Dissociation of surface properties and "intrinsic" resistance to β-lactams in *Pseudomonas aeruginosa*

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**Summary.** Carbenicillin resistance in strains of *Pseudomonas aeruginosa* isolated in Britain is mediated more frequently by "intrinsic factors" than by β-lactamase production. Intrinsically carbenicillin-resistant isolates almost invariably were more resistant to azlocillin, cefoperazone, cefotaxime, ceftazidime, chloramphenicol, tetracycline and nalidixic acid than were carbenicillin-susceptible strains. This cross-resistance to different classes of antimicrobials suggested an impermeability-based mechanism of resistance, perhaps involving the outer membrane. The structure and composition of the outer membrane of the pseudomonas cell also influences the O-serotype specificity and the susceptibility to many bacteriophages. We therefore examined these properties for possible relationships to antibiotic resistance. Carbenicillin-resistant (122) and -sensitive (127) *P. aeruginosa* strains from 24 hospitals were compared. Serotype O:1, O:3, O:6, O:10 and O:11 strains predominated in both groups. Non-typable and polyagglutinating strains were infrequent in both groups. With one possible exception, none of 18 bacteriophages showed a significant preference for carbenicillin-resistant or -sensitive strains. Variation between strains was observed in the electrophoretic profile of LPS and this could be related in part to serotype, but not to antibiotic resistance. Our results contrast with those of earlier small-scale studies which have claimed relationships between surface properties and antibiogram in *P. aeruginosa*, and suggest that interpretation of the minor changes in LPS sometimes observed in association with the development of antibiotic resistance *in vitro* requires caution.

**Introduction**

In a large-scale survey Williams *et al.* (1984a and b) found that c. 10% of isolates of *Pseudomonas aeruginosa* from British hospitals were insensitive to carbenicillin (MIC > 128mg/L). Resistance was associated with production of carbenicillin-hydrolysing β-lactamases in c. 20% of these isolates but such enzymes were absent from the remainder. The resistance of the carbenicillinase non-producing strains was attributed to 'intrinsic' factors. Intrinsic resistance involved neither changes in chromosomal β-lactamase synthesis nor modification of the penicillin-binding proteins (PBPs) and was postulated to depend on cell impermeability.

The PBPs of *P. aeruginosa* are exposed in sphaeroplasts (Livermore, unpublished observations), so the exclusion of β-lactam compounds must involve a cell structure external to the cytoplasmic membrane, such as the intact outer membrane. Most small aqueous solutes cross this layer through aqueous pores composed of 'porin' proteins and, amongst enterobacteria, there is a close correlation between porin quantity, cell permeability and resistance to β-lactams (Nikaido and Vaara, 1985). In *P. aeruginosa*, however, the amount of porin protein is surprisingly large considering the low permeability of the organism (Angus *et al.*, 1982; Hancock, 1984), and is undiminished in intrinsically-resistant strains (Livermore, 1984). To accommodate these observations, it is postulated that porin function in *P. aeruginosa* may be controlled by other outer-membrane components including the lipopolysaccharide (LPS) (Angus *et al.*, 1982; Kropinski *et al.*, 1982). The overall assembly of the outer-membrane proteins and LPS also determines surface properties such as O serotype and susceptibility to many
bacteriophages and pyocins, raising the possibility that the antibiogram of strains and their surface properties may be related. Support for this hypothesis can be drawn from the many instances in which serotype, bacteriophage-susceptibility and LPS-sugar changes have been associated with the development of resistance (and hypersensitivity) in P. aeruginosa Kropinski et al., 1982; Slack and Pitt, 1982; Darveau and Hancock, 1983; Godfrey et al., 1984; Meadow and Wells, 1985; Shearer and Legakis, 1985). However, the relationship of derived mutants to naturally-occurring resistant strains is difficult to assess and, in the present study, we have compared the surface properties of large groups of carbenicillin-sensitive and -resistant P. aeruginosa isolates, with particular emphasis on the LPS components. Serotyping and phage-susceptibility tests were performed and extracted LPS was electrophoresed to estimate its heterogeneity in each strain.

