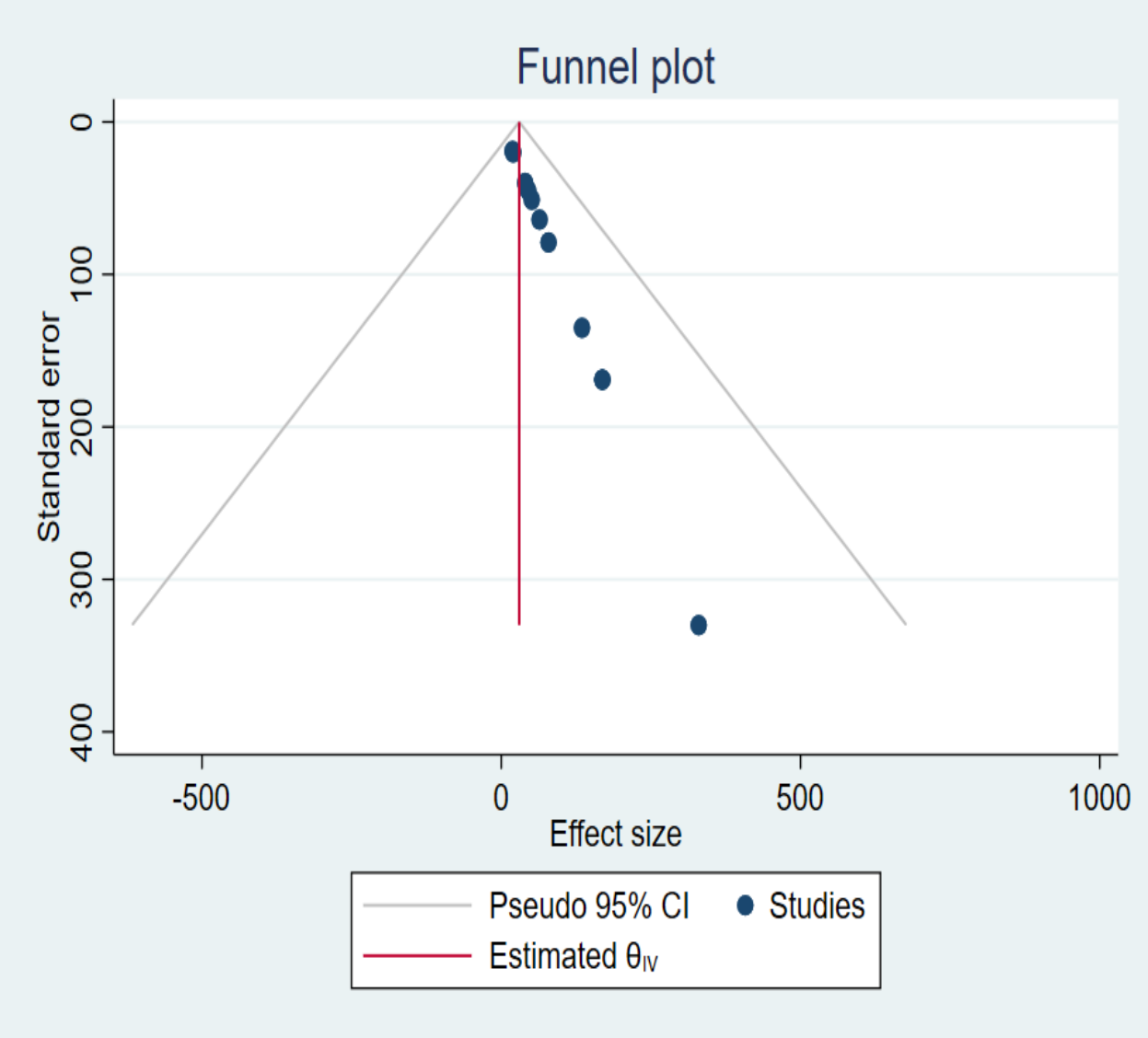

Figure 7: Funnel plot signifying the absence of publication bias in the pooled proportion of phenotypic detection of carbapenemase performance within a systematic review and meta-analysis.

