Characterization of the Mechanisms Underpinning Carbapenem Resistance in a Tertiary Urology Clinical Hospital - a Pilot Study

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Abstract

Introduction and Objectives. Multidrug resistant bacteria are a threat to healthcare both because of increased morbidity and mortality. Local epidemiology is important because it may guide interventions aimed at reducing the frequency of such infections.

Materials and Methods. A prospective study was done over the course of one month, evaluating all strains isolated that had a decreased susceptibility to at least one carbapenem. Strains were evaluated for the presence of a carbapenemase through the rapid Carbapenem Inactivation Method (rCIM). Strains that tested positive and controls were then evaluated through a broad spectrum commercial PCR system.

In the time period 50 Gram negative bacilli were isolated. After MALDI-TOF-MS identification, the 10 strains tested rCIM positive - showing the presence of a carbapenemase. Molecular characterisation showed the presence of blaNDM in 5 isolates, blaOXA-48-like in 2 isolates and blaOXA-24 in 1 isolate. One isolate did not yield a readable result.

Results and Concluions. Thus, we show that blaNDM is the dominant gene present in this pilot study. These preliminary results, which require confirmation through a larger study, shows that different medical institutions may have different genetic subtrates for carbapenem resistance, which may be due to an outbreak or the evolving landscape of resistance.

Key words: multidrug resistant bacteria, infections, carbapenemases, rCIM

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Introduction

Multidrug resistant bacteria lead to increased hospital stay and healthcare costs [1]. While resistance rates to antibiotics are generally considered to be high, in Romania, there is still little known about the epidemiology of individual medical facilities [2,3,4].

The aim of this pilot study was to investigate the molecular profile of the carbapenemase genes which lead to carbapenem resistance in clinical isolates obtained from a tertiary urology hospital in Romania.

Materials and Methods

Bacterial isolates

We performed a study of all Gram negative bacilli isolated in March 2018 from a tertiary urology hospital in Bucharest. Primary identification was done using routine biochemical tests. Standardized antibiogram was carried out using Clinical and Laboratory Standards Institute (CLSI) recommendations and all strains fitting the carbapenemase screening criteria were included in the study.

Bacterial identification

All strains were sent for identification through Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS, autoflex, Bruker). Briefly, this requires depositing a small amount of an isolated colony on the MALDI-TOF-MS target plate, after which, alpha-cyano-4-hydroxycinnamic acid (HCCA) is added and left to dry at room temperature. The target plate is then placed within the MALDI-TOF-MS system and bacterial identification is done using MALDI BioTyper (MBT) software package.

Carbapenemase activity

Strains meeting the carbapenemase screening criteria were tested for carbapenemase activity using the rapid Carbapenem Inactivation Method (rCIM). Briefly, this consists of homogenizing two 10 µL loop full of an overnight culture taken from solid media into an Eppendorf tube containing 1 mL of sterile water. For enterobacteria, two meropenem disks were added into the suspension and vortexed for 1 minute. For non-fermenters, this is substituted for one imipenem disk. The Eppendorf tube was incubated for half an hour at 37° C. After centrifugation, the supernatant is used to challenge a susceptible Escherichia coli indicator strain (ATCC 25922). If the antibiotic was hydrolysed by carbapenemases, growth of the indicator strains will be unaltered. A nephelometer is used to monitor bacterial growth. Carbapenemase production is confirmed when a growth of 0.5 McFarland units is reached within 1.5-2 hours [5].

Molecular characterisation

Molecular characterisation was performed using a commercial multiplex PCR kit with reverse-hybridization caption and identification (using a comercial kit) .This kit includes primers specific for the amplification of genes conferring resistance to β-lactam antibiotics (blaSHV and extended-spectrum blaCTX-M) and fifteen carbapenem resistance genes (KPC, SME, NMC/IMI, GES, VIM, GIM, SPM, NDM, SIM, IMP, OXA23-iike, OXA24-like, OXA48-like, OXA51-like, AND OXA58-like). It also seeks out resistance genes pertinent to Staphylococcus aureus, a gene associated with resistance to methicillin (mecA) and two genes conferring resistance to vancomycin (vanA and vanB), which were non relevant to the current study [6].

Results

Bacterial isolation and identification

Within the study timespan, 50 Gram negative bacilli were isolated and evaluated. Classic laboratory diagnosis has identified the following genre: Escherichia, Klebsiella, Pseudomonas and Acinetobacter. Non-fermenting bacilli were firstly characterised as oxidase positive or negative.

