*Epidem. Inf.* (1989), **103**, 565–576
*Printed in Great Britain*



# Multiresistant serotype O 12 *Pseudomonas aeruginosa*: evidence for a common strain in Europe

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(*Accepted 28 July 1989*)

## SUMMARY

A survey was made of serotype association and multiple antibiotic resistance in strains of *Pseudomonas aeruginosa* in Europe. Of 208 epidemiologically distinct strains from 16 laboratories in 10 countries, 48 were resistant to carbenicillin (MIC $> 128\ \mu g/ml$) and gentamicin (MIC $> 4\ \mu g/ml$), and 12 of these strains were of serotype O 12. Representative O 12 strains from different countries were compared with two multiresistant O 12 strains isolated 5 years apart, from a British burns unit and the antibiotic sensitive serotype reference strain. All O 12 strains were similar by phage and pyocin typing but lysogenic phage profiles indicated that two strains (the later burns isolate and the serotype strain) were distinct from the others. Electrophoretic characterization of outer membrane proteins, lipopolysaccharides and esterase enzymes corroborated the relationship of the strains and restriction fragment length polymorphism of DNA fragments hybridized with a cDNA probe copy of rRNA from *P. aeruginosa* provided further proof of their relatedness. We propose that the uniformity of characters of multiresistant O 12 *P. aeruginosa* in Europe is suggestive of a common origin for the strains.

## INTRODUCTION

Although *Pseudomonas aeruginosa* is intrinsically resistant to many antibiotics, a survey of British isolates by Williams and co-workers (1) showed that most strains were relatively sensitive to carbenicillin, ureidopenicillins, some third generation cephalosporins, amikacin and gentamicin. Nevertheless, antibiotic resistant strains are occasionally associated with large outbreaks of infection in hospitals and in recent years a number of reports have appeared documenting outbreaks of multiresistant serotype O 12 *P. aeruginosa* in Greece (2) Italy (3, 4) and Belgium (5, 6).

Serotype O 12 was described originally by Habs (7) and is included in the

International Antigenic Typing Scheme (IATS) (8), which forms the basis of the most widely used serotyping scheme for *P. aeruginosa*. This serotype is rare in the UK and North America, accounting for less than 0·5% of clinical isolates (9, 10). However, it is relatively common in the Indian sub-continent (11) and Egypt (12). In the UK there was a single report of an outbreak with a multiresistant strain of serotype O 12 in a burns unit by Perinpanayagam & Grundy (13) and, since then, most O 12 isolates typed at the Central Public Health Laboratory, London, have originated from this unit and they have continued to be resistant to most antipseudomonal antibiotics (Pitt, unpublished observations).

A preliminary study of epidemiological typing of multiresistant O 12 isolates from Greece showed that they were often lysed by only a single phage of the standard phage typing set and were the same pyocin type. Similar type characteristics were reported by Belgian workers (16). It was necessary therefore to determine whether the phage and pyocin types were common to other multiresistant O 12 isolates, and if so, whether further subdivision of these strains could be achieved.

We requested *P. aeruginosa* isolates that were resistant to carbenicillin and/or gentamicin from various European countries. The isolates were serotyped and phage typed and representative O 12 strains from each country were selected for further characterization. The results showed that most strains of multiresistant serotype O 12 in Europe were homogeneous in type characteristics and perhaps had a common origin.

## MATERIALS AND METHODS

*Strains of* P. aeruginosa. Five hundred and fifty-seven cultures were kindly provided by 16 laboratories in 10 European countries (Table 1). In addition, serotype O 12 isolates from an outbreak in a London (Roehampton) burns unit (13) and recent isolates from this unit were included. The serotype strains and antisera were as described (8) and the phage and respective propagating strains were 7, 16, 21, 24, 31, 44, 68, 73, F7, F8, F10, 109, 119X, 352, 1214, M4, M6, C21, C11, C18 and 188/1

*Carbenicillin and gentamicin resistance*. Cultures were grown in tryptone soy broth (Oxoid) at 37 °C for 18 h and 3 $\mu$l of an inoculum (*c.* $10^4$ c.f.u.) was applied to 2% Isosensitest (Oxoid) agar plates containing 128 $\mu$g/ml carbenicillin, or 4 $\mu$g/ml gentamicin. Plates were read after incubation at 37 °C for 18 h. Resistant strains were retained and the minimum inhibitory concentrations of carbenicillin and gentamicin were determined by agar dilution. Further studies on the range and mechanism of antibiotic resistance will be published elsewhere.

