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Typing of *Aeromonas* isolates from children with diarrhoea & water samples by randomly amplified polymorphic DNA polymerase chain reaction & whole cell protein fingerprinting

S.V. Alavandi, S. Ananthan & N.P. Pramod

Department of Microbiology, Dr. ALM Post-Graduate Institute of Basic Medical Sciences Chennai, India

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Background & objectives : ***Aeromonas*** **spp. are water-borne organisms, often associated with childhood diarrhoea. The present study was conducted to examine the epidemiological relationship among the** ***Aeromonas*** **spp. isolated from water and children with acute diarrhoea in Chennai.**

Methods : **Thirty six** ***Aeromonas*** **isolates inclusive of 16 from children with diarrhoea, 15 from domestic water samples and 5 reference strains were studied by randomly amplified polymorphic DNA polymerase chain reaction (RAPD-PCR). Twenty eight** ***Aeromonas*** **isolates, 15 from children with diarrhoea, 10 from domestic water samples and three reference strains were analysed by SDS-PAGE for their whole cell protein profiles.**

Results : **The 36** ***Aeromonas*** **isolates examined by RAPD-PCR generated RAPD fingerprints with majority of the bands ranging from about 250 to 2800 bp. The RAPD fingerprints did not correspond with the phenospecies and varied greatly among the strains within the phenospecies. Cluster analysis revealed two major groups at 75 per cent hierarchical level, comprising 18** ***Aeromonas*** **isolates, mainly recovered from domestic water samples, while the clinical isolates were scattered in different hierarchical levels in the dendrogram.**

The whole cell protein fingerprints examined by SDS-PAGE did not correspond with the phenospecies. Only four isolates of ***A. caviae*** **were found to produce similar protein fingerprints allowing them to form a cluster at about 90 per cent hierarchical level, while the rest of the isolates were scattered at various hierarchical levels in the dendrogram.**

Interpretation & conclusions : **In the present study, RAPD fingerprinting was found to be useful in distinguishing** ***Aeromonas*** **isolates recovered from clinical and domestic water supplies. However, RAPD-PCR could not distinguish the phenospecies of the genus** ***Aeromonas*****. Whole cell protein fingerprinting and cluster analysis could neither differentiate isolates from clinical and domestic water sources nor the phenospecies of the genus** ***Aeromonas*****.**

Key words *Aeromonas* - cluster analysis - dendrogram - diarrhoea - domestic water - epidemiology - protein fingerprinting - RAPD-PCR - SDS-PAGE

Aeromonads have been implicated as human pathogens over the past four decades. They have been reported to be associated with diarrhoea in children both in the developed and the developing world[1-3]. These organisms have also been found to be involved in wound infections, haemolytic uremic

syndrome, meningitis, peritonitis, respiratory tract infections and ocular infections[4].

In our earlier reports, we have described isolation of *Aeromonas* spp. from cases of childhood gastroenteritis and domestic water samples[5,6]. Several authors have reported that the aeromonads occurring in the clinical samples were different from those found in the water samples by techniques such as gas liquid chromatography of fatty acid methyl esters[7] and ribotyping[8]. Millership and Want[9] using whole cell protein fingerprinting and cluster analysis reported that epidemiologically related isolates of *Aeromonas* species were found in the same clusters.

Numerous genotyping methods such as chromosomal restriction fragment length polymorphism[10], plasmid profiling[11], ribotyping[12] and pulsed field gel electrophoresis[13] have been employed for typing *Aeromonas* spp. The restriction fragment length polymorphism (RFLP) although specific, has been found to be inadequate in providing interpretable results. Since the plasmid profiles are relatively unstable, it may not be useful in genomic typing. Ribotyping and pulsed field gel electrophoresis methods are reported to be very much useful for genomic typing. However, these methods are relatively expensive and laborious. Randomly amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) also called arbitrarily primed PCR (AP-PCR), involving use of small random primers and low stringency annealing conditions[14,15] is reported to provide better discrimination of genomic finger-prints and is easier to perform than the other genotyp-ing methods.

The present study was conducted to examine the *Aeromonas* spp. recovered from children with acute diarrhoea and domestic water samples in Chennai by RAPD-PCR and whole cell protein fingerprinting to distinguish the phenospecies of *Aeromonas* isolates and to study their epidemiological relationship.

Material & Methods

Bacterial isolates : For RAPD fingerprinting, 36 *Aeromonas* isolates inclusive of 16 from children with acute diarrhoea, 15 from domestic water samples and five reference strains (from Dr. Toshio Shimada, National Institute of Infectious Diseases, Tokyo, Japan), were used. These isolates had been phenotyped to species level earlier[5,6]. The 31 isolates from clinical sources and water comprised 5 isolates of *A. hydrophila,* 12 *A. sobria,* 13 *A. caviae* and one isolate of *A. veronii* (Table). For whole cell protein fingerprinting, 28 strains of *Aeromonas* spp., 15 recovered from children suffering from diarrhoea, 10 isolated from domestic water samples, and three reference strains comprising 7 isolates of *A. hydrophila,* 9 isolates of *A. sobria* and 12 isolates of *A. caviae* were included (Table).

Aeromonas isolates from children with acute diarrhoea were obtained during February 1997 and January 1998 and those from domestic water samples were isolated during May to October 1998.

RAPD fingerprinting :

(*i*) Extraction of genomic DNA — Bacterial DNA was extracted as per the method described by earlier workers[16]. The dry DNA pellet was suspended in 100 µl of tris-EDTA (ethylene diamine tetra acetic acid) (TE) buffer. Concentration of the DNA extracts was determined by measuring the optical density (OD) of an aliquot of the extracts (OD_{260}=1 corresponds to 50 µg DNA per ml) using an UV VIS spectrophotometer (Shimadzu, Japan).

(*ii*) Primer — Arbitrary primers of 10 bp length AP-3 (5'-TCACGATGCA-3') and AP-5 (5'-TCACGCTGCG-3'), employed previously by Talon *et al*[17] were used in the present study for RAPD profiling of the *Aeromonas* isolates. The primers were custom made and obtained from Genei (Bangalore, India).

