Detection of OXA Carbapenemase Positive *Acinetobacter* spp., Jamaica

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ABSTRACT

Objective: The global problem of resistance to carbapenem antibiotics, through the production of carbapenemases by clinically significant bacteria, continues to increase while options for antibiotic therapy remain limited. It is important to determine the mechanism of carbapenem resistance in the multidrug resistant (MDR) *Acinetobacter spp.* isolated at a tertiary care hospital, Jamaica (TCHJ) because of the implications for therapy which is the goal of this study.

Methodology: 82 MDR *Acinetobacter* spp collected at a TCHJ were identified as potential carbapenemase producers during routine susceptibility testing for a one year period from May 2009 to April 2010. These isolates were subjected to phenotypic and genotypic tests for carbapenemase detection using the modified Hodge test and PCR respectively. Multiplex PCR for OXA-23, -24, -51, -58 was performed and PFGE was used to determine if carbapenemase-positive isolates were related.

Results: PCR for OXA-carbapenemases found 13 bla_{OXA-24}, two blaOXA-23 and one blaOXA-58, establishing a prevalence of 19.5%. PFGE results showed that *Acinetobacter* spp sharing phenotypic and genotypic similarites were clonally related.

Conclusions: Carbapenemase production was found to be a cause of antibiotic resistance in MDR *Acinetobacter* spp. isolates at a TCHJ. These isolates were thought to have been derived from an outbreak or endemic strain whose presence is likely to significantly impact patient management and antibiotic policy.

Keywords: *Acinetobacter* spp, carbapenem, OXA

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INTRODUCTION

The challenge faced by clinicians to find suitable therapy for multidrug resistant (MDR) pathogens causing infections is a global one (1). At a tertiary care hospital, Jamaica (TCHJ) where this study was done, Gram-negative bacilli have been implicated in 72.5 % of infections in patients admitted to the intensive care unit (2). While *Acinetobacter* spp. isolated in the unit between 2002 and 2004 showed resistance to multiple antibiotics including the carbapenems (2), the mechanisms of resistance, crucial to antibiotic choice and appropriate infection control management were not elucidated.

Acinetobacter spp. are opportunistic pathogens that colonize human skin and dry surfaces (3). These characteristics and their ability to produce carbapenemases, allow them to resist agents such as antibiotics allowing them to establish themselves as important nosocomial pathogens (3). There have been reports in the literature of carbapenem antibiotics being used to manage *Acinetobacter* spp. infections. (2). However in recent times, as resistance to this class of antibiotic increases through the dissemination of carbapenem-hydrolysing enzymes, carbapenemases, their usefulness for the treatment of infections is increasingly compromised (3, 5). The carbapenemase genes are often transferred on plasmids that carry many other resistance genes, thereby rendering the organism resistant to multiple classes of antibiotics.

The first report of a carbapenem resistant *Acinetobacter* spp. infection was from a patient receiving care at the Royal Infirmary of Edinburgh (4). Since then carbapenem resistance in *Acinetobacter* spp. has emerged in various parts of the world, mostly as a result of the dissemination of class D OXA-type carbapenemases (4). The extent to which carbapenem resistance due to OXA carbapenemases is detected among the *Acinetobacter* spp. varies globally. Of 221 *Acinetobacter* isolates (resistant to imipenem) from 11 Chinese teaching hospitals collected between 1999 and 2005, 97.7% carried bla_{oxa23} -like genes (6). OXA-23-like

carbapenemases have been detected repeatedly within *Acinetobacter* spp. in the UK, East Asia, and South America (7). This enzyme persisted in a MDR epidemic strain that has become prevalent in UK and is known as the OXA-23 clone 1. Reports of the dissemination of OXA-24-type beta-lactamases are generally limited to Europe and the United States (7).

The production of carbapenemases is one of the main mechanisms that enable *Acinetobacter* spp. to become resistant to the more active carbapenems such as imipenem and meropenem (8). The chromosomally located OXA-51 beta-lactamase gene in *A. baumannii* may be mutated to up-regulate or hyper-produce enzyme that may result in reduced susceptibility to some carbapenems. Genes coding for related enzymes such as OXA-23 are encoded on plasmids that are readily transferable within species (9), and in response to increasing carbapenem use, the OXA-carbapenemases are now seen to be emerging as an important mechanism of carbapenem resistance among *Acinetobacter* spp. (4). Currently, the four main phylogenetic subgroups of OXA carbapenemases that are commonly found in *Acinetobacter* spp. include OXA-23,-24,-51,-58-like enzymes (9). But OXA-type enzymes are an ever expanding group and it is likely that more will be discovered as demonstrated by the fact that between 2000 and 2006, a further six novel enzymes with carbapenemase properties including OXA-143 were described in *Acinetobacter* spp. (10).