*Type identification*. Cultures were serotyped by agglutination with antisera to the IATS serotypes as described previously (9), bacteriophage typing was performed according to Asheshov (14) and pyocin typing was carried out by the revised method of Fyfe and colleagues (15).

*Induction of bacteriophage*. Lysogenic phages were induced as follows: an overnight broth culture was diluted 1 in 10 in 3 ml of sterile tryptone soy broth and incubated at 37 °C for 2 h, with shaking, in a waterbath. Mitomycin C (Sigma) was then added to a final concentration of 1 $\mu$g/ml and incubation was continued for a further 6 h at 37 °C. Chloroform (0·5 ml) was added, the culture was shaken

Table 1. *Sources of* P. aeruginosa *submitted to the survey of antibiotic resistance and serotype association*

| Town | Country | Sender | No. cultures received | No. of distinct strains |
|---|---|---|---|---|
| Linz | Austria | Dr Mittermayer | 85 | 24 |
| Vienna | Austria | Dr Rotter | 45 | 20 |
| Leuven | Belgium | Dr Verbist | 46 | 14 |
| London | England | Prof Phillips | 32 | 10 |
| Helsinki | Finland | Dr Renkonen | 50 | 31 |
| Nantes | France | Prof Drugeon | 29 | 9 |
| Nice | France | Dr Viot | 4 | 1 |
| Bonn | Germany | Dr Weidemann | 49 | 19 |
| Leiden | Holland | Prof Mouton | 38 | 13 |
| Rotterdam | Holland | Dr Wagenvoort | 50 | 9 |
| Naples | Italy | Prof Covelli | 22 | 11 |
| Udine | Italy | Dr Pitzus | 24 | 6 |
| Pordenone | Italy | Dr Santini | 27 | 5 |
| Seville | Spain | Prof Perea | 40 | 24 |
| Huddinge | Sweden | Dr Malmborg | 12 | 8 |
| Lund | Sweden | Dr Ursing | 4 | 4 |
| Total | | | 557 | 208 |

vigorously, and left to stand at room temperature for *c.* 10 min before it was centrifuged at 3000 g for 30 min. The aqueous supernatant was filtered through a 0·45 $\mu$ (pore size) membrane filter (Gelman) and phage activity was assayed by spotting a 20 $\mu$l volume of 10-fold dilutions of the filtrate on agar plates (PTA-Oxoid) which had been seeded with 4 h broth cultures of the 21 references phage-propagating strains. The plates were incubated at 32 °C for 18 h and examined for phage plaques.

*Outer membrane proteins.* The growth from one 14 cm diameter tryptone soy agar plate, that had been seeded confluently with a broth culture of the test strain and incubated at 37 °C overnight, was harvested in phosphate buffered saline (PBS) pH 7·2, and deposited by centrifugation at 5000 g for 30 min. The cells were resuspended in 5 ml of water and sonicated for 2 min at 4 °C. The lysate was centrifuged at 8000 g to deposit cell debris and the supernatant was centrifuged at 45000 g for 45 min at 4 °C. The pellet was resuspended in 1·7% (w/v) sodium lauryl sarcosinate (Sigma) in 50 mM Tris HCl pH 7·6, and left to stand for 20 min at room temperature. After centrifugation at 45000 g for 45 min at 4 °C, the supernatant was discarded and the transparent pellet was resuspended in 50–200 $\mu$l of sample buffer containing 2% (w/v) SDS, 20% (w/v) sucrose, 1% (v/v) 2-mercaptoethanol, 0·001% (w/v) bromophenol blue in 0.1 M Tris HCl, pH 6·8, and boiled for 5 min before freezing at −20 °C. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) was performed in 10% acrylamide resolving gels (16). Gels were loaded with 15–20 $\mu$l of sample and proteins were stained with Coomassie blue.

