Typing of *Aeromonas* spp. by numerical analysis of immunoblotted SDS-PAGE gels

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Summary. One hundred and three isolates of *Aeromonas* spp., collected from both environmental sources and patients, were examined by SDS-PAGE of whole cells followed by immunoblotting with polyclonal rabbit antiserum raised against whole cells of *A. sobria*. All isolates were typable, yielding 15–20 well separated bands. Reproducibility of the technique was good and discrimination excellent, yielding 30 types amongst 103 isolates. Immunoblot type was not related to biochemical phenotype. Attempts to correlate immunoblot type with serotype were unsuccessful because only 42% of the strains tested could be serotyped.

Introduction

Mesophilic members of the genus *Aeromonas* have been recognised as human pathogens for more than 30 years.¹ Aeromonads have been isolated from blood cultures in immunosuppressed and cirrhotic patients.² Less commonly, cases of cellulitis and wound infections,³ meningitis,⁴ otitis, peritonitis and endocarditis have been recorded¹ and hospital outbreaks have been described.^{5–7} These organisms have also been recognised as gastrointestinal pathogens in some studies.^{8–10}

Therefore, there is a need for a typing system that will help in understanding the epidemiology of this organism. Methods so far attempted include serotyping,¹¹ haemagglutination patterns,^{12,13} enzyme electrophoresis⁷ and whole-cell protein fingerprints.¹⁴ Serotyping has not proved very successful because many strains are not typable with currently available sera.¹¹ Haemagglutination suffers from poor reproducibility.¹³ Whole-cell protein electrophoresis has been used¹⁴ but has the disadvantage that a large number of bands (up to 60) needs to be analysed.

A further difficulty in the study of the epidemiology of the aeromonads is the considerable confusion surrounding their taxonomy. Three phenospecies, *A. caviae*, *A. sobria* and *A. hydrophila*, are generally recognised.¹⁵ However, a number of newer phenotypes, including some within the recognised groups^{16–18} and some completely new ones,¹⁹ have now been described.

Therefore, we examined immunoblotting with regard to both conventional and newer phenotypes as a means of discriminating between strains of *Aeromonas*

spp. When this method has been applied to other microbial genera, all strains have been typable,^{20–22} and reproducible electrophoretic fingerprints have been obtained, with relatively few bands which are easy to interpret.

Materials and methods

Bacterial strains

A total of 103 isolates of *Aeromonas* spp. collected from environmental sources and patients was examined (table), including six reference strains—*A. caviae* ATCC 13136, *A. schubertii* ATCC 43700, *A. veronii* ATCC 35622, *A. media* ATCC 33907, *A. sobria* ATCC 9071 and *A. hydrophila* ATCC 7965. Isolates were maintained for short periods on nutrient-agar slopes and for long term in liquid nitrogen. All strains were checked for their positive oxidase reaction, failure to grow in nutrient broth containing NaCl 6% w/v, growth in the presence of a disk containing 150 µg of O/129 (2,4 diamino-6,7-di-isopropyl pteridine), and reactions in an API 20E gallery (API systems, La Balme les Grottes, France). Further tests to assign strains to one of the phenospecies, *hydrophila*, *caviae* or *sobria*, were as previously described:²³ fermentation of lactose on MacConkey agar, growth at 42°C on horse-blood agar (HBA), haemolysis on HBA, fermentation of glucose with production of gas, fermentation of salicin and arabinose, acetylmethylcarbinol production (Voges-Proskauer) by the Barritt method,²⁴ elastase production,²⁵ aesculin hydrolysis, lysine decarboxylase by Moeller's method,²⁶ gluconate oxidation and H₂S production.

Further tests were also performed to identify newly described phenospecies.^{16,19,27} Where required, tests

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for cellobiose and mannitol fermentation and decarboxylation of arginine and ornithine were performed. Reactions in the API 20E trays for indole production and sucrose fermentation were noted and the minimum inhibitory concentration (MIC) of ampicillin was determined. An MIC of ≤ 2 mg/L was regarded as indicating sensitivity.

