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RESEARCH

Epidemiology of carbapenem-resistant Enterobacteriaceae (CRE) and comparison of the phenotypic versus genotypic screening tests for the detection of carbapenemases at a tertiary level, academic hospital in Johannesburg, South Africa

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Background: Carbapenem-resistant Enterobacteriaceae (CRE) cause significant infections and pose a threat to the viability of available antibiotics. Understanding the epidemiology of these infections will assist in guiding appropriate treatment and infection prevention and control (IPC) practices in an institution. In addition, the phenotypic carbapenemase-producing Enterobacteriaceae (CPE) screening tests are widely used in South Africa. However, there is no published literature on their performance against PCR in that setting. Therefore, CRE epidemiology and performance of the Modified Hodge with Imipenem and Imipenem + EDTA combined disk tests (CDT) was evaluated at a tertiary academic hospital in Johannesburg.
Method: A retrospective collection of data was performed. Data from January 2015 to December 2016 of all clinical isolates that were CRE OR carbapenem-susceptible Enterobacteriaceae with at least one positive CPE screening test were collected. Information collected included the ward areas from which samples were sent, specimen type that cultured CRE, CRE identification and carbapenem MIC results, phenotypic and genotypic CPE results.
Results: Certain ward areas recurred as predominant areas with CRE infection in the two-year period. The prominent sample types that cultured CRE, the predominant Enterobacteriaceae species and carbapenemases identified corresponded with national surveillance data. The predominant carbapenemase type and level of carbapenem resistance conferred changed within one year. The Hodge test performed poorly for carbapenemase detection. The CDT detected metallo-β-lactamases adequately.
Conclusion: In this study, the use of the MHT to screen for CPEs performed poorly. Continued surveillance will (i) lead to an understanding of the patient population (including infection type) affected, (ii) detect changes in the carbapenemase profiles, and (iii) inform infection prevention and control and appropriate clinical management.

Keywords: carbapenemase-producing enterobacteriaceae, CPE, screening tests, epidemiology, genotypic CPE test, imipenem, EDTA, modified Hodge test, phenotypic CPE test

Introduction

Infections due to carbapenem-resistant Enterobacteriaceae (CRE) are a growing concern both locally and internationally and pose a major threat to currently available antibiotics.[1,2] In addition, these infections have been associated with increased patient morbidity and mortality.[2,3]

Prevention and control of these infections require proper antibiotic stewardship practices, timeous identification and implementation of effective infection prevention and control (IPC) interventions.[4] It is crucial that every institution with CRE infections understands its local epidemiology so as to introduce the appropriate antibiotic and IPC protocols.[5] The level of resistance conferred, i.e. high versus low, will also guide suitable antibiotic treatment of affected patients.[2]

The mechanisms of resistance to carbapenems in the Enterobacteriaceae can be intrinsic or acquired. Intrinsic mechanisms of resistance can be due to (a) the production of chromosomal carbapenemases from the group of class A serine carbapenemases[6] or (b) efflux pumps or (c) reduction in outer membrane permeability through porin loss.[7]

The acquired mechanisms of resistance are plasmid-mediated. These are summarised in Table 1.[5,6]

Of the different resistance mechanisms, carbapenemase production remains the most common mechanism of resistance amongst the CREs.[1] Plasmid-mediated carbapenemases are easily transmitted between bacterial organisms due to the mobile nature of these genetic elements.[8] This has resulted in various local and international CRE outbreaks amongst patients in different institutions.[1,3,4] As a result, rapid identification and containment of these infections are critical.

