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MOLECULAR CHARACTERIZATION AND IDENTIFICATION OF CLINICAL ISOLATES OF CARBAPENEM RESISTANT *A. BAUMANNII*

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ABSTRACT *Acinetobacterspp* is an opportunistic pathogen causing nosocomial outbreaks and its increasing antibiotic resistance makes treatment difficult. Hence, a preliminary study was conducted on the prevalence of carbapenem resistant *Acinetobacter spp* infections at Sunrise Institute of Medical Sciences, a tertiary care hospital in Kochi, kerala. Various clinical specimens like blood, urine, abscess, vaginal swab were analyzed and the isolates were identified by 16s rDNA sequencing. 15% of the isolates was confirmed and identified as to be resistant to carbapenems.

KEYWORDS : *Acinetobacter baumanii*, Antibiotic resistance, Carbapenems, Nosocomial infections, clinical specimens, 16S rDNA sequencing, Phylogenetic tree

INTRODUCTION

Acinetobacter spp is an emerging opportunistic nosocomial Gram negative bacterial pathogen with increasing prevalence in particular the species *Acinetobacter baumannii.* Against the font that the species can survive on moist and dry surfaces, would be present in foodstuffs and on healthy skin, along with both intrinsic and acquired antibiotic resistance of *A. baumannii* account for a significant cause of outbreaks. Significant levels of morbidity and mortality have been reported with outbreaks (Tak-Chiu, 2011) and common infections include ventilator associated pneumonia and bacteremia; less frequently burn wounds and urinary tract (Bergogne, et al., 1996). *A.baumannii* is also a common cause of bloodstream infections in the intensive care setting (Wisplinghoff *et al*., 2004) and the lower respiratory tract infections and intravascular devices (Seifert *et al*., 1995; Cisneros *et al*., 1996; Jang *et al*., 2009; Jung *et al*., 2010) are reported to be the common sources. In addition wound infections and urinary tract infections have also been reported as foci of infection (Seifert *et al*., 1995). The risk factors of the infection with multidrug resistant *Acinetobacter spp* include prolonged hospital stay, exposure to an intensive care unit, receipt of mechanical ventilation, colonization pressure, exposure to antimicrobial agents, etc. (Fournier et al., 2006.). In recent years, a substantial increase in *A.baumanii* associated nosocomial pneumonia cases (Stahl et al.,2015) are reported. (Peleg *et al.,* 2008) reported that *A. baumannii* ranks with 10th among the organisms causing monomicrobial blood stream infections.

It becomes a need of novel therapeutic options owing to the emergence of isolates resistant to drugs choice like carbapenems. In recent studies done by Chang et al., 2015 also revealed high prevalence of CRAB, upto 60% of total isolates. Other researchers were also found high prevalence rate of CRAB in nosocomial infections (Hassen et al., 2014 & Khan Nhu et al., 2014).

Carbapenems are among the drug of choice for the treatment of nosocomial but resistance to this class is emerging, leading to the evolution of pan resistance strains and to the need of new therapeutic options (Quale, et al., 2003 & Van Looveren, et al., 2004). Carbapenem resistant *Acinetobacter* are becoming widespread in several regions of the world (Coelho *et al.* 2006). Mechanistically, resistance to these potential beta lactams may be due to impaired permeability resulting from altered outer membrane proteins or to alterations in the pencillin binding proteins (PBP) (Bou, et al., 2000). However, the carbapenem hydrolyzing beta lactamases that includes MBLs and oxacillinases are recognized as important contributors of carbapenem resistance in *Acinetobacter*. Resistance offered by oxacillinases is more often than MBLs (Poirel et al., 2006). There are four major OXA subgroups (OXA-51, OXA-23, OXA-40 and OXA-58) associated with *A.baumanii*.

In order to control the spread of Acinetobacter baumanii in the hospital, it is necessary to distinguish the outbreak strain. Hence this study was conducted to isolate, identify and to distinguish the antibiogram of Acinetobacter baumanii from clinical specimens.

MATERIALS AND METHODS

Preliminary isolation & identification:

The study was conducted at Sunrise Institute of Medical Sciences, Kakkanad, Kochi, Kerala and various clinical specimens (urine, blood, pus, abscess and endo-tracheal aspirations) were microbiologically screened and the isolation of *A.baumanii* were recorded and evaluated further appropriately. The specimens were cultured on 5% sheep blood agar (Biomerieux), Mac Conkey agar at 37° C for 24 to 48 hours and colonies resembling *Acinetobacter spp.* were subsequently assessed by additional cultural and biochemical evaluation with Vitek 2 compact system for identification.

Molecular Characterization:

Further molecular charecterization were conducted to confirm the identification of 5 isolates named as FEA1, FEA2, FEA3, FEA4 & FEA5 which were resistant to carbapenems. A fragment of 16S rDNA resolved from the isolates was sequenced with 8F and 1492R primers using BDT v3.1 cycle sequencing kit on ABI 3730xl genetic analyzer. And the resulted sequence was used to carry out BLAST with nr database of NCBI genbank. The first ten similar sequences were aligned using Clustal W.

Phylogenetic analysis:

Distance matrix was generated using RDP database and the phylogenetic tree was constructed using MEGA X.