(*iii*) Polymerase chain reaction — PCR kit was purchased from Genei (Bangalore) and the reaction was carried out according to the manufacturer's instructions. The PCR reaction was conducted in a final reaction volume of 25 µl containing 40 ng of DNA template extracted from the *Aeromonas* isolates, 200 µ*M* each of the four deoxy nucleotide triphosphates, 3 µ*M* $MgCl_2$, 1µ*M* primer and 0.5 units of Taq DNA polymerase.

Table. *Aeromonas* isolates employed for RAPD-PCR and whole cell protein fingerprinting

Standard strains	Clinical source	Domestic water
A. hydrophila (Ah. ATCC 7966)*	*A. sobria* (As. 38a)*	*A. caviae* (Ac.7)
A. sobria (As. CIP 224)*	*A. caviae* (Ac.359)*	*A. sobria* (As. 9)*
A. caviae (Ac.ATCC 13137)*	*A. sobria* (As. 374)*	*A. sobria* (As. 11)
A. jandaei (Aj 307-94)	*A. caviae* (Ac. 544)*	*A. hydrophila* (Ah. 12)*
A. trota (At. JCM 8315)	*A. sobria* (As. 6143)*	*A. hydrophila* (Ah.13)*
	A. sobria (As. 811)*	*A. sobria* (As. 26)
	A. hydrophila (Ah. 8143)*	*A. caviae* (Ac. 31)*
	A. caviae (Ac. 96)*	*A. hydrophila* (Ah. 62)
	A. caviae (Ac. 113)*	*A. caviae* (Ac. 65)
	A. caviae (Ac. 1154)*	*A. sobria* (As. 66)*
	A. caviae (Ac. 1164)*	*A. sobria* (As. 67)
	A. caviae (Ac. 1233)*	*A. hydrophila* (Ah. 71)*
	A. caviae (Ac. 1235)*	*A. hydrophila* (Ah. 86)*
	A. caviae (Ac. 1262)*	*A. veronii* (Av. 88)
	A. caviae (Ac. 121)*	*A. sobria* (As. 94)*
	A. sobria (As. WA1)	*A. sobria* (As. 110)*
		A. sobria (As. 115)

All isolates except Ah. 86 and As. 110 were typed by RAPD-PCR. Isolates with asterisk were typed by whole-cell protein fingerprinting

The reaction mixtures were denatured at 92°C for 2 min and then subjected to 45 cycles of denaturation at 92°C for 1 min, annealing at 36°C for 1 min and extension at 72°C for 2 min, and a final extension at 72°C for 3 min in a thermocycler (MJ Research, MA, USA).

The amplified RAPD products were electrophoresed on 1.5 per cent Tris acetate EDTA gels at 65 volts. pBR 322 DNA double digest (Apa LI/Bst NI) and Hind III digest of λ DNA were included as molecular weight markers (MBI Fermentas, Lithuania). The gels were stained with 0.5 µg per ml of ethidium bromide and photographed under UV trans-illumination.

(*iv*) Reproducibility of RAPD profile — Two isolates of *Aeromonas* (*A. caviae* Ac. 359 and *A.sobria* As. 6143) were subjected to DNA extraction twice separately and 40 ng of DNA of each extract was amplified as per the procedure given above and the RAPD fingerprints were examined on 1.5 per cent agarose gels. The experimental protocol was standardized to obtain identical profiles, and then the RAPD-PCR fingerprinting was carried out for all the isolates.

Protein profiles of Aeromonas isolates :

(*i*) Preparation of bacterial protein samples — Isolates of *Aeromonas* were grown in brain heart infusion broth (Hi Media, Mumbai, India) at 37°C for 24 h and the bacterial cells were harvested by centrifugation at 2200 *g* at 4°C for 10 min. The cell pellets were washed thrice with sterile phosphate buffered saline (PBS, *p*H 7.4) and finally suspended in 1 ml of sterile PBS. These cell suspensions were sonicated for 20 sec using a microtip probe attached

to an ultrasonicator (Vibrasonic, Germany). The sonicated samples were centrifuged at 12000 *g* at 4°C for 15 min and the supernatants were collected in fresh tubes and preserved at -20°C. The protein concentration of these extracts was determined by Lowry's method[18].

(*ii*) Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) — The samples were appropriately diluted using sample buffer to obtain a μg/μl suspension and 25μl of each extract was subjected to SDS-PAGE using 10 per cent gel[19]. Electrophoresis was performed at 20 mA when the samples were in stacking gel, and at 30mA in resolving gel. In each run, a protein molecular weight standard (Genei, Bangalore) was included. The electrophoresis was stopped when the bromophenol blue tracking dye was about 1-1.5 cm from the bottom of the gel. The protein bands were stained with Coomassie brilliant blue and photographed. The banding pattern in each sample (track) was scanned using a laser densitometer (LKB Broma, Germany) to analyse the differences in the protein fingerprints produced by different isolates.

Analysis of phylogenetic relationship : The RAPD fingerprinting and protein banding data were digitalized and phylogenetic tree was constructed using PHYLIP phylogeny inference package 3.5c computer programme (downloaded from the World Wide Web).

Results

Out of a total of 36 *Aeromonas* isolates examined for their RAPD fingerprints, 30 isolates produced four or more bands, while two isolates (As. 38a and Ac. 1233) produced three bands, one isolate (Aj. 307-94) two bands and another three isolates [two isolates of *A. caviae* from clinical source (Ac. 1164 and Ac. 121), and one isolate of *A. sobria* (As. 11) from water] did not produce any amplification (Fig. 1). The RAPD banding pattern is diagrammatically represented in Fig. 2, and it could be observed that, most isolates produced bands in the molecular weight range of about 225 to 2750 bp. *A. jandaei* (Aj. 307-94) produced only two bands one of molecular weight of about 420 and another of about 8000 bp (Fig. 1). The bands with molecular weight above 3000 bp are not shown in Fig. 2 as those would have distorted the figure. One of the clinical isolates of *A. caviae* (Ac. 544) produced two faint high molecular weight bands of about 6800 and 6930 bp in addition to seven bands in the range of about 510-2550 bp. Another isolate of *A. caviae* (Ac. 1262) also produced two faint bands in the molecular weight sizes of 4200 and 4600 bp.