Sample preparation

Cell-free extracts of whole-cell proteins were prepared from cultures grown overnight on HBA. Five colonies were suspended in 1 ml of sodium dodecyl sulphate (SDS) 1% w/v and boiled for 3 min. The samples were allowed to cool to room temperature and centrifuged at 12000 rpm for 10 min. The clear supernate was removed and the protein concentration was measured by the method of Lowry²⁸ and standardised to 100 mg/L by appropriate dilution in sample buffer. The effect of different media on the expression of proteins was examined by culturing selected strains on MacConkey agar, cystine lactose electrolyte deficient (CLED) agar and deoxycholate citrate agar (DCA). Seven strains (three *A. caviae*, three *A. hydrophila* and one *A. sobria*), were serially subcultured on HBA plates at weekly intervals for 4 months. Five protein preparations of each strain were made at weekly intervals for 4 months.

Production of antibody

Antibodies to whole-cell proteins of *A. sobria* P0027 were raised in two female half-lop rabbits: 0.5 ml of a 1:1 mixture of Freund's complete adjuvant and a cell-free extract of lysed bacteria, was injected subcutaneously at a single site on three occasions 10 days apart. Two subsequent booster injections without adjuvant were administered at intervals of 15 days and the animals were test bled. Serum was collected at 2-week intervals. Both animals were given booster injections every 3–4 weeks. All serum samples were pooled and stored at -20°C in small volumes.

Electrophoresis and blotting

An acrylamide gel was used in a discontinuous Laemmli system,²⁹ with stacking and resolving gels containing acrylamide 3% w/v and 12.5% w/v respectively. A 25- μl volume of sample was loaded on to each track. Biotinylated SDS mol. wt markers (Sigma) of 97400, 58100, 39800, 29000 and 14000 Da were used. Gels were run at a constant current of 14 mA initially for stacking gel and then at 20 mA when the tracking dye entered the resolving gel. The electrophoresed proteins were transferred to a nitrocellulose membrane (0.45- μm pore size; Sigma) by capillary blotting overnight in blotting buffer (0.15 M glycine, 0.25 M Tris base in methanol 20% w/v, pH 8.3). Membranes were transferred to a bath of fat-

free milk powder 2.5% w/v in Tris buffer plus polyoxyethylene sorbitan monolaurate (Tween 20) 0.3% v/v, pH 7.6. The blots were then incubated sequentially with rabbit antiserum (diluted 1 in 20) for 2 h, biotinylated (whole) donkey anti-rabbit antibody (Amersham) for 90 min and streptavidin-biotinylated horseradish peroxidase complex (Amersham) for 90 min. Membranes were washed three times in a solution of fat-free milk powder 0.05% w/v in Tris buffer plus Tween 20 0.3% v/v after each stage. The blots were visualised with 4-carboxy 1-naphthol (4-CIN; Sigma) 3 mg/ml in methanol and H_2O_2 0.05 w/v in phosphate-buffered saline, pH 7.0. The reaction was stopped by transferring the blot to distilled water.

Analysis of gels

The apparatus and analysis are described in detail elsewhere.³⁰ Briefly, a high resolution image was scanned into an IBM PC compatible computer *via* a video camera (Model 90, with 55 mm lens and Hoya HA50 infra-red filter; Datacopy Corp., 1215 Terra Bella Ave, Mountain View, CA 94043, USA). The image (1728 $\times$ 2240 pixels on a 256 shade grey scale) was then processed in a computerised program that located each lane and scanned its length to produce a densitometric trace corrected for background absorption and averaged across the lane width. The band positions in all lanes were also corrected for gel-to-gel variation in the positions of bands in the mol. wt standard tracks, run as controls on each gel.

These density scans (fig. 3) represented stained bands as absorption peaks along a scale representing their position along the gel corrected for gel-to-gel variations in mobility. Intense bands had high absorption values. A similarity coefficient was calculated by Fourier correlation¹⁴ for every pair of lanes. The matrix of similarities was used to construct the dendrogram shown in fig. 2, with a customised program for a sum of squares agglomerative algorithm.³¹

Serology

Thirty-six wild strains of *Aeromonas* spp. were sent to the Division of Enteric Pathogens, Central Public Health Laboratory, Colindale, London for confirmation of identity and serotyping.