The aim of this study was to determine if any OXA-carbapenemase genes could be detected among clinical isolates of MDR *Acinetobacter* spp. identified from patients at a TCHJ and to determine the relatedness of the MDR isolates.

METHODOLOGY

After obtaining ethical approval and while operating within the context in which this approval was granted, a total of 82 consecutive, de-duplicated MDR *Acinetobacter* spp. isolated from clinical specimens at a TCHJ between May 2009 and April 2010, were collected for the purposes of this study. The majority of these isolates were obtained from the intensive care unit (34%) and the surgical wards (32%). Laboratory tests for manual identification and susceptibility testing were performed according to CLSI (2009), and isolates were deemed to be MDR if resistant to one or more agents in three or more antimicrobial classes (11).

Conventional multiplex PCR was performed on all isolates to detect OXA-23, -24, -51, and -58 carbapenemase genes using primers published by Woodford *et al* (5). Pulse field gel electrophoresis (PFGE) using *Asc*I DNA restriction was performed on the *Acinetobacter* isolates which shared phenotypic and or genotypic similarities.

RESULTS

The *Acinetobacter* spp. isolates were resistant to multiple antibiotics including ampicillin cotrimoxazole, gentamicin, ceftazidime, and ceftriaxone. The table below illustrates that most isolates were only susceptible to minocycline and polymixin B.

Key: minocycline-MH; polymixin B-PB; meropenem-MEM

Table: Susceptibility pattern of the Acinetobacter isolates

Susceptibility Pattern	Percentage of isolates n=82	OXA carbapenemase gene patterns detected		PFGE type				
				1	2	3	4	Unknown
Susceptible to MH and PB ONLY	39%	OXA 51 only	22/32	-	22	-	-	-
		OXA 51 and 24	9/32	9	-	-	-	-
		OXA 51 and 23	1/32	-	-	1	-	-
Susceptible to MH, PB and MEM only	24.4%	OXA 51 only	19/20			1	4	14
		OXA 51 and 24	1/20	1	-	-	-	-
Pandrug resistant	1.2	OXA 51 only	1/1	-	-	1	-	-
Other	35.4	OXA 51 only	26/29	2	0	9	3	12
		OXA 51 and 24	2/29	-	-	2	-	-
		OXA 51 and 58	1/29	-	-	1	-	-

Table showing the percentage of *Acinetobacter* spp. isolates resistant to the panel of antibiotics tested. 54% were resistant to meropenem. Eighty one isolates were positive for bla$_{OXA-51}$, regardless of whether the isolate was susceptible or resistant to meropenem. In addition 12of these isolates also had bla$_{OXA-24}$, one had bla$_{OXA-23}$, and one had bla$_{OXA-58}$. Of these isolates but two (bla$_{OXA-24}$ and bla$_{OXA-58}$) were resistant to meropenem. One isolate had a bla$_{OXA-23}$ alone. The dendograms shown in the figure illustrate that there are at least three distinct clonally related groups of the *Acinetobacter* isolates present in the study population.
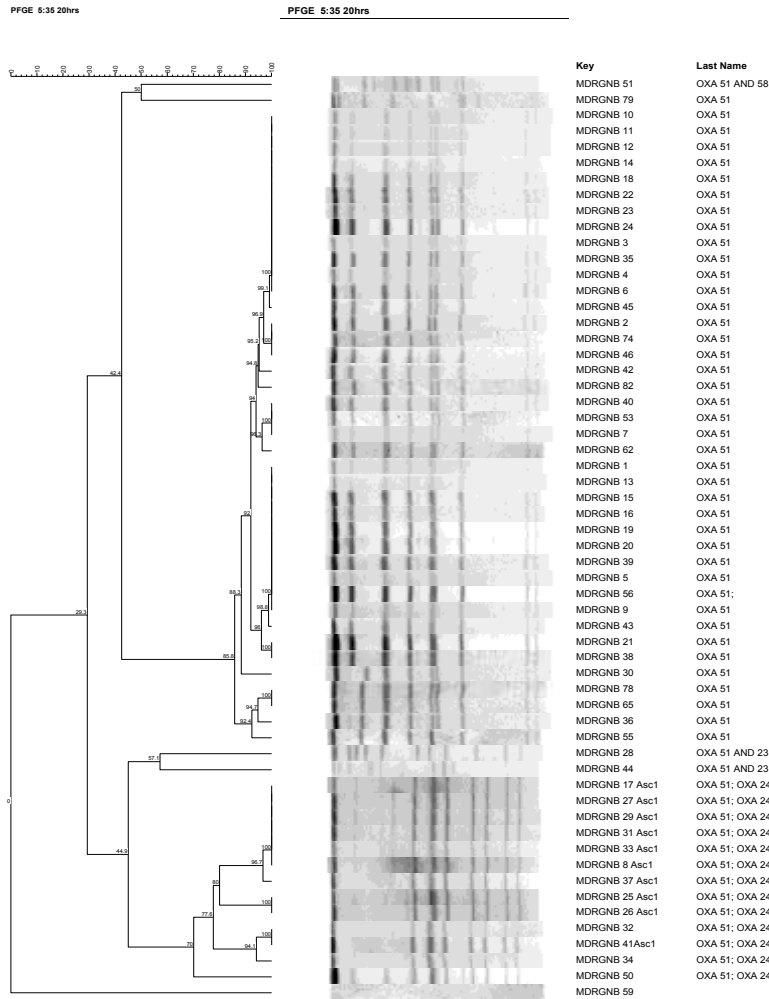
Figure: Acinetobacter PFGE results

