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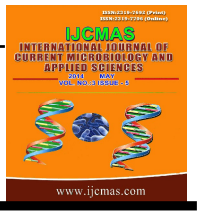

Original Research Article

Isolation and 16S rRNA Sequencing of Clinical Isolates of *Acinetobacter baumanii*

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Nosocomial pathogen, pulmonary infections, 16srRNA sequencing

A B S T R A C T

Acinetobacter is frequently isolated in nosocomial infections and is especially prevalent in intensive care units, where sporadic cases as well as epidemic and endemic occurrence are common. *A. baumannii* is a frequent cause of nosocomial pneumonia, especially of late-onset ventilator associated pneumonia. In the present study, organisms were isolated from throat swab samples of patients suspected for respiratory tract infections. The isolated organisms were identified based on biochemical tests and the sequencing 16srRNA region of the genomic DNA of the bacterial isolates was carried out to confirm their molecular identity. The sequences were then submitted to Genbank database.

Introduction

Acinetobacter spp. are gram-negative aerobic coccobacilli that are ubiquitous in nature, persistent in the hospital environment, and cause a variety of opportunistic nosocomial infections (Bergogne-Berezin *et al*, 1996). They cause various types of human infections. Of the currently known 31 *Acinetobacter* species, *Acinetobacter baumannii* is the most prevalent in clinical specimens. A number of species of *Acinetobacter* are associated with human infection yet *A. baumannii is* generally regarded as the major pathogen (Chang *et al.*, 2005; Van den Broek *et al.*, 2006). Numerous outbreaks caused by *Acinetobacter baumannii* have been reported, which are of great concern in clinical settings.

The main infection caused by this microorganism is nosocomial pneumonia, in particular ventilator-associated pneumonia in patients in Intensive Care Units (Sara Marti *et al.*, 2009). *Acinetobacter* spp. has frequently been reported to be the causative agents of hospital outbreaks. *Acinetobacter* commonly colonizes patients in the Intensive care setting. *Acinetobacter* colonization is particularly common in patients who are intubated and in those who have multiple intravenous lines or

monitoring devices, surgical drains, or indwelling urinary catheters (Cefai *et al.,* 1990). The circumstances of some outbreaks demonstrated the long survival of *Acinetobacter* in dry, inanimate environments (Wendt *et al.,* 1997).

Mortality and morbidity resulting from *A. baumannii* infection relate to the underlying cardiopulmonary immune status of the host rather than the inherent virulence of the organism. Both rates in patients who are very ill with multisystem disease are increased because of their underlying illness rather than the superimposed infection with *Acinetobacter* (Cisneros *et al.,* 2002).

Materials and Methods

Sample collection

Throat swab samples were aseptically collected from different patients visiting various multispecialty hospitals in different localities of Tamilnadu using sterile cotton swabs. Immediately after collection the samples were inoculated into nutrient broth.

Isolation and Identification of *Acinetobacter baumannii*

Characteristic colonies from the nutrient agar plates were isolated and then sub cultured to obtain pure culture. The isolated organisms were identified based on colonial morphology, microscopic study and various biochemical tests according to standard laboratory methods (Cappuccino and Sherman, 1996). Stock cultures were maintained in both agar slant and 20% sterile buffered glycerin. The non hemolytic opaque creamy colonies on blood agar and non lactose fermenting colonies on MacConkey agar were sub cultured on MacConkey agar and incubated for another 24 hrs at 37ºC (Forbes *et al.,* 2007).

16S rDNA sequencing

Genomic DNA was isolated from the three bacterial isolates and16S rRNA region of the DNA was amplified using universal 16SrRNA primers in thermal cycler. The PCR reaction conditions were initial denaturation for 5 min at 94°C, denaturation for 30 s at 94°C, annealing for 30 s at 55°C, extension at 72°C for 2 min and final extension at 72°C for 15 min. The PCR amplified products were then run on agarose gel, eluted, purified and sequenced.

BLAST analysis

The 16S rDNA sequences of the three isolates were subjected to BLAST analysis (Altschul *et al*., 1990) using NCBI BLAST tool at www.ncbi.nlm.nih.gov.

GenBank submission

The three 16S rRNA gene sequences were submitted to GenBank database using the BankIt sequence submission tool and accession numbers are awaited (BankIt ID: 1721519).