Materials and methods

Bacterial strains

All cultures were obtained during a recent multicentre survey of antibiotic resistance in P. aeruginosa (Williams et al., 1984a). We received 131 isolates that were intrinsically resistant to carbenicillin (MIC > 128mg/L), i.e., they did not produce carbenicillin-hydrolysing β lactamases, and 129 of these, from 22 hospitals, were examined in the present investigation. The intrinsic nature of their resistance was established previously (Williams et al., 1984b). The resistant strains were compared with a control sample of 130 randomly selected, carbenicillin-susceptible (MIC ≤ 64mg/L) P. aeruginosa isolates. This sample comprised four to six isolates from each of the 24 hospitals that participated in the survey. Organisms that had borderline sensitivity/resistance (MIC = 128mg/L) to carbenicillin were not examined.

Bacteriophages

The set of bacteriophages used for typing was 7, 16, 21, 24, 31, 44, 68, 73, 109, 1214, F7, F8, F10, M4, Col.11, Col.18 and Col.21. For details of the phages, see Bergan (1975).

Antibiotics

Azlocillin was obtained from Bayer Pharmaceuticals, Haywards Heath, Surrey; carbenicillin from Beecham Research Laboratories, Brentford, Middlesex; cefoperazone from Pfizer Inc., Sandwich, Kent; cefotaxime and gentamicin from Roussel Laboratories Ltd, Wembley, Middlesex; ceftriaxone from Glaxo Group Research, Greenford, Middlesex; chloramphenicol from Parke-Davis and Co., Pontypool, Gwent; nalidixic acid from Sigma Chemical Co., St Louis MO, USA; and tetracycline from Lederle Laboratories, Poole, Dorset.

Typing methods

Cultures were serotyped by slide agglutination tests with the 17 antisera of the International Antigenic Typing Scheme (Liu et al., 1983). Additional antisera were prepared against the type strains of serotypes O:2a and O:2b and O:5d (Véron, 1961) and were used for the subdivision of serotypes O:2 and O:5 (Pitt, 1981). Bacteriophage typing was performed by the methods of Asheshov (1974).

MIC determinations

MICs of antibiotics were determined by a plate dilution method as described by Williams et al. (1984).

Electrophoresis of LPS

The method of Hitchcock and Brown (1983) was adapted to determine the profiles of LPS in crude extracts of whole cells of P. aeruginosa. Cultures were grown overnight at 37°C with continuous shaking in 10-ml volumes of Antibiotic No. 3 Medium (Difco) and 1-ml volumes of these broths were used to inoculate 9 ml of fresh, warm (37°C) broth. After incubation at 37°C for 4h, 1.5-ml amounts were withdrawn and centrifuged at 10 000g for 1 min in an Eppendorf 5414 Microfuge. The pellet was blotted dry with tissue paper and resuspended in 75μl of solubilisation buffer, consisting of sodium dodecyl sulphate 2% w/v, 2-mercaptoethanol 4% v/v, glycerol 10% v/v and bromophenol blue 0.01% w/v in 1/10M Tris-HCl buffer pH 6.8. The suspensions were heated at 100°C for 10 min. Subsequently, 50 μg of proteinase K (Sigma) in 15μl of solubilisation buffer was added and the suspensions were held overnight at 55°C, then electrophoresed in the discontinuous polyacrylamide gel system of Laemmli (1970). The acrylamide and NN'-methylene bisacrylamide concentrations were adjusted to 13.5 and 0.25% w/v, respectively, in the running gel, and to 6 and 0.6% w/v, respectively, in the stacking gel. Sodium dodecyl sulphate was omitted from the running and stacking gel mixtures. Electrophoresis was performed at a constant current of 50mA/gel and LPS was visualised by silver staining (Tsai and Frasch, 1982).