There are several phenotypic and genotypic screening tests to detect carbapenemase production in CRE infected or colonised individuals. The available phenotypic tests include, (a) the Modified Hodge test (MHT) to detect carbapenemase production, (b) detection of metallo- β lactamases based on inhibition by chelating agents such as ethylene diamine etra-acetic acid (EDTA), dipicolinic acid (DPA), 1,10-phenanthroline and certain thiol compounds, (c) detection of KPCs based on inhibition by boronic acids, (d) Carba NP test (RAPIDEC®, bioMerieux, France), which is based on a colorimetric reaction due to a

Table 1: The plasmid-mediated mechanisms of resistance

Type of mechanism of resistance	Examples
(a) Carbapenemases	(1) Class A serine carbapenemases
	Klebsiella pneumoniae carbapenemase (KPC)
	Guiana extended spectrum (GES)
	(2) Class B metallo-β-lactamases (MBLs)
	Verona integron-encoded metallo-β-lactamase (VIM)
	Active on imipenem (IMP)
	Sao Paulo metallo-β-lactamase (SPM)
	Seoul imipenemase (SIM)
	German imipenemase (GIM)
	New Delhi metallo-β-lactamase (NDM)
	(3) Class D serine carbapenemases
	OXA β-lactamases (e.g. OXA-48 and variants)
(b) Amp-C beta-lactamase OR extended spectrum beta-lactamase (ESBL) production along with efflux pumps or porin loss	

drop in the pH of the media from carbapenem hydrolysis by the carbapenemase, (e) ultraviolet (UV) spectrophotometry, which measures the carbapenem hydrolysis in UV spectra and (f) matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS), which will detect the carbapenem molecule or its degradation product.[8]

The available genotypic tests detect the carbapenemase-producing genes and are performed using the following methodologies: (a) polymerase chain reaction (PCR) and (b) microarrays.[8] However, all currently available CPE screening tests have their limitations.[5] Prior organism identification is required to exclude non-Enterobacteriaceae carbapenemase-producing organisms.[5]

The MHT was initially validated and found to be highly sensitive for KPC carbapenemase detection. Pitfalls of this test are that it may give false-positive results if the organism is an AmpC β-lactamase or ESBL-producer with efflux pumps or porin loss. Also, it has failed to detect carbapenemase production in some isolates.[8]

The MBL detection methods lack sensitivity and specificity and factors such as the media (i.e. if supplemented with zinc or not) and the chelating agent used affect these parameters.[8] Also, the above two tests require a minimum of 24-hour incubation for the results and do not identify the type of carbapenemase.

The carbapenemase detection method utilising the Carba NP test can be difficult to interpret if the colour change does not match what is stipulated in the package insert.

In addition, although this test is quick, cheap and easy to perform, it has produced false-negative results in some mucoid isolates and in OXA-48-producing Enterobacteriaceae. Additionally, this test does not identify the carbapenemase produced.[8] Further evaluations of this test are required.

The MALDI-TOF MS would give a quick result. However, the cost of the equipment, interpretation of the results and false-negative results with certain OXA-48 producing strains and mucoid isolates limit its use.[8]

Spectrophotometric assays have also been proposed as a reference method for the confirmation of carbapenemase activity. However, *Enterobacter cloacae* strains expressing AmpC β-lactamase with possible efflux pump or porin loss have been misidentified as carbapenemase producers.[8]

Molecular tests, although quick and highly sensitive in detecting the carbapenemase genes, are costly, can be labour-intensive and interpretation may require skilled personnel. In addition, many of the assays have predefined gene targets and will therefore not detect novel carbapenemase genes.[9]

As a result, not one of the CPE screening tests is ideal and often a combination of phenotypic and genotypic tests is required to make the identification accurately and timeously.

Several laboratories in South Africa, either due to inaccessibility to the other CPE screening tests or due to cost constraints, utilise the MHT with or without the inhibitor-based combined disk test (CDT) of imipenem and imipenem + EDTA to identify these organisms. Also, the Clinical and Laboratory Standards Institute (CLSI) has proposed the MHT for confirmation of putative carbapenemase producers.[10] As stated, these tests have known limitations.[8] However, we do not have any published data on their performance with CPEs from our setting. This information is important to guide laboratory policy at a local and possibly national level in terms of whether these tests are adequate to screen for CPEs.

