

The Efficacy of the MicroScan® Walkaway System in Reporting Carbapenemase-Producing Enterobacteriaceae in Patients with Bacteremia, South Africa

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Abstract

Background: The emergence of Carbapenemase-producing Enterobacteriaceae (CPE) is a major public health problem worldwide. As Carbapenemase-production has emerged, treatment of infections has become more difficult leading to high mortality. Real time detection of the presence of these enzymes by *in vitro* susceptibility testing of these organisms is urgently needed to provide effective treatment and appropriate implementation of infection and prevention control measures. Automated phenotypic systems are widely used in clinical microbiology laboratories for bacterial identification and antimicrobial susceptibility testing. However, critical evaluation is needed to determine the accuracy of these systems.

Objective: Our study was set out to evaluate whether the MicroScan® Walkaway system is a reliable method for predicting CPE in patients with a Carbapenem-resistant Enterobacteriaceae (CRE) infections.

Methods: This was a cross-sectional study of CRE isolates from July 2015 to July 2016 received as part of an active CRE surveillance programme. Bacterial identification and antimicrobial susceptibility testing for Carbapenems were performed using the MALDI-ToF and MicroScan® Walkaway system, respectively. Genotypic testing was performed using the LightMix® modular Carbapenemase kits in a multiplex real-time PCR assay to confirm the presence of Carbapenemase genes.

Results: During the study period, there were 219 CRE tested. Out of 219 CRE cases, Carbapenemase genes were detected in 173 (78.9%). The most prevalent gene was *bla*_{NDM} (38.8%; n=85), followed by *bla*_{OXA-48} and variants (32.8%; n=72), *bla*_{VIM} (6.9%; n=15) and *bla*_{GES} (0.5%; n=1). The MicroScan® Walkaway system had the highest sensitivity with ertapenem (86.7%). Sensitivities for all other Carbapenems were similar, but below 65%. The positive predictive value for ertapenem was 78.9% and >80% for imipenem (86.2%), meropenem (81.3%) and doripenem (83.7%). Overall, testing for all Carbapenems had a sensitivity of 89%, positive predictive value of 79% and specificity of 10.9%, amongst them imipenem having the highest specificity at 60.9%.

Conclusion: The MicroScan® Walkaway system is sensitive, but lacks specificity. However, it shows to be an efficient diagnostic adjunct to the LightMix® modular multiplex real-time PCR assay for predicting CPE in a patient with a CRE infection depending on the Carbapenem used.

Keywords: Antimicrobial resistance surveillance; Carbapenemase-producing Enterobacteriaceae; Carbapenems-resistant Enterobacteriaceae; MicroScan® walkaway system; Light Mix® modular multiplex real-time PCR assay

Introduction

Enterobacteriaceae are Gram-negative bacteria that commonly colonize the gut of humans and can cause life threatening infections such as cystitis and pyelonephritis with bloodstream infections, pneumonia, meningitis and endocarditis in both community and hospital settings [1,2]. Although the majority of infections occur among hospitalized patients with immuno-compromising and underlying illnesses, community-acquired infections are increasingly

reported [3]. Carbapenem-resistant Enterobacteriaceae are resistant to multiple antibiotic classes and infections with these organisms often results in high mortality rates. Extensive and inappropriate utilization of antibiotics to treat infections has greatly contributed to the development and spread of CREs [4].

Most CRE acquire resistance through plasmids harboring β -lactamase enzymes, including Carbapenemases. Carbapenemase-producing Enterobacteriaceae (CPE) are capable of hydrolysing Carbapenems and all other β -lactam antibiotics [5-7]. Although plasmid-mediated resistance is not the only mechanism of resistance among CRE, it is the most epidemiologically important due to the high efficiency at which Enterobacteriaceae exchange plasmids among each other [7].

