ANTIBIOGRAM AND GENOTYPING OF *PSEUDOMONAS AERUGINOSA* ISOLATED FROM HUMAN, ANIMAL, PLANT, WATER AND SOIL SOURCES IN NORTH INDIA

Ram Janam, Anil K Gulati and Gopal Nath

Department of Microbiology, Institute of Medical Sciences,
Banaras Hindu University, Varanasi, India

Abstract. The present study was designed to determine antibiotic resistance rates and patterns and its correlation with enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) whole genome analysis of *Pseudomonas aeruginosa* obtained from clinical and environmental sources. In order to determine the possibility of clonality in the bacteria and to track the resistance markers for a better understanding of the epidemiology of drug resistance. A total of 500 strains, 100 each from clinical, water, animal, plant and soil sources were subjected to antibiogram analysis by disc diffusion method. Seventy-five randomly selected strains, 15 each of the five sources were subjected to ERIC-PCR analysis. Clinical isolates were more resistant to combinations of very high numbers of drugs as compared to isolates from other sources. Weak clonality was observed in *P. aeruginosa* by ERIC-PCR method with 80% of the clinical strains belonging to only 3 clones. It could be concluded that it is the drug selection pressure in clinical environment that is causing the accumulation of drug resistance against many antimicrobials. Furthermore, *P. aeruginosa* does have clonal expansion. Further studies are warranted to confirm the results.

Keyword: *Pseudomonas aeruginosa*, MDR, ERIC- PCR, metallo β-lactamase, imipenem, India

INTRODUCTION

The medical community has been witnessing a growing epidemic of infections due to multiple drug resistant (MDR) gram-negative bacteria involving almost whole globe (Hsueh *et al*, 2002; Landman *et al*, 2002; Canton *et al*, 2003; Sharma *et al*, 2005). Recently epidemiology of bacteria resistant to majority of the available antimicrobial agents has become focus of numerous surveillance studies (Tambic *et al*, 2002; Jones 2003).

Pseudomonas aeruginosa is a known notoriously difficult organism to deal with using antibiotics and disinfectants (Hancock, 1998). This organism has a remarkable ability to acquire antibiotic resistance genes, to persist in the hospital environment and to spread easily from patient to patient (Navon-Venezia *et al*, 2005). Outbreaks due to multi drug

Correspondence: Prof Gopal Nath, Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India.
Tel: +91-542-307516*41; Fax: +91-542-367568
E-mail: gopalnath@gmail.com

resistant (MDR) *P. aeruginosa* infection in hospitals despite efficient infection control policies may be due to advanced invasive procedures adopted in intensive care units (ICUs). Such situations have also resulted in the dissemination of only a few particular persistent resistant bacterial clones in hospitals (Scott and Pitt, 2004). In India, *P. aeruginosa* was the commonest species isolated from ventilator-associated pneumonia (VAP) patients in ICUs (55%) and from wound infections (59%) with high mortality rate ranging from 16% to 46% (Pawar *et al*, 2003; Agnihotri *et al*, 2004; Agarwal *et al*, 2005; Pandey *et al*, 2005). Emergence of MDR *P. aeruginosa* in many hospitals across the country is of great concern (Agarwal *et al*, 2005; Pandey *et al*, 2005; Sharma *et al*, 2005). Most recent development is the addition of metallo-β-lactamase (MBL) to already existing pool of many other β-lactamases in this bacterium (Garau *et al*, 2005). A few studies have employed molecular typing methods such as enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) and PCR-ribotyping to characterize *P. aeruginosa* in India and abroad (Agarwal *et al*, 2002, 2005). A recent investigation had employed fluorescent amplified fragment length polymorphism (fAFLP) for elucidation of *P. aeruginosa* isolates obtained from endophthalmitis patients (Kenchappa *et al*, 2005). These studies have focused on either clinical or environmental isolates of hospital premises. It is of interesting to know the drug resistance pattern and genotypic relatedness of *P. aeruginosa* isolates collected from various environmental and clinical sources for better understanding of its epidemiology.

The present study was undertaken to determine drug resistant pattern of *P. aeruginosa* isolated from various environments with and without high antibiotic selection pressure *ie*, clinical and environmental sources, respectively.

MATERIALS AND METHODS

Collection and isolation of *P. aeruginosa*

A total of 100 different strains of *P. aeruginosa* from each of the following 5 sources were collected and isolated as described below.

Clinical specimens. The Sir Sunder Lal Hospital of Banaras Hindu University India is a 1,000-bed hospital that caters tertiary level health services to eastern part of northern India. Clinical isolates of *P. aeruginosa* were obtained from a variety of clinical specimens: pus, blood, urine, high vaginal swab (HVS), CSF, endotracheal tube (ETT) and wound swab. The specimens were inoculated within 2 hours of collection on MacConkey agar (MA) and blood agar (BA) plates and incubated at 37ºC for 18 to 24 hours.

Animal dung. Dung specimens were collected from pets and stray cattle in and around Varanasi City. Five grams of dung were inoculated into 5 ml of fresh Acetamide selective broth and incubated at 37ºC for 18 hours. From the broth, subcultures were made on MA and cetrimide agar.

