

Original Research Article

Whole Cell Protein Profiles of *Pseudomonas aeruginosa* Strains Isolated at a Tertiary Care Diabetic Specialty Hospital in Chennai, Tamilnadu, India

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ABSTRACT

Keywords

Pseudomonas aeruginosa,
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Investigation

Whole Cell Protein Analysis (WCPA) by SDS-PAGE permitted subdivision of the strains into groups, to such a convincing level to imply that the technique may be valuable in typing strains isolated during outbreaks of infection in hospital.

Introduction

Pseudomonas aeruginosa is often associated with immunosuppression, chronic abscesses, diabetic foot ulcer, and iatrogenic meningitis. The occurrence of this opportunistic pathogen offering resistance to commonly used antibiotics has become a threatening challenge to medical practitioners in recent years. Outbreaks of infection caused by *Pseudomonas aeruginosa* have become a growing concern in tertiary care hospitals all across the world (Wiblin, 1997; Pollack, 1995; Kluytmans, 1997; Noonai *et al.*, 1985).

Pseudomonas aeruginosa shows a high

degree of resistance with relevance to cephalosporins, quinolones and aminoglycosides. Clinical hospital environmental isolates were processed and identified as per the standard operative procedures. The strains were also tested for sensitivity to the antibiotics by the Kirby Bauer disc diffusion method, with discs procured from Hi-media.

Pseudomonas aeruginosa is the most common gram negative bacterium found in nosocomial infections (Wiblin *et al.*, 1997; Pollack, 1995; Kluytmans, 1997; Noonai *et al.*, 1985).

Kugelberg *et al.* (2005) concluded that, although DNA restriction enzyme typing is the “gold standard” for *Pseudomonas aeruginosa*, due to consideration of the time duration required for carrying out the analysis, it may not be possible to fully depend on such time-consuming methods. In such a situation, epidemiological and phenotyping data to describe a susceptibility profile will come to the rescue during times of epidemics, considering the need to identify the problematic *P. aeruginosa*, the opportunistic organism causing nosocomial infections, including wound, urinary and respiratory tract infections. The most important risk-group for the patients with cystic fibrosis in whom the *Pseudomonas aeruginosa* could prove to be the cause of morbidity; and in diabetic foot ulcer patients, it may lead to amputation of the limb, and hence the need for quick diagnosis and early treatment deserves a careful consideration, especially during epidemics.

Zamowski *et al.* (1988) have studied the characteristics of 42 bacterial strains of the genus *pseudomonas*, based on protein fingerprinting, using the Sodium Dodecyl Sulphate (SDS)- Polyacrylamide Gel Electrophoregrams (PAGE) of cell-free extracts.

Densitometrical analyses revealed a unique and distinct characteristic of the species being studied. The isolates of samples were identifiable as four main groups, and twelve sub-clusters. Therefore, this method was considered as an effective and reliable method, from the viewpoint of classification of bacteria, and for showing similarities as well as variability among them. Cosras *et al.* (1989), evaluated the reliability of SDS-PAGE method in typing the protein patterns of *Escherichia cloacae*, and concluded that the SDS-PAGE gave similar results to the

findings achieved with conventional typing methods, and that all strain groups recognised by combined sero/phage typing procedures were also identified by SDS-PAGE method.

In addition, it revealed the protein, subdivided into a group of four serotype O3 isolates that were difficult to be distinguished by phage typing. The authors, through their extensive study, were able to conclude that the high resolution achievable by SDS-PAGE in protein analysis could provide an effective method of typing *Escherichia cloacae*.

Khan *et al.* (1996), undertook a preliminary study of fingerprinting of *Pseudomonas aeruginosa*, by adopting SDS-PAGE procedures. The 42-strains of *Pseudomonas aeruginosa* samples were isolated from broncho-alveolar lavage fluid from intubated patients admitted to the Intensive Care Unit (ICU), of the All India Institute of Medical Sciences (AIIMS), New Delhi, during December 1993 and June 1994.

After obtaining typical biochemical profiles for the samples, efforts were made to perform antimicrobial susceptibility against Ceftazidime, Ampicillin, Cefotaxime, and Ciprofloxacin. Pyocin typing of these 42-strains were performed by Scrape, and Streak Method, using 22-indicator strains. The Whole Cell Protein Profile, obtained by the SDS-PAGE procedures indicated the presence of the 45 per cent bands of different molecular weights from 340kDa to 14.3kDa. On the basis of Dice Index of similarity, the strains could be grouped into 20 types. Since all the strains could be typed, satisfactorily, the system was considered to have an adequate typeability aspect. As the repeated testings of strains indicated reproducibility, the SDS-PAGE procedure could be considered satisfactory.

