Persistence of ISAbal- blaOXA-23 Acinetobacter baumannii in Intensive Care Unit, University Hospital

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Abstract

Acinetobacter baumannii is an important nosocomial pathogen which is intrinsically resistant to the major antimicrobial agents available and poses high risk particularly in immunocompromised patients. Carbapenem remains as the effective antimicrobial agent for the treatment of A. baumannii infections. However, carbapenem-resistant A. baumannii is increasingly reported worldwide. In 2006-2009, a total of 175 carbapenem-resistant A. baumannii strains were isolated from patients, hands of healthcare workers and environment in the intensive care unit, University Malaya Medical Centre. Therefore, this study was performed to determine the carbapenem resistance genes and the genetic relatedness among the strains. Imipenem-EDTA double-disk synergy and combined disk tests showed none of the strains were metallo-β-lactamase producers. blaOXA-51 was present in all the strains while blaOXA-23 was found in 174 strains (99%). Insertion element, ISAbal was found upstream of the blaOXA-23 gene. Southern hybridisation revealed that blaOXA-23 gene was chromosomal and/or plasmid-mediated. To our knowledge, this is the first report of the plasmid- and chromosomal-mediated OXA-23-producing carbapenem-resistant A. baumannii in Malaysia. REP-PCR analyses showed close genetic relatedness among the strains isolated from patients, environment and hands of healthcare worker. OXA-23-producing A. baumannii has persisted in the ICU environment throughout the 2006-2008 and new genotype strains were observed in 2009. Persistence and spread of the plasmid- and integron-harboured A. baumannii in the environment could prolong problem in the hospital infections control.

Introduction

Acinetobacter baumannii is an important opportunistic nosocomial pathogen and constitutes a major problem in hospitals worldwide. A. baumannii is intrinsically multidrug-resistant and has limited treatment options particularly in immunocompromised patients. Increasing of carbapenem-resistant A. baumannii strains is worrying as carbapenem remains the treatment options for A. baumannii infections.

Among the β-lactamase classes, class B (metallo-β-lactamas, MBLs) and class D (oxacillinases, OXAs) enzymes play a major role in conferring resistance to carbapenem in A. baumannii (Poirel et al., 2006). OXA enzymes are more prevalent in carbapenem-resistant A. baumannii compared to MBL enzymes (IMP, VIM, SIM, GIM and SPM) (Poirel et al., 2006). The OXA enzymes in A. baumannii are categorised into four groups, OXA-23, OXA-24, OXA-51 and OXA-58 enzymes. The OXA-23, OXA-24 and OXA-58 can either be plasmid- or chromosomal-mediated while OXA-51 is chromosomally intrinsic in A. baumannii (Peleg et al., 2008). Plasmid-encoded oxacillinase OXA-23, OXA-24 and OXA-58 have been reported in A. baumannii in some European countries (Poirel et al., 2006; Peleg et al., 2008). Presence of the insertion sequences, ISAbal upstream of the oxacillinase genes could enhance expression of the genes and resulted in carbapenem resistance has been described in A. baumannii (Segal et al., 2005).

Pulsed-field gel electrophoresis (PFGE) is known as the gold standard for molecular subtyping of bacterial nosocomial pathogens. However, repetitive sequence-based PCR (REP-PCR) is simpler, faster and can be applied in estimating epidemiological relatedness of A. baumannii strains in a defined setting (Snelling et al., 1996; van Belkum et al., 2007).

Aims

The aims of this study were to determine the carbapenem resistance genes and genetic relatedness between the A. baumannii strains isolated from patients, hands of healthcare worker (HCW) and environment in the Intensive Care Unit (ICU), University Malaya Medical Center (UMMC) over a 4-year period (2006 to 2009). The data obtained will be helpful in understanding the spread and persistence of A. baumannii, leading to a better control of infections in the ICU, UMMC.

Materials and methods

A total of 175 carbapenem-resistant A. baumannii strains (clinical, n=167; environment, n=7; hands of healthcare worker, n=1) collected from ICU, UMMC in 2006-2009 were studied. MBLs phenotype of the strains was screened by imipenem-EDTA double-disk synergy test (Yong et al., 2002) and combined disk test methods (Lee et al., 2003). Two different multiplex PCR assays were carried out to detect the MBL genes (blaGIM, blaGPM, blaGSM, blaIMP andblaVIM) (Ellington et al., 2007) and OXA genes (blaOXA-23, blaOXA-24, blaOXA-51 and blaOXA-58) (Woodford et al.,
The presence of the insertion element ISAbal was screened by PCR (Segal et al., 2005). PCR mapping was carried out to determine the insertion element ISAbal upstream of blaOXA-23 and blaOXA-51 (Turton et al., 2006a). Plasmid DNA of the strains was extracted using alkaline lysis method according to Birnboim and Doly, 1979. The sizes of the plasmid were determined with Escherichia coli 39R and V517 as plasmid size markers. To localise the blaOXA-23 gene on chromosomal and/or plasmid DNA, Southern hybridisation studies were performed using the plasmid DNA, S1 nuclease with PFGE and 1-Ceu I restricted chromosomal DNA according to the manufacturer’s guidelines (Roche Diagnostics, Mannheim, Germany). The genetic relatedness of the strains was studied by REP-PCR fingerprinting (Snelling et al., 1996). A dendrogram based on REP-PCR fingerprints was generated using the algorithms unweighted pair group arithmetic means methods (UPGMA) and Dice’s coefficient using BioNumeric Version 6.0 (Applied Maths, Belgium) software.

Results and Discussion

None of the carbapenem-resistant A. baumannii was MBL-producers. All the strains harboured the blaOXA-51 gene. blaOXA-51 gene is intrinsic to A. baumannii and presence of this gene in all the strains has confirmed the utility of this gene as a reliable marker for A. baumannii identification (Turton et al., 2006b). The blaOXA-23 gene was detected in 174 (99%) strains with insertion element ISAbal present upstream. This findings indicate that carbapenem resistance particularly imipenem in the strains was probably due to overexpression of the blaOXA-23 induced by the promoter sequences located upstream ISAbal. Hybridisation studies revealed blaOXA-23 gene were hybridised on chromosomal and/or plasmid DNA of the strains. This is the first report of the plasmid- and chromosomal-mediated OXA-23-producing carbapenem-resistant A. baumannii in Malaysia. Presence of the blaOXA-23 gene on plasmid indicated there is a possible of gene transmission between the strains in the hospital environment. REP-PCR analysis of 175 carbapenem-resistant A. baumannii strains generated 53 distinct reproducible REP types (F_D= 0.70-1.00) with discriminatory index D=0.96. Twenty-one genotypes (A - U) were designated based on 90% similarity cut-off value. Genotype C was predominant (n= 50), followed by genotype R (n= 28), Q (n= 22) and O (n= 15). The environmental strains shared similar genotype with the clinical strains, 3 strains were subtyped into genotype C and four strains into genotype O. Strain isolated from the hands of a HCW were also subtyped into genotype C. The genotype C strains have persisted in the ICU ward throughout 2006 - 2009 and new genotype R strains were determined in 2009.

Conclusion

Carbapenem resistance in the A. baumannii strains were mediated by ISAbal-blaOXA-23 gene with its diverse locations on the plasmid and chromosome. Persistence genotypes C and newly emerged genotype R strains were identified in the ICU. There is a need for more effective antimicrobials and infection control measures to control the dissemination of carbapenem-resistant A. baumannii strains within the hospital.

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References


