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COMPARISON OF SCREENING METHODS FOR CARBAPENEMASE PRODUCING ORGANISMS IN THE CLINICAL LABORATORY

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COMPARISON OF SCREENING METHODS FOR CARBAPENEMASE PRODUCING ORGANISMS IN THE CLINICAL LABORATORY

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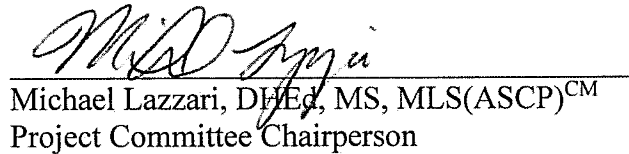
We recommend acceptance of this project in partial fulfillment of the candidate's requirements for the degree of Master of Clinical Microbiology

The candidate has completed the oral defense of the project.



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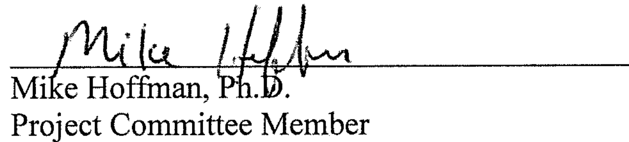

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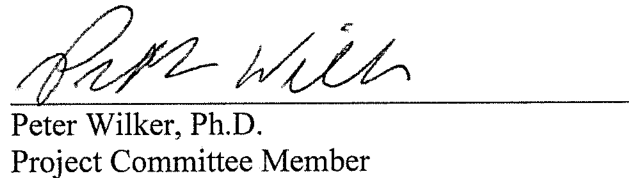

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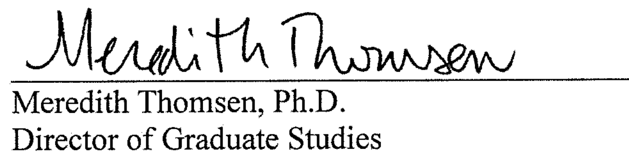

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ABSTRACT

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Carbapenemase producing organisms (CPOs) are an emerging threat in healthcare facilities. Infections with CPOs lead to higher healthcare costs for both patients and providers. An increase in these organisms could lead to a post-antibiotic era that causes serious injury in what was once viewed as a routine infection. Outbreaks have been reported since 2001, with the largest outbreak hitting the USA in 2011. The spread of CPOs spans most of the world where numbers are tracked and reported. Resistant organisms become even harder to track since different resistant genes reside in different locations (KPC, NDM, OXA-48, etc.). Screening for CPOs can help the nosocomial spread by placing the patient in isolation. This review will give an overview of the different ways to screen and detect CPOs. It will also compare the pros and cons of each method such as cost, turn around time and sensitivity. Determining the most accurate and cost efficient method can help to alleviate stress on providers, patients and clinical laboratories.

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INTRODUCTION

Antibiotic resistance is one of the biggest threats facing the healthcare industry. Patients are at a higher risk of longer stays at hospitals because of antibiotic resistance, which leads to higher costs. The total economic burden on the U.S. due to antibiotic resistant infections is estimated at 20 billion dollars (Golkar, Bagasra, & Pace, 2014). There is also an increased mortality and morbidity directly linked to increased resistance. This threat keeps escalating and could lead to a post-antibiotic era where any infection could lead to serious injury or death (Alanis, 2005). Among the bacteria that pose a threat to the healthcare industry, there are many different types of resistance patterns. These resistance patterns are determined from antibiotic susceptibility testing. Gram-positive organisms include methicillin and vancomycin resistant *Staphylococcus aureus* (MRSA and VRSA, respectively), and vancomycin resistant *Enterococcus* sp. (VRE). Groups of gram-negative organisms include extended-spectrum beta-lactamase (ESBL) producing organisms and carbapenemase-producing organisms (CPOs). CPOs prevent physicians from prescribing last resort drugs known as carbapenems (Rhomberg, & Jones, 2009). This means that physicians may have no antibiotics left in their arsenal to treat the infection. This review paper discusses the typical phenotypic methods being used in clinical laboratories to help limit the spread of CPOs.

Resistance Mechanisms

Bacteria use many different mechanisms to combat antibiotics. Bacteria may gain resistance by picking up DNA encoding resistance determinants or spontaneous mutations. In general, there are three ways that bacteria can combat antibiotics. The first involves either decreasing uptake of the antibiotic or increasing the discharge of the drug from inside the bacterium. The organism can decrease uptake of a drug by altering proteins in the outer membrane. Bacteria can also up-regulate efflux proteins, causing the antibiotic to be pushed out of the cell (Sritharan, Swathi, Chikala, & Ratnakar, 2016). The second mechanism is modifying the target that the drug would adhere to. An example of this is the modification of the 50S rRNA subunit. The modification of the 50S rRNA subunit renders linezolid-based antibiotics useless (Long & Vester, 2011). One of the most infamous examples of target modification is in *S. aureus.* This organism acquired a mobile genetic element encoding for an altered penicillin binding protein (PBP2a, *mec*A) with a decreased affinity to β-lactams (Malouin & Bryan, 1986). The third mechanism, and the theme of this review paper, is the enzymatic inactivation of antibiotics. Organisms can harbor chromosomal or plasmid based genes that produce enzymes, such as carbapenemases, involved in inactivating antibiotics. This paper will focus on the detection of plasmid-based genes, since they cause the biggest threat to infection control within a healthcare system (Bennett, 2008).

