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Isolation of Multiple Drug Resistant (MDR) Bacteria from Hospital Environment

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A B S T R A C T

A multiple drug resistant (MDR) bacteria are a class of bacteria which is resistant to different classes of antibiotics and cause severe complications in humans as well as animals. This study investigated for isolation and identification and the antimicrobial resistance patterns of *P. aeruginosa* clinical isolates obtained from hospitalized patients. The results confirmed the occurrence of drug resistant strains of *P. aeruginosa*. In this study, molecular identification of 16SrRNA was performed and phylogenetic tree was constructed using a nearly complete sequence within the 16S rDNA gene *P. aeruginosa*. The result shows that the isolate belongs to the species *P. aeruginosa*. Amikacin, ciprofloxacin and Ceptazidine were found to be the most effective antimicrobial drugs. Therefore calls for a very judicious, rational treatment regimens prescription by the physicians to limit the further spread of antimicrobial resistance among the *P. aeruginosa* strains

Introduction

Pseudomonas aeruginosa founds in hospital environments and is a common problem in the environment since it is the second most common causative agent of nosocomial infections. The bacterium possesses a wide range of secretion systems, which secrets numerous proteins relevant to the pathogenesis of clinical strains (Hardie *et al*. 2009). *Pseudomonas* (Neu, 1983) are motile (one or more polar flagella), rod shaped aerobe and Gram-negative bacteria. They are found almost everywhere, in soil, water, plants and animals. *P. aeruginosa* is an opportunistic human pathogen most commonly affecting immuno-compromised patients.

It accounts for the majority of human infections. Pathogenic *Pseudomonas* is found throughout the body, most commonly in the urinary tract, respiratory tract, blood and wounds (Emori and Gaynes, 1993). *P. aeruginosa* is a type of bacterium that has the ability to develop resistance (Poirel *et al*., 2004) to antibiotics rather rapidly over several generations. This resistance present in some strains makes *P. aeruginosa* very difficult to treat infected host, such as a human or other animal (Drenkard, 2003). Adding to its ability to develop drug resistance is its flexibility that allows it to thrive in various environments, especially medical environments and moist

environments containing water. It acquires resistance genes and specific mutations that enhance its survival in the presence of antibiotics. Its genome was sequenced completely in 2000 and was recorded as having 6.3 million base pairs. Such a notably large genome encodes many proteins contributing to its versatility in adaptation, virulence function, and antimicrobial resistance (Livermore, 2002). To avoid the problem of variable phenotype for identification to provide more accurate species genotypes identification method is required. However, the taxonomic complexity, doubtful phylogeny, and lack of genomic sequence data of the dozens of species within the broad genus *Pseudomonas* present an obstacle to genotypic identification. The size of a 16S rRNA gene is about 1,540 base pairs (bp).

In the past two decades, the rapid emergence of resistance to antimicrobial agents has been one of the most important clinical calamities. According to Fridkin *et al.* (1999) stated that the production of a variety of beta-lactamases, outer membrane permeability, and combinations of multiple mechanisms of resistance, the gram negative bacteria have acquired resistance to antibiotics such as extended-spectrum Cephalosporins, Monobactams, Carbapenems and beta-lactam-beta-lactamase inhibitor combinations. Survival study assessed in vitro susceptibility of commonly used antibiotics against clinical isolates of *P. aeruginosa* provides estimations of the rate of MDRPA infections (Karlowsky *et al.*, 2003).

The MDR (Multiple Drug Resistance) bacteria (Cloete, 2003) were isolated from hospitals in Bangalore and through microbiological and biochemical analysis organism Pseudomonas was identified and isolated (Dotsch *et al.*, 2009). In order to study the multiple drug resistance, antibiotic discs containing 8 different classes of antibiotics was used. By paper disc plate method the antibiotic resistance was confirmed. The present study was to study the resistance pattern of *P. aeruginosa* treated with various antibiotics which was isolated from Government Hospital in Bangalore, India.

Materials and Methods

Source

Samples were collected for investigation Government Medical Hospital, a centrally located Bangalore, Karnataka. Specimens were collected from various purulent swabs, infected urine and blood, hospital beds, linen, other furniture surfaces etc. patients who were hospitalized for more than one week duration. The samples were investigated for bacterial identification. Only one isolate was considered and include in the study.

Isolation of Bacteria

The samples were inoculated onto blood chocolate and MaCconkey agar plates. All plates were incubated both aerobically and anaerobically at 37°C overnight. Emergent colonies were identified according to standard bacteriological methods (Cheesbrough, 2006) and biochemical tests were carried out (Collee *et al.*, 1996).