Pastar *et al.* (2013) reported that the resistance to Methicilin treatment is increased due to the combined presence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in the polymicrobial wound, and that *Pseudomonas aeruginosa* causes an influence on *Staphylococcus aureus* to enhance its virulence factor, thereby causing a delay in healing.

Many investigators have studied the complex problem of resistance exhibited by the *Pseudomonas aeruginosa* to antibiotic treatment (Clark *et al.*, 1998; Reller *et al.*, 1974; Hasef, 1996).

Meenakshisundaram *et al.* (2015), investigated the 104 samples isolated from 75 patients of Diabetic Foot Ulcer (DFU) ailment who were receiving treatment in a tertiary diabetic care specialty hospital in Chennai, Tamilnadu, India, and reported that the prevalence of *Pseudomonas aeruginosa* in diabetic foot ulcer wounds was in the order of 17.3% in the polymicrobial samples; and that the resistances offered by *Pseudomonas aeruginosa* to the different antimicrobial drugs were varying, namely, at 38% to Ampicillin, 22% to Co-Amoxyclav, 11% to Piperacillin, 66 % to Cefotaxime, 33% to Ceftazidime, 44% to Ceftriaxone, 33 % to Gentamicin, 44% to Ciprofloxacin, and 5.5% to Imipenem.

They reported that, in the 104- polymicrobial samples isolated from 75 Diabetic Foot Ulcer (DFU) patients, the bacterial content was in different proportions, namely, 22.2% of *Escherichia coli*, 17.3% of *Pseudomonas aeruginosa*, 17.3% of *Staphylococcus aureus* (coagulase positive), 10.6% of *Klebsiella spp.*, 10.6% of Coagulase negative *Staphylococcus* (CONS), 9.6% of *Proteus spp.*, 5.8 % of *Streptococcus spp.*, 3.8% of *Corynebacterium spp.*, and 2.9% of

Enterococcus. It was interesting to note that, among the *Pseudomonas aeruginosa* population present in the isolates, only 66% was resistant to Cefotaxime, 44% of it was resistant to Ceftriaxone, and 44% of it was resistant to Ciprofloxacin.

Some investigators have reported that an early identification of the causative agent is an important factor for helping the correct diagnosis, as otherwise, the bacterium exhibiting resistance to certain drugs would become more resistant, if the precious time is lost before finalising the correct identification of the causative agent through a suitable method of analysis (Lakshminarayanan *et al.*, 2013; Murugan *et al.*, 2015).

From this viewpoint, the SDS-PAGE method seems to be having quite a few merits.

All the above research findings by various investigators would only highlight the necessity of assessing the presence of a dangerous bacterial species such as *Pseudomonas aeruginosa* in human body, early enough and with a reliable accuracy, in identifying it, without any ambiguity,

Syed Arshi *et al.* (2014) carried out whole cell protein analysis, using SDS-PAGE method and categorised 60 isolates into 16 groups (S1-S16), on the basis of bonding patterns.

Shankar Esakkimuthu *et al.* (2006) reported that the Outer Membrane Proteins (OMP)-profiles were different for environmental strains compared to strains of clinical samples, suggesting a probability that the origin of infections by *P. aeruginosa* could be due to infection of patients prior to their admission to the hospital.

They reported that this particular organism, namely, *Pseudomonas aeruginosa* is capable of causing life-threatening infections, including septicemia in already debilitated individuals.

The aim of the present study was to examine, in depth, the suitability of the popular SDS-PAGE method for the Whole Cell Protein Analysis (WCPA), on *Pseudomonas aeruginosa*, the opportunistic pathogen.

For this purpose, experiments were conducted, for evaluating the Whole Cell Protein Profile Typing of *Pseudomonas aeruginosa*, in clinical samples and environmental samples, in a reputed Tertiary Diabetic Care Specialty Hospital, in Chennai City.

Seven strains were grown in MacConkey's agar, blood agar and nutrient agar for 48 hours, at 37 degrees Centigrade temperature, before biochemical and sensitivity tests were performed, as per standard protocol.

Materials and Methods

Diabetic Foot Ulcer pus samples were obtained from the deepest part of the ulcer, after due scrubbing of the wound with sterile moistened gauze, from patients attending Dr.V.Mohan's Diabetic Specialties Research Centre, Gopalapuram, Chennai-600 028, during a period of 5-months from May 2005 to September 2005. Surveillance was carried out in the hospital from floor, sink, water-tap, door handles of wards, bed, trolley, kidney tray, gauze pack, foot-rest, electric switches, curtains, cupboards etc, to obtain environmental samples of *Pseudomonas aeruginosa* that were native to the nosocomion. Carey Blair transport media was used to transport the swabs. A total of 18 isolates of *Pseudomonas aeruginosa* were isolated from a selected group of

hospitalized patients from May 2005 to September 2005, fitting into Wagner's grade 2 to 5 of Diabetic Foot Ulcer (DFU).