Emergence and Discovery of Carbapenemases

Carbapenems are a set of broad-spectrum β-lactam antibiotics that are used against a wide variety of bacterial infections. The drug acts to inhibit cell wall production, leading to death of the cell. Yigit et *al.* (2008) published a paper in 2001

describing a *Klebsiella pneumoniae* isolate that was presenting moderate to high levels of resistance to imipenem and meropenem, two of the most highly used carbapenems in the hospital setting. An investigation into this organism led to the discovery of a new gene known as *K. pneumoniae* carbapenemase-1 (KPC-1). This gene produced a carbapenemase that was able to render carbapenem-based drugs useless. It was also discovered that this gene was plasmid based, meaning that it could be horizontally transferred to other organisms. KPC-1 could be purified to prove activity against carbapenem drugs, as well as to extended-spectrum cephalosporins and aztreonam. This specific KPC-1 (sequence type 258) is thought to be responsible for the endemic spread of carbapenem resistance in the U.S. (Banerjee & Humphries, 2016). In 2010 Kumarasamy et *al.* (2010) published a report describing the emergence of a new carbapenemase being produced by Enterobacteriaceae, consisting mostly of *K. pneumoniae* and *Escherichia coli*. This carbapenemase was called New Delhi metallo-b-lactamase-1 (NDM-1) and was also found to be plasmid based. Since this description, there have been over 975 articles published describing carbapenemase activity, epidemiology and treatment (Savard & Pearl, 2014). Other carbapenamases such as VIM, IMP, OXA-48 and OXA-181 have been described in papers (Rodriguez-Martinez et al. 2009, Limbago et al. 2011; Poirel et al 2012; Potron et al., 2011). Most of these genes were discovered in the clinical laboratory due to an altered antibiogram, and then studied further in a research setting. To this day, novel carbapenemases are being discovered and studied to help prevent the spread of resistance in hospital settings.

Ambler Classes

Carbapenemases belong to three of four known β-lactamase classes, known as Ambler classes (Table 1). An example of an Ambler class A protein is the KPC enzyme, Ambler class B or metallo-β-lactamases (MBLs) contains the NDM enzyme, among others, and Ambler class D contains OXA enzymes. Each class of enzymes causes resistance to first through fourth generation cephalosporins, older β-lactamase/β-lactamase inhibitors (BLBLIs) and carbapenems (Vasoo, Barreto, & Tosh, 2015). Ambler class C organisms contain AmpC enzymes, which are chromosomal and can be treated using cefepime. The chromosomal nature of these Ambler class C enzymes means there is little threat of horizontal spread to other organisms within a healthcare setting (Thomson, 2010).

Table 1. Examples of Ambler Classes

Ambler Class	Resistance Genes	Resistance to Other Drugs
A	IMI, SME, KPC, GES	β-lactams, aztreonam, cephalosporins, carbapenems
B	NDM, IMP, VIM, GIM	β-lactams, cephalosporins, carbapenems
C	AmpC	Penicillins, some cephalospoirins
D	OXA (23,40,48,50,51, etc.)	Penicillins, cephalosporins (variable), carbapenems,

Outbreaks and Spread of CPOs

There are two main ways that a patient can acquire a CPO. The first is patient-to-patient transmission, and the second being the emergence of resistance from a once susceptible gram-negative bacterium (Richter & Marchaim, 2016). Most of the larger outbreaks, including the ones below, are thought to occur from patient-to-patient transmission. Varying sizes of CPO outbreaks are being seen all over the globe. The type of enzyme responsible for the outbreak depends on the location. KPC production is more

widely seen in North and South America. NDM and OXA producing organisms are endemic and predominant in India. Figures 1 and 2 show a breakdown of KPC and NDM distribution, respectively (Lee et al., 2016). OXA producing organisms tend to have a lower hydrolyzing profile, which makes them harder to track. This leads to a lack of information regarding where OXA producing organisms are the most prevalent (Bakthavatchalam, Anadan, & Veeraraghavan, 2016).

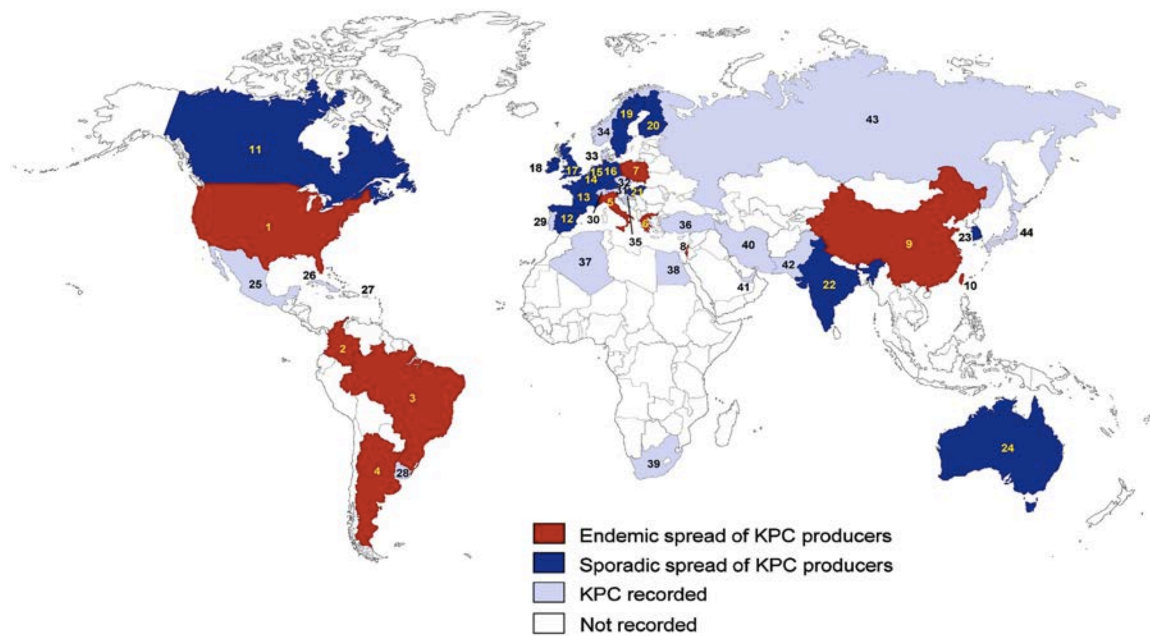

Figure 1. KPC producers around the world through 2016. Numbers relate to the spread of KPCs from 1 (discovered in the USA) to 44 (last found in Japan).