Water samples. Water samples (25 ml) were collected from a nearby sewer (Assi drain) and Ganga river. Approximately 5 ml of each of the samples were inoculated into 5 ml of fresh selective Acetamide broth and incubated at 37ºC for 18 hours. From the broth, subcultures were made on CA and MA.

Plant specimens. Roots of local herbs and shrubs were collected from nearby villages of Varanasi City and also from decaying vegetables. The roots and leaves were cut

into small pieces and inoculated into 5 ml of fresh selective Acetamide broth and incubated at 37°C for 18 hours. From the broth, subcultures were made on CA and MA.

Soil specimens. Soil samples were collected from villages around Varanasi City mostly from cultivated farms. Five grams of each soil specimen were inoculated into 5 ml of fresh selective Acetamide broth and processed as described above.

Identification of the suspected colonies

The colonies resembling *P. aeruginosa* were further tested by standard Gram's staining, motility, oxidase and catalase production and other biochemical methods following the standard protocol (Phillips *et al*, 1969).

Antimicrobial susceptibility test

Five hundred isolates of *P. aeruginosa* were isolated from five different sources (water, clinical, plants, soil and animal). Antimicrobial susceptibility test were performed according to CLSI guidelines for the disk diffusion method (Bauer *et al*, 1966; NCCLS, 2000). All isolates were tested for their susceptibility to amikacin (AMK, 30 µg/ml), ampicillin A (AMP,10 µg/ml), ampicillin/sulbactam (AMS,10/10 µg/ml), amoxyclav (AMC,10 µg/ml), aztreonam (ATM,30 µg/ml), ceftazidime (CAZ,30 µg/ml), cephotaxime (CTX,30 µg/ml), ceftriaxone (CRO,30 µg/ml), cefoperazone (CPZ,75 µg/ml), cefuroxime (CXM,30 µg/ml), cephalexin (LEX,30 µg/ml), ciprofloxacin (CIP,5 µg/ml), furazolidone (FZN,50 µg/ml), gentamicin (GEN,10 µg/ml), imipenem (IMP,10 µg/ml), lincomycin (LCM,15 µg/ml), meropenem (MEM,10 µg/ml), netilmicin sulphate (NET,30 µg/ml), piperacillin/tazobactam (PTZ,100/10 µg/ml), tobramycin (TOB,10 µg/ml) and trimethoprime/sulfomethoxazole (TMP, SMZ,1.25/23.75 µg/ml).

Genotyping of the strains

Isolation of genomic DNA. Cultures grown for 18 hours in LB broth were used for DNA extraction using the standard chloroform phenol method (Gouvea *et al*, 1991). DNA concentration was measured by absorbance at 260 nm.

DNA amplification by ERIC-PCR

The forward primer (5'-ATGTA-AGCTCCTGGGGATTCAC-3') and reverse primer (5'AAGTAAGTGACT-GGGGTGAGCG-3') (Versalovic *et al*, 1991) were used to amplify repetitive sequences present in the chromosomal DNA of *P. aeruginosa* isolates. ERIC-PCR was carried in 25 µl, volume comprising of 100 ng of *P. aeruginosa* DNA, 1.5 µl (10 pmol) of each primer and 1 µl (25 mM) of dNTPs, 2.5 µl of 10x PCR assay buffer and 0.33 µl (3 U/µl) of *Taq* DNA polymerase (M/s Bangalore Genie, Bangalura, India). Filtered Mille-Q water was added to the mixture to make a final volume of 25 µl. Reactions were carried out using a programmable thermocycler (M/s Biometra, Germany) according to the following thermocycling conditions: 94°C for 7 minutes, 34 cycles of 30 seconds at 94°C, 38°C for 1 minute, 72°C for 5 minutes and final step of 72°C for 15 minutes. All reagents used for PCR master mixture were from Bangalore genie (Balgaluru, India). Each set of PCR amplification was performed with negative control containing distilled water and a positive control containing *P. aeruginosa* DNA isolated from a reference strain (ATCC27853).

Gel electrophoresis

After PCR, 2 µl aliquot of 6x loading buffer (0.1% bromophenol blue, 50% glycerol) was mixed with 10 µl of each of the PCR solution and the mixture was electrophoresed in 1% agarose gel containing 0.5 µg/µl ethidium bromide in 1x TBE buffer

(40 mM Tris-borate, 1 mM EDTA, pH 8.0). DNA size markers of 100 bp and 1kb (MBI, Fermentas, Burlington, ON, Canada) were used. The gels were photographed after electrophoresis under UV light to record results. Representative gel photographs of ERIC-PCR are shown in Fig 1 A-E. Reproducibility was monitored by comparing the results of repeated ERIC-PCR carried out with the same strains.

Gel analysis

All the gel images were analyzed under UV light in a gel documentation system (Alpha Innotech, San Leandro, CA). Band sizes, band attributes and standard molecular weights were assigned according to molecular weight markers. Numerical index of the discriminatory ability of ERIC was calculated by applying Simpson's Index of Diversity equation (Navon-Venezia *et al*, 2005) as follows:

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{s} n_j(n_j - 1)$$

Where N is the total number of strains in the sample population, S the total number of types described, and nj the number of strains belonging to the jth type.