Based on the RAPD fingerprints in the agarose gels, a phylogenetic tree (dendrogram) was constructed (Fig. 3). At 90 per cent hierarchical level, 18 phenons could be defined. Among the various *Aeromonas* spp., only three isolates of *A. caviae* at phenon 14, formed a small cluster, while, all the other *Aeromonas* isolates were found dispersed in various clusters, indicating dissimilarity of their RAPD fingerprints. At 75 per cent hierarchical level (marked by dotted box), 18 *Aeromonas* isolates, mainly recovered from domestic water samples, were clustered.

RAPD fingerprinting and cluster analysis has revealed genetic diversity among the *Aeromonas* spp. and further, it is found that the fingerprints did not correspond to phenospecies of *Aeromonas*. However, the isolates recovered from domestic water supplies and clinical samples could be distinguished more or less clearly, although, four clinical isolates were also clustered along with two larger branches of isolates from domestic water sources.

Twenty eight *Aeromonas* isolates comprising 7 strains of *A. hydrophila*, 9 strains of *A. sobria* and 12 strains of *A. caviae* when subjected to SDS-PAGE of their whole cell protein extracts produced 30-35 bands (Fig. 4). The protein fingerprints could be deciphered clearly upon scanning the gels using the laser densitometer (Fig. 5). Each peak represented a stained protein band. The absorbance values of the protein bands ranged from 0.38-0.74. The data obtained on the number of peaks and relative mobility of the bands were used for construction of a dendrogram. The dendrogram revealed 14 phenons at about 85 per cent hierarchical level (Fig. 6). Out of the 7 isolates of *A. hydrophila*,

