ISSN:0975-542X

Multi-Drug Resistant Capability of *Pseudomonas Aeruginosa* Isolates from Nasocomal and Non-Nasacomal Sources

Hemalatha. N[1] and P. Dhasarathan[2*]

[1]Research and Development centre, Bharathiyar University, Coimbatore – 641046.

[2]Dept. of Biotechnology, Prathyusha Institute of Tech. and Management, Thiruvallur – 25

Abstract:

P. aeruginosa were isolated from pus and urine sample of hospitalized patient and confirmed morphologically and biochemically. Two different pigment pyocycin (greenish colour) and pyoverdin (UV-fluorescing pigment) were analysed for photostability and its antibacterial activity. Two strains were sensitive to pyocin produced by urine isolate. Three strains of *Pseudomonas aeruginosa* were isolated from Urine, Pus sample and soil. It is confirmed morphologically and biochemically. *Pseudomonas aeruginosa* was confirmed by its growth in Cetrimide agar (Inhibitor of all other Gram Positive and Gram Negative bacteria) and its pigment production. Environmental and pathogenic isolates were analysed with various antibiotics for their multi drug resistance. Different molecular sized plasmid were isolated and transformed in DH 5α cells. β-Lactamase providing genes might be present in plasmids, and it was confirmed with plasmid isolation from transformed cells. The bla gene coding beta lactamase having the size of 1 kb was amplified.

Keywords: *Pseudomonas, PCR, antibiotics, susceptibility and drugs.*

Introduction:

Pseudomonas is frequently present in hospital environment especially in moist places such as bowls, drain cleaning buckets and humidifiers (Quinteros *et al.* 2003). It can also found growing in eye drops ointments and weak antiseptic solution. *Pseudomonas aeruginosa* and *Pseudomonas pseudomallei* are very important medicinal strains. *Pseudomonas maltophillia* is occasionally medicinal important strain and other *Pseudomonas* species may also cause disease (Champs *et al*. 1993 and Jacqueline *et al.* 2004).

Pseudomonas pseudomallei is naturally found in rice paddy fields, the mud of the river banks and surface stagnant water. It is causes melioidosis (Pneumoenteritis) in human and animals. The *P. pseudomallei* invading to host organism through open wound, but can also be inhaled. In melioidosis, pus filler nodules and abscesses form in the lungs, spleen, liver joints, skin or subcutaneous tissues. Severe diarrhea and vomiting may occur and occasionally septicaemia, fever and a rash are usually present due to cause of *P. pseudomallei*.

Pseudomonas aeruginosa possesses a considerable degree of natural resistance to antibiotics. Antibiotics such as aminoglycosides (Gentamicin, amikacin), Cephalosporins (cefotaxime, ceftazidime, cefoperazone), fluoroquinolones (ciprofloxacin, ofloxacin, perfloxacin), penicillins (piperacillin, ticarcillin, azlocillin) are non sensitive to *Pseudomonas* strains. The genes for drug resistance are present on both the bacterial chromosome and plasmids. For localized infections, topical colistin, polymyxin B or 1% acetic acid may be useful to control *Pseudomonas* strains (Elizabeth and Jean, 2002). In the present investigation characterize the multi drug resistant ability of *Pseudomonas aeruginosa* isolates from hospital and hospital free environment.

Plasmid resistance genes often code for enzymes that destroy or modify drugs; for eg ; the hydrolysis of penicillin or the acetylation of chloramphenicol and aminoglycosides drugs. Plasmids – associated genes have been implicated in resistance the aminoglycosides, Chloramphenicol, Penicillin and Cephalosporins, Erythromycin, Tetracycline, Sulfonamids and others (Mallea *et al.* 2000). Once a bacterial cell possesses an R-plasmid, the plasmid may be transferred to other cells quite rapidly through normal gene exchange processes such as conjugation, transduction and transformation (Emanuela *et al.* 2002).

Materials and Methods:

Isolation of test strains: The bacterial isolates were isolated from patients hospitalized for more than five days in Melur Government Hospital and from hospital free environmental soil sample. Ten pus samples named as P_1-P_{10} and ten

urine samples named as U_1-U_{10} were carefully collected, enriched in peptone broth and plated on Cetrimite agar. After 1 day of incubation at 37°C, the green colored colonies were selected and sub-cultured in nutrient broth. Simultaneously non- pathogenic *P. aeruginosa* were isolated from soil. The enriched cultures were used for further analysis.

Characterization isolated bacterial strains: Various standard biochemical tests (Simple staining, Gram staining, growths on TSI agar, citrate utilization, Oxidase test and Urease test) were performed to characterize isolated bacterial strains and motility confirmed by Hanging drop method.

Antibiotic susceptibility test: The isolated bacterial strains antibiotic susceptibility was screened by Tube dilution method and Disc diffusion method.