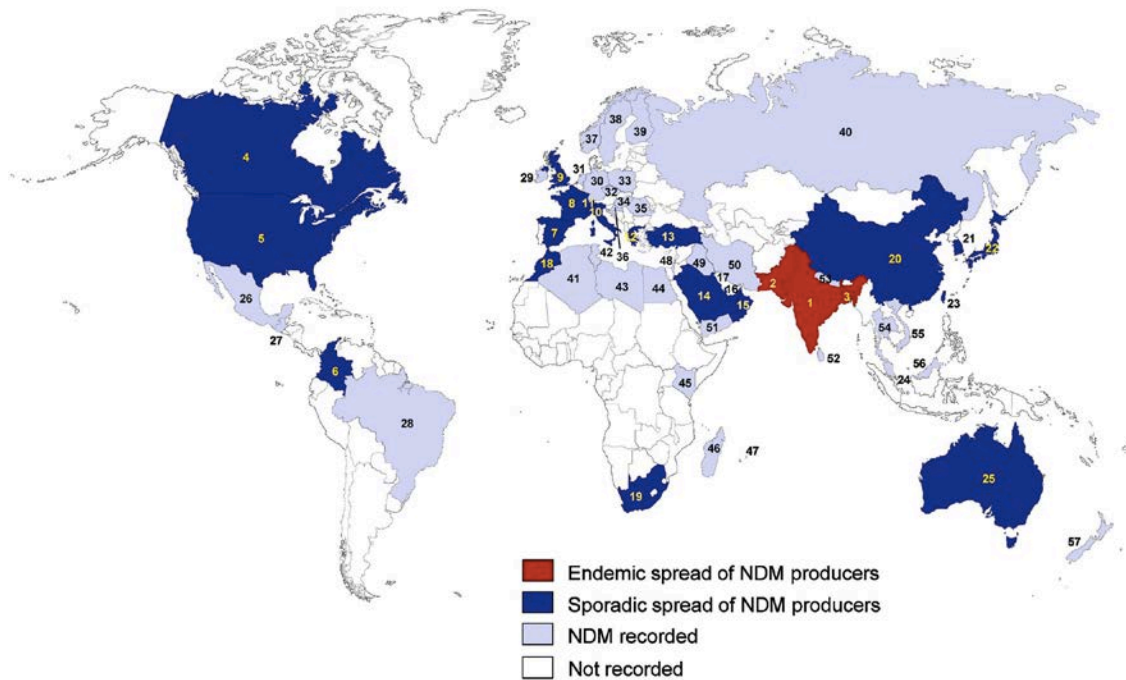

Figure 2. NDM producers around the world through 2016. Numbers relate to the spread of NDMs from 1 (discovered in India) to 57 (last found in New Zealand).

One of the largest outbreaks of KPC-producing *K. pneumoniae* occurred at the U.S. National Institutes of Health (NIH) Clinical Center. Eighteen patients were infected with KPC, eleven of which died from the infection and other underlying conditions. The outbreak was contained by the use of strict infection control techniques. Pulse field gel electrophoresis was used to confirm that the same strain was being passed from patient to patient (Snitkin et al., 2012). Another example of an outbreak of CPOs occurred in the Shandong province in China. This outbreak was caused by NDM-1 producing *K. pneumoniae*. The two separate outbreaks occurred in the same neonatal unit. The first outbreak affected 4 patients in August of 2012 and the second affected 14 patients in September of 2013 (Jin et al., 2015). All isolates were confirmed positive by the Modified Hodge Test.

Need for Screening CPOs

First and foremost, the main reason for screening for CPOs is to prevent the nosocomial spread of carbapenem resistance. When a CPO is discovered, infection control can step in quickly to place that patient in precautions, preventing the spread of resistance genes through hand washing and donning a gown and gloves before entering the affected patient’s room (Savard & Perl 2014). As stated earlier, invasive infections (e.g., bloodstream infections) caused by CPOs have been associated with high mortality rates (up to 40-50% in some studies) (Mariappan, Sekar, & Kamalanathan, 2017). This means that acting quickly is important to prevent spread and treat the infected patient with the correct antibiotics. This may also mean that the patient is transferred to a facility that can supply the correct care and contact precautions.