Identification of Isolates using 16srRNA

Molecular phylogeny of bacteria was determined by amplifying genomic 16S rRNA region. Two primers specific to 16S rRNA region used in this study were 519F and 1385R in order to amplify approximately 850bp sequence of bacterial 16srRNA gene (Edward *et al.*, 2004). The

DNA sequences were analyzed using BLASTn (nucleotide Basic Local Alignment Search Tool) facility of National Centre for Biotechnology Information (NCBI). The BLAST results were used to find out evolutionary relationship of bacteria. Altogether twenty sequences, including sample were used to generate phylogenetic tree. The tree was constructed by using MEGA 5 software (Saitou and Nei, 1987).

Determination of Antibiotic Sensitivity

The test organism *P. aeruginosa* was taken with a sterile loop, suspended in sterile water. The turbidity of the suspension was adjusted to 0.5 McFarland's standard (1.5×10^8 CFU/mL). It was then spread on the surface of Mueller-Hinton agar (MHA) plate using sterile cotton swab. A total of 19 antibiotics in the following concentrations Amikacin, Netilmycin, Cefadroxil, Sparfloxacin, Ceftriaxone, Ciprofloxacin, Gentamycin, Cefotaxine, Cefoperazone, Lomefloxacin, Ampicillin+Sulbactam, Ceptazidine, Ampicillin, Chloramphenicol, Bacitracin, Erythromycin, Gentamycin, Polymyxin-B, Tetracycline. A stock antibiotic solution of 100mg/100ml was prepared. A volume of 30µl of antibiotic was added to each well aseptically and the plate was incubated at 37°C for 24hrs. The zone of inhibition was measured and with reference to the standard table it was determined whether the bacteria was sensitive, intermediate and resistant (Bauer *et al*., 1966).

Results and Discussion

Isolation and Identification of Bacteria

Based on the morphological and biochemical characters it was identified as *Pseudomonas* species. Pseudomonas sp. was gram negative, which shows positive result on catalase and oxidase, Gelatin liquefaction, N_2 Reduction test. Organism shows motile, the bacteria showed bluish pigmented colony due to pyocyanin and colonies appeared circular 1mm in diameter flat and soft in consistency in Cetrimide Agar. Urease, Indole, Methyl Red, Voges-Proskauer and citrate test revealed as negative. In Blood Agar, colonies show colourless zone due to beta hemolysis. In Mac Conkey agar plate, colonies appeared colourless indicated *Pseudomonas* sp. as nonlactose fermemter respectively. Hence to identify and confirm the Pseudomonas sp. at molecular level, 16S rRNA gene region was amplified and sequenced. Genomic DNA was extracted from Pseudomonas sp. by the standard method. PCR amplification of 16S rRNA gene region by using universal primer, the obtained PCR product resulted in 1451 bp. By using BLAST analysis, 98 sequences of NCBI data gave 99% similarity. Phylogenetic tree generated by NCBI tool proves that this organism genetically related with other organisms (Fig. 1). On the basis of the position of sequence of the given bacterial samples in the phylogenetic tree, the closest similarity for the sample labeled as *Pseudomonas* was found to be *Pseudomonas aeruginosa*.

Determination of Antibiotic Sensitivity

The zone of inhibition was observed (Fig2) and measured. *P. aeruginosa* isolates showed maximum resistance pattern to Ampicillin, Chloramphenicol, Bacitracin, Erythromycin, Gentamycin, Tetracyclin, Polymyxin B and Ciprofloxacin and sensitive to amikacin, Ciprofloxacin, Ceptazidine and intermediate to Ceftriaxone, Gentamycin, Cefoperazone respectively. The resistance pattern of the *P. aeruginosa* to various antibiotics tested was shown (Table 1). The emergence of infectious diseases poses a serious threat to public

health worldwide, and the increasing rate of the appearance of antibiotic –resistant strains in a short period of time both Gram positive and Gram-negative microorganisms is a major public health concern (Levinston and Jawetz, 2000).