PFGE of *Acinetobacter baumannii* identified two epidemic lineages, and some sporadic isolates at TCHJ. The most prevalent involved 40 isolates that shared >85% similarity and were positive for only OXA-51, a gene common to all *A. baumannii*; this PCR profile was shared by only one other isolate unrelated by PFGE. The second epidemic cluster comprised 13 isolates, all of which tested positive for the OXA-24 carbapenemase gene in addition to OXA-51. The two *A. baumannii* positive for the OXA-23 carbapenemase and the single isolate positive for the OXA-

58 carbapenemase, were unrelated to each other and to the two epidemic clusters. While the latter appear to be new introductions, the major clusters comprised isolates derived from multiple years, which indicates that they formed parts of ongoing outbreaks or it may be that these strains have already become endemic in the absence of adequate infection control.

DISCUSSION

Multiplex PCR identified OXA carbapenemase genes as a common mechanism of carbapenem resistance among isolates of *Acinetobacter* spp. at a TCHJ. Almost all (98.7%) of these isolates had the bla$_{OXA51}$ gene typically found in *Acinetobacter baumannii* species (5). The gene may be unique to this species and could be used as a means of rapidly identifying them (5). The presence of bla$_{OXA51}$ does not automatically mean its phenotypic expression as it has to be regulated by insertion sequences such as ISA*ba*1 (5). This was not evaluated and is one of the limitations of this study this study. A further limitation is that all the possible mechanisms for carbapenem was not explored.   Although the impact of the regulation and over expression of the bla$_{OXA51}$ gene has not been fully determined, recent data suggests that it is involved in carbapenem resistance (10). The high prevalence of carbapenem resistance seen among these *Acinetobacter* spp. could therefore be related to the presence and expression of blaOXA51 however further studies are needed to confirm this finding.

Reduced susceptibility or total resistance to carbapenems is usually seen when alleles encoding OXA-23 like, OXA-24 like and OXA-58 like genes are present in *Acinetobacter* spp. This was true for the 5 isolates that had these genes present.

PFGE revealed at least three groups of *Acinetobacter* spp. that were highly related which suggests that they either were derived from an outbreak or were endemic strains (12).

Longitudinal epidemiological studies are needed to determine this. Among the clonally related isolates, 38% *were* from the intensive care unit and 28% came from surgical wards. The rest were evenly distributed throughout the rest of the hospital.

CONCLUSION

The increased resistance to carbapenem antibiotics remains a cause for concern as this class of antibiotics remains one of the few therapeutic options for the treatment of infections caused by MDR resistant organisms (1). As resistance to these antibiotics increases, therapeutic options become more limited. Isolates that become endemic in a healthcare setting pose a risk of infection patients who receive care at that institution resulting in increased morbidity and mortality (4). An understanding of the prevalence and mechanism of carbapenem resistance in a local setting is important to drive antibiotic policies to limit the emergence of further resistance while ensuring adequate therapy (1). As many of the genes coding for some of the OXA carbapenemases are present on mobile genetic elements, infection control will play a key role in limiting the spread of some of these resistance genes. This is particularly important in the context of decreased antimicrobial therapeutic options (1, 5).

AUTHORS' NOTE

There are no financial or personal relationships with other persons or organizations that would inappropriately influence this study.

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