*Lipopolysaccharides.* The growth from a 9 cm nutrient agar plate was harvested in 3 ml of PBS and sonicated for 2 min at 4 °C. After removal of cell debris by low-speed centrifugation (8000 g), the lysate was centrifuged at 30000 g and the pellet

was resuspended in 500 $\mu$l of sample buffer and boiled for 10 min. To 100 $\mu$l of sample was added 50 $\mu$g of proteinase K (BDH) in 20 $\mu$l of sample buffer and this was incubated for 4–6 h at 60 °C in a waterbath. Extracts were stored at −20 °C prior to SDS–PAGE which was performed as for the outer membrane proteins. Lipopolysaccharides were visualized by a modified silver stain (17) with carbonate developer (0·28 M sodium carbonate containing 0·05% (v/v) formaldehyde).

*Isoenzymes.* Isoenzymes were examined by previously described methods (18, 19). The overnight growth, at 37 °C, from a 9 cm nutrient agar plate culture of a strain was harvested in 3 ml of PBS and the cells were disrupted with ultrasound for 2 min in an ice-bath. The debris was removed by centrifugation at 5000 g for 30 min at 4 °C and the supernatant was collected and stored at −70 °C. For enzyme electrophoresis, a 6·5% acrylamide resolving gel was used but SDS was omitted from all buffers. Gels were loaded with 25 $\mu$l of sample. The $\alpha$-naphthyl acetate esterase was detected by a standard enzyme kit (Sigma 91.A). Preliminary experiments with the set of serotype strains of *P. aeruginosa* showed that of 16 enzymes tested, the $\alpha$-naphthyl acetate esterase was the most heterogeneous in relative mobilities and this enzyme was examined in the collection of O 12 strains.

*Ribosomal RNA gene fragment polymorphism.* Strains were grown with shaking in 20 ml of tryptone soy broth supplemented with 0·4% (w/v) yeast extract (TSY) for 16 h at 37 °C. Cultures were centrifuged at 8000 g for 10 min and cell pellets were lysed with 5 M guanidium thiocyanate and treated with 7·5 M ammonium acetate, chloroform, and the DNA was precipitated with 2-propanol (20). The DNA was washed three times in 70% (v/v) ethanol, dried *in vacuo*, and redissolved in sterile distilled water to a concentration of 500 $\mu$g/ml.

Ribosomal RNA was prepared from the IATS serotype O 12 strain which was grown in 200 ml of TSY broth and incubated for 16 h at 32 °C with shaking. The cells were deposited at 8000 g for 30 min and rRNA (16S+23S) was prepared by the method of Chirgwin (21). The sample was washed three times in 70% (v/v) ethanol, dried *in vacuo*, and redissolved in sterile distilled water to 100 $\mu$g/ml.

Samples of DNA (5 $\mu$g) were digested with 8–10 units of restriction enzymes *Eco* R1, *Hin*d III, and *Sma* I using buffers and reaction conditions recommended by the manufacturer (BRL-Gibco, Paisley, UK). Electrophoresis was carried out in 25 × 20 cm horizontal 0·8% (w/v) agarose gels in Tris borate buffer (Tris base 89 mM, boric acid 89 mM, EDTA 2 mM, pH 8·3) at 30 V for 20 h. Gels were stained with ethidium bromide and viewed with ultra-violet illumination.

Digested DNA fragments were transferred from gels to Hybond-N membranes according to the manufacturer's instructions (Amersham International, Amersham, UK). Prehybridization and hybridization was carried out at 42 °C (22). The probe was a biotinylated cDNA prepared from total ribosomal RNA of the O 12 serotype strain using the reverse transcriptase reaction described previously (23).

Hybridization bands on membranes were visualized with the 'Bluegene' non-radioactive nucleic acid detection system (BRL-Gibco).

## RESULTS

*Survey of antibiotic resistance.* Each of the 557 cultures of *Pseudomonas aeruginosa* received from participating laboratories was serotyped and phage typed. Sequential and related isolates of the same strain were excluded and this resulted in the identification of 208 distinct strains.