Mechanisms for Carbapenem resistance in Enterobacteriaceae include hyper-production of extended spectrum beta-lactamases (ESBLs), class c-β-lactamases (AmpC), mutations in porins and alterations or up-regulation of efflux pumps [6]. The most common Carbapenemases worldwide include the metallo-β-lactamases New Delhi-metallo-β-lactamase (NDM), Imipenemase β-lactamases (IMP), Verona integron encoded metallo-β-lactamase (VIM), Guiana extended spectrum β-lactamases (GES), and Oxacillinase β-lactamase (OXA-48 types) [8-14]. The presence of genes encoding these enzymes has been identified in South African isolates in both public and private sector hospitals [15-17] and the emergence of these genotypes in our setting poses a difficult challenge to clinical microbiologists, infection and prevention control (IPC) practitioners, and treating clinicians [18,19].

Infections caused by CRE often fail respond to first line treatment, resulting in delayed treatment and high mortality rates of up to 50%, and higher costs due to prolonged hospitalization [20]. Compounding the public health importance of these infections is the ability of CPE to exhibit resistance to multiple antimicrobial classes, limiting treatment options for patients. Of particular concern is resistance to Carbapenems, which are considered the last resort antibiotics for the treatment of Gram-negative bacterial infections, threatening their usefulness in treating invasive and non-invasive infections [21].

Standard Antimicrobial Susceptibility Testing (AST) methods have limited sensitivity and specificity for detecting CPE, resulting in delayed appropriate treatment and causing a high rate of clinical failures [22]. Timely and accurate detection of CPE among infected patients is important when deciding on the most appropriate antibiotic treatment and IPC measures, which are crucial in preventing outbreaks and limiting further emergence and spread of these resistant genotypes. The MicroScan® Walkaway automated system is currently the most reliable commercially available system providing accurate results in the detection of resistance compared to the Vitek-2® compact and BD Phoenix™ systems [23-25]. Although genotypic methods are the gold-standards due to their accuracy in detecting the presence of the Carbapenemase genes, they have limitations when used as real-time diagnostic tools and are costly to implement routinely. An automated system that is able to provide accurate *in vitro* susceptibility of organisms and correctly predict infections with CPE for appropriate implementation of IPC measures and patient treatment is therefore needed. Our study set out to evaluate whether the MicroScan® Walkaway system is a reliable method for predicting CPE in a patient with CRE infection.

Methods

This was a cross-sectional study of patients with CRE bloodstream infections between July 2015 and July 2016, admitted to public-sector hospitals in the Gauteng, Western Cape, and Free State and KwaZulu-Natal provinces. The isolates were submitted by sentinel site laboratories to the Antimicrobial Resistance Laboratory (AMRL) at the National Institute for Communicable Diseases (NICD), as part of enhanced surveillance programme that enrolls all patients with laboratory confirmed CRE bloodstream infections.

Case definition

We defined CRE as blood culture isolates submitted to AMRL and identified as Enterobacteriaceae (*Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., *Serratia* spp., *Escherichia coli*, *Providentia* spp.) and

were non-susceptible to any of the Carbapenems (ertapenem, meropenem, imipenem and doripenem). CPE was defined as any Enterobacteriaceae confirmed positive for Carbapenemase production by the LightMix® modular multiplex real-time PCR assay.

Antibiotic susceptibility testing and detection of Carbapenemase encoding genes

Organism identification and antimicrobial susceptibility profiles of submitted isolates were confirmed by the reference laboratory. Organism identification was confirmed by the Microflex MALDI-ToF (Bruker Daltonik, GmbH). Antimicrobial susceptibility testing for ertapenem, meropenem, imipenem and doripenem was performed on the MicroScan® Walkaway system (Siemens, USA) using the Gram-negative MIC Panel Type 44 (ertapenem 0.5-1 µg/mL; doripenem 1-4 µg/mL; imipenem 1-8 µg/mL; meropenem 1-8 µg/mL). Interpretation of susceptibility was done according to the Clinical and Laboratory Standards Institute (CLSI) guidelines 2016 [26].

For the detection of carpanemase genes, DNA was first extracted using a crude boiling method at 95°C for 25 minutes. The supernatant was harvested and screened for the presence of *bla*NDM, *bla*KPC, *bla*OXA-48 and its variants (*OXA* 162, 163, 244, 245, 247, 181, 204 and 232), *bla*GES (*GES*-1-9, 11), *bla*IMP (*IMP*-9, 16, 18, 22, 25) and *bla*VIM (*VIM*-1-36) genes using a LightMix® modular multiplex real-time PCR assay (LightCycler 480 II, Roche Applied Science, LightCycler 480 Probes Master kit and the individual TibMol Biol LightMix® modular Carbapenemase kits, Roche Diagnostics, USA).