The aim of this study was therefore to:

(1) Elucidate the local epidemiology of CRE infections at a tertiary-level, academic hospital in Johannesburg, in order to identify the affected ward areas, patient populations and characterisation of carbapenemases produced so that targeted IPC strategies and appropriate treatment can be instituted.

(2) To evaluate the accuracy of currently utilised phenotypic CPE screening tests (the MHT & inhibitor-based CDT of imipenem and imipenem + EDTA) in comparison with the genotypic PCR test results.

(3) Lastly, based on the study results, to make recommendations on appropriate CPE screening tests that should be introduced at local and possibly at national level in the National Health Laboratory Service (NHLS).

Methods

A retrospective collection of the data was performed using the NHLS Laboratory Information System (LIS) for all CRE as well as carbapenem-susceptible Enterobacteriaceae with a positive result on one or both of the CPE phenotypic screening tests, cultured from clinical samples of patients at a tertiary-level, academic hospital in Johannesburg. The data collected from the LIS were for the period January 1, 2015 to December 31, 2016 and included (a) ward areas from which the samples were sent, (b) the specimen type that cultured the organism, (c) the

organism identification (to species level), (d) carbapenem MICs (determined by E-tests) to elucidate high-level versus low-level resistance and (e) the carbapenemase screening test results (i.e. the MHT and CDT of imipenem and imipenem + EDTA). The CPE screening tests were performed simultaneously with the carbapenem E-tests in suspected CRE isolates from antibiotic disc diffusion testing to ensure quicker turnaround time of results. In this study, high-level resistance is defined as any CRE isolate with ≥ 1 carbapenem MIC result/s with a 'resistant' category as per the CLSI guidelines document.[10] Low-level resistance is defined as carbapenem MIC results that were in the susceptible range with a positive CPE phenotypic screening test (i.e. MHT ± imipenem and imipenem + EDTA CDT) or carbapenem MIC results in the 'susceptible to intermediate' categories, with at least one carbapenem agent in the 'intermediate' category and without any carbapenem agent in the 'resistant' category according to the CLSI guidelines document,[10] regardless of the CPE phenotypic screening test results.

Convenience sampling was employed so that all available CRE isolate results from 2015 to 2016 were analysed.

PCR testing is routinely performed on all suspected or confirmed CRE isolates, from selected NHLS laboratories, at the Antimicrobial Resistance Unit (AMRU) at the National Institute of Communicable Diseases (NICD) in Sandringham, Johannesburg. This test is performed using the Roche kits (LightCycler 480 Probes Master kit (Roche Diagnostics, IN, USA) and the commercially available individual LightMix Modular kits (Roche Diagnostics, IN, USA) and LightCycler 480 II instrument (Roche Applied Science, Germany) and is a multiplex, real-time PCR. It detects the following carbapenemase genes bla_{NDM}, bla_{KPC}, bla_{OXA-48} and variants, bla_{GES}, bla_{IMP} and bla_{VIM}. The PCR results, for the stipulated period, were recorded and stored on the database at the Infection Control Services (ICS) laboratory in Johannesburg. These results were retrospectively collated and compared with the phenotypic CPE screening test results.

Data analysis

The descriptive epidemiology was analysed using a frequency table and graphically represented in pie or bar charts. The accuracy of the currently utilised screening tests in comparison with the PCR test was assessed using two-by-two tables.

Ethics clearance

Ethics clearance was obtained from the Human Research Ethics Committee (HREC) of Witwatersrand University for this study.

Results

Based on the above stipulated criteria, a total of 259 isolates were analysed over the two-year period.

Certain ward areas recurred in the 'Top 5 areas with the highest CRE prevalence' over the two years namely the Adult ICU and Paediatric Oncology wards (Figures 1 and 2).