DISCUSSION

The systematic review and meta-analysis aimed to evaluate the diagnostic method utilized for detection of CPE. The emergence of antibiotic resistance within the Enterobacteriaceae family has been extensively documented and now presents a substantial menace to healthcare delivery (Almugadam et al., 2018). Due to their elevated antibiotic resistance profiles, CPE pose formidable therapeutic challenges, as they possess the capability to enzymatically degrade all beta-lactam antibiotics, including carbapenems, thereby rendering them inefficacious (Huttner et al.,2013). The heightened prevalence of CPE may also contribute to increased mortality rates, prolonged hospitalizations, and heightened utilization of healthcare resources (Wailan et al., 2016). Consequently, the determination of the aggregated prevalence of CPE assumes pivotal importance. It serves as a crucial stride in providing insights into the temporal and geographical distribution of carbapenem resistance, as well as the magnitude of this predicament. This information, in

turn, is instrumental in formulating a comprehensive national public health strategy aimed at countering the burgeoning threat posed by these developing pathogens.

In this comprehensive review and meta-analysis, the combined occurrence that comprised of MHT, Carba NP test, and mCIM was 40.53% (95% CI; 24.79 to 57.44). The observed elevated rate of resistance to carbapenems might be attributed to several factors including prior exposure to antimicrobial agents, a history of hospitalization, Extended hospitalizations, the use of invasive medical equipment, older age, and the existence of serious underlying medical issues (Van et al., 2018). Furthermore, it is plausible that the unregulated prescription of antibiotics without considering their vulnerability patterns, or the introduction and dissemination of carbapenem-resistant bacterial strains from regions with high resistance rates, could contribute to this phenomenon. The repetitive, inappropriate, and inaccurate utilization of antimicrobial medications in empirical treatment, when combined with insufficient infection control measures, it can also contribute to the rising occurrence of Carbapenem-resistant Enterobacteriaceae in the community. The pooled estimate higher when compared to data with the reports from 4.9% in Kuwait (Jamal et al.,2015), 5.19% in Lebanon (Hamze et al.,2018), 5.74% in Malaysia (Zaidah et al.,2017), 5.1% in Senegal (5.1%) (Makhtar et al.,2017), and the 4.6% in United Arab Emirates (Moubareck et al., 2018). Nevertheless, The reported pooled prevalence was found to be lower than the figure of 54.1% reported in Egypt by (Kotb et al.,2020) This variance can be attributed to several factors, including differences in the methods used for antibiotic susceptibility testing (AST), variations in the target population, variations in sample types, the number of bacterial isolates studied, the criteria used to classify the presence of carbapenemase-producing isolates, discrepancies in antibiotic usage policies, and differences in geographical locations. Furthermore, these differences may also arise from variances in local antibiotic prescription practices and the effectiveness of infection control programs in various healthcare facilities, as observed in the study by (Dahab et al., 2017).

Subgroup analysis was undertaken to assess potential variations in phenotypic detection methods for ascertaining the existence of diversity among chosen studies. The studies in question employed three distinct methods: the Carba-NP test, MHT, and the mCIM. Among these, the highest aggregated proportion of phenotypic identification of carbapenemase activity was observed in studies employing mCIM, with a prevalence of 58.20% (95% CI: 14.23–95.26%). Conversely, the lowest proportion was recorded in studies utilizing the Carba NP method, where it stood at 27.79% (95% CI: 10.72–49.07%). Studies employing the MHT method yielded a pooled proportion of 34.62% (95% CI: 20.92–49.74%) for the phenotypic detection of carbapenemase activity. The highest aggregated proportion of phenotypic detection of carbapenemase activity, as reported in studies utilizing the mCIM, is a significant finding that has important implications for the diagnosis and management of CPE. The prevalence of 58.20% (95% CI: 14.23–95.26%) observed in these studies highlights the effectiveness of the mCIM in identifying CPE strains. This finding is consistent with the growing recognition as a valuable tool in the detection of carbapenemase activity among Enterobacteriaceae. Several studies (Nordmann et al.,2019: Iovleva et al.,2017) have contributed to our understanding of the prevalence of CPE and the diagnostic methods employed to detect them. Notably, research in the field of clinical microbiology has emphasized the need for accurate and timely identification of CPE due to their significant implications for patient care and infection control. The mCIM, as one of the phenotypic detection methods, has garnered attention for its ability to detect carbapenemase activity in CPE strains effectively.