Data analysis

Data analysis was done using STATA statistical software (Version 14; StataCorp LP Texas USA). The proportion of isolates categorized as CRE by the MicroScan® Walkaway system was compared to the proportion characterized as CPE by the multiplex real-time PCR assay. The sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) of the MicroScan® Walkaway system were calculated.

Results

During the study period, a total of 219 non-repetitive CRE isolates were obtained from 100 (45.7%) male and 98 (44.7%) female patients. The median age was 30 years (range of 1-83 years). Of these isolates, 75.8% (n=166) were *Klebsiella* sp., 10.9% (n=24) were *Enterobacter* sp., 7.8% (n=17) were *Serratia marcescens*, 5% (n=11) were *Escherichia coli* and 0.5% (n=1) *Providencia rettgeri* (Table1).

Based on the MicroScan® Walkaway system, isolates were highly resistant to Carbapenems, showing non-susceptibility rates of >50% to Ertapenem. Among all species, the highest ertapenem non-susceptibility rates ranged from 83.7% (139/166) for *Klebsiella* spp. to 100% (1/1) for *P. rettgeri*. Organisms were least resistant to doripenem (129/219, 58.90%), however, *Klebsiella* spp. (60.8%, 101/166) and *Serratia marcescens* (100%, 17/17) exhibited high levels of non-susceptibility compared to *Enterobacter* spp. (25.0%, 6/24) and *Escherichia coli* (36.4%, 4/11).

Prevalence and distribution of Carbapenemase genes

According to LightMix® modular Carbapenemase multiplex real-time PCR assay, 78.9% (173/219) of the CRE isolates harbored Carbapenemase genes (Table 2). Of these, 38.8% (85) were *bla*NDM-

positive, 32.8% (72) were *bla*OXA-48 and variants-positive, 6.9% (15) were *VIM*-positive, 0.5% (1) was *bla*GES-positive and 21.0% (46) did not harbor any of the Carbapenemase genes tested for. None of the isolates were found to be positive for the *bla*IMP and *bla*KPC genes. Notably, five isolates harbored more than one Carbapenemase gene, including combinations *bla*NDM and *bla*OXA-48 (n=2), *bla*OXA-48 and *bla*VIM (n=1), and *bla*NDM and *bla*VIM (n=2).

Correlation of the MicroScan® Walkaway system with the LightMix® modular Carbapenemase multiplex real-time PCR assay in detecting Carbapenem resistance

Based on the MicroScan® Walkaway system, 190 (86.7%), 130 (59.4%), 134 (61.2%) and 129 (58.9%) of study isolates were non-susceptible to ertapenem, imipenem, meropenem and doripenem, respectively (Table 3). A total of 46 (21.0%) isolates that were non-susceptible to Carbapenems by the automated system did not harbor Carbapenemase genes. The highest proportion 40 (21.05%) of non-susceptible isolates that did not harbor genes were those with ertapenem non-susceptibility, compared to other antibiotics. Isolates that showed non-susceptibility rates of >60% to doripenem, imipenem and meropenem were mostly positive for the *NDM* gene (Table 3). Similar patterns were not observed with all other Carbapenemases, however, majority of the isolates harboring the *OXA-48* genes (28.4%, 54/190) were non-susceptible to ertapenem.

The sensitivity, specificity, positive and negative predictive values of the MicroScan® Walkaway system against the LightMix® modular Carbapenemase multiplex real-time PCR assay are shown in Table 4. The phenotypic method had the highest sensitivity with ertapenem (86.7%; 95% confidence interval [CI] 80.7-91.4). Sensitivities for all other Carbapenems were similar, but below 65%. The positive predictive value for ertapenem was 78.9% (95% CI 79.0-91.6) and >80% for imipenem, (86.2%; 95% CI 72.5-84.5) meropenem (81.3%; 95% CI 73.3-87.5), and doripenem (83.7%; 95% CI 76.2-89.6). Overall, testing for all Carbapenems had a sensitivity of 89% (83.4-93.9) and a positive predictive value of 79% (95% CI 72.6-84.5). Specificity of the test for all Carbapenems combined was low (10.9%; 95% CI 3.62-23.6), with imipenem having the highest specificity at 60.9% (95% CI 45.4-74.9).