CRE isolates were cultured from a varied number of sample types (i.e. blood culture, urine, fluid/pus, rectal swab, sputum, catheter tip, pus swab, tracheal aspirate and tissue). The predominant sample types that cultured CRE in 2015 and 2016 were blood cultures, urine and fluid or pus.

The three predominant members of the Enterobacteriaceae family identified, in both years, as CRE were *Klebsiella pneumoniae* (*K. pneumoniae*), *Enterobacter cloacae* (*E. cloacae*) and *Escherichia coli* (*E. coli*) (Figure 3).

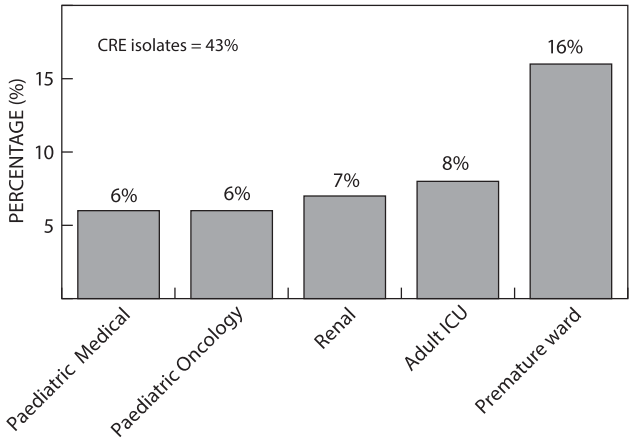


Figure 1: Top 5 areas with the highest CRE prevalence (2015).

The following carbapenemase genes were identified from the CRE isolates over the two-year period: bla_{NDM}, bla_{OXA-48} and variants, bla_{GES} and bla_{VIM}. In 2016, seven isolates with two carbapenemase gene types were detected (i.e. 6- $bla_{NDM\ +OXA-48}$ and variants, and 1 $bla_{NDM\ +\ VIM}$). In addition, there was a shift from bla_{NDM} in 2015 to bla_{OXA-48} and variants in 2016. Four percent (10/259) of the isolates did not have a PCR result as the samples were not sent to the NICD for PCR testing or were lost in transit. Also, several isolates were negative for the carbapenemases, i.e. 28% (71/249) of all isolates over the two-year period (Figure 4).

Regarding the different carbapenemases and the type of carbapenem resistance seen, i.e. low-level versus high-level resistance, 247 isolates were analysed out of the 259 isolates (10 isolates did not have PCR results and 2 isolates did not have the carbapenem MIC results).

In 2015, amongst the NDM-identified isolates, low-level resistance was seen in 12% (6/50) and high-level resistance was seen in 88% (44/50); amongst the OXA-48 and variants, low-level resistance was seen in 46% (6/13) and high-level resistance in 54% (7/13); with VIM isolates, low-level resistance was seen in 80% (4/5) and high-level resistance in 20% (1/5) and 100% (1/1) of GES isolates expressed high-level resistance. In isolates with

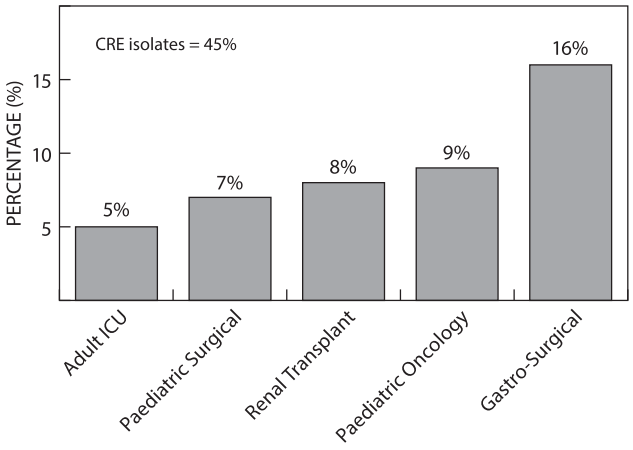


Figure 2: Top 5 areas with the highest CRE prevalence (2016).

