Molecular detection of carbapenemase-producing genes in referral Enterobacteriaceae in South Africa: A short report

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Enterobacteriaceae are a large group of Gram-negative, rod-shaped bacteria. They are the bacteria most frequently isolated from clinical specimens and may account for up to 80% of all clinically significant isolates from Gram-negative bacilli and up to 50% of all clinically significant bacteria. Among all CPE, the most common enzyme was NDM-1. NDM-1 was first described in 2008 in a Swedish patient returning from the Indian subcontinent. NDM-1 has subsequently been reported worldwide, with most early cases of NDM-1 diagnosed in the UK having epidemiological links with the Indian subcontinent.

Objective
To demonstrate the presence of carbapenemases in Enterobacteriaceae over a 4-year period, based on a referral system for confirmation of CPE genes.

Methods
Carbapenem non-susceptible, clinically significant isolates from the Enterobacteriaceae family were submitted to the Antimicrobial Resistance Laboratory (AMRL) at the National Institute for Communicable Diseases (NICD), Johannesburg, South Africa (SA), for confirmation of carbapenemase-producing genes from 2012 through 2015. Referral of isolates by public and private microbiology laboratories was based on non-susceptibility to carbapenems according to defined criteria for antimicrobial susceptibility testing results were sent to a reference laboratory. A proportion of isolates had limited demographic, epidemiological and clinical data available. Organism identification was reconfirmed using reference laboratory methods, and the presence of carbapenemases was confirmed with a real-time polymerase chain reaction. We analysed 1 503 significant isolates received for confirmation from the National Health Laboratory Service and some private laboratories during 2012 - 2015 and confirmed one or more carbapenemase-producing genes in 68% of isolates, the most common organism being Klebsiella pneumoniae (60%). The most common carbapenemase genes were blaNDM, followed by blaOXA-48 and its variants. blaNDM and its variants demonstrated non-susceptibility to ertapenem in 89% of the isolates when analysed by the phenotypic method, and to ceftazidime in 34%. Overall, the detection rate for carbapenemases in K. pneumoniae blood isolates in the public sector was 1.9% during the 4-year period. This report indicates the presence of CPE in SA, and it is important for all healthcare workers to be aware of this major public health threat so that infection prevention and control measures can be implemented to prevent the spread of CPE in healthcare facilities.

Enterobacteriaceae cause both nosocomial and community-acquired infections and are increasingly becoming multidrug resistant (MDR) to antimicrobial agents. The past few decades have seen the rapid emergence and spread of antimicrobial resistance genes on plasmids and chromosomes. The focus given to transmission of carbapenem resistance owing to easily transmissible concentration (MIC) testing methods interpreted by the Clinical and Laboratory Standards Institute guideline. Submission practice from public laboratories was based on a guideline from the reference
laboratory at the NICD and from private laboratories on a voluntary basis.

Organism identification was reconfirmed using automated systems (VITEK 2 [bio-Mérieux, France] and/or Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry [MALDI-ToF, Bruker Daltonik GmbH, Germany], and antimicrobial susceptibility testing (AST) was done using the MicroScan Walkaway system (Siemens, USA) at the AMRL. For molecular methods, DNA was extracted from purity plates using a crude boiling method at 95°C for 25 minutes. The supernatant was harvested and screened for $\text{bla}_{\text{NDM}}$, $\text{bla}_{\text{OXA-48}}$, $\text{bla}_{\text{IMP}}$, and its variants, $\text{bla}_{\text{TAX-97}}$, $\text{bla}_{\text{VIM}}$ and $\text{bla}_{\text{GES}}$ using a real-time polymerase chain reaction (LightCycler 480 II, Roche Applied Science, Germany), the LightCycler 480 Probes Master kit (Roche Diagnostics, USA) and individual LightMix Modular kits (Roche Diagnostics, USA). Kit-positive controls as well as in-house controls were used in all assays ($\text{bla}_{\text{NDM}}$, ATCC BAA21246; $\text{bla}_{\text{IMP}}$, ATCC BAA1705; $\text{bla}_{\text{TAX-97}}$, and its variants, NCTC13442; $\text{bla}_{\text{VIM}}$, clinical isolate; $\text{bla}_{\text{GES}}$, NCTC 13476; and $\text{bla}_{\text{GES}}$, clinical isolate). Sterile water was used as a negative control.

Statistical analysis
Where appropriate, we calculated frequencies and percentages and used the $\chi^2$ test/Fisher’s exact test to compare categorical variables. Statistical analyses were performed using STATA 14 (StataCorp, USA).

