Original Article

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

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ABSTRACT

Background and Objective: *Acinetobacter*, has been identified as an important pathogen in nosocomial outbreaks with high levels of emerging drug resistance. The present study was conducted in *Acinetobacter spp* to find out the utility of the multiplex PCR assay, which may be used as a useful technique in the early detection & prevention of bla_{OXA-23} and bla_{OXA-58} gene harbouring in clinical isolates taken from the patients coming to a tertiary care hospital.

Material and Methods: Strains of *Acinetobacter* collected from different clinical samples were subjected to antimicrobial susceptibility testing. Strains which were found showing resistance to imipenem by both disk diffusion and minimum inhibitory concentration (MIC), were analysed for the presence bla_{OXA-23} and bla_{OXA-58} (CLASS D) by using multiplex PCR.

Results: Among 175 strains of *Acinetobacter* collected from the clinical samples, 45 strains showed imipenem resistance, both by disk diffusion and MIC out of which 19(42.2%) were positive for bla_{OXA-58} gene and all strains 45(100%) were positive for bla_{OXA-23} gene.

Conclusion: The present study shows that there is dissemination of genes produced carbapenem resistant in the *Acinetobacter* isolates. This scientific evidence can be used to limit the spread of such strains in hospital settings as well as in the community, and also may help in initiating specific hospital infection control measures.

Keywords:
OXA carbapenemases, Imipenem, *Acinetobacter,* PCR

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INTRODUCTION

Acinetobacter baumannii, an emerging pathogen of healthcare centers, shows intrinsic as well as acquired drug-resistance mechanisms.[1] *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.[2] Because of frequent resistance to the aminoglycosides and third generation cephalosporin, carbapenem are widely used for managing *Acinetobacter* infections.[3] The emergence of carbapenem resistance in *Acinetobacter* spp is a significant public health concern because of limited option of antibiotic treatment.[4] Carbapenamases found in *Acinetobacter* may belong to class B (Metallo enzymes) or class D (OXA enzymes).[5] The OXA carbapenamases of *Acinetobacter* is divided into four phylogenetic subgroups namely bla_{OXA-23} like, bla_{OXA-24} like, bla_{OXA-51} and bla_{OXA-58}. A study done in India has reported the emergence of OXA carbapenemases in different enterobacterial species and also in *Acinetobacter*.[6]

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

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South India. Also the study was carried out to find out if there can be a dissemination of carbapenem resistant bla_{OXA-23} and bla_{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 in-patients 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_{OXA-23} and bla_{OXA-58} carbapenemases encoding genes as shown below[7]

OXA 23-(453bp)-F-AGTATTGGGGCTTGTGCT
R-AACTTCCGTGCCTATTTG

OXA 58-(233bp)-F-ATGCAAAGTGAATTGCAACG
R-CCCCAGCCACTTTTAGCATA

Amplifications conditions followed in the methodology were, initial denaturation at 94^0 C for 3 mins, 30 cycles of 94^0 C for 1 min, 55^0C for 1 min,72^0C for 1 min and final extension at 72^0 C for 5 mins.

RESULTS

175 strains of *Acinetobacter w*ere 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_{OXA-23} gene among which 19 (42.2%) of them were also positive for bla_{OXA-58} gene (Figure 2).

Figure 1: Minimum Inhibitory Concentration (MIC) method for Imipenem

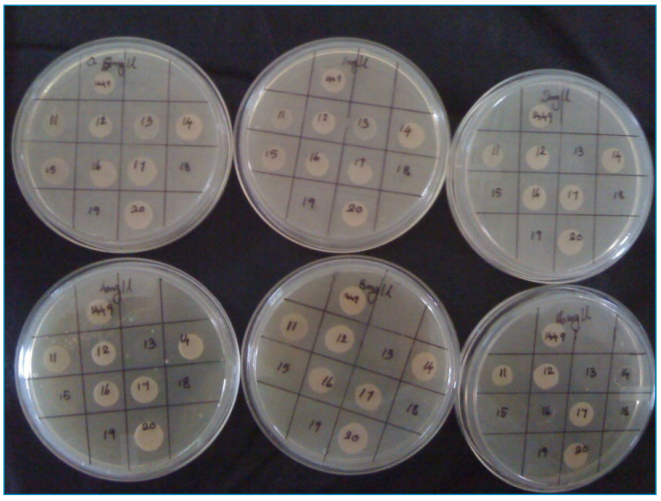
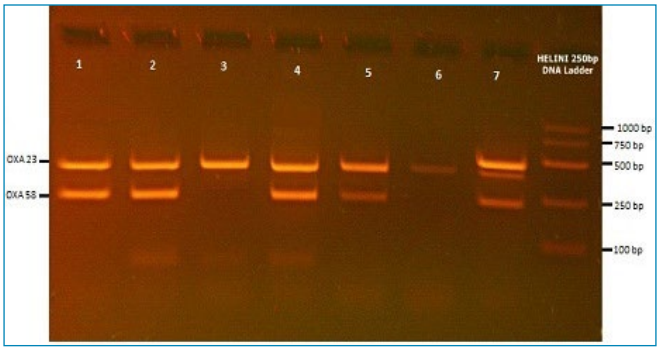


Figure 2: Detection of bla_{OXA-23} and bla_{OXA-58} gene by molecular methods



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.[8] 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 enterobacteriacae 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 *Acinetobacte*r. Thus the cost effective multiplex PCR technique may be very helpful to detect carbapenemase 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

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