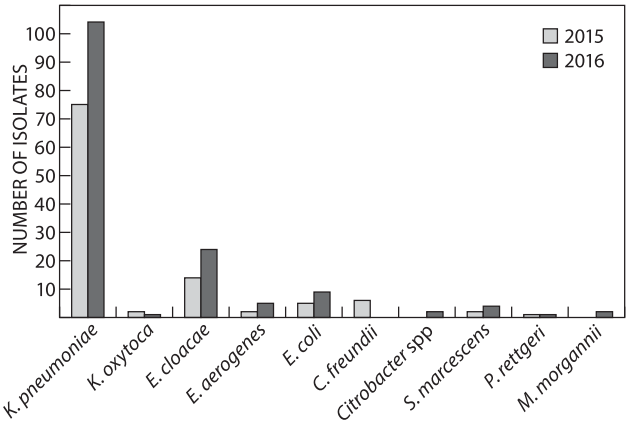


Figure 3: Predominant members of the Enterobacteriaceae family identified as CRE.

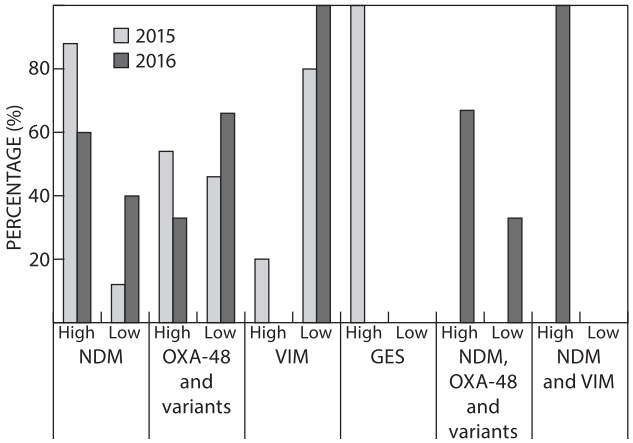


Figure 5: Carbapenemase and level of carbapenem resistance.

negative PCR results, low-level resistance was seen in 48% (14/29) and high-level resistance in 52% (15/29).

In contrast, in 2016, low-level resistance in the NDM isolates increased by 28% in comparison with 2015: low-level resistance was 40% (10/25) and high-level resistance decreased to 60% (15/25); in isolates with OXA-48 and variants, low-level resistance increased by 20% to 66% (47/71) and high-level resistance decreased to 33% (24/71). All VIM isolates expressed low-level resistance (4/4). In the isolates where two carbapenemase genes were detected, such as NDM & OXA-48 and variants, low-level resistance was seen in 33% (2/6) and high-level resistance in 67% (4/6); the one NDM and VIM isolate expressed low-level resistance (Figure 5). In isolates with negative PCR results, low-level resistance increased by 19% to 67% (28/42) and high-level resistance decreased to 33% (14/42)

A subset analysis of the isolates with OXA-48 and variants that expressed the highest numbers of low-level resistance amongst the carbapenemases was performed. All of the 2015 isolates had at least one carbapenem in the intermediately susceptible range. However, in 2016, 55% (26/47) of isolates had at least one carbapenem MIC in the intermediately susceptible range. The remaining 45% (21/47) had carbapenem MICs that were susceptible and only the Hodge test was positive, which prompted the PCR test. Of note, 76% (16/21) of the carbapenem-susceptible and Hodge-positive isolates had a meropenem MIC > 0.12 ug/ml. The remaining 24% (5/21) of isolates had a meropenem MIC < 0.12 ug/ml.