MALDI-TOF-MS identification shows a larger spectrum of bacterial genera than what could be identified by classic techniques (Figure 1). Thus, we have a greater diversity underpinning Enterobacter, Citrobacter, Morganella, Providencia and Serratia species.

Carbapenemase activity and identification

After eliminating chromosomal carbapenemase producers (Stenotrophomonas spp) and Myroides spp., 44 strains were analysed. Nine isolates were identified as carbapenemase producers: 7 Klebsiella pneumoniae strains, 1 Enterobacter cloacae and 1 Acinetobacter baumannii strain. Six control strains were chosen in order to evaluate the presence of carbapenemases which would be negative in the phenotypic carbapenem inactivation test: 3 Escherichia coli, 1 Enterobacter hormachei, 1 Enterobacter cloacae and 1 Pseudomonas aeruginosa strain (Figure 2).

The molecular diagnosis showed the presence of blaNDM in 5 isolates, blaOXA-48-like in 2 isolates and blaOXA-24 in 1 isolate. Thus, 4 Klebsiella pneumoniae

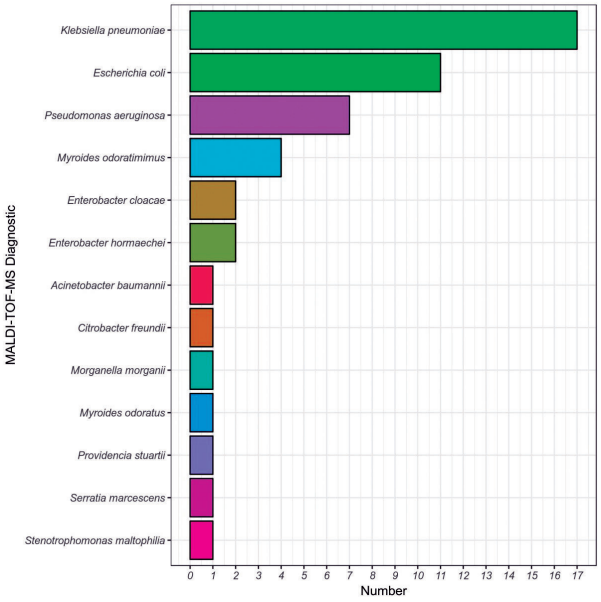


Figure 1.
MALDI-TOF-MS identification of clinical isolates

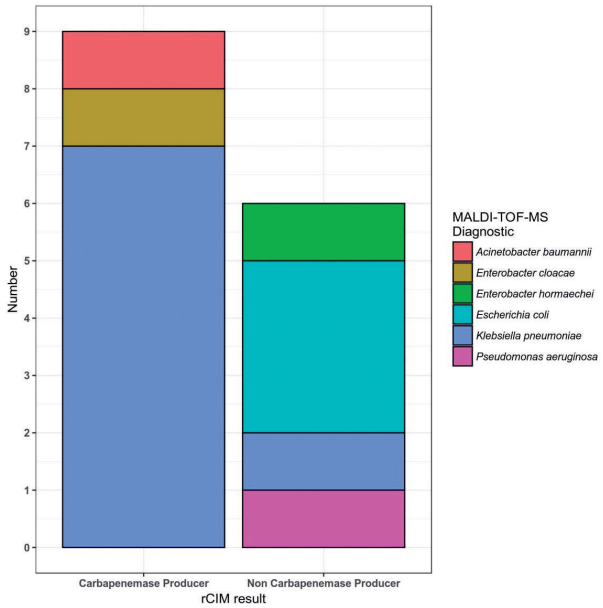


Figure 2. Strains belonging to each genera that were included in the molecular analysis

as well as the Enterobacter cloacae strain were positive for blaNDM, 2 K. pneumoniae strains were blaOXA-48-like positive. The Acinetobacter baumannii strain was positive for blaOXA-24 and blaOXA-51, the latter being a naturally occurring carbapenemase. One hyper-mucoid Klebsiella pneumoniae strain did not yield a readable result