Organism identified	Antibiotic n (%)			
	Ertapenem	Imipenem	Meropenem	Doripenem
<i>Klebsiella</i> spp.(N=166)	139 (83.7)	103 (62.0)	104 (62.7)	101 (60.8)
<i>Escherichia coli</i> (N=11)	11 (100)	4 (36.4)	5 (45.5)	4 (36.4)
<i>Enterobacter</i> sp.(N=24)	22 (93.7)	5 (20.8)	8 (33.3)	6 (25.0)
<i>Serratia marcescens</i> (N=17)	17 (100)	17(100)	16 (94.1)	17 (100)
<i>P. rettgeri</i> (N=1)	1 (100)	1 (100)	1 (100)	1 (100)
Total (219)	190 (86.76)	130 (59.36)	134 (61.19)	129 (58.90)

Table 1: Carbapenem non-susceptibility of Enterobacteriaceae isolated from patients with bloodstream infections from July 2015 to July 2016.

Organism	Carbapenemase gene n (%)				
	NDM	OXA-48*	VIM†	GES‡	Negative
<i>Klebsiella</i> spp.(N=166)	65 (39.2)	59 (35.5)	14(8.4)	1 (0.6)	27(16.3)
<i>Escherichia coli</i> (N=11)	1 (9.0)	8 (72.7)	0 (0)	0 (0)	2 (18.2)
<i>Enterobacter</i> spp. (N=24)	5 (20.8)	5 (4.2)	1 (4.2)	0 (0)	13 (54.2)
<i>Serratia marcescens</i> (N=17)	13 (76.5)	0 (0)	0 (0)	0 (0)	4 (23.5)
<i>P. rettgeri</i> (N=1)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Total	85 (38.8)	72 (32.8)	15(6.9)	1 (0.5)	46 (21)

Table 2: Distribution of Carbapenemase genes among CRE isolated from patients with bloodstream infections from July 2015 to July 2016. *Includes *OXA-48* and variants (*OXA 162, 163, 244, 245, 247, 181, 204* and *232*), †*VIM* (*VIM-1-36*), ‡*GES* (*GES-1-9, 11*).

Non-susceptible isolates by the MicroScan® Walkaway system	Carbapenemase genes n (%)				
	NDM n (%)	OXA-48 & variants n (%)	VIM n (%)	GES n (%)	Negative n (%)
Ertapenem (N=150)	84 (44.2)	54 (28.4)	11 (5.8)	1 (0.5)	40 (21.05)
Imipenem (N=112)	82 (63.0)	14 (10.8)	15 (11.5)	1 (0.8)	18 (13.8)
Meropenem (N=109)	82 (61.2)	17 (12.7)	9 (6.7)	1 (0.7)	25 (18.6)
Doripenem (N=108)	82 (63.5)	14 (10.9%)	11 (8.5)	1(100)	21 (16.3)

Table 3: Carbapenemase production according to the MicroScan® Walkaway system versus the LightMix® modular Carbapenemase multiplex real-time PCR assay.

Carbapenems	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive predictive value % (95% CI)	Negative predictive value % (95% CI)
Ertapenem	86.7 (80.7-91.4)	13 (4.94-26.3)	78.9 (79-91.6)	20.7 (22-42.2)

Imipenem	64.7 (57.1-71.8)	60.9 (45.4-74.9)	86.2 (72.5-84.5)	31.5 (7.99-39.7)
Meropenem	63 (55.3-70.2)	45.7 (30.9-61)	81.3 (73.7-87.5)	24.7 (16-35.3)
Doripenem	62.4 (54.8-69.7)	54.3(39-69.1)	83.7 (76.2-89.6)	27.7 (18.9-38.2)
All Carbapenems	89 (83.4-93.3)	10.9 (3.62-23.6)	79 (72.6-84.5)	20.8 (7.13-42.2)

Table 4: Sensitivity, specificity, positive and negative predictive values of the MicroScan® Walkaway system.