Results

Species identification

Among the wild strains there were 53 isolates of *A. caviae* including four clusters of isolates from the same source and 26 single strains; 21 isolates of *A. hydrophila* including five clusters and nine single strains; and 15 single isolates of *A. sobria*. There were 18 isolates from patients and 34 food and environ-

Fig. 1. Immunoblot of five *Aeromonas* strains. Tracks 1 and 2, strain E0089 (*A. hydrophila*); 3 and 5, strain E0087 (*A. hydrophila*); 6 and 7, strain P0042 (*A. caviae*); 9 and 10, strain P0029 (*A. caviae*); 11 and 12, strain P0027 (*A. sobria*). Tracks 4, 8 and 13 show mol. wt markers of sizes 97 400, 58 100, 39 800, 29 000 and 14 000 Da.

mental isolates of *A. caviae*, five clinical and 16 food and environmental isolates of *A. hydrophila*, and 11 clinical and three environmental isolates of *A. sobria*. Eight isolates could not be identified with any recognised, named group. Additional tests for newly described phenospecies did not reveal any isolates corresponding to the description of *A. schubertii*, *A. veronii* or *A. trota*.

All strains were typable by SDS-PAGE and immunoblotting, giving fingerprints with 15–20 well separated bands. Representative banding patterns of five *Aeromonas* strains are shown in fig. 1.

Immunoblot typing

Variation due to differences in staining, band width and residual variation in band position was investigated. Pairs of tracks derived from the same batch of sample and electrophoresed on the same and different gels were compared. Similarity coefficients for 68 replicate pairs analysed on the same gel showed a mean within-pair similarity of 94(SD4)%. By contrast, comparison of 86 replicate pairs analysed on different gels gave a mean within-pair similarity of 83(SD6)%. Clearly, between-gel variation reduced replicate similarities by 10% and it seemed reasonable, therefore, to correct all within-gel similarities by a

factor of 0.9, to allow direct comparison of within- and between-gel derived similarities in the calculation of the dendrogram (fig. 2).

Discrimination was also investigated. Comparison of pairs of strains of the same phenotype but epidemiologically distinct origin showed mean within-pair similarities of 55% for 87 within-gel pairs, and 43% for 87 between-gel pairs; SDs were larger for these heterologous comparisons at 17% and 14%, respectively.

On the basis of these means and SDs, isolates showing similarities > 91% (within-gel comparison) or 76% (between-gel comparison) were regarded as indistinguishable. Isolates showing similarities < 75% (within-gel comparison) or < 65% (between-gel comparison) were regarded as distinct. This left a range of 91–75% (within-gel comparison) or 76–65% (between-gel comparison) within which results were regarded as equivocal. Despite the width of this range only 10% of isolate comparisons proved equivocal.

Reproducibility

Protein preparations of each of the five strains made at intervals throughout the 4 months showed that banding patterns did not alter after repeated subculture; 91 between-gel comparisons of pairs of

Fig. 2. Dendrogram of all the analysed strains of *Aeromonas* spp. including deliberate repeats. OTU, operational taxonomic unit, i.e., isolates and replicates. Colonial variants are indicated by strain number and either: l and d, light and dark variants or s and r, smooth and rough colony variants.

tracks from these different protein preparations had a mean similarity of 83 (SD6)%, which was the same as that for between-gel comparisons of the same protein preparation. However, changes in culture medium did affect banding patterns. Additional bands in the region 29–14.3 kDa were visible after culture on MacConkey,

CLED or DCA medium, compared to profiles on HBA (results not shown).

When the data were represented as a dendrogram (fig. 2), all the replicate samples and colonial variants clustered at a hierarchical level of $\geq 95\%$. This level defined 30 immunoblot types, of which some examples

Fig. 3. One-dimensional density plots of the immunoblots of the five *Aeromonas* strains shown in fig. 1: x axis, linear on scale of log₁₀ mol. wt (actual weights are shown for clarity); y axis, intensity is in arbitrary units, with 255 indicating maximum brightness. **a**, Strain E0089, *A. hydrophila*; **b**, strain E0087, *A. hydrophila*; **c**, strain P0042, *A. caviae*; **d**, strain P0029, *A. caviae*; **e**, strain P0027, *A. sobria*. Strains E0089 and E0087, and P0042 and P0029 are considered to be the same.

are shown in fig. 3. These plots illustrate the differences between isolates considered to be one strain, and isolates considered to be different strains.

Serology

Of the 36 strains sent for serotyping, 21 (58%) were non-typable. Among the remaining strains, 12 belonged to one of two serogroups (table).