Screening for CPOs

Screening for CPOs starts with screening Enterobacteriacae and non-fermenting gram-negative bacilli. The first carbapenemase was discovered in an *Enterobacter cloacae* isolate in 1990 in a French hospital. The gene was named NOR-1 and was placed into Ambler class C. This carbapenamase was found to be chromosomally encoded, something that has been an ongoing problem with *Enterobacter* sp. The first plasmid-borne carbapenemase (IMP-1) was discovered in a *Serratia* sp. isolate in 1991 in Japan (Nordmann & Poirel, 2002). Typical organisms that are screened by laboratories include *E. coli, Klebsiella* sp., *Enterobacter* sp., *Serratia* sp., *Proteus mirabilis,* and many other Enterobacteriacae (Lutgring & Limbago, 2016). Non-fermenting organisms that are routinely screened include but are not limited to *Pseudomonas aeruginosa* and *Acinetobacter* sp. (Kumar et al., 2012). Screening these organisms is crucial for

infectious disease specialists to prevent outbreaks. An example of a healthcare facility that has a screening procedure already in place is Allina Health in Minneapolis, MN. All gram-negative organisms that could harbor a carbapenemase are routinely tested for susceptibilities. The susceptibility system will use algorithms to flag an organism if it suspects that it could contain a carbapenemase. The organism is then freshly isolated and additional phenotypic testing is conducted. Types of phenotypic testing will be discussed later in this paper. Clinical laboratories can also offer screening tests such as the Carba-R. This test involves a rectal swab that gets tested genetically for typical carbapenemase genes in that geographic location. Since this test is very expensive, smaller laboratories send the sample to larger clinical laboratories such as Mayo Laboratories.

A very important thing to know about gram-negative bacilli is that they can harbor both chromosomal and plasmid-based carbapenemases (Queenan & Bush, 2007). This is important because carbapenemases produced chromosomally are passed on to the next generation through vertical transfer. The fact that these genes cannot be transferred horizontally lowers the risk of rapid spread of a carbapenamase gene. Plasmid-based transfer on the other hand, allows for the horizontal transfer of genes within the same generation of bacteria. Plasmids being passed from an organism harboring a carbapenemase to a carbapenem susceptible organism is a real phenomenon that has happened in many healthcare units throughout the world (Di Carlo et al., 2011; Souli et al., 2010,; Maltezou et al., 2009). This risk is even more concerning due to the fact that mortality rates associated with CPOs is over 50% (Mariappan, Sekar, & Kamalanathan, 2017).

Antibiotic Treatment of CPOs

Treatment of CPOs is difficult and in some cases next to impossible. Physicians are faced with such choices as treating with past drugs that were thought to have high toxicities such as polymyxin B, a derivative of colistin, or using a combination therapy of drugs that tested as resistant. In recent years scientists have tried to develop an algorithm to help with the treatment of CPOs (Morrill et al., 2015). These algorithms must take into mind the drugs that are usually on hand for physicians to use. Some combinations that have been used in recent studies include rifampin (or tigecycline) and polymyxin B to treat *Acinetobacter baumannii* (Muthusamy, Sudhishnaa, & Boppe, 2016) and a triple-antibiotic combination of amikacin plus meropenem/rifampicin plus polymyxin B for *Pseudomonas aeruginosa* infections (Tängdén, 2014). The development of new drugs to help treat CPOs is a struggle for pharmaceutical companies. Even with governmental subsidies to help with the development, companies do not find much financial benefit in a drug that is used for only a short amount of time. While the development of new drugs is needed, the time from creation to FDA approval can take up to ten years. Companies must go through multiple clinical trials, spend upwards of 800 million dollars, all without the guarantee of FDA approval (Conly & Johnston, 2005). If the drug does reach approval by the FDA, there is also the risk that the drug will become obsolete because of resistance or the development of a better drug. A great example of this was the release of ampicillin to the market in 1961 for the treatment of *Salmonella* sp. By the end of 1962 there were already cases of ampicillin resistant *Salmonella* sp. infections (Tran-Dien, Le Hello, Bouchier, & Weill, 2018).

Physicians have found success in treating carbapenemase producing *E. coli* and *K. pneumoniae* infections with recently released drugs. New antibiotics that have been developed and released in 2014-2016 include ceftolozane/tazobactam and ceftazidime/avibactam. Both are novel β-lactam/β-lactamase inhibiting combination antibiotics. A study in 2014 showed a 100% eradication rate of *K. pneumoniae* intra-abdominal infections when treating with ceftolozane/tazobactam. Meropenem achieved eradication in only 88% of cases (Sorbera et al., 2014; Lagace-Wiens, Walkty, & Karlowsky, 2014).

METHODS FOR DETECTION OF CPOS

Testing for CPOs is a fickle science that can lead to both false positives and negatives (Normann et al., 2012). Automated systems like VITEK 2 (bioMérieux), MicroScan (Siemens Healthcare), and Phoenix (Becton Dickinson) use algorithms within their database to suggest the presence of a carbapenemase or AmpC producing organism. The algorithm used includes such parameters as identification, MIC value, testing value of other drugs and user input/thresholds. Laboratories will use these suggestions to conduct phenotypic and/or genotypic confirmatory testing. Each testing method comes with downfalls such as price, false positives or negatives, or turn-around-time. Popular methods and their advantages and disadvantages will be discussed briefly in the next section.

Phenotypic Testing

Most clinical laboratories use phenotypic testing for confirmation of carbapenemase activity. This includes tests such as the Modified Hodge Test (MHT), Carbapenem Inactivation Method (CIM), CarbaNP and Modified CIM (mCIM). All four

of these tests are based on the hydrolysis of a carbapenem (usually meropenem or imipenem) by the bacterium in question (Amjad et al., 2011; Van der Zwaluw et al., 2015; Nordmann, Poirel, & Dortet, 2012).