Antimicrobial Susceptibility:

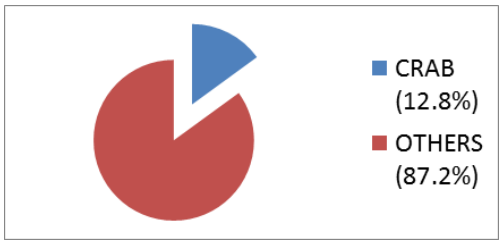
Similarly , the Clinical and Laboratory Standards Institute (CLSI 2017) recommendations were followed to determine the antibiotic susceptibility of the confirmed isolates of *Acinetobacter* along with AST N280 cards on Vitek 2 compact system based determination. Accordingly a total of thirteen (13) antibiotics were included for the determination of susceptibility patterns.

RESULTS

A total of 39 isolates of *Acinetobacter* spp. from different clinical specimens were subsequently processed through vitek 2 compact system for further identification. *A. baumanii* was confirmed to be the most abundant species (84 %), followed by *A. lowfii*, *A.junii* and *A. ursingii* and that a majority of the isolates were identified from patients with urinary tract infection.

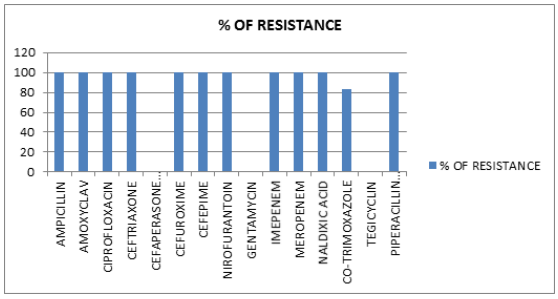
Susceptibility to antimicrobial agents: In this study, 12.8 % of the 39 *A baumanii* isolates were noted to be carbapenem resistant *A baumanii* (CRAB) (Fig 1).

Fig 1:



A resistance to most of the antibiotics was observed to be common among the identified CRAB isolates. In particular, of the 15 various antibiotics tested, the CRAB isolates had 100% resistance to all the 11 antibiotics (Ampicillin, Amoxyclav, Ciprofloxacin, Ceftriaxone, Cefuroxime, Cefepime, Nitrofurantoin, Imepenem, Meropenem, Naldixic acid, Piperacillin tazobactam) and were found to be multi drug resistant. However, Tigecyclin and gentamycin were the only drugs against which the isolates were susceptible. The antibiogram-resistogram patterns of the Carbapenem Resistant *A. baumanii* is given in fig 2.

Fig 2



5 carbapenem resistant isolates (FEA1, FEA2, FEA3, FEA4 & FEA5) were further subjected to 16s rRNA sequencing and fragment of 16S rDNA gene was amplified by PCR. A single discrete PCR amplicon band of 1500 bp was observed when resolved on Agarose Gel (Fig 3).

Fig 3: Gel Image of 16s rRNA

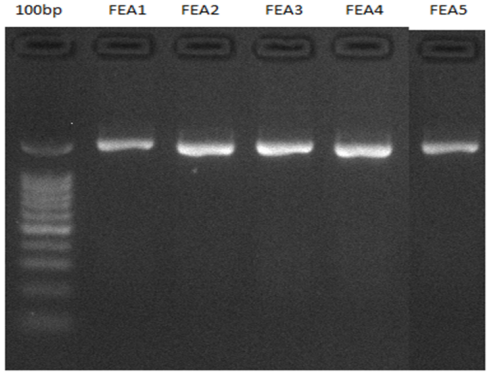
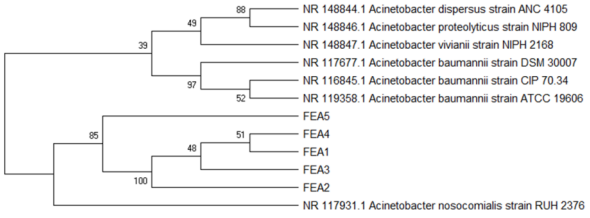


Figure 1: 1.5% Agarose gel showing amplification with 16s primers

Consensus sequence of 1200 bp 16S rDNA gene was generated from forward and reverse sequence data using aligner software. The 16S rDNA gene sequence was used to carry out BLAST with the nrdatabase of NCBI genbank database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. A distance matrix was generated using RDP database and the phylogenetic tree was constructed using MEGA X (fig 4) and all the five strains were identified and confirmed as *Acinetobacter baumanii* based on sequencing and phylogenetic studies.

Fig 4: Evolutionary relationships of taxa



DISCUSSION

It was further found that the source of the isolates of the present evaluation was different compared to previous studies. In particular, most (48%) of the *Acinteobacter* isolates of the study was identified from urine samples while only 12.8% was from blood specimens. On the other hand, Manikal *et al*. (2000) reported as much as 17% of the isolates from blood samples. In another study of Rit and his colleagues in 2012 among 4180 clinical isolates 74.02% *A.baumannii* and 25.98% other types of *Acinetobacter* have been diagnosed. Similarly, 30% and 27% of susceptibilities were reported by them against gentamycin and trimethoprim sulfamethaxazol in contrast to as high as 100 & 83% of susceptible isolates of the present study. In 2015, Ghajavand et al., reported as 53.5% of the isolates were resistant to amikacin, 83.7% to tetracyclin, 86% to ceftazidime, 90.7% to trimethoprim sulfametoxazol, 93% to cefepime, imipenem, meropenem, and ampicillin–sulbactam. Though, the study is being undertaken currently and is progressive, the findings of the study had thrown a light on the continuing menace due to carbapenem resistant *A. baumanii.*

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