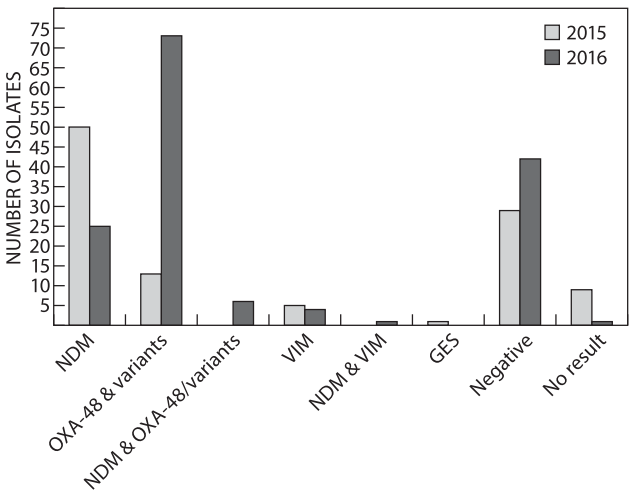


Figure 4: PCR-identified carbapenemase genes from CRE isolates.

In terms of the performance of the CPE phenotypic screening tests in comparison with the PCR test, which detects the carbapenemase genes, 95% (247/259) of the isolates had both phenotypic and genotypic results and these isolates were therefore used for the comparative analysis.

The imipenem and imipenem + EDTA CDT used to detect the metallo-β-lactamases had a sensitivity of 92.3%, specificity of 96.1%, positive predictive value (PPV) of 93.3% and negative predictive value (NPV) of 95.5%.

The MHT for the detection of carbapenemases had a sensitivity of 88.6%, specificity of 36.6%, PPV of 77.6% and NPV of 56.5%. For the detection of metallo-β-lactamases, the test had a sensitivity of 83.5%, specificity of 19.8%, PPV of 37.8% and NPV of 67.3%. And, lastly, for the detection of the OXA-48 and variants, the sensitivity was 93.3%, specificity 25.4%, PPV 41.7% and NPV 86.9%.

Discussion

During the two-year study period, several ward areas in the hospital became endemic for CRE infections. Notably, two ward areas recurred, over the two years, in the 'Top 5 areas with the highest prevalence of CRE', namely the Adult ICU and the Paediatric Oncology ward. Possible explanations for these two wards being affected, among other reasons, could be the critical nature of illness in their patients and the prolonged hospitalisation required by many of them.[1] However, antibiotic usage and IPC practices in these two areas need to be assessed going forward.

Of all sample types from which CRE were cultured, blood cultures in 2015 and urine cultures in 2016 were the most predominant. This is possibly due to the fact that these sites are exposed to indwelling devices that get colonised by these organisms, increasing risk of infection.[11]

The three commonest Enterobacteriaceae identified (i.e. *K. pneumoniae*, *E. cloacae* and *E. coli)* and the predominant carbapenemase genes identified (i.e. bla_{NDM} and bla_{OXA-48} and variants) are in keeping with the national surveillance data from the National Institute for Communicable Diseases (NICD) on carbapenemase-producing Enterobacteriaceae for 2015 and 2016.[12]

Of note, by the end of 2016, rates of *Serratia marcescens* CRE infections superseded that of *E. coli*, nationally. Other carbapenemase genes were also identified in isolates from this study such as bla_{VIM} (in 2015 & 2016) and bla_{GES} (only in 2015). Additionally, although few in numbers, certain isolates carried genes for two carbapenemases namely, $bla_{NDM+OXA-48}$ and variants, and $bla_{NDM+VIM}$.

It is unclear why a shift from bla_{NDM}, as the predominant carbapenemase in 2015, to bla_{OXA-48} and variants in 2016 occurred within the hospital. Factors that may have contributed to the shift in carbapenemases such as change in antibiotic practices in the different units or whether there is increased ease of transmissibility of bla_{OXA-48} and variants in comparison with bla_{NDM} between bacteria were not assessed. However, it is alarming that this change in carbapenemase profile occurred within a time-span of only one year. This is worth investigating further.