Table 1, which serves as a foundational reference point for our discussion, encapsulates essential information extracted from eleven diverse studies encompassing different geographic regions and timeframes. These studies, primarily conducted in Africa and Asia, have delved into the pressing issue of CPE detection. It's important to note that while most studies were carried out in these continents, two exceptions hail from Canada and the United States, underscoring the global relevance and significance of this research topic (Ambretti et al., 2019). The temporal scope of these investigations' spans from 2015 to 2023, reflecting the ongoing nature of research dedicated to understanding and mitigating the spread of CPE. Over an average study duration of 28 months, researchers diligently collected an impressive cohort of 5228 carbapenemase isolates, ultimately identifying 1121 as positive for carbapenemase production. These numbers emphasise the pervasive nature of CPE and emphasize the pressing need for effective detection methods to combat this global health concern (Aljeldah et al., 2022). Of particular interest is the diverse array of phenotypic detection methods employed by these studies. The Carba NP test, MHT, and mCIM represent three distinct yet pivotal approaches to identify carbapenemase activity within

Enterobacteriaceae. Each of these methods operates on unique mechanisms and criteria, contributing to the multifaceted nature of CPE identification (Yamada et al., 2016; Kuchibiro et al., 2018). Furthermore, it's noteworthy that despite the phenotypic diversity in detection methods, all the included studies uniformly adopted PCR as the molecular technique of choice to conclusively confirm the presence of carbapenemase genes. PCR, a well-established and highly sensitive molecular biology tool, allows for the specific and definitive detection of genetic markers associated with antibiotic resistance, reinforcing the robustness of the findings (Harris et al.,2023).

In Figure 7, The funnel plot exhibited clear asymmetry, which could suggest the possibility of publication bias. Publication bias arises when studies with particular outcomes are more inclined to get published leading to a skewed representation in the available literature, while those with other outcomes, often non-significant or null findings, may remain unpublished. This can skew the overall effect size estimated by a meta-analysis, as it may overrepresent studies with significant results. To assess the impact of this potential publication bias, a thorough analysis was conducted. The Egger correlation test is a statistical method used to quantitatively evaluate publication bias. In the study, results of the Egger correlation test yielded a p=value of 0.2248. Importantly, this p-value suggests no significant publication bias. In other words, there is no strong statistical evidence to conclude that publication bias significantly affected the results of the meta-analysis. Furthermore, z-score of 3.06, often associated with the Egger test, provides reassuring evidence that there is no substantial publication bias in the included studies. This suggests that smaller or less significant studies are not disproportionately absent from the analysis, indicating a more balanced representation of research findings in the literature. This corroborates the findings of the Egger test, further supporting the conclusion that publication bias did not exert a substantial influence on the results of the meta-analysis (Ropovik et al.,2021). Crucially, it's noteworthy that despite the apparent asymmetry observed in the funnel chart, the estimated value of the combined effect size, a key outcome in meta-analysis, did not change significantly. This stability in the effect size estimation suggests that even if there were potential publication bias, its impact on the overall results was minimal. In essence, the meta-analysis results remained relatively stable and trustworthy.

A genotypic detection meta-analysis was not conducted in this study because all included studies uniformly adopted PCR as the molecular technique for confirming the presence of carbapenemase genes. This uniformity in methodology could introduce publication bias, as studies confirming the presence of carbapenemase genes using PCR are more likely to be published, potentially skewing the overall results. Additionally, the homogeneity in the use of PCR limits the variability in the data and may not provide significant additional insights beyond confirming the presence of these genes, thus reducing the information gain. Furthermore, the findings would primarily represent the accuracy of PCR in detecting carbapenemase genes but may not capture the broader landscape of genotypic methods used globally, limiting the generalizability of the meta-analysis findings to regions or settings where alternative genotypic methods are more prevalent. Therefore, given the uniformity of genotypic methods across the included studies, the focus of the analysis was appropriately placed on phenotypic detection methods and their effectiveness.

This systematic review and meta-analysis have several limitations. Firstly, there is significant heterogeneity among the included studies due to variations in study methodologies, including differences in antibiotic susceptibility testing methods, target populations, sample types, bacterial isolates, and definitions of carbapenemase-producing isolates. This heterogeneity can introduce bias and affect the generalizability of the findings. The geographic bias towards Africa and Asia in the selected studies may limit the applicability of the results to regions with different antibiotic prescribing practices and infection control measures. In addition, the diverse timeframes of the studies, spanning from 2015 to 2023, could reflect evolving antibiotic resistance patterns influenced by changes in policies and practices. The use of various phenotypic detection methods and the reliance on PCR for gene confirmation may introduce variability in the reported prevalence, as each method has unique sensitivities and specificities. Furthermore, the study's inclusion criteria were limited to publications in English, potentially introducing language-related bias into the research.