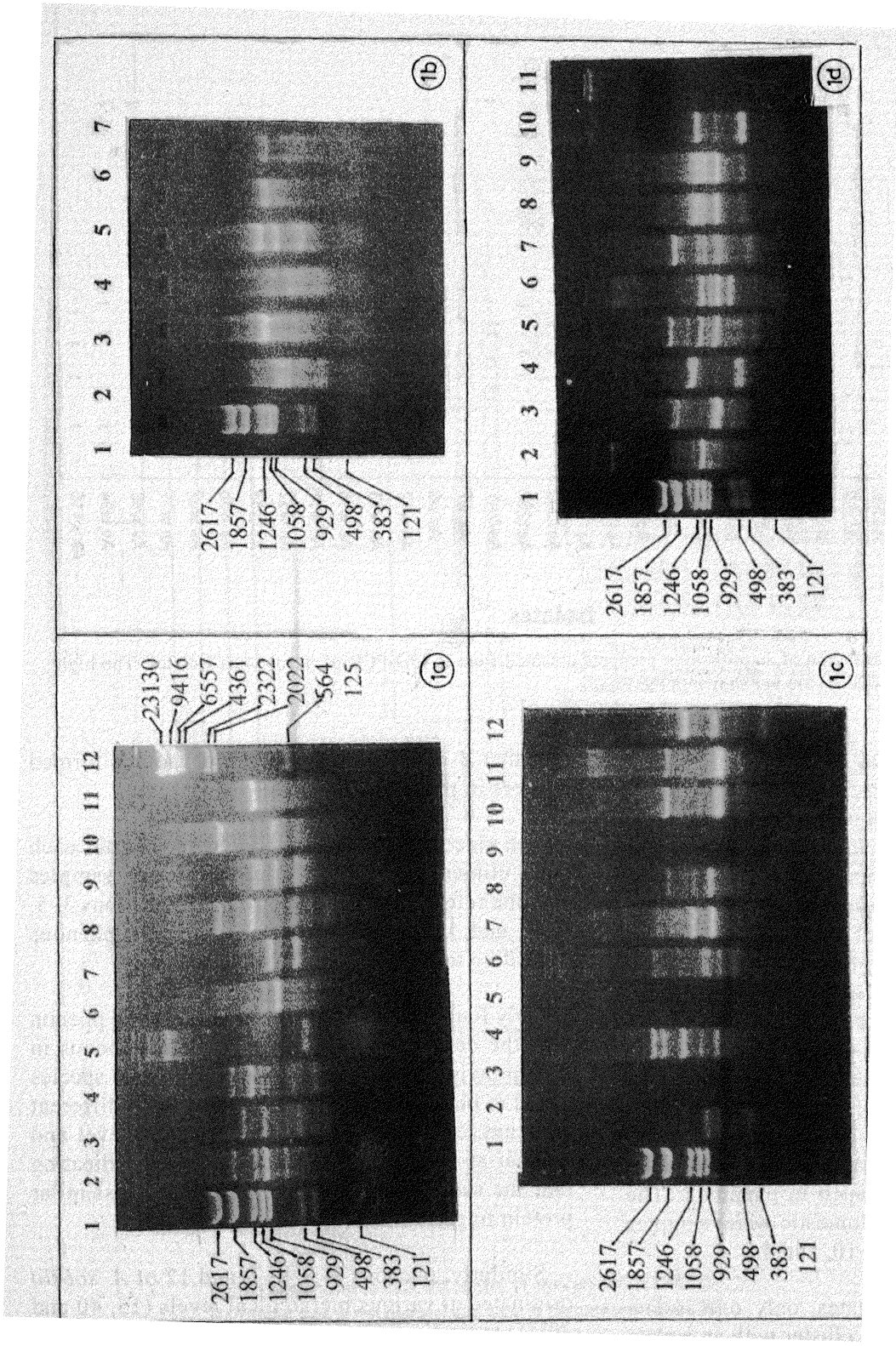


Fig 1. RAPD fingerprints of *Aeromonas* isolates **Fig. 1a.** Lane 1 : Molecular weight marker [pBR 322 DNA double digest (Apa LI/Bst NI)], Lane 2 : *A. hydrophila* (Ah. ATCC 7966), Lane 3: *A. sobria* (As. CIP 224), Lane 4 : *A. caviae* (Ac. ATCC 13137), Lane 5 : *A. jandaei* (Aj. 307-94), Lane 6 : *A. trota* (At. JCM 8315), Lane 7: *A. sobria* (As. 38a), Lane 8 : *A. caviae* (Ac 359), Lane 9 : *A. sobria* (As. 374), Lane 10 : *A. caviae* (Ac. 544), Lane 11 : *A.sobria* (As. 6143), Lane 12 : Molecular weight marker (λ DNA Hind III digest). **Fig. 1b.** Lane 1 : Molecular weight marker [pBR 322 DNA double digest (Apa LI/Bst NI)], Lane 2; *A. sobria* (As. 811), Lane 3: *A. hydrophila* (Ah. 8143), Lane 4: *A. caviae* (Ac. 96), Lane 5 : *A. caviae* (Ac. 113), Lane 6 : *A caviae* (Ac. 1154), Lane 7 : *A. caviae* (Ac. 1235). **Fig. 1c.** Lane 1 : Molecular weight marker [pBR 322 DNA double digest (Apa LI/Bst NI)], Lane 2 : *A caviae* (Ac. 1233), Lane 3: *A. caviae* (Ac. 1164), Lane 4 : *A. caviae* (Ac. 1262), Lane 5 : *A. caviae* (Ac.121), Lane 6 : *A. sobria* (As. WA1), Lane 7: *A. caviae* (Ac. 7), Lane 8 : *A. sobria* (As. 9), Lane 9 : *A. sobria* (As. 11), Lane 10 : *A. hydrophila* (Ah. 12), Lane 11 : *A. hydrophila* (Ah. 13), Lane 12: *A. sobria* (As. 26). **Fig. 1d.** Lane 1 : Molecular weight marker [pBR 322 DNA double digest (Apa LI/Bst NI)], Lane 2 : *A.caviae* (Ac. 31), Lane 3 : *A. hydrophila* (Ah. 62), Lane 4 : *A. caviae* (Ac. 65), Lane 5 : *A. sobria* (As. 66), Lane 6 : *A.sobria* (As. 67), Lane 7 : *A. hydrophila* (Ah. 71), Lane 8 : *A. veronii* (Av. 88), Lane 9 : *A.sobria* (As. 94), Lane 10 : *A. sobria* (As. 115), Lane 11 : Negative control.

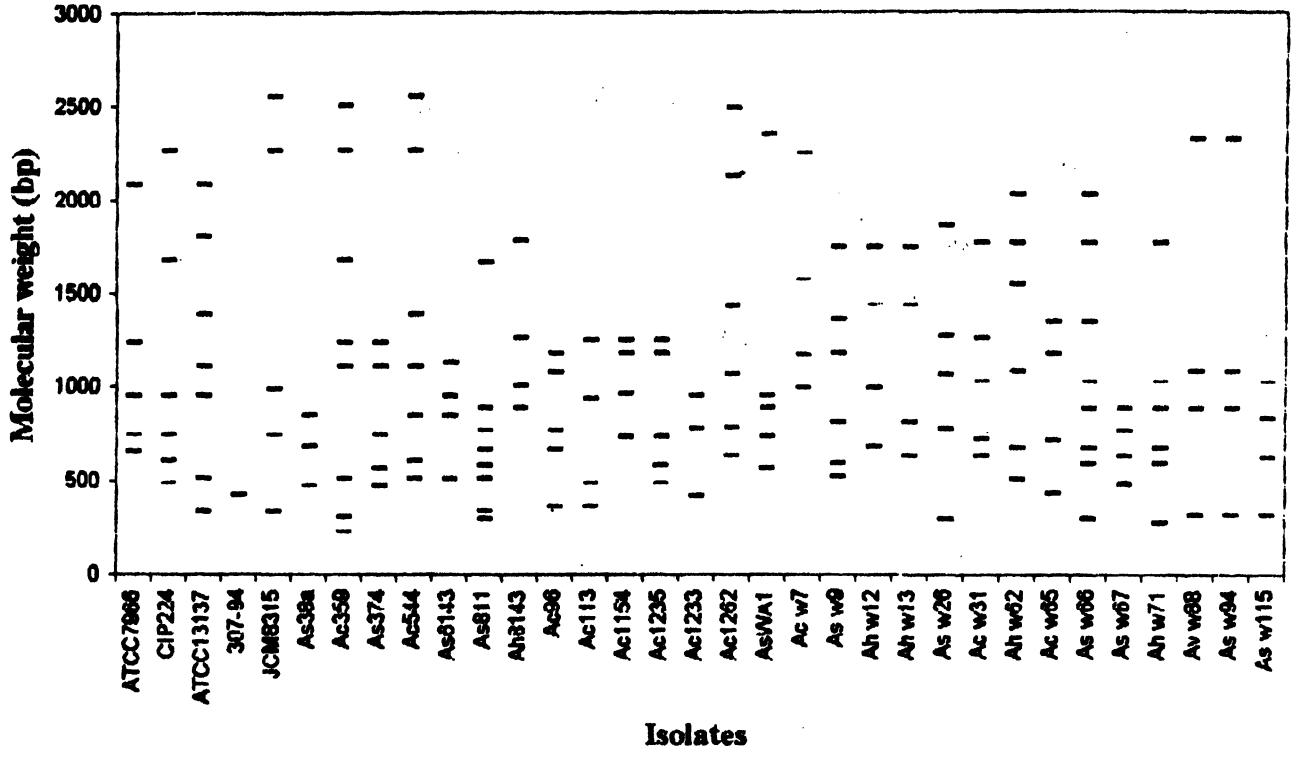


Fig. 2. Diagrammatic representation of amplification products obtained from RAPD-PCR of *Aeromonas* isolates. The high molecular weight bands (>3000bp) are not shown in the Figure.

phenons of four isolates was defined at 5 per cent hierarchical level (phenons 1, 2, and 14) and among the remaining three isolates, two isolates were clustered along with *A. sobria* in phenons 5 and 13 and one separately in phenon 10. At 85 per cent hierarchical level, the remaining 11 phenons were defined (3-13). A majority of the *A. caviae* isolates were clustered in phenons 4, 8, 9 and 11, while one isolate was clustered with phenon 3 with *A. sobria*, and another with phenon 13, comprising of one isolate of *A. sobria* and one isolate of *A. hydrophila*. The *A. sobria* isolates were clustered in phenons 3, 5, 6, 7, and 12.