Interestingly, low-level carbapenem resistance doubled in 2016 (seen in 62% of the isolates) in comparison with 2015 (seen in 31% of the isolates). This increase was seen amongst bla_{NDM}, bla_{VIM} and notably in the majority of bla_{OXA-48} and variants isolates. Low-level carbapenem resistance in bla_{OXA-48} and variants isolates is expected, as it is a known characteristic of this carbapenemase.[13] The study results, however, highlight that the type of carbapenemase identified may not predict the level of antibiotic resistance as the resistance profile for the different carbapenemases, including bla_{OXA-48} and variants, changed over the two-year period.

Ironically, 43% (3/7) of the isolates harbouring two carbapenemase genes, namely $bla_{NDM+OXA-48}$ and variants, and $bla_{NDM+VIM}$, also conferred low-level resistance. One would assume that the presence of more than one resistant gene would increase the potential for carbapenem resistance. In addition, the isolates negative for a carbapenemase on PCR testing also showed an increase in low-level resistance over the two-year period.

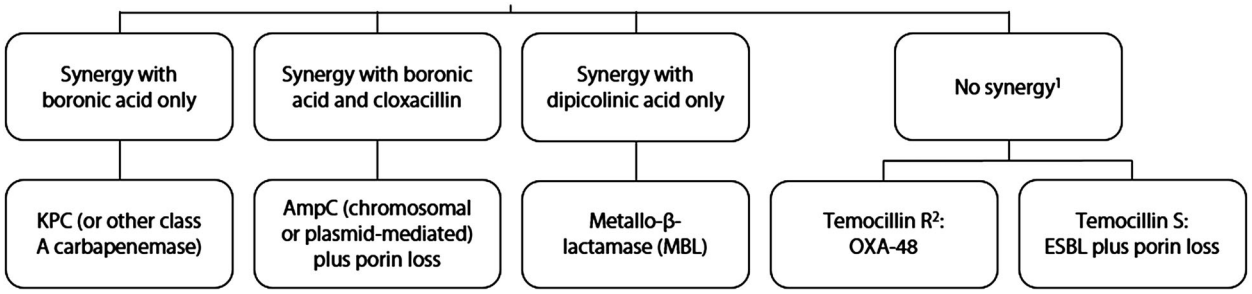
It has become evident through several publications that factors such as knowledge of the CRE site of infection, the patient profile regarding associated co-morbidities and the level of carbapenem resistance in the isolates assists with optimal treatment of the patient. Unfortunately, there are no randomised controlled trials assessing antibiotic treatment options for CRE infections. Much of the existing evidence is from case reports, case series or small retrospective studies.[2] Combination therapy is associated with improved survival in the critically ill patients or those with severe CRE infections.[14] The benefits of combination therapy include reduction of initial inappropriate antimicrobial therapy, potential synergistic effects and suppression of emerging resistance.[15] In addition, several retrospective studies have observed improved mortality outcomes with carbapenem-based combination therapy compared with non-carbapenem-based therapy.[16–19] However, the efficacy of carbapenem-based combination therapy appears to be MIC dependent. The lower the carbapenem MIC the greater the mortality benefit for patients on carbapenem-based combination therapy. This benefit is seen in CRE isolates with MIC values $\leq$ 8 ug/ml.[20] With high carbapenem MIC values $\geq$ 16 ug/ml, non-carbapenem-based combination treatment options would need to be considered in the critically ill or patients with severe infections.

There is evidence that monotherapy may be considered in urinary tract infections and, possibly, in other sites of infection, provided there is good source control of the infection and absence of other co-morbidities in the patient. Monotherapy, particularly, with the aminoglycosides and fosfomycin in urinary tract infections, has led to bacterial clearance of CRE.[2] There are several case reports of fosfomycin efficacy in CRE-associated urinary tract infections.[21–24] Intravenous fosfomycin has also been found to be efficacious in complicated urinary tract infections with CRE.[2]

Therefore, a good understanding of the CRE epidemiology within an institution can guide empiric and targeted antibiotic treatment protocols in the affected ward areas. However, if more than one CPE strain is circulating in a particular ward, antibiotic treatment should be tailored per patient taking into consideration the patient profile, site of infection, carbapenemase type and the level of carbapenem resistance conferred, as discussed earlier.