Clustering

Immunoblot groups were analysed with regard to serotype, phenotype and epidemiological clustering. Two strains of *A. caviae* in immunoblot groups 2 and 4 were both serogroup AX24 and two strains of *A. hydrophila* in immunoblot group 17 were serogroup 3. Otherwise there was little correspondence between serogroup and immunoblot group. Strains of serogroup O3 were in immunoblot groups 3, 10, 12, 17

Table. Isolates examined by immunoblotting

Strain no.	Species (Serotype)	Source	Immunoblot group
F0168	Unknown phenotype	Raw chicken	1
R0227	<i>A. media</i>	ATCC 33907	1
P0149	<i>A. caviae</i>	Female, 74 years, faeces	1
E0143	<i>A. caviae</i>	Kitchen, basement	1
F0145	<i>A. caviae</i>	Cockles	1
P0150	<i>A. caviae</i>	Male, 17 years, faeces	2
E0125*	<i>A. caviae</i> (AX24)	Hospital A, tap	2
E0108	<i>A. caviae</i>	Hospital A, tap	2
E0110*	<i>A. caviae</i> (AX24)	Hospital A, storage tank	2
E0117	<i>A. caviae</i>	Hospital B, storage tank	2
E0120	<i>A. caviae</i>	Hospital B, storage tank	2
P0016	<i>A. caviae</i>	Female, 86 years, faeces	3
E0090	<i>A. caviae</i>	Hospital B, storage tank	3
E0093*	<i>A. caviae</i>	Hospital B, storage tank	3
E0094*	<i>A. caviae</i>	Hospital B, storage tank	3
E0096	<i>A. caviae</i>	Hospital B, storage tank	3
E0097*	<i>A. caviae</i> (AX24)	Hospital B, storage tank	3
E0119	<i>A. caviae</i>	Hospital B, storage tank	3
E0104	<i>A. caviae</i>	Hospital C, storage tank	3
E0106*	<i>A. caviae</i> (03)	Hospital C, storage tank	3
E0088	<i>A. caviae</i>	Vapour baths	3
E0082*	<i>A. caviae</i> (AX24)	Hospital A, storage tank	4
E0083*	<i>A. caviae</i> (AX24)	Hospital A, storage tank	4
E0095	<i>A. caviae</i>	Hospital B, storage tank	4
8814	<i>A. caviae</i>	Maidstone Public Health Laboratory	5
R0224	<i>A. caviae</i>	ATCC 13136	5
E0176	Unknown phenotype	Garden puddle	6
P0170	<i>A. hydrophila</i>	Male, 80 years, faeces	6
P0116	<i>A. caviae</i>	Male, 42 years, faeces	7
P0131	<i>A. caviae</i>	Hospital D, ITU, perineal swab	8
E1322*	<i>A. caviae</i>	Hospital D, ITU, tap	8
W1326*	<i>A. caviae</i>	Hospital D, ITU, tap	8
F0134*	<i>A. caviae</i>	Hospital D, ITU, enteral feed	8
P0029*	<i>A. caviae</i>	Female, 64 years, faeces	8
P0042	<i>A. caviae</i>	Female, 83 years, faeces	8
P0103	<i>A. caviae</i>	Female, 69 years, faeces	8
E0113*	<i>A. caviae</i>	Stream, N. Wales	8
P0156	<i>A. sobria</i>	Male, 17 years, faeces	9
P0154	<i>A. caviae</i>	Male, 16 years, faeces	9
E0129*	<i>A. caviae</i>	Hospital B, storage tank	10
P0026*	<i>A. caviae</i>	Male, 1 year, faeces	10
E0128*	<i>A. caviae</i>	Hospital D, storage tank	10
P0148	<i>A. caviae</i>	Female, adult, faeces	10
F0135*	<i>A. hydrophila</i> (03)	Hospital D, ITU, enteral feed	10
E0163	<i>A. sobria</i>	Pond	10
P1801	Unknown phenotype	Female, adult, wound	11
E1661	Unknown phenotype	River Avon	11
P0179	Unknown phenotype	Female, 90 years, faeces	11
E0165	Unknown phenotype	Pond	11
E0109*	<i>A. caviae</i> (AX24)	Hospital B, kitchen tap	12
E0121*	<i>A. caviae</i> (AX23)	Hospital B, storage tap	12
E0107	<i>A. caviae</i>	Hospital C, storage tap	12
P1231*	<i>A. caviae</i> (03)	Hospital D, ITU, throat swab	12
P1232	<i>A. caviae</i>	Hospital D, ITU, throat swab	12
E0111	<i>A. caviae</i>	Domestic tap	12
E0142	<i>A. caviae</i>	Pond	12
E0112	<i>A. hydrophila</i>	Stream, N. Wales	12
E0114	<i>A. hydrophila</i>	Stream, N. Wales	12
E0141	<i>A. hydrophila</i>	Pond	12
P0169	<i>A. hydrophila</i>	Female, 54 years, faeces	13
E0174	<i>A. caviae</i>	Bath drain	13
R0228	<i>A. sobria</i>	ATCC 9071	13
P0171	<i>A. sobria</i>	Female, 74 years, faeces	14
P0178	<i>A. sobria</i>	Male, adult, wound	14
R0226	<i>A. veronii</i>	ATCC 35622	14
E0144*	<i>A. hydrophila</i>	Hospital D, tap	15
P0139	<i>A. sobria</i>	Male, 74 years, faeces	15
P0147	<i>A. sobria</i>	Male, 71 years, faeces	15
E0162	<i>A. sobria</i>	Pond	15
P0027	<i>A. sobria</i>	Male, 80 years, faeces	16
E0118	<i>A. sobria</i>	Hospital B, storage tank	16
E0089*	<i>A. hydrophila</i> (03)	Hospital B, storage tank	17