At the breakpoint concentrations of 128 $\mu$g/ml carbenicillin and 4 $\mu$g/ml gentamicin, 113 of the 208 strains were resistant to one or both antibiotics; 76 were resistant to carbenicillin, 79 to gentamicin, and 48 to both antibiotics. The MIC of carbenicillin and gentamicin for resistant strains was $> 1024$ $\mu$g/ml and $> 8$ $\mu$g/ml respectively. The origin and serotypes of the multiresistant strains are shown in Table 2. Serotype O 12 strains were found in all but one of the centres. Different strains, distinguishable by phage typing, of serotypes O 2, O 4, O 6, and O 10 were also multiresistant as were four strains that reacted only with the polyvalent serum II, which contained antisera to the antigenically related serotypes O 2, O 5 and O 16. Two multiresistant strains were polyagglutinating, that is, reacted with three or four antisera to unrelated O antigens, and two strains were serologically not typable.

*Typing of selected O 12 strains.* A panel of strains was assembled (Table 3) comprising one serotype O 12 strain from each of the 10 centres surveyed, two multiresistant O 12 strains from the Roehampton burns unit, one, (strain 19) was isolated in 1982 and the other (strain 20) in 1987. The IATS serotype strain O 12 was included as a reference control (strain 23).

Phage 68 was active on 11 of the 14 selected O 12 strains (Table 3) but with the exception of the occasional weak reaction of phage 119X, no other phage reactions were observed. On account of the poor sensitivity of the strains to typing phages the induced phage profile was examined. All strains contained phages but only three patterns of lysis of the reference propagating strains were found. Strain 20 and the serotype strain were distinct and each gave a lytic profile markedly different from that of the remainder. All of the survey strains were pyocin type 1, subtype h, but the burns unit strain 20 differed slightly in subtype pattern. Conventional typing methods were therefore unable to distinguish further between the O 12 strains, with the exception of strains 20 and 23, and so additional methods of strain discrimination were applied to the panel of strains.

The outer membrane protein profiles of all strains were typical for *P. aeruginosa* and only strain 20 showed a marginally different pattern from that given by all other clinical strains and the serotype strain (Fig. 1).

Morphological heterogeneity of lipopolysaccharides determining the serotype was examined and with the exception of strains 16 (Naples) and 20, the high-molecular-weight 0 repeating units of all strains were identical as evidenced by the correspondence of ladder bands with those of the serotype strain. Strain 16 and 20 showed bands in the middle portion of the gel that coincided with bands in all strains (Fig. 2). Both strains 16 and 20 were agglutinated to the same titre as the serotype strain by the homologous antiserum indicating that the absence of visual high-molecular-weight bands did not reduce the sero-reactivity of these strains. The relative mobilities of the $\alpha$-naphthyl acetate esterase enzyme of the panel of strains were similar but strains 20 and 23 were clearly distinguishable from the others (Fig. 3).

Table 2. *Origin and serotypes of epidemiologically distinct* P. aeruginosa *strains resistant to both carbenicillin (128 μg/ml) and gentamicin (4 μg/ml)*

| Town | Country | No. strains | O serotypes | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | PA* | II† | NT‡ |
| Linz | Austria | 3 | . | . | . | . | . | . | . | . | . | . | . | 1 | . | . | . | . | . | . | 1 | 1 |
| Leuven | Belgium | 8 | . | . | 1 | 3§ | . | . | . | . | . | 1 | 1 | 1 | . | . | . | . | . | 1 | . | . |
| London | England | 2 | . | . | . | . | . | . | . | . | . | . | . | 1 | . | . | . | . | . | . | 1 | . |
| Helsinki | Finland | 4 | . | . | . | . | . | . | . | . | . | 1 | . | 1 | . | . | . | . | . | . | 1 | 1 |
| Nantes | France | 5 | . | . | . | 1 | . | . | . | 1 | . | 1 | . | 1 | . | . | . | . | . | . | 1 | . |
| Nice | France | 1 | . | . | . | . | . | . | . | . | . | . | . | 1 | . | . | . | . | . | . | . | . |
| Bonn | Germany | 3 | . | 2§ | . | . | . | . | . | . | . | . | . | 1 | . | . | . | . | . | . | . | . |
| Leiden | Holland | 4 | . | 1 | . | . | . | 1 | . | . | . | . | 1 | 1 | . | . | . | . | . | . | . | . |
| Rotterdam | Holland | 5 | . | . | . | . | . | 2§ | . | . | . | 2§ | . | 1 | . | . | . | . | . | . | . | . |
| Naples | Italy | 1 | . | . | . | . | . | . | . | . | . | . | . | 1 | . | . | . | . | . | . | . | . |
| Udine | Italy | 5 | . | . | . | 2§ | . | . | . | . | . | 1 | . | 1 | . | . | . | . | . | 1 | . | . |
| Pordenone | Italy | 3 | . | . | . | . | . | 1 | . | . | . | . | 1 | 1 | . | . | . | . | . | . | . | . |
| Seville | Spain | 4 | . | 1 | . | . | 1 | . | . | . | . | . | 1 | . | 1 | . | . | . | . | . | . | . |