In this study, the performance of the CDT (imipenem and imipenem + EDTA) to detect metallo-β-lactamases proved to be optimal. However, although the MHT had good sensitivity in detecting the metallo-β-lactamases and OXA-48 and variants, it had poor specificity, PPV and NPV for these carbapenemases. This test is currently recommended by CLSI for CPE detection whereas European Committee For Antimicrobial Susceptibility Testing (EUCAST) does not recommend this test for this purpose.[10,25] The MHT needs to be replaced by more accurate tests in the NHLS.

As mentioned before, due to the limitations of each of the phenotypic and genotypic CPE screening tests, it is important that combinations of tests are performed to overcome the limitations of the individual tests. A suggestion would be to utilise CDT as



[1]Combination of several carbepenemases can also contribute to no synergy – eg MBL and KPC in combination. Molecular testing is usually necessary in such cases.
[2]High-level temocillin resistance (>128 mg/l, zone diameter <11 mm) is a phenotypic marker of OXA-48.

Figure 6: Carbapenemase-producing Enterobacteriaceae (CPE) screening using combined disk tests.[25]

suggested by EUCAST[25] (see algorithm in Figure 6) and then to confirm the presence or absence of the respective carbapenemase by a PCR method.

Although the above-mentioned algorithm will detect CPE accurately, it also has limitations. CDTs require a minimum of 18 hours' culture incubation for accurate results and the currently available PCR tests detect specific carbapenemases. Novel or variant carbapenemase genes will not be identified.

Another concerning finding from the study was in the subset analysis of the 2016 $bla_{\text{OXA-48}}$ and variants isolates with low-level carbapenem resistance: 45% (21/47) of these isolates had susceptible carbapenem MICs and it was solely the positive MHT that prompted PCR testing; 24% (5/21) of these isolates had a meropenem MIC of $\leq$ 0.12 ug/ml. This is interesting because the current EUCAST guidelines consider meropenem to have the best combination of both sensitivity and specificity in detecting CPEs and recommend that further CPE screening is only required if the meropenem MIC is > 0.12 ug/ml.[25] Based on these results, further evaluation of the EUCAST recommendation is required.

Limitations of the study include the fact that (1) it was a retrospective collection of data, so isolates may not have been included in the database or information was missing as mentioned with the 12 isolates in this study; also (2) in the study, 247 isolates were used in the analysis to ascertain the accuracy of the phenotypic CPE screening tests. This is a small sample size. However, our finding that the MHT performs poorly in CPE detection and is no longer a recommended test for this purpose has been supported by several international publications and the current EUCAST guidelines.[8,9,25]

Conclusion

Understanding the CRE epidemiology in this tertiary academic hospital in terms of the type of patients affected, the sites of infections, carbapenemases identified and the level of carbapenem resistance conferred provides critical information that did not exist previously. This study also illustrated how quickly the carbapenemase profile and expression of the level of carbapenemase resistance can change within an institution. Continued dissemination of this type of information is important to guide hospital clinicians and IPC practitioners on the appropriate treatment and control of these infections within this hospital.

Based on the study results, the MHT performed poorly for the detection of metallo-β-lactamases and OXA-48 and variants. This test should no longer be recommended as a CPE screening test. In addition, the current EUCAST recommendation to screen for CPE only if the meropenem MIC is > 0.12 ug/ml needs further evaluation as OXA-48 and variants can be missed if this criterion is used.

CPE screening and confirmation requires a combination of phenotypic and genotypic tests to increase accuracy of detection. CDT as recommended by EUCAST followed by PCR confirmation of the carbapenemase gene appears to be the most reliable testing algorithm to follow.

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