Tube dilution method: The antibiotic stock has been prepared by dissolving 1mg /ml in sterile water. Mueller Hinton broth has been prepared and distributed as 5 ml/tube. Eight dilutions have been carried out with each antibiotic (20-160 µl). Mueller Hinton broth without culture and antibiotic, tube with culture and without antibiotic has been taken as control. Antibiotic stock solutions were added in the concentration of 20, 40, 60, 80, 100, 120, 140, 160 µg/ml to sterile Mueller Hinton Broth, then 50µl of log phase culture (pathogenic and non pathogenic) has been added to all the tubes and incubate at 37°C for a day.

Disc diffusion method: Mueller Hinton agar plates were prepared. Both the pathogenic and non- pathogenic isolates were aseptically swabbed on the surface of the agar completely to make a lawn. Before applying the disc, the agar surface was allowed to dry for about five minutes. The antibiotic disc was taken by a sterile forceps and placed over the agar plate at centre least 15mm from the edge of the plate. The disc was gently pressed to give a better contact with the agar. Four different antibiotic discs at the same distance apart from each other have been placed on the agar plate. The plates were then incubated for 24 hrs at 37° C. After two days the zone of inhibition around the antibiotic disc has been observed and recorded. Multidrug resistant character may be plasmid encoded. It was characterized by transformation.

Plasmid Isolation: The plasmid was isolated from the pathogenic *P. aeruginosa* by two methods alkaline lysis method and fast plasmid mini preparation. Multidrug resistant character may be plasmid encoded. It was characterized by transformation.

Results:

Isolation: Out of ten pus samples, two bacterial isolates (P1 and P5) were (*Pseudomonas aeruginosa*) isolated and confirmed, out of ten urine samples, one bacterial isolate (U1) was isolated and confirmed. Non-pathogenic bacteria isolate (NP1) was isolated from the environment soil sample by serial dilution the soil sample and then plated on cetrimide agar medium. Totally of four isolates were selected and maintained on cetrimide agar medium.

Characterization: The selected isolates were characterized by different biochemical tests. The results of the different biochemical tests performed with the four isolates are given in Table 1.

Antibiotic susceptibility assay: Antibiotic susceptibility test was performed by tube dilution and disc diffusion method.

Tube Dilution Method: The results for the tube dilution method were given in the table 2 and 3. From the observations the pathogenic isolates were identified as resistant to many antibiotics.

Disc diffusion method: The results of the different antibiotic disc performed with the two strains (U_1 and NP) are given in Table 4. Sensitivity was identified by the zone of inhibition.

Pyocin susceptibility assay: Pyocin photostability was observed in different incubation condition *i.e.,* at low

Table 1: Biochemical characterization of *P. aeruginosa* isolated from clinical and non clinical sources.

Biochemical Tests	Pathogenic strain			
	U1	P1	P5	NP 1
Oxidase	+	+	+	+
Urease	-	-	-	-
Citrate	+	+	+	+
TSI agar (Alkaline slant)	-	-	-	+

Table 4: Antibiotic assay of *Pseudomonas aeruginosa* sample U_1 and NP using Disc Diffusion method.

S. No.	Antibiotic	U_1 (diameter of the zone)	NP (diameter of the zone)
1	Ciprofloxin	31 mm	13 mm
2	Cefatazidine/calvalic acid	26 mm	26 mm
3	Cefazolin	Resistant	25 mm
4	Bacitracin	Resistant	19 mm (s)
5	Tetracycline	Resistant	Resistant
6	Chloramphenicol	15 mm (s)	15 mm (s)
7	Amoxycillin	Resistant	Resistant
8	Streptomycin	Resistant	Resistant
9	Amikacin	19 mm (s)	19 mm (s)
10	Erythromycin	Resistant	Resistant
11	Ampicillin	Resistant	Resistant

temperature and high temperature and at different times. U_1 isolate, NP isolate was susceptible to pyocin and P1 isolate was resistant to pyocin of P_2 isolate.

Plasmid from isolated strains: The plasmid was isolated from the culture by Alkaline lysis method and Fast plasmid mini preparation method. Isolated plasmid was separated on 1% agarose and stained with Ethidium Bromide (5µg/ml). The molecular size of the plasmid has been calculated by comparing with λ DNA / *Hind III* marker. Three bands were observed and had molecular size of 23, 19 and 9kb respectively.

Transformation: Two colonies were observed in LB agar plates containing Ampicillin in the concentration of 100µg / ml.

Plasmid isolation from transformed cells: Plasmids were isolated from transformed *E.coli* cells and separated in 1% Agarose gel electrophoresis. Plasmid separated had the molecular size of 19kb.