Dendrogram construction and genetic relatedness

Dendrogram for cluster analysis of all the isolates were constructed using NTSYS pc2.0 software (Applied Biostatistics, Setauket, NY) and unweighted pair-group method arithmetic mean (UPGMA) (Romesburg, 1984). The dendrogram was constructed on the basis of the banding pattern produced by ERIC-PCR. A binary table or a haplotype matrix for each strain was constructed by linearly composing presence (1)/absence (0) data derived from analysis of the gel/ antibiogram. Microsoft Excel (Microsoft, Redmond, WA) was used for putting strains in rows and the markers in columns. The haplotype matrix made in Microsoft Excel was incorporated in NTSYS pc2.0. SIMINT program was used to compute similarities or dissimilarities in the form of average taxonomic distance, which was used to perform sequential, agglomerative, hierarchical and nested (SAHN) clustering. A dendrogram thus was constructed from the similarity matrix obtained in the SIMINT program by the unweighted pair-group method with arithmetic mean (UPGMA) (Rohlf, 1994).

RESULTS

Antibiotic resistance

The clinical isolates were observed to be most resistant against the majority of anti-pseudomonal antibiotics: AMK, GEN, CAZ, TZP, CIP, MEM and IMP with the exception of GEN, TOB and ATM (Table 1). The isolates from water samples were the most resistant among the non clinical samples. Isolates from soil specimens were the least resistant against all the antibiotics tested. The highest levels of resistance were against GEN and ATM in water sample isolates while those from animal dung were against TOB.

Among the non-pseudomonal antibiotics, the highest rate of resistance was obtained against synthetic cephalosporins in clinical isolates: CTX (57%), CRO (60%) and CPZ (61%) whereas for other non-pseudomonal antibiotics such as AMP, AMS, AMC, CXM, LEX, FZ, LCM and TMP the resistance rates were comparable in all the groups of strains isolated from clinical as well as environmental samples. Further, when MDR *P. aeruginosa* was defined as the strains resistant to ≥ 1 agent in ≥ 3 antibiotic categories used against the bacterium, 24% of the clinical and 8% of water sample isolates were observed to

Table 1
Resistant patterns of 500 *Pseudomonas aeruginosa* isolates from different sources.

Antibiotic category	Antimicrobial agent	Clinical (100)	Water (100)	Animal (100)	Soil (100)	Plant (100)
Aminoglycosides	Gentamicin	56	96	4	0	2
	Amikacin	44	7	3	0	0
	Tobramycin	60	9	82	0	21
	Netilmicin sulphate	49	5	15	0	2
Antipseudomonal cephalosporin	Ceftazidime	56	20	7	3	8
Antipseudomonal penicillin+ inhibitor	Piperacillin/Tazobactam	39	1	1	0	0
Antipseudomonal fluoroquinolone	Ciprofloxacin	61	6	0	0	0
Antipseudomonal carbpenems	Meropenem	62	6	0	0	0
	Imipenem	8	1	0	0	0
Monobactams	Aztreonam	70	80	7	13	22
Other penicillins	Ampicillin	96	100	99	100	100
	Amoxyclav	93	98	87	100	100
	Ampicillin/Sulbactam	89	89	97	97	100
	Cephalexin	95	95	100	99	100
	Cefuroxime	97	97	100	100	100
Other cephalosporins	Cefoperazone	61	10	4	1	0
	Cefotaxime	60	38	7	3	4
	Ceftriaxone	57	14	4	3	4
Other antimicrobials	Trimethoprim + Sulfamethoxazole	96	97	97	91	100
	Lincomycin	96	96	100	100	100
	Furazolidone	94	94	94	94	100

be MDR. However, none of the isolates of animal dung, plant and soil was found to be MDR. Resistance to IMP was observed in 8% of the clinical isolates and 1% of the water sample isolates

There were two major clusters when dendrogram was generated on the basis of their antimicrobial sensitivity against 21 antibiotics (Fig 2). The largest cluster comprised of 63 isolates, which were resistant to relatively less number of antibiotics and of diverse origins. The second cluster,