Discussion

CREs has shown to increase among Gram-negative bacteria due to the acquisition of genes producing Carbapenemases. An automated system that is able to provide accurate *in vitro* susceptibility of organisms and correctly predict infections with CPE for appropriate implementation of IPC measures and patient treatment is therefore desirable. In this study, we evaluated whether the MicroScan® Walkaway system is a reliable method for predicting CPE in patients with a CRE infection. In our study setting, the MicroScan® Walkaway system has shown to be an efficient diagnostic screening tool for predicting CPE in patients with CRE infections. It was found that *bla*^{NDM} and *bla*^{OXA-48} and its variants were the most predominant Carbapenemase genes in our hospital settings.

Similar to previous South African studies, we found that *Klebsiella* sp., *Enterobacter* spp. and *Serratia marcescens* were the most common CREs in our study [22]. A high proportion (78.9%) of CRE isolates were carbapenemase-producers, with resistance mostly mediated by *bla*^{NDM} and *bla*^{OXA-48} and variants. This is in agreement with other studies conducted in South Africa which found a high prevalence these genes in both the public and private sector hospitals [17,22]. Just below a quarter of CRE isolates were found negative for Carbapenemases. It should be noted that false-negative results may be due to the fact that only six Carbapenemases are detected by the LightMix® modular Carbapenemase multiplex real-time PCR assay [22,27], thus limiting the ability to detect other carbapenemases and derivatives responsible for the phenotypic resistance [22,28,29]. Alternatively, resistance may have been conferred by other mechanisms of resistance such as AmpC and/or ESBL, efflux pumps and porin mutations.

Further studies should be conducted to elucidate other mechanisms of resistance among non-susceptible isolates that do not harbor Carbapenemases. Although the PCR assay allows for detection of *bla*^{IMP} and *bla*^{KPC}, and these genes have been found in South African isolates [22]. They were not detected in our study population and possibly these genes are not circulating genotypes in our study sentinel sites.

Overall, the sensitivity of the MicroScan® Walkaway system in detecting CPEs was high when using all Carbapenems for testing. However, the overall specificity was low, indicating its inability of the system to adequately detect non-CPEs. Sensitivity was highest when using ertapenem, but the lowest specificity was obtained with this antibiotic. The system had the highest specificity with imipenem, followed by doripenem. The sensitivities and specificities in our study are in keeping with a similar study conducted using varied specimen types, where an ertapenem sensitivity of 98% (versus the current study of 86.7%) was observed [22]. In a previous study, He et al. reported sensitivity and specificity of MicroScan® Walkaway using imipenem, meropenem and ertapenem as 93.8% and 42.4% respectively, which is similar to the results in our study [28].

All four Carbapenems showed a positive predictive value of >78%, and imipenem had the highest positive and negative predictive values. As both positive and negative predictive values measures are influenced by prevalence, it is important to note that the predictive values found in our study are only applicable to settings where the prevalence of CPEs among CREs is similar to that in our study. Our results suggest that the MicroScan® Walkaway system for AST testing is reliable in detecting and predicting CPEs among CRE, and testing with ertapenem and imipenem provides the best overall accuracy. The utilisation of only ertapenem and imipenem with the MicroScan® Walkaway may be considered for predicting CPEs among bacteremia patients in order to reduce testing cost.

Conclusion

The MicroScan® Walkaway system is sensitive, but lacks specificity. However, it shows to be an efficient diagnostic adjunct to the LightMix® modular multiplex real-time PCR assay for predicting CPE in a patient with a CRE infection depending on the Carbapenem used.

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Authors' Contributions

MR, ASM and OP conceived and designed the study and experiments, MR performed the experiments, analyzed the data and wrote the manuscript. LS participated in data analysis and review of the manuscript. All authors read, edited and approved the final manuscript.

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NICD funding was obtained for this study.

Conflict of Interest

The authors declare that they have no conflict of interests.

Ethics approval and consent

Permission to conduct this study was obtained from the Human Research Ethics Committee R14/49, University of Witwatersrand, Johannesburg, South Africa, clearance certificate M10464.

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