Table.1 Determination of Zone of Inhibition of *P. aeruginosa* against antibiotics

Antibiotics	Zone of inhibition(mm)	S/I/R	Strength(mcg)
Amikacin	17	S	30mcg
Netilmycin	12	R	30mcg
Cefadroxil	0	R	30mcg
Sparfloxacin	12	R	5mcg
Ceftriaxone	17	I	30mcg
Ciprofloxacin	25	S	5mcg
Gentamycin	14	I	10mcg
Cefotaxine	0	R	30mcg
Cefoperazone	20	I	75mcg
Lomefloxacin	16	R	5mcg
Ampicillin+Sulbactam	0	R	25mcg
Ceptazidine	18	S	20mcg
Ampicillin	6	R	10
Chloramphenicol	4	R	30
Bacitracin	0	R	30
Erythromycin	0	R	15
Gentamycin	2	R	10
Polymyxin-B	7	R	300 Units
Tetracycline	8	R	30

Fig.1 Phylogenetic tree for *Pseudomonas aeruginosa* using partial 16S rRNA gene sequence

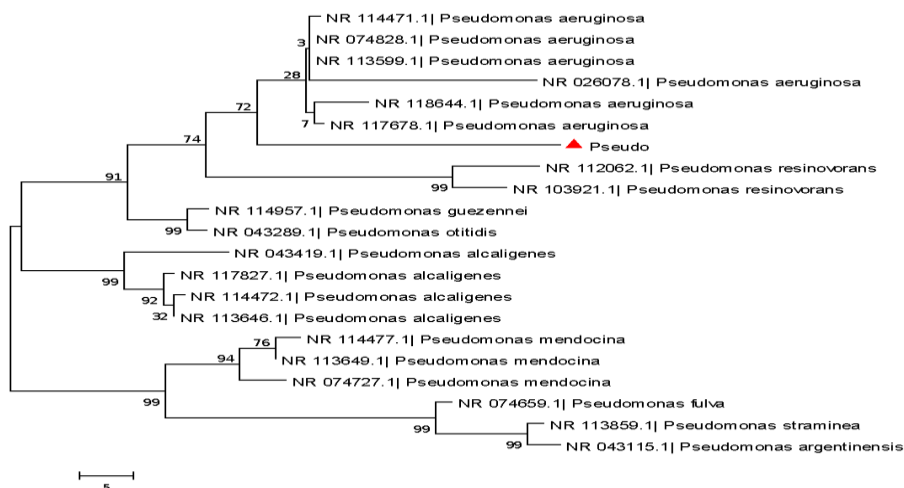
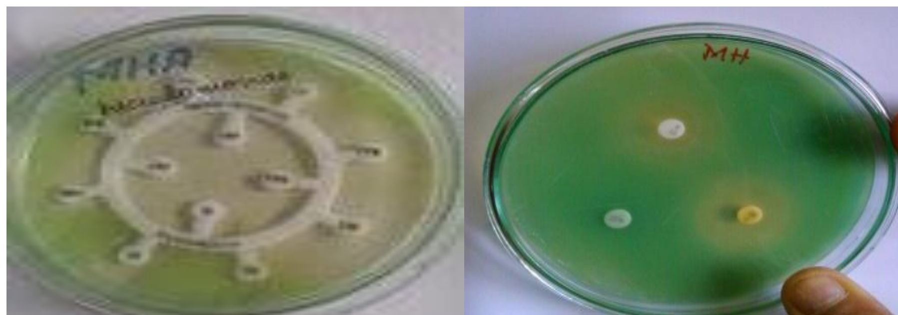


Fig.2 Antibiotic sensitivity test of *P. aeruginosa*



Treatment of infectious diseases becomes more challenging with each passing year. This is especially true for infections caused by the opportunistic pathogen *P. aeruginosa*, with its ability to rapidly develop resistance to multiple classes of antibiotics (Aloush *et al*., 2006). The ability of *P. aeruginosa* to survive on minimal nutritional requirements and to tolerate a variety of physical conditions has allowed this organism to persist in both community and hospital settings. *P. aeruginosa* presents a serious therapeutic challenge for treatment of both community-acquired and nosocomial infections, and selection of the appropriate antibiotic to initiate therapy is essential to optimizing the clinical outcome. Unfortunately, selection of the most appropriate antibiotic is complicated by the ability of *P. aeruginosa* to develop resistance to multiple classes of antibacterial agents, even during the course of treating an infection (Dimatatac, 2006). A more accepted approach is to treat serious *P. aeruginosa* infections with a combination of antibacterial agents. Although synergistic interactions are an important aspect for some drug combinations, the primary focus of combination therapy against *P. aeruginosa* is preventing the emergence of resistance.

In conclusion, this study illustrated that the *Pseudomonas aeruginosa* strain which was obtained from hospital environment were resistant to various antibiotics. The high rate of antibiotic resistance among *P. aeruginosa* strains is very alarming and can be responsible for serious infections. So identification of more such strains and taking efforts to reduce the rate of transfer between different strains are important goal for treatment of *P. aeruginosa* infections in future.

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