* Polyagglutinating strain.
† Strain agglutinated by polyvalent serum II (O 2, O 5, O 16) only.
‡ Not typable.
§ Strains distinguishable by phage typing.

Table 3. *Phage sensitivity, pyocin type and lysogenic phage state of serotype O 12 strains of* Pseudomonas aeruginosa

| Strain no. | Source | Phage sensitivity | Pyocin type | Lysogenic state* |
|---|---|---|---|---|
| 1 | Linz | NT† | 1/h | 1235715 |
| 2 | Leuven | 68 | 1/h | 1235715 |
| 4 | Nantes | 68 | 1/h | 1235715 |
| 6 | Rotterdam | 68 | 1/h | 1235715 |
| 8 | Leiden | 68/119x‖ | 1/h | 1235715 |
| 10 | London | 68 | 1/h | 1235715 |
| 12 | Udine | 68/119x‖ | 1/h | 1235715 |
| 14 | Bonn | 68 | 1/h | 1235715 |
| 16 | Naples | NT | 1/h | 1235715 |
| 17 | Pordenone | 68 | 1/h | 1235715 |
| 19‡ | Roehampton | 68 | 1/h | 1235715 |
| 20§ | Roehampton | NT | 1/c | 3335711 |
| 23 | IATS-O 12 | 68 | 1/h | 3137717 |
| 25 | Athens | 68 | 1/h | 1235715 |

* Mnemonic code.
† Not typable.
‡ Outbreak strain 1982.
§ Outbreak strain 1987.
‖ Weak reaction.

Figure 4 shows the Southern blot patterns of restriction endonuclease digests of DNA hybridized with the cDNA probe. The clinical isolates, with the exception of strains 12 and 20, gave identical patterns but all isolates clearly differed from the serotype strain in all three digests. Strain 12 (Udine) differed in two (*Eco* RI and *Hind* III) of the digests. The reason for this was not clear, since this strain conformed to the general pattern of bands given by the majority of the strains. The patterns shown by strain 20 differed in *Sma* I and *Eco* RI digests but the *Hind* III digest pattern was uniform.

Typing by a variety of methods therefore showed that the majority of the multiresistant O 12 strains were homogeneous but of the clinical strains, only strain 20 and possibly strain 12 was distinguishable and these were distinct from the antibiotic-sensitive reference serotype strain.

## DISCUSSION

Our interest in the epidemiology of multiresistant serotype O 12 *P. aeruginosa* was first stimulated by the finding that strains of this serotype in Athens, Greece (2) were indistinguishable by phage and pyocin typing from an O 12 strain which was endemic in an English burns unit in 1982 (13). Furthermore, reports had appeared describing a high incidence of O 12 strains in Rome and Palermo, Italy (4) and Jolimont, Belgium (5) with frequencies ranging from 11·7 to 42% respectively of all *P. aeruginosa* isolated. This was in direct contrast to the very low frequencies of O 12 strains in the UK and the failure to find any members of this serotype among 122 carbenicillin resistant strains selected from 24 British hospitals (24).