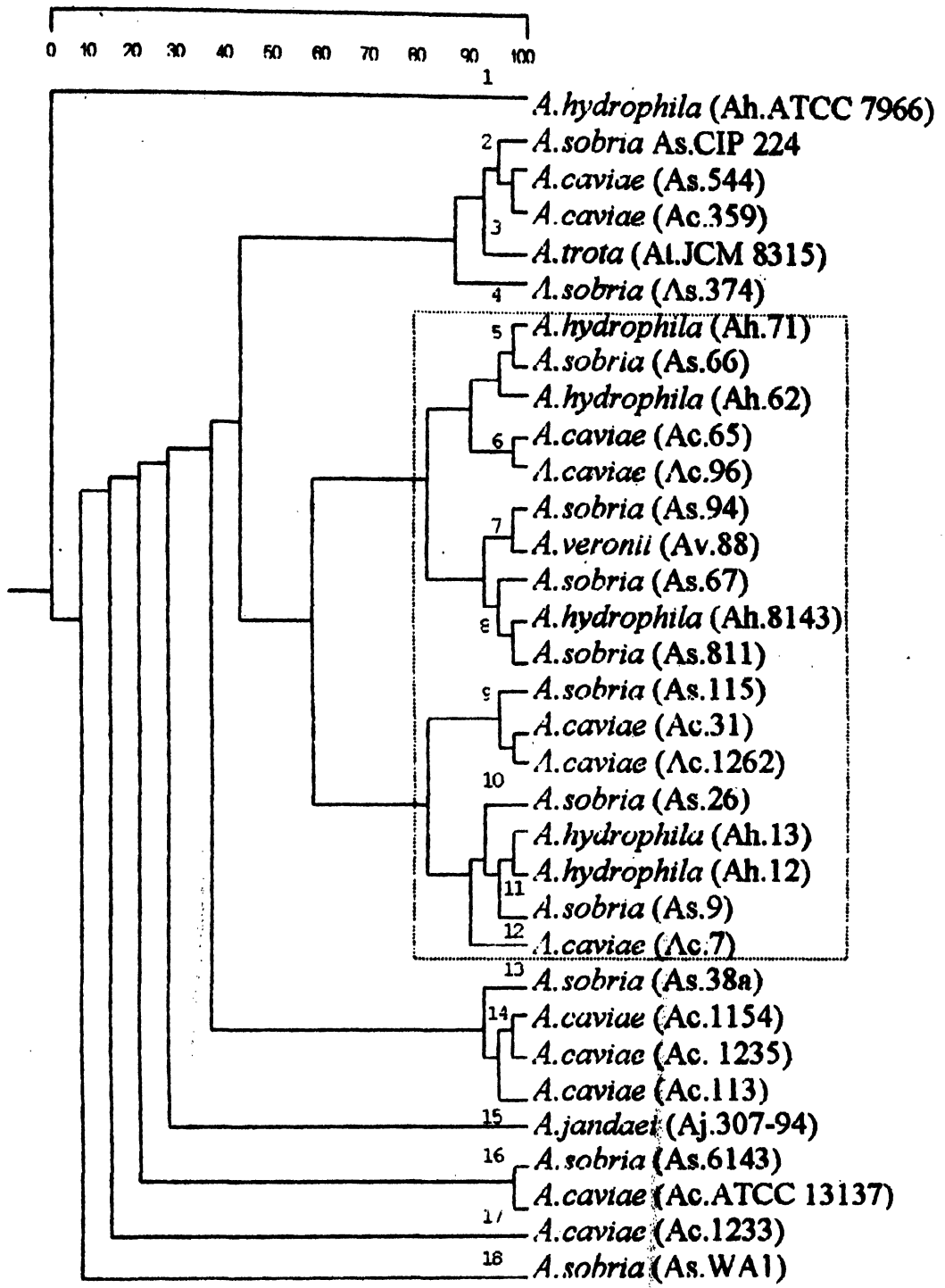
The single isolate of *A. hydrophila* from clinical source formed a separate branch in phenon 1. The *A. hydrophila* isolates from domestic water samples were clustered in phenons 2, 10, and 14.

Among the *A. caviae* isolates, only one isolate from clinical source formed a cluster with an isolate from domestic water sample in phenon 11. While all the other *A. caviae* isolates of clinical source formed clusters in phenons 4, 8, and 9.

The nine *A. sobria* isolates inclusive of four each from clinical source and domestic water samples and one reference strain were found in phenons 3, 5, 6, 7, and 12 and did not form separate phenons according to their source of isolation.

Only four isolates of *A. caviae* clustered in phenon 9 of the dendrogram appear to be homogeneous in their protein fingerprints. However, the same species found in phenons 4 and 11 originated from different branches, one at 15 per cent hierarchical level and another at 40 per cent hierarchical level, indicating that the same phenospecies can produce dissimilar protein fingerprints.

Similarly, phenons 3, 5, 6, 7, and 12 of *A. sobria* originated at various hierarchical levels (15, 40 and 70%), indicating occurrence of same phenospecies with differing protein profiles.



Dotted box shown to indicate cluster of environmental isolates
Horizontal axis is the hierarchical level in percentage similarity

Fig 3. Dendrogram of *Aeromonas* isolates from clinical and domestic water samples based on their RAPD fingerprints.