however, had only 12 isolates that were resistant to 16-21 antibiotics. Of these only 3 (25%) were from water while the rest were of human origin. In cluster-I, there were 3 groups of identical strains based on their antibiogram: Ia consisted of 12 strains, 11 (91%) of animal dung origin and 1 from soil and all were resistant to 9 similar spectrum of antibiotics; cluster IIb consisted of 23 strains, 11 from plants, 9 from soil, 2 from clinical and 1 from water and all were resistant to 8 similar antibiotics. The
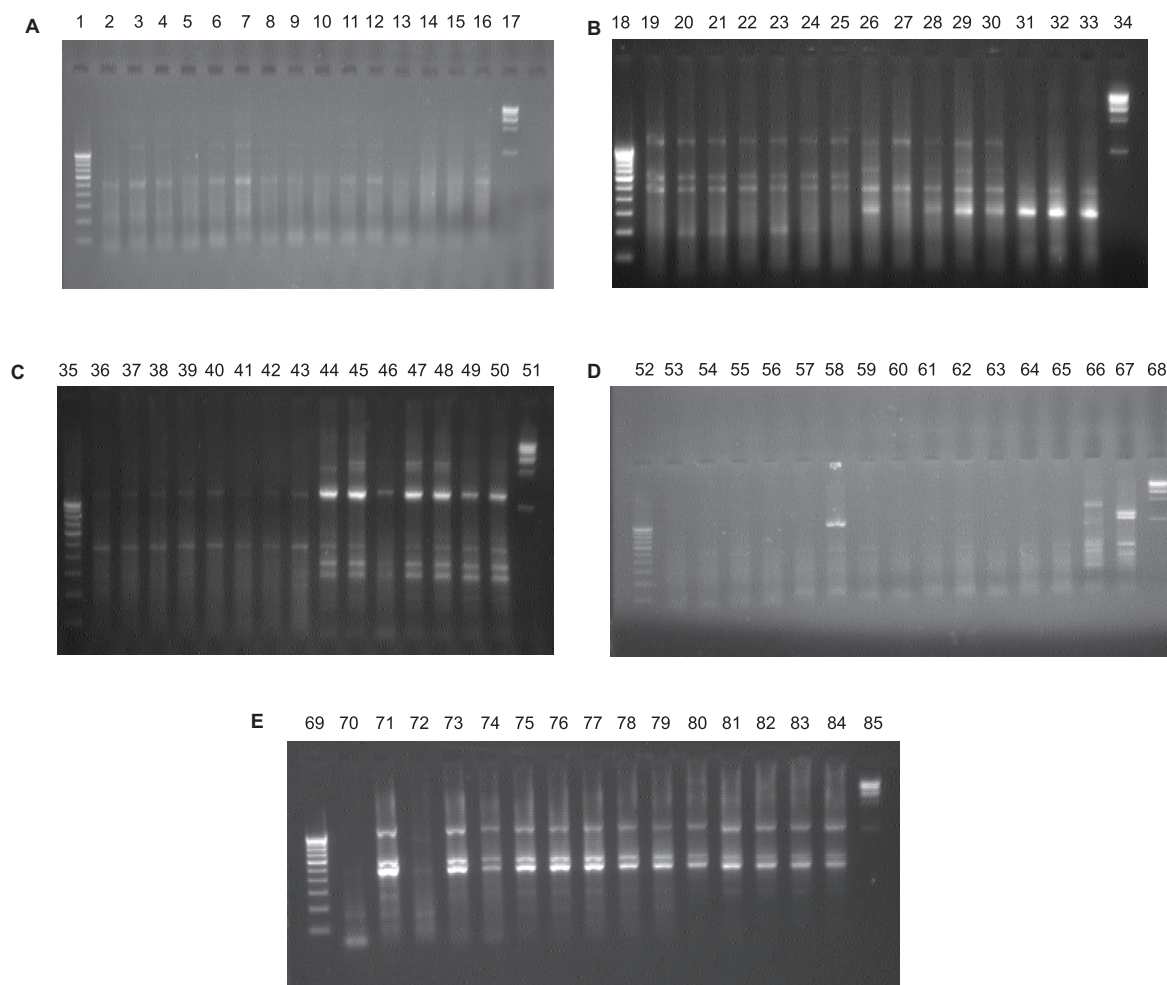
Fig 1–Representative gel showing whole genome analysis of *Pseudomonas aeruginosa* isolates from different sources (clinical, water, animal, soil and plants) by ERIC primers. A: Lane 1 100 bp DNA ladder; lane 17 1kb DNA ladder; lanes 2 to 16: *P. aeruginosa* isolates from clinical specimens. B: Lane 18 100bp DNA ladder; lane 34 1kb DNA ladder; lane 19 to 33; *P. aeruginosa* isolates from water specimens. C: Lane 35 100bp DNA ladder; lane 51 1kb DNA ladder; lane 36 to 50; *P. aeruginosa* isolates from animal specimens. D: Lane 52 100bp DNA ladder; lane 68 1kb DNA ladder; lanes 53 to 67; *P. aeruginosa* isolates from soil specimens. E: Lane 69 100 bp DNA ladder; lane 85 1kb DNA ladder; lanes 70 to 84; *P. aeruginosa* isolates from plant specimens

third group was resistant to 9 drugs and the isolates were from plants (3), soil (2) and water (1).