Modified Hodge Test

Amjad et al. (2011) explains the MHT method as: A 0.5 McFarland dilution of the *Escherichia coli* ATCC 25922 in 5 ml of broth or saline is prepared. A 1:10 dilution is then lawned on to a Mueller Hinton agar plate. A 10 µg meropenem or ertapenem susceptibility disk is placed in the center of the test area. Test organism was streaked in a straight line from the edge of the disk to the edge of the plate. The plate is then incubated overnight at 35±2°C in ambient air for 16–24 hours. Figure 3 below shows positive and negative results using the MHT. The MHT is inexpensive, less than $0.50 per test, and most laboratories will already carry everything necessary for testing. The MHT must be incubated overnight, which delays the placement of patients into isolation, if positive. The MHT also has a history of false positives, something that cannot be taken lightly in the infectious disease setting. False negatives are also experienced with the MHT, especially in NDM producing isolates (Carvalhaes et al., 2010; Girlich, Poirel, & Nordmann, 2012). Meropenem, ertapenem and imipenem have all been used in testing for the MHT. It has been determined in multiple studies that the meropenem disk is the best option for accurate results (Gauthier, Bonnin, Dortet, & Naas, 2017). The MHT has been shown to have good sensitivity rates for all species of Enterobacteriaciae that are screened for carbapenemase activity. The test struggles when analyzing specificity, leading to many false positives in *Enterobacter* spp, *Citrobacter freundii* and other non-*Klebsiella* spp. (Mathers, Carroll, Sifri, & Hazen, 2013). Multiple studies have calculated

specificity of the MHT to be lower than 65% (Mathers, Carroll, Sifri, & Hazen, 2013; Bayramoglu et al., 2016; Song et al., 2015). Results from research from the past few years have shown that tests such as the CIM and CarbaNP perform much better than the MHT (Song et al., 2015). With this in mind, many labs have removed the MHT as the primary test for confirmation.

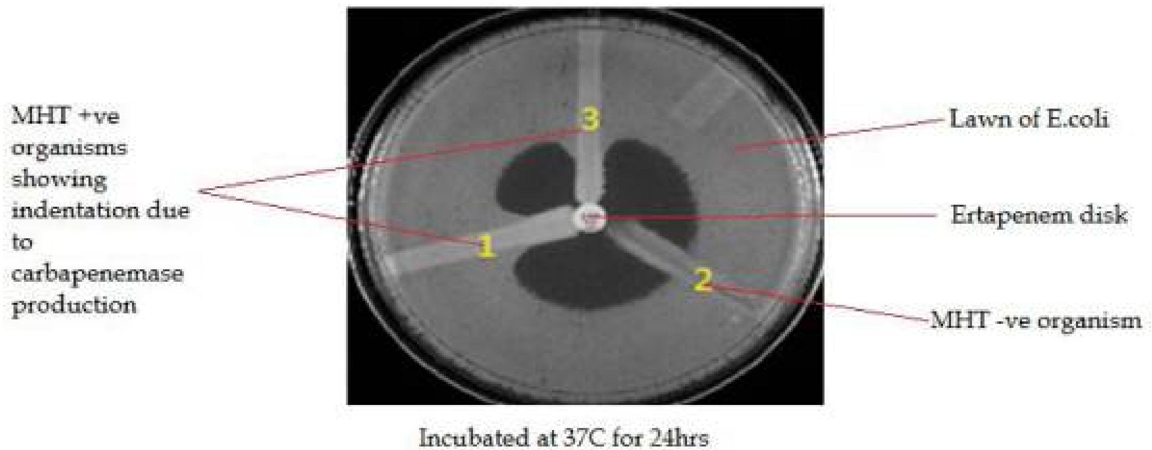

Figure 3. The Modified Hodge test performed on a Muller Hinton Agar plate. (1) MHT positive result (2) MHT negative result; and (3) a clinical isolate, positive result.

Carbapenem Inactivation Method (CIM)

The CIM is based on hydrolysis of a meropenem disk by the organism in question. Van der Zwaluw et al. (2015) explained the method as such: A suspension is made by suspending a full 10 µl inoculation loop of culture, taken from a Mueller-Hinton or blood agar plate in 400 µl water. Subsequently, a susceptibility-testing disk containing 10 µg meropenem (Oxoid Ltd, Hampshire, United Kingdom) is immersed in the suspension and incubated for a minimum of two hours at 35°C. After incubation, the disk is removed from the suspension using an inoculation loop, placed on a Mueller-Hinton agar plate inoculated with a susceptible *E. coli* indicator strain at 0.5 McFarland (ATCC

29522) and subsequently incubated at 35°C. Figure 4 below shows positive and negative results. The CIM is very inexpensive; it also has an ease of reading when compared to the MHT. The CIM must be incubated for at least 12 hours for best results, which leads to a delay in results compared to molecular testing and CarbaNP. Meropenem, ertapenem and imipenem have all been used in testing for the CIM. It has been determined in multiple studies that the meropenem disk is the best option, which is the same as the MHT. Cost is another positive of the CIM, usually being less than $0.50 per test.