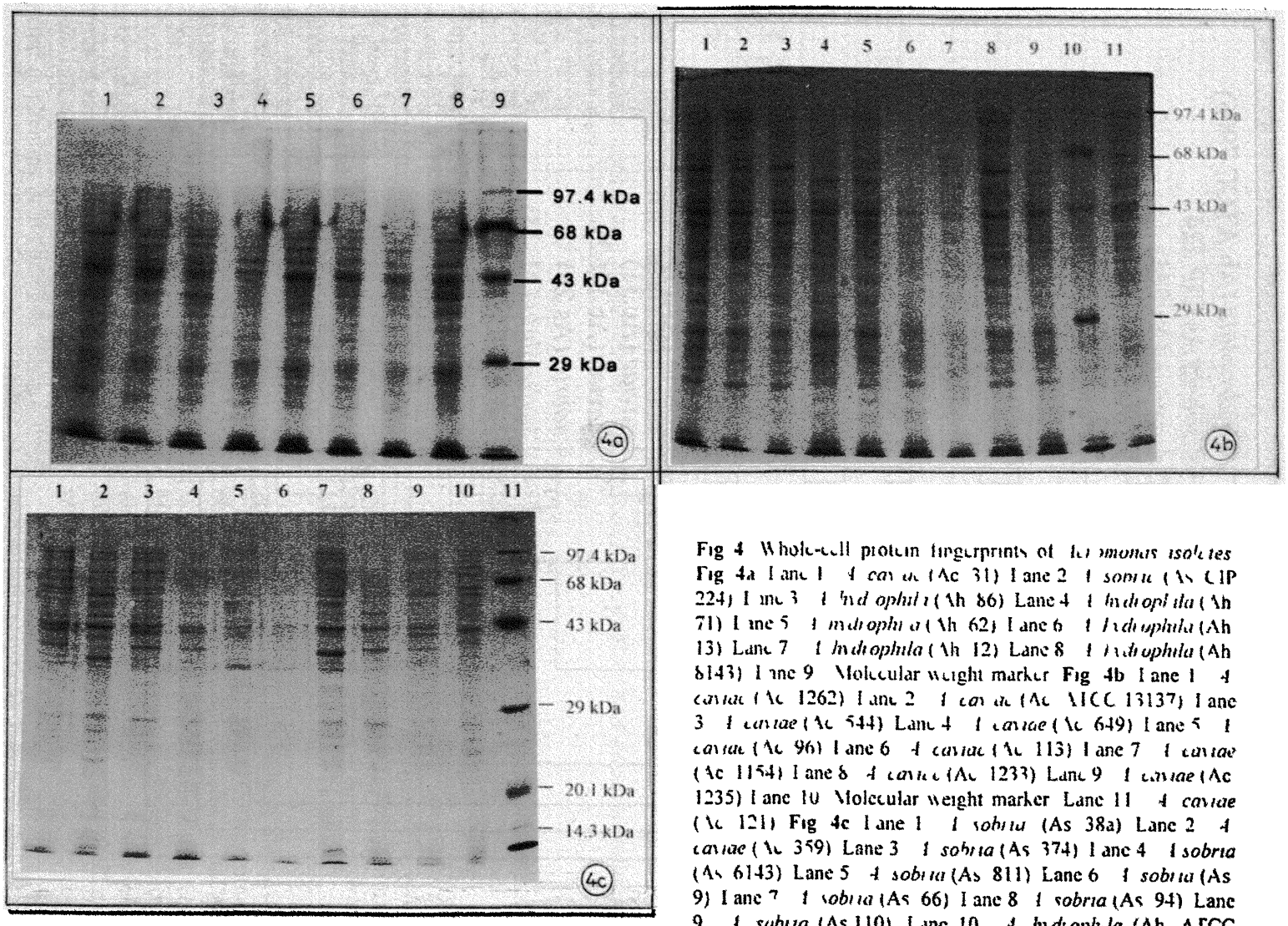


Fig 4 Whole-cell protein fingerprints of *Aeromonas isolates* Fig 4a Lane 1 *A. caviae* (Ac 31) Lane 2 *A. sobria* (As CIP 224) Lane 3 *A. hydrophila* (Ah 86) Lane 4 *A. hydrophila* (Ah 71) Lane 5 *A. hydrophila* (Ah 62) Lane 6 *A. hydrophila* (Ah 13) Lane 7 *A. hydrophila* (Ah 12) Lane 8 *A. hydrophila* (Ah 8143) Lane 9 Molecular weight marker Fig 4b Lane 1 *A. caviae* (Ac 1262) Lane 2 *A. caviae* (Ac ATCC 13137) Lane 3 *A. caviae* (Ac 544) Lane 4 *A. caviae* (Ac 649) Lane 5 *A. caviae* (Ac 96) Lane 6 *A. caviae* (Ac 113) Lane 7 *A. caviae* (Ac 1154) Lane 8 *A. caviae* (Ac 1233) Lane 9 *A. caviae* (Ac 1235) Lane 10 Molecular weight marker Lane 11 *A. caviae* (Ac 121) Fig 4c Lane 1 *A. sobria* (As 38a) Lane 2 *A. caviae* (Ac 359) Lane 3 *A. sobria* (As 374) Lane 4 *A. sobria* (As 6143) Lane 5 *A. sobria* (As 811) Lane 6 *A. sobria* (As 9) Lane 7 *A. sobria* (As 66) Lane 8 *A. sobria* (As 94) Lane 9 *A. sobria* (As 110) Lane 10 *A. hydrophila* (Ah ATCC 7966) Lane 11 Molecular weight marker