Table. (cont.)

Strain no.	Species (Serotype)	Source	Immunoblot group
E0077*	<i>A. hydrophila</i> (03)	Hospital D, staff residences tap	17
E0087*	<i>A. hydrophila</i> (016)	Hospital D, staff residences tap	17
F0130	<i>A. hydrophila</i>	Hospital D, ITU, enteral feed	18
1381*	<i>A. hydrophila</i>	Hospital D, ITU, equipment	18
1382*	<i>A. hydrophila</i>	Hospital D, ITU, equipment	18
R0229	<i>A. hydrophila</i>	ATCC 7965	18
E0091*	<i>A. hydrophila</i>	Hospital B, storage tank	19
E0092*	<i>A. hydrophila</i>	Hospital B, storage tank	19
0200	Unknown phenotype	Unknown	20
P0045	<i>A. sobria</i>	Male, 3 years, faeces	21
P0051	<i>A. sobria</i>	Male, adult, faeces	21
P0074*	<i>A. sobria</i>	Male, adult, faeces	21
P0084	<i>A. hydrophila</i>	Female, 21 years, faeces	22
P0041*	<i>A. hydrophila</i>	Male, 4 months, faeces	23
P0031*	<i>A. caviae</i>	Female, age unknown, faeces	24
P0032*	<i>A. caviae</i>	Male, age unknown, faeces	24
P0048	<i>A. hydrophila</i>	Male, 4 years, faeces	24
1140	<i>A. sobria</i>	Maidstone Public Health Laboratory	25
E0063	<i>A. hydrophila</i>	Public baths	25
E0079*	<i>A. hydrophila</i>	Public baths	25
R0225	<i>A. schubertii</i>	ATCC 43700	26
E0072	Unknown phenotype	Hospital E, tap	27
E0068*	<i>A. hydrophila</i> (03)	Tap water	27
P0044	<i>A. caviae</i>	Female, 76 years, faeces	27
P0043	<i>A. caviae</i>	Male, 20 years, faeces	28
E0071	<i>A. caviae</i>	Tap water	28
E0028	<i>A. caviae</i>	Male, 22 years, faeces	29
E0078	<i>A. caviae</i>	Hospital E, storage tank	29
P0055	<i>A. sobria</i>	Male, 4 years, faeces	30
P0046*	<i>A. caviae</i>	Female, 3 months, faeces	30

*Isolates sent to reference laboratory for confirmation of identity and serotyping.

ITU, intensive care unit; P, patient; F, food isolate; E, water isolate; R, reference strain. All water isolates were from N.E. London unless otherwise stated.

or 27, and some immunoblot groups (12, 17) contained strains of different serotypes. The greater discriminatory capacity of immunoblotting was shown with environmental isolates from hospital A where strains E0082, E0083, E0110, E0125 and E0109 all belonged to serogroup AX24 but were differentiated into three immunoblot groups.

