Rapid detection of gene encoding oxa carbapenemases in Acinetobacter using multiplex PCR

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INTRODUCTION

Acinetobacter baumannii, an emerging pathogen of healthcare centers, shows intrinsic as well as acquired drug-resistance mechanisms. Acinetobacter spp are gram negative non-fermentative bacteria. Clinical manifestations of Acinetobacter infections includes hospital acquired pneumonia, blood stream infections, urinary tract infection, meningitis and wound infection. Because of frequent resistance to the aminoglycosides and third generation cephalosporin, carbapenem are widely used for managing Acinetobacter infections. The emergence of carbapenem resistance in Acinetobacter spp is a significant public health concern because of limited option of antibiotic treatment. Carbapenamases found in Acinetobacter may belong to class B (Metallo enzymes) or class D (OXA enzymes). The OXA carbapenamases of Acinetobacter is divided into four phylogenetic subgroups namely blaOXA-23 like, blaOXA-24 like, blaOXA-51 and blaOXA-58. A study done in India has reported the emergence of OXA carbapenemases in different enterobacterial species and also in Acinetobacter.

Thus, the aim of the present study was to determine antibiotic susceptibility profile, antibiotic resistance genes and genetic mechanism of carbapenem resistance of A.baumannii in clinical isolates at a tertiary care hospital in...
South India. Also the study was carried out to find out if there can be a dissemination of carbapenem resistant bla\textsubscript{OXA-23} and bla\textsubscript{OXA-58} genes in Acinetobacter isolates, enabling us to limit the spread of such strains in hospital settings as well as in the community, and also help in initiating specific hospital infection control measures.

**MATERIALS AND METHODS**

The Strains of Acinetobacter were isolated from inpatients coming to the tertiary care hospital in South India, were collected from different samples i.e., sputum, tracheal aspirate, wound swab, blood, urine etc. All clinical isolates, identified to be non lactose fermenting, glucose non-acidifier, Gram negative bacilli, catalase positive, oxidase negative and citrate positive were taken up for the study.

**DETECTION OF IMIPENEM RESISTANT**

Antimicrobial susceptibility testing was done following Kirby Bauer disk diffusion method using routine drugs including imipenem as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Modified Hodge test and Imipenem EDTA disk synergy test was used to detect carbapenemase production from isolates of Acinetobacter spp and further tested by Minimum inhibitory concentration (MIC) by agar dilution method. The antimicrobial concentration ranges tested were from 0.03 to 128 g/ml for imipenem.

**DETECTION OF GENES BY PCR**

DNA extraction was done using multiplex PCR assay on imipenem resistance strains of Acinetobacter, by both disk diffusion and agar dilution method to detect bla\textsubscript{OXA-23} and bla\textsubscript{OXA-58} carbapenemases encoding genes as shown below

\[
\text{OXA 23-(453bp)-F-AGTATTGGGGCTTGTGCT} \\
\text{R-AACTTCCGTGCCTATTTG}
\]

\[
\text{OXA 58-(233bp)-F-ATGCAAAGTGAATTGCAACG} \\
\text{R-CCCCAGCCACTTTTAGCATA}
\]

Amplifications conditions followed in the methodology were, initial denaturation at 94\(^\circ\) C for 3 mins, 30 cycles of 94\(^\circ\) C for 1 min, 55\(^\circ\) C for 1 min,72\(^\circ\) C for 1 min and final extension at 72\(^\circ\) C for 5 mins.

**RESULTS**

175 strains of Acinetobacter were isolated from different clinical samples. Among the 175 strains, 61 were found to be resistant to imipenem EDTA disk synergy test. Of these 61 strains, 45 showed resistance to imipenem by MIC agar dilution method (Figure 1). Subjecting these 45 strains of Acinetobacter to Multiplex PCR, showed that all the 45 (100\%) strains were positive for bla\textsubscript{OXA-23} gene among which 19 (42.2\%) of them were also positive for bla\textsubscript{OXA-58} gene (Figure 2).

**DISCUSSION**

The high antimicrobial resistance of Acinetobacter spp emerged as a nosocomial pathogen worldwide. The need for strategic measures to deal with this challenge is to find a solution to minimize antimicrobial misuse within both clinical and non-clinical settings has been stressed by many medical professionals. In 1993,
acquired OXA carbapenemases was reported for the first time and subsequently after that emergence and spread of OXA enzymes have been reported worldwide.9

The bla_{OXA-23} gene is one of the most prevalent carbapenemases encoding genes reported worldwide, which can be located on chromosomes of *Acinetobacter* plasmids.10 Similarly in the present study all the strains were found to be positive for bla_{OXA-23}. Many reports have indicated that in United Kingdom, that bla_{OXA-23} and bla_{OXA-58} are most frequently detected in *Acinetobacter*. And as reported by earlier studies, bla_{OXA-58} may be present along with bla_{OXA-23} which is responsible for reduced susceptibility to carbapenem group of drugs, the finding is very much similar to our study.

NDM-1 metallo-β-lactamase was detected among enterobacteriaceae and also in *Acinetobacter baumannii* especially in India and Pakistan. A study conducted in India showed the co-existence of bla_{OXA-23} and NDM-1 in clinical isolates of *Acinetobacter baumannii*.11 In our study we used a cost effective multiplex PCR technique and observed only the emergence of bla_{OXA-23} and bla_{OXA-58} in imipenem resistant *Acinetobacter* isolates.

With increase in drug resistance in *Acinetobacter*, resistance surveillance has become increasingly important. Hence both the phenotypic and genotypic methods are important to detect the carbapenem resistance in *Acinetobacter*. Thus the cost effective multiplex PCR technique may be very helpful to detect carbapenem resistant genes since we get the results within a short duration.12 Technique like multiplex PCR would also help us to monitor the emergence and spread of carbapenem resistant *Acinetobacter* spp.13 OXA-type carbapenemases which will further limit chemotherapeutic options that threatens the public health.14

**CONCLUSION**

The study successfully demonstrates the utility of cost effective multiplex PCR assay as a useful technique in the detection of bla_{OXA-23} and bla_{OXA-58} harbouring clinical isolates of *Acinetobacter*. Because of the difficulty in treating patients infected with OXA carbapenemases genes harbouring bacterial pathogens, it is necessary to identify such strains as soon as possible. Moreover, studying the epidemiology of such resistant strains helps us to limit the spread of such strains in hospital settings as well as in the community, and also helps in initiating specific hospital infection control measures.

**CONFLICTS OF INTEREST**

None

**References**