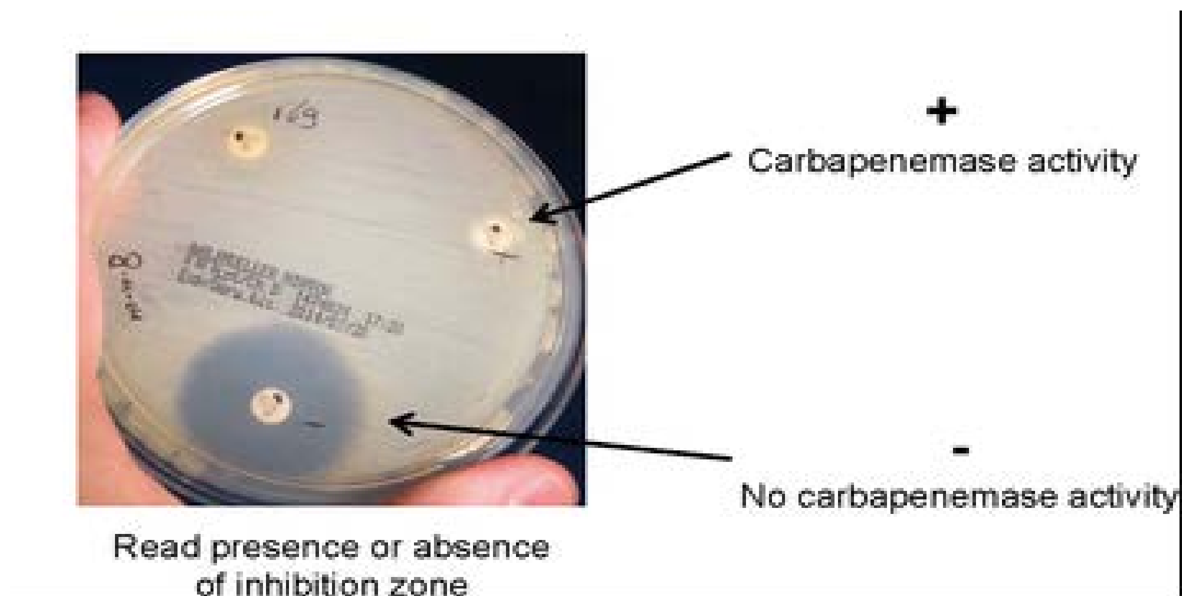

Figure 4. Demonstration of the results of the CIM. Growth to the disk indicates a positive result. A zone of inhibition (>14mm) indicates a negative result.

Modified Carbapenem Inactivation Method

Using the CIM as inspiration, the modified CIM (mCIM) was developed by S.D. Das, P. Patel, L.R. Peterson, K. Mangold, and R.B. Thomson, (unpublished data) and was validated by a CLSI working group. The mCIM closely follows the method of the CIM, with a couple of major changes. Instead of incubating the meropenem disk/isolate in

sterile water, the disk/isolate is incubated using tryptic soy broth (TSB). The other major change is an increase in incubation time of disk and organism from two hours to four hours (±15 minutes). The mCIM was developed to attempt to increase the detection of OXA-48-type carbapenemases. Investigators have reported detection rates of OXA-48-type carbapenemases by the CIM to be anywhere from 50-91%. Pierce et al (2017) of CLSI were tasked with validation of this new method. This was an extensive validation that consisted of two stages. The first stage used one laboratory to test 115 isolates from 11 different genera. The isolates were tested using whole genome sequencing (WGS) at the CDC and UCLA to determine resistance genes. The second stage of testing used 9 separate laboratories to analyze 61 isolates from 10 different genera. The first stage of testing produced a sensitivity of 99% (91/92) and a specificity of 100% (23/23). One of the nine labs also compared the mCIM to CIM testing. The testing laboratory discovered the mCIM to have a higher sensitivity (93% [95% CI, 74% to 99%]) to the CIM (82% [95% CI, 61% to 93%]) and to equal the CIM in specificity (100%).

CarbaNP

CarbaNP testing is based on *in vitro* hydrolysis of imipenem by a bacterial lysate, which is detected by changes in pH values using the indicator phenol red (red to yellow/orange). Nordmann, Poirel, & Dortet (2012) first described the method as such: One calibrated loop (10 µl) of the tested strain directly recovered from the antibiogram is suspended in a Tris-HCl 20 mmol/L lysis buffer (B-PERII, Bacterial Protein Extraction Reagent; Thermo Scientific Pierce, Rockford, IL, USA), vortexed for 1 minute and further incubated at room temperature for 30 minutes. This bacterial suspension is then centrifuged at 10,000 × *g* at room temperature for 5 minutes. Thirty µl of the supernatant,

corresponding to the enzymatic bacterial suspension, is then mixed in a 96-well tray with 100 µl of a 1-ml solution made of 3 mg of imipenem monohydrate (Sigma, Saint-Quentin Fallavier, France), pH 7.8, phenol red solution, and 0.1 mmol/L $ZnSO_4$ (Merck Millipore, Guyancourt, France). The phenol red solution is prepared by mixing 2 ml of a phenol red (Merck Millipore) solution 0.5% (wt/vol) with 16.6 ml of distilled water. The pH value is then adjusted to 7.8 by adding drops of 1 N NaOH. A mixture of the phenol red solution and the enzymatic suspension being tested is incubated at 37°C for a maximum of 2 hours. Figure 5 below shows an example of a commercially produced test.

The CarbaNP test can produce false negatives when it is tested against OXA-48-type carbapenemases. The test is subject to the interpretation of the scientist reading the results (color change) and is more expensive than the CIM and MHT ($4-5 per test). A key benefit to the CarbaNP test is the much shorter turn around time than the CIM and MHT (Rudresh et al., 2017). Results can be acquired in as short as 30 minutes, this is much faster than both the CIM and MHT, which must be incubated overnight for best results (Tamma et al., 2017). Researchers have compared this method to the MHT and CIM. The CarbaNP method compares similarly to the CIM, with the exception of a couple non-fermenting isolates that were falsely negative when testing with the CarbaNP (Tiget, Patel, & Melano, 2016). The CarbaNP has been shown to test more accurately than the MHT, with specificities of 100% and 65% respectively (Mathers , Carroll, Sifri, & Hazen, 2013; Song et al., 2015). Van der Zwaluw et al. (2015) compared the CIM and CarbaNP using meropenem for testing. The study used PCR testing consisting of NDM, KPC, OXA-48-like, VIM and IMP as a confirmation method. There was a concordance of 100% for Enterobacteriaceae and 98.8% for non-fermenters between PCRs that detect

carbapenem encoding genes and carbapenemase activity detected by CIM. The CarbaNP+ performed similarly, but struggled with a couple of non-fermenting isolates (Van der Zwaluw et al., 2015).