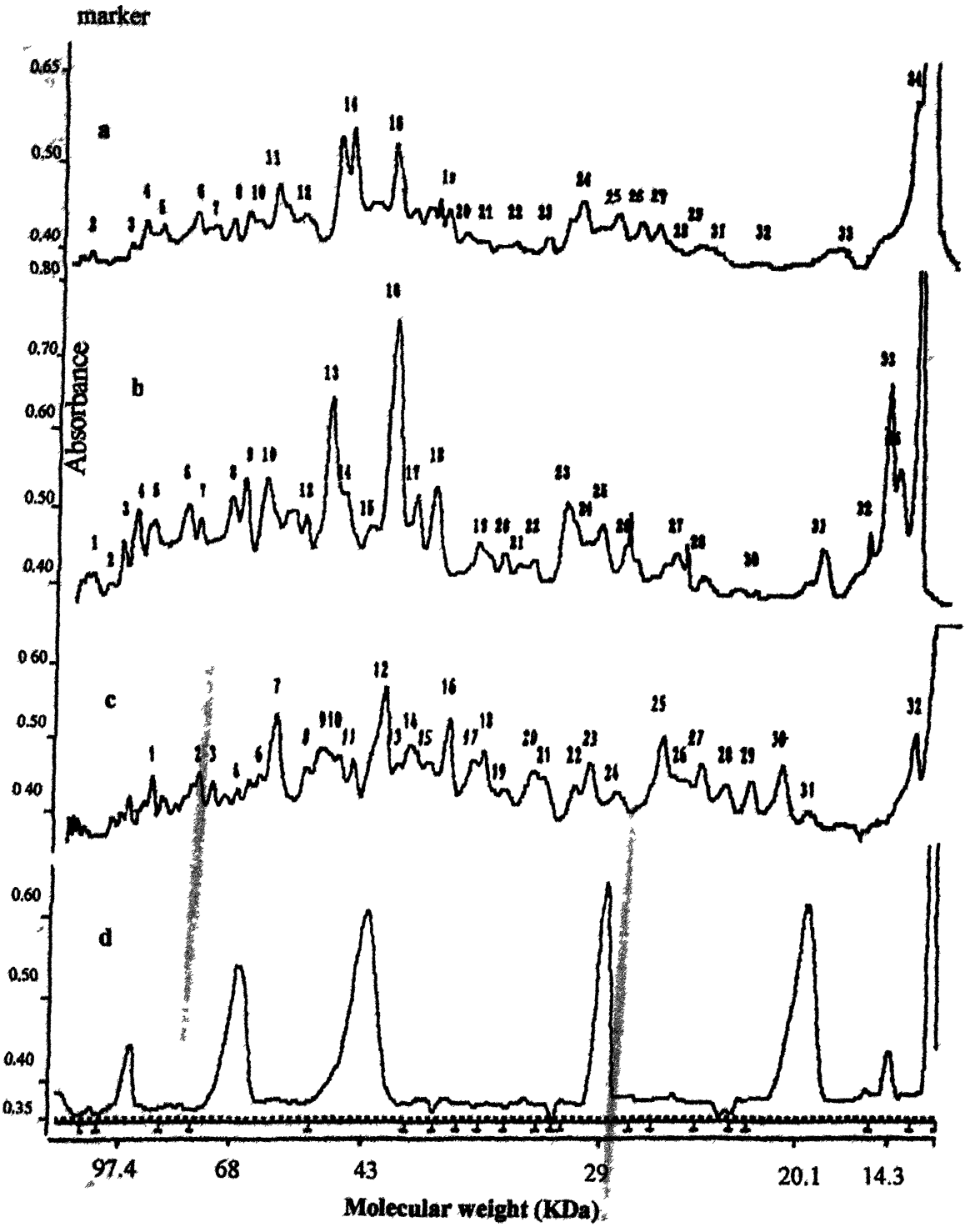


Fig. 5. One dimensional density plots of whole cell protein fingerprints of *Aeromonas* spp. **a:** *A hydrophila* (Ah. 8143), **b:** *A. sobria* (As. 38a), **c:** *A.caviae* (Ac. 544) **d:** molecular weight marker.

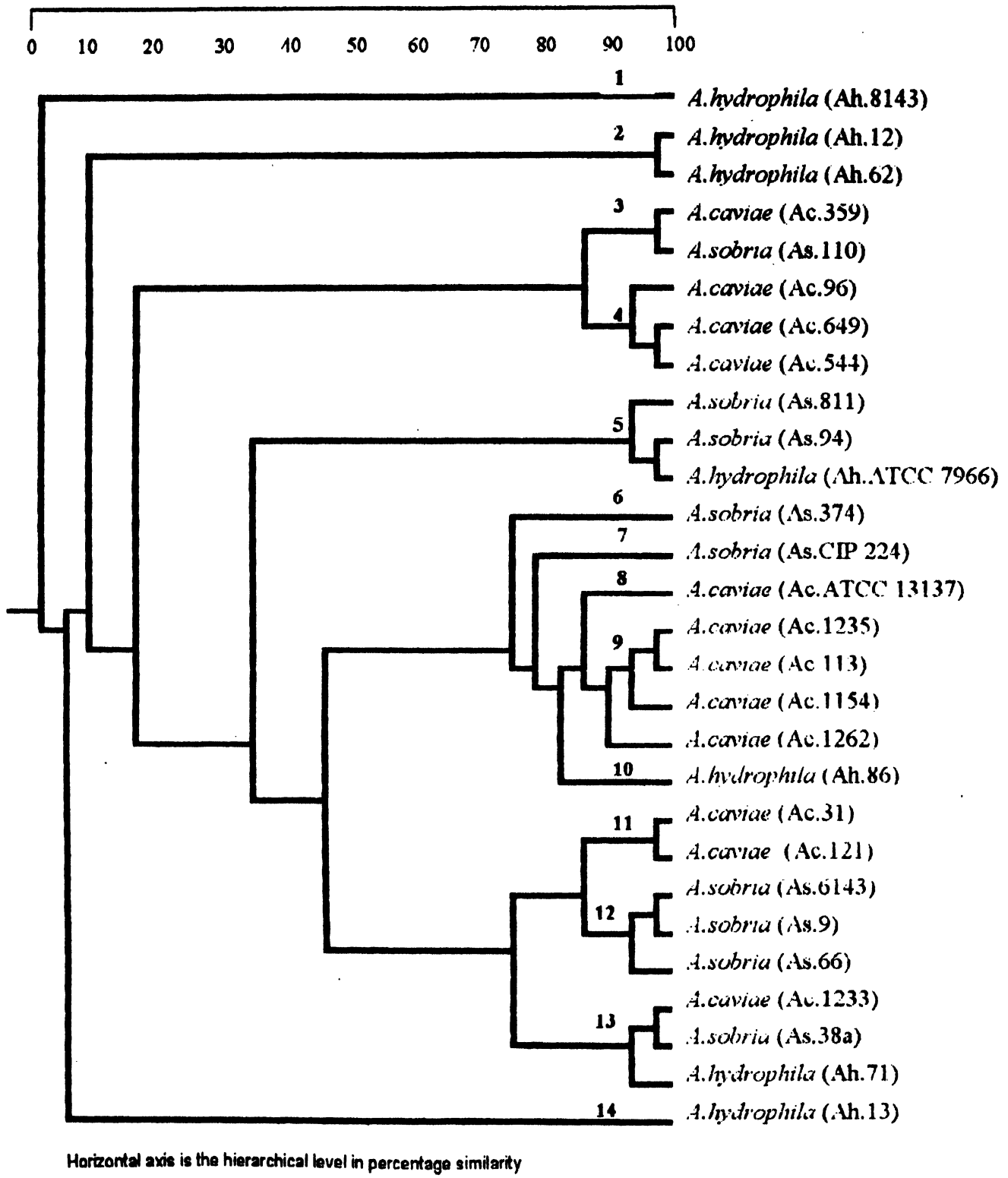


Fig. 6. Cluster analysis of *Aeromonas* isolates based on whole cell protein profile.