Statistical test

The χ² test was used to test the significance of differences in the serotype distributions and bacteriophage susceptibilities of the carbenicillin-resistant and sensitive groups of P. aeruginosa strains.
Results

Elimination of replicate organisms

Both the carbenicillin-resistant and -sensitive groups of isolates appeared to contain some replicate cultures. These were defined as organisms from the same hospital that gave identical serotype reactions and antibiotic-susceptibility patterns and were indistinguishable by phage typing. We identified seven apparent replicates amongst the resistant organisms and three in the sensitive group, and these were excluded from further studies. Thus, 122 carbenicillin-resistant and 127 sensitive strains remained.

Susceptibility of carbenicillin-resistant and -sensitive strains to other antibiotics

The distributions of MICs of azlocillin, cefoperazone, cefotaxime, ceftazidime, nalidixic acid, chloramphenicol, tetracycline and gentamicin for both groups of strains are presented in table I. The carbenicillin-resistant organisms were more resistant than the sensitive group to all the other antibiotics tested except gentamicin. The only exceptions to this cross-resistance pattern were occasional carbenicillin-sensitive strains that were highly resistant to one or more of tetracycline, chloramphenicol and nalidixic acid.

Serotype distribution of resistant and sensitive isolates.

There was little association between antibiogram and O serotype (table II). The same serotypes — O:1, O:3, O:6, O:10 and O:11 predominated in both the resistant and sensitive groups. Strains that reacted also with the polyvalent II antiserum pool were encountered at high frequency in each group. Many of these “group II” strains reacted specifically with one of the individual antisera contained in this pool (i.e., O:2a, O:2b, O:5 or O:16) and appear under these individual headings in table II,

Table I. Distribution of MICs of β-lactam and non-β-lactam antibiotics for carbenicillin-resistant and -sensitive P. aeruginosa

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Group of strains</th>
<th>Number of strains for which MIC (mg/L) was</th>
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<th>0.25</th>
<th>0.5</th>
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<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>32</th>
<th>64</th>
<th>128</th>
<th>256</th>
<th>512</th>
<th>&gt;512</th>
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<tr>
<td></td>
<td>Cb'</td>
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</tr>
<tr>
<td></td>
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<tr>
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<tr>
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<td>7</td>
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<td>2</td>
<td>7</td>
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<tr>
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<td>Cb'</td>
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<td>43</td>
<td>54</td>
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<tr>
<td>Gentamicin</td>
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<td>29</td>
<td>50</td>
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<td>8</td>
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<tr>
<td></td>
<td>Cb'</td>
<td></td>
<td>3</td>
<td>51</td>
<td>52</td>
<td>20</td>
<td>1</td>
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</table>

*Cb' = carbenicillin resistant; Cb' = carbenicillin sensitive.
Table II. Serotypes of carbenicillin-resistant and -sensitive *P. aeruginosa*

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Resistant group (122)</th>
<th>Sensitive group (127)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>5 (4.1)</td>
<td>12 (9.4)</td>
</tr>
<tr>
<td>2a</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>2b</td>
<td>0 (0)</td>
<td>3 (2.4)</td>
</tr>
<tr>
<td>3</td>
<td>9 (7.4)</td>
<td>13 (0.2)</td>
</tr>
<tr>
<td>4</td>
<td>3 (2.5)</td>
<td>4 (3.1)</td>
</tr>
<tr>
<td>5d</td>
<td>5 (4.1)</td>
<td>2 (1.6)</td>
</tr>
<tr>
<td>6</td>
<td>31 (25.4)</td>
<td>35 (27.5)</td>
</tr>
<tr>
<td>7</td>
<td>2 (1.6)</td>
<td>0 (0)</td>
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<td>2 (1.6)</td>
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</tr>
<tr>
<td>9</td>
<td>5 (4.1)</td>
<td>3 (2.4)</td>
</tr>
<tr>
<td>10</td>
<td>18 (14.8)</td>
<td>15 (11.8)</td>
</tr>
<tr>
<td>11</td>
<td>18 (14.8)</td>
<td>9 (7.1)</td>
</tr>
<tr>
<td>13</td>
<td>0 (0)</td>
<td>2 (1.6)</td>
</tr>
<tr>
<td>16</td>
<td>4 (3.3)</td>
<td>8 (6.3)</td>
</tr>
<tr>
<td>Pool* I</td>
<td>1 (0.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>II</td>
<td>4 (3.3)</td>
<td>4 (3.1)</td>
</tr>
<tr>
<td>IV</td>
<td>0 (0)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>PA†</td>
<td>9 (7.4)</td>
<td>6 (4.7)</td>
</tr>
<tr>
<td>NT‡</td>
<td>5 (4.1)</td>
<td>5 (3.9)</td>
</tr>
</tbody>
</table>