Figure 5. Example of a FDA cleared CARBA NP test produced by Biomerieux.

MALDI-TOF

Recently, MALDI-TOF has been used in the research setting for the detection of carbapenemases. To detect a carbapenemase using this method the bacterium must first be mixed with 20 mM Tris-HCl buffer (pH 6.8) and a carbapenem and incubated for a period of time (3-6 hours). The solution is then centrifuged and the supernatant is measured. The MALDI-TOF is able to detect the presence or absence of peaks to carbapenems and sodium carbonate. By using flexAnalysis 3.0 software (Bruker Daltonics GmbH, Bremen, Germany) scientists are able to determine if/how the peaks of the carbapenem in question were altered when incubated with an organism. A decrease in the carbapenem peaks means the drug was degraded by the presence of a carbapenemase. While this method has a quick turnaround time (around 4 hours), there is no approval of this method using CLSI guidelines (Hrabák et al., 2011). Most clinical laboratories using a MALDI-TOF need this instrument exclusively for organism identification. With that in mind, this method would require an additional MALDI-TOF instrument, something outside of the budget of most clinical laboratories.

Molecular Testing

Some examples of molecular assays include PCR, microarrays and whole genome sequencing (WGS). Molecular assays would be used prior to susceptibility testing to give the provider rapid results. Expensive molecular testing is only ordered by infectious disease doctors due to the increased cost. This result would then be followed up with standard susceptibility testing.

There are commercially available or laboratory developed PCR methods. Commercial assays include the Cepheid Xpert Carba-R, eazyplex SuperBug ID and BD Check-Direct CPE assays. All three of these assays test for KPC, NDM, VIM and OXA-48 (Xpert Carba-R 2016; Hyplex SuperBug ID 2012; Check-Direct CPE Screen 2017). Sensitivities for these assays are great, ranging from 97-100%. A major downfall with PCR testing is that all of these assays are limited to research use only (RUO). RUO testing will not be reimbursed by the Centers for Medicare and Medicade Services (CMMS) to same level as an FDA approved test. A laboratory should research which resistance genes are prominent in their area when choosing a PCR assay. PCR assays only contain targets for certain genes. If genes are not prevalent in that region, there is no need to bring in that platform.

Commercial microarrays include Luminex Verigene, BioFire film array, and Check-Points CPO assay. They all have reported sensitivities nearing 100% from multiple studies (Ward et al., 2015; Lau et al., 2015). A benefit of microarray technology is that a laboratory can test for dozens to hundreds of targets, compared to the 4-8 using PCR (Lutgring & Limbago, 2016). Many laboratories are already using an instrument

from BioFire or Verigene for such things as blood and stool pathogen identification. Adding carbapenemase screening (prior to susceptibility testing) to these instruments could lower costs to the laboratory since the instrument is already in use.

WGS is a very exciting and innovative technology that could screen an entire bacterial genome for resistance genes. Infectious disease units could use WGS to quickly screen a patient for hundreds of resistance genes, which could help to alter treatment and prevent outbreaks. At this time WGS is quite expensive, but will hopefully drop in price as development continues. Due to the increased cost of about $120 per organism and a lack of defined coverage from the Centers for Medicare & Medicaid Services, laboratory and infection control leaders must determine if use would be high enough to justify these molecular platforms.

Table 2. Comparison of Methods for Detection of CPOs

Method	**Cost**	**Sensitivity**	**Specificity**	**Ease of Use**	**TAT**
MHT	$	90-100%	60-70%	Medium	20-24 H
CIM	$	50-90%	95-100%	Easy	14-20 H
Modified CIM	$	95-100%	95-100%	Easy	14-20 H
CarbaNP	$$	70-80%	95-100%	Medium	5-6 H
Molecular	$$$	95-100%	95-100%	Easy	0.5-4 H

Chromogenic agars

Companies are starting to produce screening agars and broths that can help in the detection of CPOs. Some examples of media being produced include Thermo Fisher Brilliance CRE, InTray Colorex KPC and bioMerieux chromID Carba. Chromogenic agars use colony growth along with color morphology to identify the production of a carbapenemase, along with helping to provide a genus. The agars have sensitivities ranging from 56-96% (Wilkinson et al., 2012). Most agars are still in development and are not yet FDA approved for use in a clinical laboratory. This method could see a jump

in use if CPO rates increase, such as with MRSA screening methods. The question still remains as to how well they would work in the screening of patients, let alone as a confirmation method. HiCrome KPC agar was compared to the MHT using organisms that were screened for carabapenem resistance by Kirby Bauer disk diffusion. Of the 202 isolates that were tested by MHT, 155 (76.73%) showed. The HiCrome method produced 178 (88.11%) positives that matched the manufacturers claims. Although both methods found large numbers of CPOs, they only matched in 132 of the isolates (65.34%). This test did not use molecular methods to confirm a carbapenamase enzyme in any of the positive organisms (Chaturvedi & Banashankari, 2017). The fact that these methods did not match in identification of CPOs leaves questions about chromogenic agars. The agars are selective for carbapenem resistant organisms, but this does not mean that they produce a plasmid-based carbapenemase. While identification of carbapenem resistant orgainsms is helpful, gram-negative organisms like *Proteus* sp. and *Serratia* sp. are intrinsically resistant to carbapenems. Extra laboratory resources would be needed to test these positive organisms for carbapenemase production, defeating the purpose of a quick screening method.