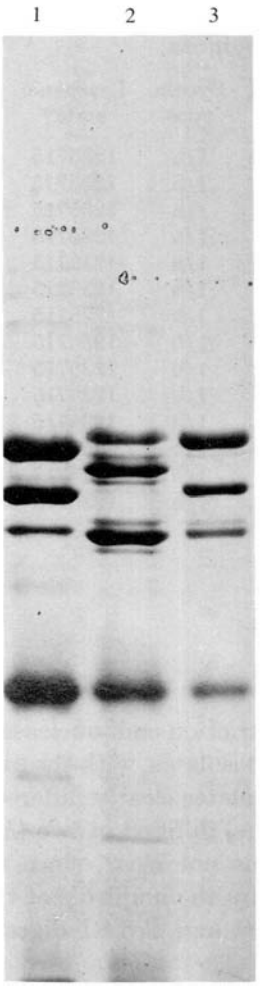


Fig. 1


Fig. 2

Fig. 1. Outer membrane proteins of *P. aeruginosa* O 12 strains. Lane 1, strain no. 20. 2, Serotype strain. 3, Strain nos. 1, 2, 4, 6, 8, 10, 12, 14, 16, 17, 19, 25 (see Table 3 for origin).

Fig. 2. Lipopolysaccharide profiles of *P. aeruginosa* O 12 strains. Lane 1, serotype strain. 2, Strain nos. 16, 20. 3, Strain nos. 1, 2, 4, 6, 8, 10, 12, 14, 17, 19, 25 (see Table 3 for origin).

The traditional methods for the typing of *P. aeruginosa* are in the main relatively discriminating (25), but the uniformity of phage lytic patterns and pyocin types of the O 12 strains was considered to be insufficient evidence to allow confirmation of strain relatedness. All O 12 strains were lysed by only one or two of the standard typing phages, or not at all, and pyocin typing failed to discriminate further between strains.

The conservation of phage and pyocin types among O 12 isolates was also reported by Allemeersch and colleagues (6) who found 93% belonging to phage type 68/119X, 68, or 119X, or were phage non-typable, and that 75% were of


Fig. 3. Esterase enzyme profiles of *P. aeruginosa* O 12 strains. Lane 1, strain no. 20. 2, Serotype strain. 3, Strain nos. 1, 2, 4, 6, 8, 10, 12, 14, 16, 17, 19, 25 (see Table 3 for origin).

pyocin types 1 or 45, which are similar in inhibition pattern (26). Most Italian O 12 strains were also of phage type 68 or 68/F8 and pyocin type (3). Pyocin type 1 is a common type worldwide and phage 68 is the most active of the Lindberg typing set (25) and this phenotype is in our experience, frequently found in other serotypes.

Most strains of *P. aeruginosa* are lysogenic and a single strain may carry as many as 10 temperate phages (27). The lysogenic profile of strains is widely used for the typing of staphylococci (28) and although the method is rarely employed for typing *P. aeruginosa*, a wide range of lytic patterns can be demonstrated within the species (10). The similarity of the lysogenic profile of the O 12 strains was therefore not expected if the strains were distinct.

The outer membrane proteins of *P. aeruginosa* are morphologically homogeneous in SDS–PAGE (29) so any major variations in banding pattern of strains grown under standard conditions can be considered indicative of structural differences in the outer membrane (namely strains 20 and 23). However lipopolysaccharide banding profiles in SDS–PAGE are not necessarily consistent for a given serotype of *P. aeruginosa* (24) and heterogeneity has been clearly