Clinical isolates showed the highest number of strains (73%, 11/15) resistant to ≥ 11 drugs followed by water samples (40%, 6/15) and plant (7%, 1/15). Each of the 15 *P. aeruginosa* isolates from both the soil and animal sources were resistant to ≤ 10 antibiotics only.
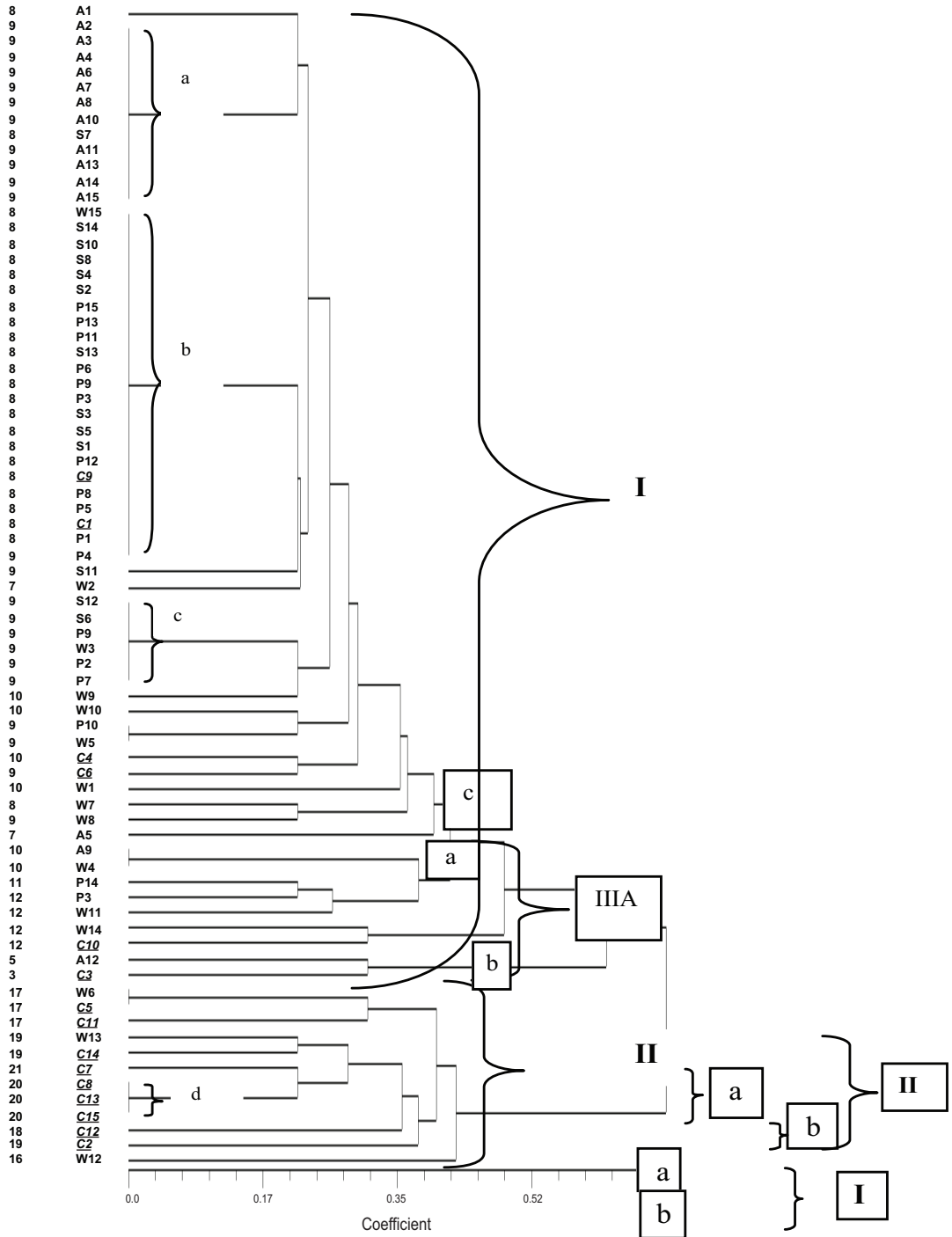
Fig 2–Dendrogram of *Pseudomonas aeruginosa* based on ERIC-PCR. Fifteen random selected samples from clinical, water, animal, plant and soil were analyzed.
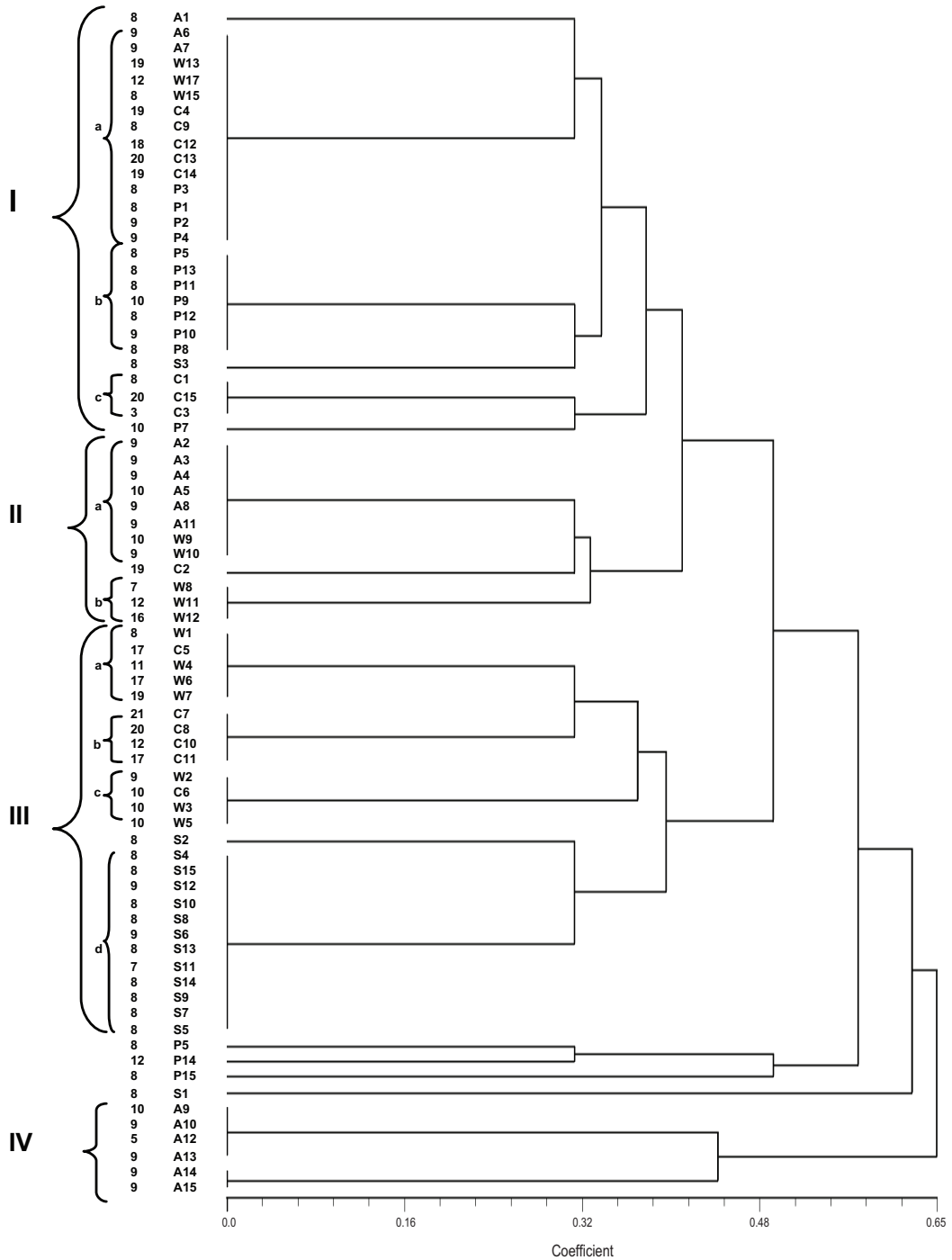
Fig 3–Dendrogram of *Pseudomonas aeruginosa* based on ERIC-PCR. Fifteen random selected samples from clinical, water, animal, plant and soil were analyzed.