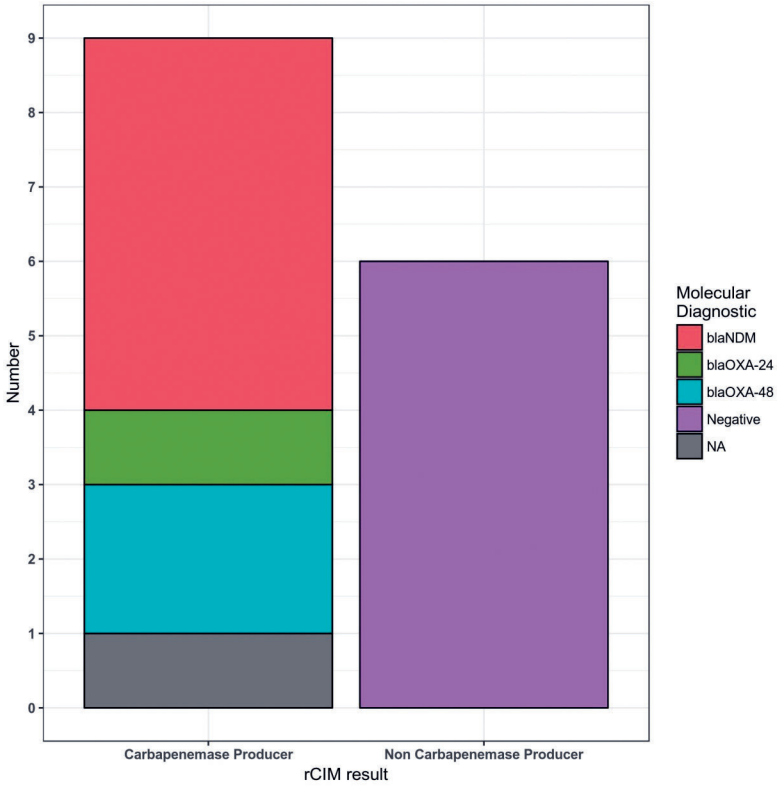


Figure 3. Carbapenemase genes identified in rCIM positive strains and accompanying negative controls. One hyper-mucoid Klebsiella pneumoniae strain did not yield a readable result.

Discussion

This pilot study investigates the resistance to carbapenems identified in clinical strains isolated from a tertiary urology clinic in Bucharest, Romania. While a small number of strains were evaluated, it shows the presence of blaNDM in a number of clinical isolates from both Klebsiella and Enterobacter genera. This may be due to clonal spread in the community or local transmission. Klebsiella pneumoniae isolates also harboured blaOXA48-like which is the dominant carbapenemase found in recent epidemiologic studies in Romania.

Of note, we identified blaOXA24 in an Acinetobacter baumannii isolate.

The molecular profile underpinning carbapenem resistance is becoming more important in both enterobacteria and non-fermenters, due to the targeted activity of new antibiotics or new combinations of antibiotics and beta lactamase inhibitors. The spectrum of activity is shown in Table 1 (adapted from [7]).

The study carries a number of limitations. The most important are the small number of isolates tested and the

Table 1. Activity of novel antibiotics or combination antibiotic - beta lactamase inhibitors against carbapenemases in Enterobacteria and non-fermenters. Adapted from [7]

Name of antibiotic	Enterobacteria				Pseudomonas spp.	Acinetobacter spp.
	KPC	MBL	AmpC	O X A - 4 8 like		
Ceftazidim-avibactam	+	-	+	+/-	-	-
Ceftolozan-tazobactam	-	-	-	-	+	-
Imipenem-relebactam	++	-	++	-	+	-
Meropenem-varbobactam	++	-	++	-	+/-	-
Cefepim-zidebactam	++	++	++	++	++	-
Aztreonam-avibactam	++	++	++	+	+	-
Cefiderocol	++	++	++	++	++	+

absence of a result for one of the isolates. Taking into account that this is a pilot study which is aimed at generating preliminary data, it needs to be followed up with a much larger study which should include a larger number of isolates and different methodologies to evaluate the strains.

The study also has a number of strengths: the use of a broad spectrum multiplex PCR, targeting both very widespread carbapenemases (the big five: blaKCP, blaNDM, blaVIM, blaIMP and blaOXA-48) as well as less encountered carbapenemases (minor carbapenemases, like blaGES, blaGIM, etc.) [8]. This is also a proof of concept that the rCIM is a feasible method that may be used in a medium-low resource setting, to complement the study of resistance mechanisms.

Conclusion

This pilot study shows the landscape of carbapenemase production in clinical isolates obtained from a tertiary care center. The results need to be confirmed by a larger study, including more samples, over a wider period of time and different methodology to investigate the resistance.

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