It also appeared that immunoblot group was independent of phenotype, whether or not newer phenotypes were included. There was no clear segregation of the different phenotypes and some immunoblot groups (1, 6, 12, 13, 14, 15, 25, 27 and 30) had strains of more than one phenotype.

The epidemiological grouping partially correlated with immunoblot groups (table). Strains E0112 and E0114 isolated from water in N. Wales belonged to immunoblot group 12; strains E0077 and E0087 isolated from staff residences in a N.E. London hospital belonged to immunoblot group 17; strains E0063 and E0079 from public baths in N.E. London belonged to immunoblot group 25. The majority of isolates from hospital B were in immunoblot group 3, and those from the intensive care unit of hospital D in group 8. However, among the other clusters of environmental isolates only two or three were grouped together by immunoblotting.

Discussion

All isolates were typable with 15–20 well separated bands, rather than the 30–40, sometimes overlapping, bands visible in a whole-cell protein fingerprint. Visual analysis of limited numbers of fingerprints was, therefore, simplified. However, all electrophoretic methods have the problem that, unlike conventional typing methods where, for instance, a bacterium either agglutinates with an antiserum or it does not, bands may occur anywhere on an electrophoresis track. Data are produced in analogue rather than digital form, and although similar strains may group together and dissimilar strains appear different, there is no absolute cut-off between them. Therefore, a computer analysis was performed to quantify similarities between isolates.

Results were reproducible, if fingerprints were analysed either within or between gels. It is well known that even with very careful attention to electrophoretic conditions, some allowance must be made for variation in positions of the same band in different gels,³² which is usually done by increasing the limits over which a band in different fingerprints is considered to be the same. Although Fourier correlation has several advantages, especially in its tolerance of left and right

shifts of the whole pattern, increasing the band detection limits cannot be used. Thus to combine within- and between-gel analysis it is necessary to apply a correction factor to the similarity coefficients.

Known replicates clustered at or above the 95% hierarchical level. At this level, 30 phenons were defined. If phenotype is also considered, the combination with immunoblotting is highly discriminatory. There was partial correlation between immunoblot type and clusters of phenotypically similar isolates from the same source. This is not surprising; a multiplicity of *Aeromonas* strains in environmental isolates has been described previously.⁷ A number of drinking water isolates were from different hospital buildings which may, at times, have been served by the same reservoir, thus explaining the occurrence of the same immunoblot types in different environmental clusters. There was some evidence that human and environmental strains from the intensive care unit of hospital D were similar. However, none of these strains was isolated from faeces in spite of some of them being found in enteral feeds. There was little evidence that human faecal isolates were acquired from the water supply as in only one case were phenotypically similar isolates of the same immunoblot type isolated from both water and human faeces, despite the probability of some strains being present in the tap water.

It has often been found in other genera that whole-cell protein fingerprints are related to phenotype.³³ However, this does not appear to be the case for aeromonads³⁴ or with the immunoblot fingerprints presented here. The finding is not changed by analysis with regard to the newer phenotypes, since no isolates

were thereby redesignated. Although attempts have been made to identify all the known hybridisation groups (HG) phenotypically,³⁵ usually the *A. caviae* group (including *A. media* and *A. eucrenophila*) and the *A. hydrophila* group (including *A. salmonicida*) are not subdivided.³⁶ *A. veronii* (*A. veronii* biotype *veronii*) is similar to *A. hydrophila* but cellobiose negative, arginine dihydrolase negative and ornithine decarboxylase positive. *A. schubertii* ferments few sugars but is lysine decarboxylase positive. *A. trota* is similar to *A. sobria* but sucrose negative and ampicillin sensitive. *A. jandaei* is another sucrose-negative species but differentiation from *A. sobria* relies on a very limited number of tests. Therefore, it was not formally included in our analysis; nevertheless, no isolates corresponding to the original description¹⁷ were found.

Conventional serogrouping was not helpful in identifying clusters of strains because only 42% were typable and the remainder belonged to a few serotypes. Therefore, we could not make a meaningful comparison with our results. As well as increased typability, the use of antisera for immunoblotting has a further advantage over conventional serological typing in that a single serum source, which does not require cross-adsorption, may be used for all strains.

Our results show that immunoblotting is a useful method for typing *Aeromonas* spp., with good discrimination and satisfactory reproducibility. Computerised analysis removes the subjective element and between-gel comparisons may be achieved.

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