Isolation, identification and characterisation were carried out as per procedures indicated by Konneman, et al 1998. Antibiogram was performed using commercially available antibiotic discs with *Pseudomonas aeruginosa* ATCC 27853 as control.

Preparation of whole cell protein of *P. aeruginosa* – protocol

A single colony of *Pseudomonas aeruginosa* was inoculated in 25 ml of Brain Heart Infusion Broth (BHIB), incubated in shaker-incubator, overnight at 37 degrees Centigrade. The overnight broth culture was centrifuged at 4000 G for 15minutes (REMI Centrifuge, INDIA). The supernatant was discarded and the pellet mixed with 10 ml of Tris-ethylene diamine tetra acetic acid buffer, and centrifuged at 4000 G for 10 minutes, sonication of cells was done after adding 50ul of Tris-EDTA buffer with 8* 15-s bursts in an MSE sonic oscillator fitted with a titanium probe with amplitude of 1.4nm. The cells were maintained in an icebox, and bursts were performed for 3 minutes with 30 seconds interval for cooling (Vibronics-ultronics, India). The sonicated suspension was centrifuged at 1000 G for 10 minutes. The supernatant suspended in 10ul of 1% SDS, was boiled for 10 minutes. Protein estimation was done as per Lowry method, with crystalline bovine serum as standard SDS-PAGE PROTOCOL.

Vertical anionic electrophoresis was carried out in polyacrylamide slab gel, with 0.025 Tris-0.2M glycine electrode buffer, pH 8.3, Sodium Dodecyl Sulfate (10%) was prepared, and allowed to set; 20 ul of each of the whole cell protein was mixed with 20 ul of the sample buffer, and solubilized by

boiling for 5 minutes; 30ul of each of this was loaded into individual wells; a protein marker – range 340kDa to 14.3kDa obtained from GENEI, Bangalore was added; Voltage was set at 50V using a constant – voltage power supply till bromophenol blue (Biotech-Yercaud, India) tracking dye placed in the upper tank reach bottom of gel, approximately 5 hrs. The gel slab was removed after the run, and was subjected to staining procedure with 2% Coomassie brilliant blue R-250, for 4 hrs at 370 C; the gel was de-stained, with several changes of the same solvent, without dye to be stored in 7% acetic acid; the gel was then observed for different protein fractions in gel documentation system (BIO-RAD Systems, France) attached to U-VP White/UV Trans-illuminator.

Results and Discussion

Whole Cell Protein Profiles of *Pseudomonas aeruginosa*, from different diabetic foot ulcer specimen are presented in figure 1.

All typing systems for epidemiological investigation, which have a very important role, and which should have established type ability and reproducibility, may also be considered useful for confirming and for being sensitive enough to distinguish organisms that are amenable for identifications. In the former case, visual comparisons of similar, but not identical, and with the merit of proven value on the gel tracks, might be considered adequate, as a tool for identification of the organisms.

Biotyping can be considered as an easy method for screening, as an epidemiological tool. However, cell envelope protein typing is a more sensitive method to detect differences, even though the method is more

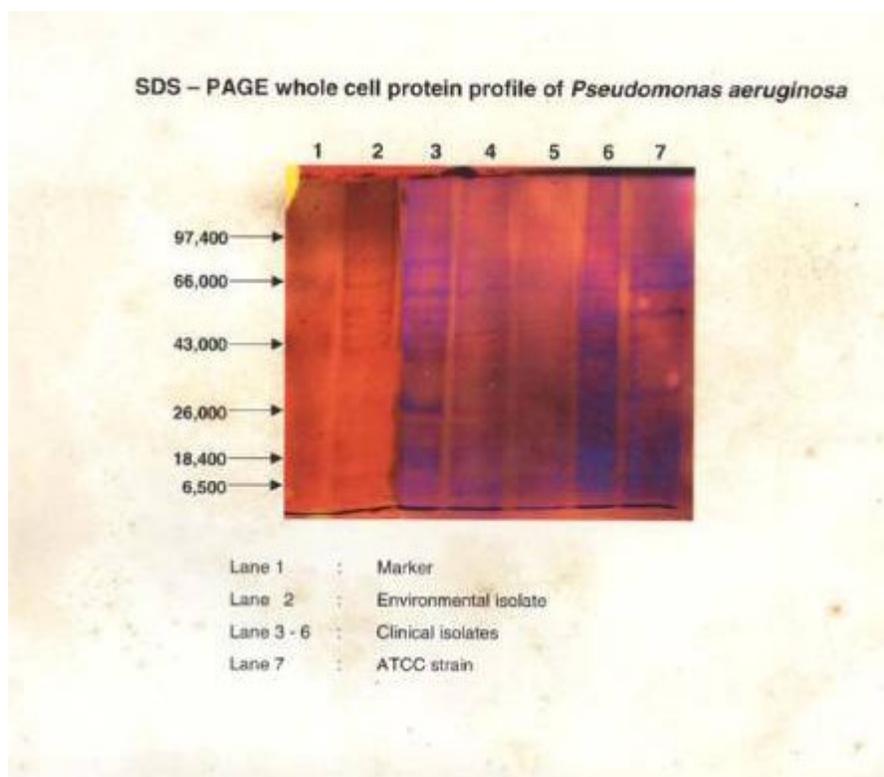
laborious to carry out, and to interpret the results.