Genotyping of randomly selected *P. aeruginosa* strains

By ERIC-PCR amplification 1 to 6 bands ranging from 50-2,800 bp could be generated. By UPGMA analysis, there were 3 major clusters and 1 minor cluster when all the 75 *P. aeruginosa* isolates were analyzed (Fig 3). A total of 9 strains were found on separate branches. Cluster-I had 27 strains comprising of 8, 3, 3, 13 and 1 of clinical, water, animal, plant and soil origins, respectively. Genotypically (ERIC-PCR), there were 3 different clones. Clone Ia had 5 clinical, 3 water, 2 animal 4 plant origins. These identical strains were resistant to 8-20 different antibiotics. There were 7 strains in clone Ib and all of them were of plant origin with resistance varying from 8-10 antibiotics. The third clone was of 3 strains and all were of clinical origin with resistance ranging from 3-20 antibiotics.

The second cluster comprised of 12 strains with 2 genotypically identical groups. Group IIa consisted of 8 strains (water 2, animal 6) and resistance varying from 9 to 10 antibiotics while the other clone IIb had all the 3 strains from water. Among these strains resistance ranged against 7-16 antibiotics.

The third cluster (III) consisted of a total of 26 strains (clinical 6, water 7 and soil 13). There were 4 identical groups of strains. Group IIIa had all the 12 strains of soil origin and were resistant to 7-9 drugs. Group IIIb had 1 strain of clinical origin resistant to 17 drugs and 4 strains from water with resistance to 8-19 antibiotics. The third group of this cluster (IIId) had 4 strains (clinical 1, water 3) with resistance to 9-10 drugs. The last clone of the cluster III comprised of 12 strains, all of them were from soil with resistance against 7-9 antibiotics.

The fourth cluster (VI) had a total of 6 strains and all were of animal origin with resistance to 5-10 antibiotics. Of the 15 clinical isolates analyzed 12 (80%) belonged to 3 clones only. The discriminatory index (DI) of ERIC-PCR in this study was 0.9132.

DISCUSSION

P. aeruginosa is ubiquitous in the biosphere and metabolically very versatile. This species is known for its high levels of intrinsic as well as acquired resistance to antimicrobials. The resistance in this species may due to low permeability of its cell wall, its genetic capacity to express a wide repertoire of resistance mechanisms, mutation in chromosomal genes regulating resistance genes and acquisition of additional resistance genes from other organisms by horizontal gene transfer via plasmids, transposon and bacteriophages (Davison, 1999).