Amplification of BLA gene: 1 kb amplicon was observed in 1% Agarose gel electrophoresis inferring the amplification of bla gene in plasmid**.**

Strains of *P. aeruginosa* isolated from clinical samples and soil were diversified. Their diversification may be present in genetic differences in their pyocin metabolising gene and drug resistant genes (Delphine *et al*. 2000 and Karden *et al*. 2000). It can be further studied after sequencing their genes. Resistance to extended spectrum cephalosporins commonly develops in *Pseudomonas aeruginosa*, during therapy due to selection of mutants producing high levels of the chromosomal bush group 1 β lactamase (Arpin *et al.* 2003). β lactamases were investigated by an isoelectric focusing overlay technique which simultaneously determined isoelectric points (pIs)and substrate or inhibitor profiles. All strains isolated by Johann et al. (1997) produced an inducible bush group 1 β lactamase (pI 8.3). Three

different SHV derived ESBLs were transferred by transconjugation to *E.coli* C600 N and amplified by PCR.

Ampicillin resistant genes were found in plasmids of both Pathogenic and non-pathogenic strains. Genetic homology and difference can be studied after sequencing their bla genes (Claeys *et al.* 2004). The difference may also be present in Beta-Lactamase of both the strains. Epidemiology can be studied with PFGE analysis of all isolates.

References:

[1] Arpin. C, Veronique Dubois, Laure Coulange, Catherine Andre, Isabelle Fischer, Patrick Noury, Frederic Grobost, Jean-Philippe Brochet, Jacqueline Jullin, Brigitte Dutilh, Gilberte Larribet, Isabelle Lagrange, and Claudine Quentin. (2003) Extended-Spectrum β-Lactamase-Producing Enterobacteriaceae in Community and Private Health Care Centers. Antimicrob Agents Chemother. 47(11): 3506–3514.

[2] Champs C., C. Henquell, D. Gueron, D.Sirot. (1993) Clinical and bacteriological study of Nosocomial infections due to E. aerogene resistance to imipenem. Journal of Clinical Microbiology. 31(1) : 123-7.

[3] Claeys, Thierry De Baere, Georges Wauters, Patricia Vandecandelaere, Gerda Verschraegen, An Muylaert, and Mario Vaneechoutte. (2004) Extended-Spectrum β-lactamase (ESBL) producing Enterobacter aerogenes phenotypically misidentified as Klebsiella pneumoniae or K. terrigena. BMC Microbiol. 4: 49.

[4] Delphine Girlich, Laurent Poirel, Amornrut Leelaporn, Amal Karim, Chanwitt Tribuddharat, Michael Fennewald, and Patrice Nordmann. (2001) Molecular epidemiology of the integron-Located VEB-1 ESBL in Nosocomial Enterobacterial isolates in Bangkok, Thailand. J Clin Microbiol. 39(1):175-182.

[5] Elizabeth Pradel and Jean- Marie Pag. (2002) The AcrAB-TolC Efflux pump contributes to Multidrug resistance in the Nosocomial Pathogen Enterobacter aerogenes. Antimicrob agents Chemotherapy. 46(8) : 2640-2643.

[6] Emanuela Dell' Amico, Mariagrazia Perilli, Bernardetta Segatore, Maria Rosaria de Massis, Ciro Bianchi, Francesco Luzzaro, Gian Maria Rossolini, Antonio Toniolo, Giuseppe Nicoletti, and Gianfranco Amicosante. (2002) Molecular characterization of ESBL produced by Nosocomial isolates of Enterobacteraiceae from an Italian Nationwide survey. J Clin Microbiol. 40(2):611-614.

[7] Jacqueline Chevalier, Jerome Bredin, Abdulla Mahamoud, Monique Mallea, Jacques Barbe and Jean-Marie Page. (2004) Inhibitors of Antibiotic efflux in resistant Enterobacter aerogenes and Klebsiella pneumoniae strains. Antimicrobial agents and Chemotherapy. 48(3). 2001 –2007.

[8] Johann D. Pitout, Kenneth S. Thomson, Nancy D. Hanson, Anton F. Ehrhardt, Philip Coudron, and Christine C. Sanders. (1998) Plasmid-mediated resistance to Expanded-Spectrum Cephalosporins among Enterobacter aerogenes strains. Antimicrob agents Chemotherapy. 42(3): 198 – 205.

[9] Karen E. Preston, Christopher C. A. Radomski, and Richard A. Venezia. (2000) Nucleotide sequence of the Chromosomal ampC gene of Enterobacter aerogenes. J. Antimicrobial Agents chemotherapy, 44(11): 3158 – 3162.

[10] Mallea M, Chevalier J, Eyraud A, Pages JM. (2002) Inhibitors of antibiotic efflux pump in resistant Enterobacter aerogenes strains. Biochem Biophys Res Commun. 24: 293 - 295.

[11] Quinteros M., M. Radice, N. Gardella, M. M. Rodriguez, N. Costa, D. Korbenfeld, E. Couto, G. Gutkind, (2003) Extended-Spectrum β-Lactamases in Enterobacteriaceae in Buenos Aires, Argentina, Public Hospitals. Antimicrob Agents Chemother. 47(9): 2864–2867.