Among the three species, *A. hydrophila* isolates appeared to produce highly heterogeneous protein fingerprints as revealed by their distribution in different phenons in the dendrogram.

The SDS-PAGE whole cell protein fingerprinting and cluster analysis of *Aeromonas* species has revealed that the aeromonads of epidemiologically unrelated sources could not be differentiated. Protein fingerprints of the same phenospecies formed clusters at different hierarchical levels and hence, the protein fingerprints do not correspond with the phenospecies on most occasions.

Discussion

Among the 31(+5 references strains) *Aeromonas* isolates subjected to RAPD-PCR, 16 were from clinical origin, isolated from children suffering from acute diarrhoea. Fifteen of these isolates were recovered as the sole enteropathogen, while from one child *Shigella flexneri* was isolated along with *A. sobria*[5]. Nine of these isolates were cytotoxic to CHO cells, produced β-haemolysin and induced secretory response in the Ussing chambers[5]. *A. caviae* and *A.sobria* were the two predominant species found in the domestic water samples and faeces of children suffering from diarrhoea[5,6].

Majority of the *Aeromonas* isolates produced amplification products ranging from about 250 to 2800 bp size. RAPD fingerprints of similar banding pattern with bands in this molecular weight range have been demonstrated previously in a number of studies[17,20,21]. Some earlier studies have indicated that the differences in the method of DNA extraction, DNA concentration and differences in the electrophoresis time lead to variation in the RAPD fingerprints[22,23]. The method of DNA extraction employed in the present study has been reported to provide better typability of the *Aeromonas* isolates compared to boiling method of DNA extraction[16]. The RAPD pattern of two isolates examined to check the reproducibility of the banding pattern gave identical fingerprints when subjected to two separate experiments involving DNA extraction and amplification.

The human pathogenic strains and environmental strains of mesophilic *Aeromonas*, to a large extent are similar with respect to their physiological and biochemical characteristics and are usually transmitted to humans through water[24]. In our earlier studies[5,6] also, similar phenospecies of *Aeromonas*, were found in both the sources. However, the strains from the two sources varied in their RAPD fingerprints. The dendrogram constructed based on the RAPD-PCR fingerprints showed that the isolates recovered from the domestic water supply formed two major clusters with four strains from the clinical source interspersed, while the clinical isolates and the reference strains formed several small branches, indicating their difference with those from aquatic source. Several authors have also reported differences among aeromonads occurring in the clinical and environmental samples. Havelaar *et al*[7] analysed 187 isolates of *Aeromonas* strains from human diarrhoeal stools and 263 strains from drinking water samples in The Netherlands by biotyping in conjunction with gas liquid chromatography of cell wall fatty acid methyl esters and reported dissimilarity among these strains. Similarly, aeromonads isolated from public water supply in Iowa City were found by ribotyping to be unrelated to those isolated from patients with gastroenteritis[8]. DNA-DNA hybridization studies along with whole cell protein profiles studied by SDS-PAGE indicated that most *Aeromonas* strains recovered from clinical material belonged to the DNA hybridization group 1 (HG 1), which was not found in the fresh water and drinking water samples[25]. Kirov *et al*[26] also reported predominance of aeromonads of HG1 in clinical samples, while the environmental isolates belonged to HG 3. The observations by these investigators and the results obtained in the present study with respect to RAPD fingerprinting indicate that differences exist between the clinical and environmental strains of *Aeromonas* species, and that only some strains occurring in water are potentially enteropathogenic. A detailed investigation is required to ascertain what makes some of these strains pathogenic. Although Kanagawa-type of phenomenon observed in *Vibrio parahaemolyticus* is not reported in *Aeromonas*, passaging in the ligated rabbit ileal loops could induce enterotoxigenicity in the strains of *Aeromonas* spp[27].

Protein fingerprinting has been reported to usually correlate with phenospecies[28]. In the present study,

the whole cell protein fingerprints of only four isolates of *A.caviae* strains appeared to correspond to phenospecies as observed by their cluster formation in phenon 9 of the dendrogram. However, other *A.caviae* isolates were also found in clusters at 15-40 per cent hierarchical levels. Similarly, *A. sobria* isolates were found in four different clusters at 15-70 per cent hierarchical levels. The *A. hydrophila* isolates formed small separate clusters at 5-10 per cent hierarchical levels. These results indicate that the strains among the same phenospecies can produce different SDS-PAGE fingerprints. Hence, whole cell protein fingerprinting could not distinguish phenospecies of genus *Aeromonas*. These results corroborate with the earlier studies by Millership and Want[9], who also found that the whole cell protein fingerprints did not correlate with phenotypes. Further, the whole cell protein fingerprinting and cluster analysis could not distinguish epidemiologically unrelated isolates of aeromonads.

In the present study, it has been found that the RAPD fingerprinting could to a large extent differentiate the clinical strains of *Aeromonas* spp. from the environmental isolates. The RAPD fingerprinting and cluster analysis has shown that the isolates recovered from these sources are epidemiologically unrelated, and that the *Aeromonas* genus by itself is a highly heterogeneous group. However, whole cell protein fingerprinting and cluster analysis could not distinguish the aeromonads recovered from children with acute diarrhoea and domestic water samples. Further, both techniques could not distinguish the phenospecies of the genus *Aeromonas*.

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Reprint requests : Dr S. Ananthan, Professor, Department of Microbiology, Dr ALM Post-Graduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai 600 113, India.