Ethical considerations
Laboratory-based antimicrobial resistance surveillance for nosocomial bacteria was approved by the Human Research Ethics Committee (Medical) at the University of the Witwatersrand, Johannesburg (clearance certificate no. M10464).

Results
We analysed 1 503 clinically significant Enterobacteriaceae isolates received for carbapenemase-producing gene confirmation. The age distribution of patients with carbapenem-resistant Enterobacteriaceae (CRE) infections showed two significant peaks, in children aged 0 - 5 years and in adults aged 30 - 40 years ($p<0.05$). Blood was the most common specimen type (25%), followed by urine (22%). We confirmed ESBL in 93% of isolates by the automated MIC method. Furthermore, carbapenemase-producing genes were confirmed molecularly in 68% of isolates (1 021/1 503). Of all Enterobacteriaceae, the most common was $K.\ pneumoniae$ (60%), followed by $E.\ coli$ (14%) and $S.\ marcescens$ (6%).

The detection rate of carbapenemases among all $K.\ pneumoniae$ blood isolates from public laboratories during the 4-year period was 1.9%. Based on voluntary referral practice, we could not estimate the prevalence of carbapenemases for all Enterobacteriaceae isolates and specimen types in SA.

Discussion
This short report describes emerging resistance to carbapenems in Enterobacteriaceae over a 4-year period in SA. We detected the presence of major carbapenemases, i.e. NDM, OXA-48 and VIM. In a previous laboratory-based antimicrobial resistance surveillance study conducted in 2010 - 2012, no isolates containing $\text{bla}_{\text{NDM}}$ or $\text{bla}_{\text{OXA-48}}$ were found, and $\text{bla}_{\text{IMP}}$ and $\text{bla}_{\text{VIM}}$ were confirmed in <0.1%. The present report suggests rapid dissemination of these genes once they are introduced into the environment, and we describe its longitudinal nature, which is in line with the global dissemination.

The vast majority of the referral isolates produced ESBL, which is to be expected owing to the MDR patterns of these organisms and compares with a previous surveillance report. Enterobacteriaceae extensively exhibit MDR patterns, which enable them to persist and spread rapidly in healthcare settings.

Our results indicate that $\text{bla}_{\text{NDM}}$-positive isolates could be missed in 11% of isolates owing to susceptibility to ertapenem, yet 43% were sensitive to imipenem and 57% to meropenem, which demonstrated that AST methods cannot be used for screening of these enzymes. This differs from an Indian study that showed 75% sensitivity to ertapenem and 84% sensitivity to imipenem and meropenem.[14] Poirel et al.[15] pointed out menace behaviour of these enzymes and the difficulties this poses for phenotypic detection. As we report here, some of these enzymes will be overlooked, particularly if carbapenems are used as indicators for AST resistance screening.

There are various approaches to the control of MDR organisms. Carmeli et al.[16] indicated that the objective for control should be eradication, while others such as Thurlow et al.[17] considered that a wider approach is needed once endemicity is established. Thurlow et al.[17] also suggested that reducing the burden of CPE on patients’ skin should be explored further as a way of reducing cross-transmission at long-term healthcare hospitals, where endemicity is most likely. Clinicians and infection control practitioners should be aware of the presence of carbapenemases in Enterobacteriaceae and its implications for infection prevention and control in the SA setting.

Study limitations
This report has a number of limitations. Owing to lack of policies and voluntary
practice in sending isolates for confirmation to the reference laboratory, we were unable to determine the national prevalence of CPE or to establish whether there has been an increase in CPE in SA. Changes in submission practices and/or increased awareness of CPE infections undoubtedly influenced the number of isolates referred to the reference laboratory. However, this analysis reports a minimum estimate of the presence of CPE organisms and carbapenemase-producing genes in the country. Missing demographic, epidemiological and clinical data reduced our ability to analyse laboratory data in more meaningful ways. Surveillance for CPE through the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA) surveillance platform was introduced at 12 sentinel sites in four SA provinces in 2015. Future analysis of surveillance data for CPE should provide a more representative estimate of their prevalence and distribution in SA.

Conclusions

This report indicates the presence of CPE in SA. It is essential for all clinicians to be aware of this major public threat and be prepared to act if CPE occurs in patients. At all healthcare facilities, the importance of enforcing infection prevention and control measures to prevent the spread of CPE should be emphasised. Importantly, antimicrobial stewardship programmes should be implemented at facility level to prevent selection pressure on bacterial organisms to develop resistance.

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