Combining Screening and Phenotypic Methods

Other laboratories are taking screening and confirmation a step further by using multiple tests in unison. The idea of using a screening broth in combination with a confirmation method like the mCIM could help labs in preventing false negatives and positives. One example of this combination approach was done by Song et al (2015), using the MHT and carbapenemase inhibiton test (CIT) for detection of CPOs. The CIT uses phenylboronic acid and EDTA to detect both CPEs and MBL-producing

Pseudomonas spp. via inhibition. The study used 63 Enterobacteriaciae and 46 *Pseudomonas* spp. that contained KPC, VIM, GES, NDM, IMP and OXA-48. Thirty-five carbapenem non-susceptible organisms that contained no carbapenemase were also used in this study. By using all of these tests in unison, there was 100% specificity in the detection of KPC, MBL and OXA-48-like-producing isolates. The sensitivity was 100% for KPC and OXA-48-like isolates and 94% for MBL producing isolates. This type of combination testing works great in a research laboratory where dedication can be put towards one study. The idea of this dual test working in a clinical laboratory is different. Doing two tests for one organism would increase cost, technician time and turn around time. A clinical laboratory must investigate which CPOs are prevalent in their region and determine if this combination testing is justified.

Tracking CPOs

Healthcare facilities, state health departments, and national public health agencies all have systems in place to track the isolation of new CPOs. It is critical that healthcare laboratories inform state health departments of new cases and sends them the isolates (Guh et al., 2015). The state departments will then test the isolated organism for resistance genes, which can help to track outbreaks due to such genes as KPC and NDM. In 2015 the federal government implemented the initiative to Combat Antibiotic Resistant Bacteria (CARB) (Office of the Press Secretary, 2015). This means more screening from public health departments, with the help of local laboratories. State departments such as the Minnesota Department of Health have requested that any gram-negative organisms with an increased imipenem or meropenem resistance (MIC $\geq$2) be sent to their lab for additional testing (Minnesota Dept. of Health, 2017). The hope is to

identify resistance trends, change antibiotic use patterns, and isolate patients before the spread gets out of control.

The Clinical and Laboratory Standards Institute (CLSI) creates resources such as the M100. The M100 includes recommendations for testing of standard antibiotics against commonly isolated human pathogens. CLSI also gives protocols to follow when antibiotic resistance is encountered. The recommendation for CLSI goes as follows: For Enterobacteriacae that test resistant to carbapenems, and any isolates that test susceptible to a carbapenem but demonstrate reduced susceptibility either by disk diffusion or MIC testing, performing a phenotypic test for carbapenemase activity, the Modified Hodge Test (MHT), CIM or CarbaNP, is recommended (CLSI, 2017). In 2016 CLSI released lower breakpoints (minimum inhibitory concentration) for testing of carbapenem drugs. With these lower breakpoints laboratories do not necessarily have to screen for CPOs. Infectious disease specialists and laboratory leadership will have more subjectivity regarding when confirmatory phenotypic testing is needed (CLSI, 2016).

A positive result from a screening test can indicate that a carbapenemase is being produced by the organism (Maurer et al., 2015). The provider and infection control must be alerted immediately so that the patient can be placed in isolation. Along with isolation, they will receive a special dedicated staff, decolonization protocols and a surveillance program (Richter & Marchaim, 2016). The drug regiment will also change since carbapenems tend to be the drug of choice in cases where many other combinations have failed. Each screening test has the risk of false positives, which can incorrectly place a patient in isolation. The infectious disease team must take this into mind when deciding to put a patient in isolation.

Every state health department has different requirements for clinical laboratories, most of which include the reporting and submittal of organisms with increased MICs to carbapenems. A state health department, such as the Minnesota Department of Health (MDH), will collect the isolates and test them for the genes discussed above (KPC, NDM, etc.). This will help the state to monitor for outbreaks and help to contain them as quickly as possible. The state health departments must report their findings to the Centers for Disease Control (CDC), which monitors outbreaks on a national level (CDC, 2011). This testing may be labor intensive at each level, but is worthwhile to help prevent outbreaks.

SUMMARY AND PERSPECTIVES

The lowering of breakpoints to carbapenems by CLSI has alleviated some stress on clinical laboratories. Even with the lowering of breakpoints, there is still a need by infectious disease personnel in a hospital setting to screen for carbapenemase production. The clinical microbiology laboratory must find a confirmatory test that is cost effective, along with being rapid and accurate. Options include the CIM, mCIM, MHT and carbaNP tests, with other testing such as chromagars, genetic testing and combined tests being too expensive or too time consuming at this time. Tests will continue to be developed that help the clinical laboratory to rapidly detect carbapenemase activity in a cost effective matter. Laboratory leadership must remember that increased spending on the laboratory side can lead to shorter hospital stays for patients, which can save millions of dollars for healthcare in the long run. More testing must also be conducted on non-fermenting organisms such as *Pseudomonas* and *Acinetobacter* sp., which also contain carbapenemases, but are not screened in many laboratories. CLSI does not recommend

the testing of these non-fermenters at this time due to the increased amount of false positives and negatives. Until a standard test is discovered, hospital staff must stay diligent and continue to use the correct precautionary measures to prevent the spread of deadly resistance genes.

The detection and prevention of CPOs all leads to a larger scale discussion that needs to take place by healthcare leaders. Extra effort must be given to teach patients and providers to not prescribe antibiotics when they are not needed. The patient can also help with this movement. They must understand that being prescribed an antibiotic is not always the correct choice. Aside from education for patients and providers, companies that develop antibiotics, antivirals and antifungals must continue this research even if it is not the most profitable of areas. The FDA and other government agencies must encourage drug companies to continue research through subsidies and rewards. This then loops back to the healthcare facilities to prevent over prescription of these drugs and prescribe only as a last resort. If everybody in healthcare serves their part to identify, prevent and treat the spread of CPOs and other antibiotic resistant organisms to the best of their ability, these trends can be stopped and even reversed.

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