This is the first study of its kind where we have looked into the drug resistance pattern and genotyping in the *P. aeruginosa* strains of clinical as well as environment origin to explore the possibility of clonality and source of drug resistance genes. The observations made in the present study indicate that the selection pressure exerted by a variety of antimicrobials in clinical environment has led to an extensive accumulation of drug resistance in the clinical isolates. Furthermore, detection of 24% of *P. aeruginosa* strains as multidrug resistant and 8% resistant to imipenem is alarming. While it seems that isolates from animals, soil and plants were not under much selection pressure, resistance in some of the water sample isolates against many of the anti-pseudomonal agents may be linked with clinical sources. However, high degree of resistance against

TOB in 82% of the animal isolates, 96% and 80% against GEN and ATM, respectively in water sample isolates deserve further exploration.

Apart from natural presence of high level of the above or related antimicrobials in water generating high selection pressure, plasmid mediated transfer of some of the drug resistance markers in aquatic system may be another possible explanation as plasmid bearing GEN resistance in *P. aeruginosa* of clinical origin has already been demonstrated (Kato *et al*, 1982). Presence of metallo-β-lactamase gene along with other β-lactamases in the genome of *P. aeruginosa* has already been reported (Woodford *et al*, 1998; Chaibi *et al*, 1999; Vahaboglu *et al*, 2001). Moreover, MBL resistance gene has been reported on plasmids in many of the gram-negative bacteria, which may worsen the state of antimicrobials in combating the bacterial infections (Kumarasmy *et al*, 2010). Intrinsic resistance to a majority of non-pseudomonal agents with the exception of some of the synthetic cephalosporins *viz*, CXM, CTX and CRO, in all the isolates of the bacteria irrespective of their origin indicates presence of structurally similar/ related molecules in the environment.

There are reports suggesting lack of clonality in clinical isolates of *P. aeruginosa* as they are indistinguishable from environmental isolates genotypically, chemotaxonomically and functionally (Rahme *et al*, 1995; Foght *et al*, 1996; Alonso *et al*, 1999) consistent with our findings. The clinical isolates based on ERIC-PCR analysis could be seen distributed in all the 3 major clusters grouped with strains from water, plant, animals and soil. However, it is interesting to note that despite the wide variations in antimicrobial susceptibility patterns, there were 10 clones by ERIC-PCR analysis. Furthermore, 80% of the clinical isolates could be linked with only 3 such clones. Do these 3 clones have some special virulence markers? This question needs to be addressed by detailed genomic and proteomic studies of these strains.

This is the first study involving different environmental strains where clonality has been observed. Clonal occurrence of the bacteria is further supported by the observation of 80% of plant isolates as single clone in cluster III-d. Similarly, cluster IV had 6 strains (40%,) of animal origin 4 and 2 being identical. Therefore, based on these observations, the clonal nature of *P. aeruginosa* cannot be denied, although, more studies are needed. The viable non-clonal epidemic/endemic population of *P. aeruginosa* in clinical environment may occur due to the fact that the bacteria cause infection primarily in immunocompromised host or tissue and thus any of the environmental strain can colonize and multiply as it was growing in the non human environment.

In conclusion, despite the strain diversity among clinical isolates, extremely high level of accumulation of drug resistance suggests that efficient and sustained control measures and proper antibiotic policy is a must. In addition, the clonality in *P. aeruginosa* and its implications in clinical infection need to be addressed.

ACKNOWLEDGEMENTS

REFERENCES

Agarwal G, Kapil A, Kabra S K, Chandra R, Das B, Diwedi S N. Phenotypic and genotypic variants of *Pseudomonas aeruginosa* isolated

from children with cystic fibrosis in India. *Indian J Med Res* 2002; 116: 73-81.

Agarwal G, Kapil A, Kabra SK, Das BK, Diwedi SN. Characterization of *Pseudomonas aeruginosa* isolated from chronically infected children with cystic fibrosis in India. *BMC Microbiol* 2005; 5: 43.

Agnihotri N, Gupta V, Joshi RM. Aerobic bacterial isolates from burn wound infections and their antibiograms a five-year study. *Burns* 2004; 30: 241-3.

Alonso A, Rojo F, Martinez JL. Environmental and clinical isolates of *Pseudomonas aeruginosa* show pathogenic and biodegradative properties irrespective of their origin. *Environ Microbiol* 1999; 1: 421-30.

Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disc method. *Am J Clin Pathol* 1966; 45: 493-6.

Canton R, Coque TM, Baquero F, *et al*. Multi-resistant Gram-negative bacilli: from epidemics to endemics. *Curr Opin Infect Dis* 2003; 16: 315-25.

Chaibi EB, Sirot D, Paul G, Labia R. Inhibitor-resistant TEM lactamases: phenotypic, genetic and biochemical characteristics. *J Antimicrob Chemother* 1999; 43: 447-58.

Davison J. Genetic exchange between bacteria in the environment. *Plasmid* 1999; 42: 73-91.

Foght JM, Westlake DWS, Johnson WM, Ridgway HF. Environmental gasoline-utilizing isolates of *Pseudomonas aeruginosa* are taxonomically indistinguishable by chemotaxonomic and molecular techniques. *Microbiology* 1996; 142: 2333-40.