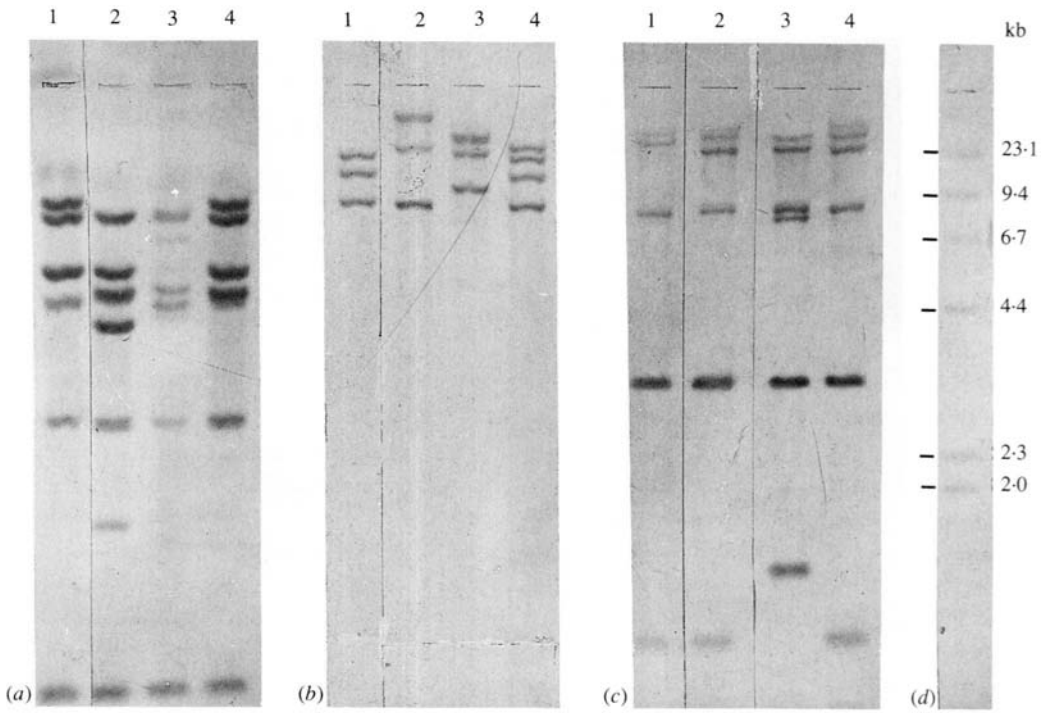


Fig. 4. Southern blot patterns of restriction digests of DNA from *P. aeruginosa* O 12 strains hybridized with a biotinylated cDNA probe transcribed from total rRNA from *P. aeruginosa* serotype strain O 12: (*a*) *Sma* I, (*b*) *Eco* RI, (*c*) *Hin*d III (*d*) *Hin*d III digest of biotinylated lambda phage DNA. Lane 1, strain no. 12. 2, Strain no. 20. 3. Serotype strain. 4. Strain nos. 1, 2, 4, 6, 8, 10, 14, 16, 17, 19, 25 (see Table 3 for origin).

demonstrated within chemotypes of salmonella lipopolysaccharides (30). Nevertheless, the banding profile of the clinical O 12 strains corresponded to that of the reference serotype strain and the only variation observed was in the expression of high-molecular-weight 0 repeating units associated with serotype specificity.

Multi-locus enzyme polymorphism has been used to characterize strains of various species (18) and the relative mobilities of esterase enzyme in particular have been applied to strain differentiation in epidemiological studies (19). This study supports the use of enzyme polymorphism as an epidemiological test and they provided supportive evidence that the European isolates were homogeneous.

Recently, probes of the genetic relatedness of strains have been described for a number of bacterial species and include the use of cloned random DNA sequences (31), rRNA (32, 33) and cDNA transcripts of rRNA prepared using reverse transcriptase (23). We used a *P. aeruginosa* rRNA probe in the knowledge that we were indexing genetic variation in only relatively few loci in a small region of the chromosome of the O 12 strains. However as strains 20 and 23 gave profiles distinct from the majority, this corroborated the results obtained by other methods. On the other hand the genetic relatedness of the strains that demonstrated identity in banding pattern is not proven. These have to be interpreted in the context of patterns produced by unrelated strains and the

number of rRNA genes in the species. Preliminary studies of epidemiologically related and distinct strains have shown that most strains produce only three or four bands which may or may not correlate with the epidemiology. Further studies of the number of digests probed are also indicated given the finding here that strains may give similar bands with two restriction enzymes but be distinguishable by a third enzyme digest.

There are many precedents for the international spread of resistant bacterial strains and the origin of O 12 *P. aeruginosa* is at present unknown but the criteria of Orskov & Orskov (34) of 'bacterial cultures isolated independently from different sources at different times, but showing so many identical phenotypes and genetic traits that the most likely explanation is a common origin' appears to have been met with these strains.

ACKNOWLEDGEMENTS

We thank the following for submitting strains and data to the survey: Drs I. Covelli, H. Drugeon, A. Malmborg, H. Mittermayer, R. Mouton, E. Perea, R. Perinpanayagam, I. Phillips, E. Pitzus, O. Renkonen, M. Rotter, G. Santini, J. Ursing, L. Verbist, M. Viot, J. Wagenvoort, B. Weidemann. Our thanks also to Pauline Nicholson for isoenzyme electrophoresis.

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