Walia *et al.* (1988) had found that there could be no difference in environmental and clinical isolates of *Pseudomonas aeruginosa* in the whole cell protein profile. There was difference in solubisible protein profile. Only a few studies were done on the whole cell protein of *Pseudomonas aeruginosa*, during those years, and it would not have been possible to come to any definable direction, in this aspect.

The variance in protein profiles among the isolates was usually used as a guide to assess the similarity between the genus species and strains of bacteria. The findings of Walia et.al, was considered as a satisfactory demonstration of this concept. In their work, as they achieved the results by combining SDS-PAGE dendogram, obtained from the numerical analysis that were originated from a variety of proteins banding patterns of strains. Subsequently, Celebi *et al.* in 2004, used the same SDS-PAGE method, for determining the taxonomical and epidemiological studies in urophatogenic *Escherichia coli*.

The SDS-PAGE method, usually combined with dendogram derived from the numerical analysis of whole cell protein patterns of the strains, has been studied extensively to investigate the similarities or differences among the genera, species, and the strains as well as shown by Adwan and Adwan, (2004), who determined the level of similarity between each isolate, in the form of dendogram similarity, and evaluated the matrix of similarity values for the corresponding isolates.

Figure.1 In the Whole Cell Protein SDS-PAGE patterns, the Molecular Weight Standards are shown on the left-hand-side of the slide (Figure 1). Lane 1: Molecular weight marker,; Lane 2: Environmental isolate; Lane 3- 6: Clinical isolates; Lane 7: ATCC strain



Besides the work of characterization, and identification of microbial aspects, using the molecular techniques, it is possible to assess the microbial characteristics using the chemical characterization. The studies by Kanso and Patel (2003), Sembiring and Goodfellow (2010) correspond to the first case; and the study of Schroll *et al.* (2001) corresponds to the latter case.

Generally, it has been established by some investigators that the character of the chemical analysis based on comparison of the protein profiles can be used for rapid microbial identification (Berber, 2004). Cellular Protein profiles using SDS-PAGE method, is one of the chemical character methods, which is widely used for strengthening the process of a reliable identification, because of the fact that it is

through this method, that it becomes possible to generate complex, specific, stable and reproducible protein banding patterns, so that it becomes possible to interpret and compare the tested strains with reference to the reference- strain (being used as the standard), as reported by Ghazi *et al.* (2004),

Considering the limitations of molecular studies, in particular, Wayan Suardana *et al.* (2013) studied the protein profiles analysis of chemical characterisation of *Escherichia coli* 0157:H7 samples, isolated from human origin as well as from animal origin, to indicate the reliability of the method of SDS-PAGE.

Adwan and Adwan (2004) reported that the SDS-PAGE method can be usually

combined with dendrogram, derived from the numerical analysis of whole cell protein patterns of strains, and that it can be used extensively to study the similarity or difference prevailing among the genera, species, and also the strains.

Khan *et al.* (1996), studied the whole cell protein profiles of 42 strains of *P. aeruginosa*, isolated from clinical samples, using the SDS-PAGE method, and reported the presence of protein band, ranging from 340kDa to 14.3kDa. On the basis of Dice Index of similarity, the strains could be grouped into 20 types, the system has been glorified as the method having the adequate typeability and good reproducibility.

In conclusion, the Whole Cell Protein Profiles, for the environmental isolates and clinical isolates were different, revealing the following:

- i) the SDS-PAGE method is sensitive enough to bring out the difference between clinical samples and environmental samples,
- ii) the infection spreads from DFU-patients to the hospital environment through their body-contacts with wash-basin, toilet-seats, door-handles, bed-spreads, clothes, floor surfaces, etc,
- iii) The infection spreads through the staff attending on the patients.
- iv) There is reason to believe that the source of infection need not be necessarily the hospital, from where the specimens were collected.

It can also be construed that the growth patterns are flourishingly different when the particular bacteria is in the polymicrobial cluster, within the wound, than being left alone in the environment, in the absence of an entertaining host-cell, thereby making the difference large enough between the clinical isolates and the environmental isolates.

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