Garau G, Bebrone C, Anne C, Galleni M, Frere JM, Dideberg O. A metallo-β-lactamase enzyme in action: Crystal structure of the monozinc carbapenemase CphA and its complex with biapenem. *J Mol Biol* 2005; 345: 785-95.

Gouvea V, Allen JR, Glass RI, *et al*. Detection of group B and C rotaviruses by polymerase chain reaction. *J Clin Microbiol* 1991; 29: 519-23.

Hancock RE. Resistance mechanisms in *Pseudomonas aeruginosa* and other nonfermentative gram- negative bacteria. *Clin Infect Dis* 1998; (suppl 1): S93-925.

Hsueh PR, Liu CY, Luh KT. Current status of antimicrobial resistance in Taiwan. *Emerg Infect Dis* 2002; 8: 132-7.

Jones RN. Global epidemiology of antimicrobial resistance among community-acquired and nosocomial pathogens: a five-year summary from the SENTRY Antimicrobial Surveillance Program (1997-2001). *Semin Respir Crit Care Med* 2003; 24: 121-34.

Kato T, Sato Y, Iyobe S, Mitsuhashi S. Plasmid mediated gentamicin resistance of *Pseudomonas aeruginosa* and its lack of expression in *Escherichia coli*. *Antimicrob Agents Chemother* 1982; 22: 358-63.

Kenchappa P, Rao KR, Ahmed N, *et al*. Fluorescent amplified fragment length polymorphism (FAFLP) based molecular epidemiology of hospital infections in a tertiary care setting in Hyderabad, India. *Infect Genet Evol* 2006; 6: 220-7.

Kenchappa P, Sangwan VS, Ahmed N, *et al*. High-resolution genotyping of *Pseudomonas aeruginosa* strains linked to acute post cataract surgery endophthalmitis outbreaks in India. *Clin Microbiol Antimicrob* 2005; 4: 19.

Kumarasamy KK, Toleman MA, Timothy RW, *et al*. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect Dis* 2010; 10: 597-602.

Landman D, Quale JM, Mayorga D, *et al*. City-wide clonal outbreak of multiresistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in Brooklyn, NY: the preantibiotic era has returned. *Arch Intern Med* 2002; 162: 1515-20.

National Committee for Clinical Laboratory Standards (NCCLS). Performance standards for antimicrobial disk susceptibility tests. 7th ed. Approved standards: M2-A7 and supplemental tables. M 100-S 10 (M2).

Wayne, PA: NCCLS, 2000.

Navon-Venezia S, Ben-Ami R, Carmeli Y. Update on *Pseudomonas aeruginosa* and *Acinetobacter baumannii* infections in the healthcare setting. *Curr Opin Infect Dis* 2005; 18: 306-13.

Pandey A, Malenie R, Asthana AK. Beta-lactamase producing *Pseudomonas aeruginosa* in hospitalized patients. *Indian J Pathol Microbiol* 2005; 48: 530-3.

Pawar M, Mehta Y, Khurana P, Chaudhary A, Kulkarni V, Trehan N. Ventilator-associated pneumonia: Incidence, risk factors, outcome, and microbiology. *J Cardiothorac Vasc Anest* 2003; 17: 22-8.

Phillips I. Identification of *Pseudomonas aeruginosa* in the clinical laboratory. *J Med Microbiol* 1969; 2: 9-16.

Rahme LG, Stevens EJ, Wolfort SF, Shao J, Tompkins RG, Ausubel FM. Common virulence factors for bacterial pathogenicity in plants and animals. *Science* 1995; 268: 1899-902.

Rohlf FJ. NTSYS-pc. Numerical taxonomy and multivariate analysis system, Version 1.80. Setauket, NY: Exeter Software, 1994.

Romesburg HC. Cluster analysis for researchers. Belmont: Lifetime Learning Publications, 1984.

Scott FW, Pitt TL. Identification and charac-terization of transmissible *Pseudomonas aeruginosa* strains in cystic fibrosis patients in England and Wales. *J Med Microbiol* 2004; 53: 609-15.

Sharma R, Sharma CL, Kapoor B. Antibacterial resistance: current problems and possible solutions. *Indian J Med Sci* 2005; 59: 120-9.

Shashikala, Kanungo R, Srinivasan S, Devi S. Emerging resistance to carbapenems in hospital acquired *Pseudomonas* infection: A cause for concern. *Indian J Pharmacol* 2006; 38: 287-8.

Tambic AA, Tambic T, Kalenic S, Jankovic V. Surveillance for antimicrobial resistance in Croatia. *Emerg Infect Dis* 2002; 8: 14-8.

Vahaboglu H, Coskunkan F, Tansel O, *et al*. Clinical importance of extended-spectrum β-lactamase (PER-1-type)-producing *Acinetobacte*r spp and *Pseudomonas aeruginosa* strains. *J Med Microbiol* 2001; 50: 642-5.

Versalovic LJ, Koeuth T, Lupski JR. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Res* 1991; 19: 6823-31.

Woodford N, Adebiyi AMA, Palepou MFI, Cookson BD. Diversity of VanA glycopeptides resistance elements in enterococci from humans and nonhuman sources. *Antimicrob Agents Chemother* 1998; 42: 502-8.