According to (Caliskan et al.,2023) recent advancements in molecular techniques and automated platforms have significantly enhanced the cost-effectiveness and efficiency of identifying β – lactam resistant genes. One notable example of this advancement is the adoption of Luminex technology, which relies on color-coded microspheres in a flow cytometry assay. This method allows for the precise detection of specific alleles, antibodies, or peptides from individual bacteria colonies. Furthermore, the implementation of a

multiplex oligonucleotide ligation -PCR procedure further enhances the detection of β - lactam resistance genes and their variants. This procedure exhibits remarkable sensitivity and specificity rates, achieving 100% and 99.4%, respectively. All these advancements are accomplished within a relatively short timeframe of just 5 hours. Additionally, the incorporation of the LAMP technique, incorporating the use of hydroxy naptol blue dye (LAMP-HNB) and microarray technologies, has demonstrated significant promise in the precise identification of carbapenemase-encoding genes. This method has achieved exceptional levels of specificity, surpassing 90% and reaching 100% respectively. An inventive method known as the multiplexed paper-based Bac-PAC assay has also emerged. This approach allows for the characterization of the antibiotic resistance profiles of different CRE strains through colorimetric readings, providing a valuable tool in the fight against antibiotic resistance.

This study assessed CPE prevalence and detection methods across eleven studies from Africa, Asia, and North America (2015-2023). Among 5228 isolates, 1121 were CPE-positive. Various phenotypic detection methods, including Carba NP, MHT, and mCIM, supplemented by PCR, were employed. The pooled proportion for phenotypic CPE detection was 40.53%, with heterogeneity. Subgroup analysis identified mCIM as the most effective method (58.20%), and Carba NP the least sensitive (27.79%). Despite hints of publication bias in funnel plots, statistical tests found no significant bias.

CONCLUSION AND RECOMMENDATION

In conclusion the new technologies have advantages including quicker findings, improved precision, and the potential for point-of-care testing, which leads to quicker patient care decisions and less transmission. However, they have drawbacks, such as the need for standardization, cost-effectiveness, and integration into existing healthcare systems. Additionally, to diligently tackle the counter-evolving treats, it is crucial to keep an eye on new CPE strains and their defence mechanism.

The outcome of this study underscores the importance of standardizing detection methods, adopting international guidelines, and promoting collaboration in future research efforts. It emphasizes the need for global representation in CPE prevalence studies to provide a comprehensive understanding of this emerging threat. Longitudinal studies are recommended to track CPE trends over time and evaluate intervention effectiveness. Integrating advanced molecular techniques with phenotypic methods can enhance detection accuracy. Furthermore, healthcare facilities should prioritize infection control and antibiotic stewardship to address the public health challenge posed by CPE effectively. These recommendations aim to guide research and public health efforts in combating CPE.

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APPENDIX 1

Table A1: Joanna Briggs Checklist for Prevalence Studies (Available from https://jbi.global/critical-appraisal-tools).

First Author	Was the sample frame appropriate to address the target population? (Yes = 1; No = 0)	Were the participants sampled in an appropriate way? (Yes = 1; No = 0)	Was the sample size adequate? (Yes = 1; No = 0)	Study subjects and setting described in detail. Yes = 1; No = 0	Was the data analysis conducted with sufficient coverage of the identified sample? (Yes = 1; No = 0)	Were valid methods used for the identification of the condition? (Yes = 1; No = 0)	Was the condition measured in a standard, reliable way for all participants? (Yes = 1; No = 0)
Lefebvre et al., 2015	1	0	1	1	0	0	0
Olowo-Okere et al 2020	1	1	1	1	1	1	1
Beresford and Maley 2019	1	0	1	1	0	1	1
Belouad et al 2023	1	1	1	1	0	0	0
Davari et al 2022	1	1	1	0	1	0	0
Mulla et al 2016	1	0	1	1	1	0	1
Hosoda et al 2021	1	1	1	1	1	0	0
Li et al 2019	1	0	1	1	1	1	0
Ghaith et al 2019	1	0	1	1	0	1	0
Shrief et al 2022	1	0	1	1	1	1	1
Velasco et al 2017	1	1	1	1	1	1	1