Results and Discussion

A. baumannii can survive on the human skin or dry surfaces for weeks. It is the second most commonly isolated non-fermenting bacteria in human specimens. *A. baumannii* infections are uncommon but, when they occur, usually involve organ systems that have a high fluid content (e.g. respiratory tract, CSF, peritoneal fluid, urinary tract), manifesting as nosocomial pneumonia, infections associated with continuous ambulatory peritoneal dialysis (CAPD), or catheter-

associated bacteruria. *Acinetobacter* pneumonias occur in outbreaks and are usually associated with colonized respiratory support equipment or fluids. Nosocomial meningitis may occur in colonized neurosurgical patients with external ventricular drainage tubes (Chen *et al.,* 2005). Go and Cunha (1999) summarized that *Acinetobacter* commonly colonizes skin, oropharynx secretions, respiratory secretions and urine. *Acinetobacter* uncommonly colonizes the gastrointestinal tract and is associated with nosocomial pneumonias, bacterimias and wound infections. *Acinetobacter* infection is rarely associated with meningitis, endocarditis (native valve infective endocarditis and prosthetic valve endocarditis). Bacterial cultures were isolated from throat samples of patients suspected for lower respiratory tract infection. Of the different cultures obtained, three *Acinetobacter baumannii* isolates were named as SKP-1, SKP-2 and SKP-3. The three isolates were found to be Gram negative cocco-bacilli and non motile. All the strains were gram negative and non motile. These strains had the capacity to produce acid from glucose and lactose. All strains were positive to Simmons citrate, catalase and oxidative fermentation (Table-1). The negative reactions were: the acid production from sucrose, H2S on TSI and gas production, mannitol, indole and oxidase (Sofia *et al.,* 2004).

Sequencing of the 16S rRNA region of the Genomic DNA of the three bacterial isolates revealed that the isolate SKP-1 has 400 Base pairs (bp), isolate SKP-2 has 3354bp and SKP-3 has 295 base pairs respectively. In the present investigation, the BLAST analysis of the 16s rRNA region of the DNA sequences of the three bacterial isolates revealed 99% similarity to *A. baumannii* and thus the molecular identity of the three isolates were confirmed. Sundar and Nasrin (2010) reported that the 16SRNA and the subsequent blast analysis confirm the identity of clinical isolates of UTI pathogens isolated from Nagercoil township of Tamil Nadu.

Acinetobacter baumannii isolates SKP-1

1 tggggagtgt tgggtaagtc ccccaagagc ccaacccttt tcttacttgc aacaatttcg
61 gatgggaact ttaaggatac tccagtgaca aaactgagga aggcgggggc gacgtcaagt
121 catcatggcc ctacggccag ggctacacac gtgctacaat ggtcggtaca aagggttgct
181 acacagcgat gtgatgctaa tgaaaaaaag ccgatcgtag tccggattgg agtctgcaac
241 tcgactccat gaagtcggaa tcgctagtaa tcgcggatca gaatgccgcg gtgaatacgt
301 tcccgggcct tgtacacacc ccccgtcaca ccatgggagt ttgttgcacc agaagtagct
361 agcctaactg caaagagggc ggtaccatcg gttgaccaag

Acinetobacter baumannii isolates SKP-2

1 gcaactttgg atggaattaa ggatctccag tgcaaatgga agaaggcggg gcgacgtcaa
61 gtcatatggc cttacggcca gggctacaca cgtctacaat ggtcggacaa agggttgcta
121 cacagcgatg tgtttttgg aagggaaagc cgatcgtagt ccggattgga gtctgcaact
181 cgactccatg aagtcggaat cgctagtaat cgcggatcag atgcccggtg aatacgttcc
241 cgggccttgt acacccccc cgtctcacca tggga gtttg ttgcaccaga agtagctagc
301 ctaactgcaa agagggcggt accaacggtt ccccg

Acinetobacter baumannii isolates SKP-3

1 ctcgactcca tgaagtcgga atcgctagta atcgcggatc agatgcccgg tgaatacgtt
61 cccgggcctt gtacacaccc cccgtctcac catgggagtt tgttgcacca gaagtagcta
121 gcctaactgc aaagaggcgg taccacggtt ggccc ggggg gaagatcttc cttgtacgta
181 aaatgatgca agaagtggtg actgcaccat catgtgcgca tgactctaga gatctctcta
241 gctcagcagt atcgatgcga ctggcgtacc tatcacatag ctataagggt cgcc

Table.1 Biochemical tests for the strains of *Acinetobacter baumannii*

S.No	Biochemical tests	Result
1	Glucose	+
2	Lactose	+
3	Sucrose	-
4	H_2S Production	-
5	Gas Production	-
6	Mannitol	-
7	Motility	-
8	Citrate	+
9	Indole	-
10	Catalase	+
11	Oxidase	-

The organism still remains as a major threat to the life of the people because of its spread, the degree of lower respiratory tract infection and resistance to most of the new generation antibiotics.

The scientific community should concentrate on identifying the drug targets in the virulent regions especially in the OMPA region and design drugs which efficiently bind to these targets and thereby preventing the emergence of multidrug resistant strains of the bacterium in the future (Sundar *et al*., 2013).

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