* Agglutinated by antisera pool, not by any individual antiserum.
† PA = polyagglutinating, i.e., agglutinated by 2 or more unrelated antisera.
‡ NT = not typable, i.e., not agglutinated by any antiserum.

...but some strains failed to react with these sera and were classified as O:11. Serotype O:1 strains were twice as frequent in the sensitive group as in the resistant group and serotype O:11 strains were encountered twice as frequently in the resistant group but in neither case was this difference statistically significant (p > 0.05; χ² tests). Non-typable organisms (not agglutinated by any antiserum tested) and polyagglutinating strains (agglutinated by two or more antisera to unrelated serotypes) were encountered with equal infrequency in both the carbenicillin-resistant and -sensitive groups.

Lysis of carbenicillin-resistant and -sensitive *P. aeruginosa* by bacteriophages

With the exception of phage Col. 21, none of the phage was significantly more or less active on carbenicillin-resistant or -sensitive strains (p > 0.05; χ² tests, table III). Statistical analysis did suggest that Col.21 was more often lytic for carbenicillin-sensitive organisms than for carbenicillin-resistant strains (p < 0.05; χ² test) but we are cautious of this result because of the small total number of strains lysed by this bacteriophage (13 out of 249 strains tested).

Some relationship was apparent between the serotypes of strains and their bacteriophage-susceptibility. Strains of serotypes O:3, O:6 and O:9 generally were susceptible to five or more bacteriophages, whereas the mean numbers of lytic reactions with strains of serotypes O:10 and O:11 and with non-typable strains were three or fewer.

Typing of highly resistant organisms

The typing results with the most resistant *P. aeruginosa* strains (carbenicillin MIC ≥1024mg/L) were analysed separately (table IV). Eight strains fell within this category; they exhibited a diverse scatter of serotypes and bacteriophage susceptibilities. Five (63%) of these strains were lysed by bacteriophage 7 compared with only 40 out of 122 (32.8%) and 44 out of 127 (34.6%) of the whole resistant and sensitive groups, respectively.

Electrophoresis of LPS

Studies in our laboratories have shown that the LPS profiles obtained with *P. aeruginosa* by the
Table IV. Serotypes and phage sensitivity of highly carbenicillin-resistant *P. aeruginosa* strains

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>Source</th>
<th>Serotype</th>
<th>Carbenicillin MIC (mg/L)</th>
<th>Bacteriophage causing lysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>M76</td>
<td>Aberdeen</td>
<td>O:11</td>
<td>2048</td>
<td>1214</td>
</tr>
<tr>
<td>M642</td>
<td>Edinburgh</td>
<td>O:6</td>
<td>1024</td>
<td>7, 24, M4, Col.11</td>
</tr>
<tr>
<td>M917</td>
<td>Harlow</td>
<td>O:5d</td>
<td>1024</td>
<td>None</td>
</tr>
<tr>
<td>M1251</td>
<td>Liverpool</td>
<td>O:9</td>
<td>1024</td>
<td>7, 21, 24, 68</td>
</tr>
<tr>
<td>M1426</td>
<td>Nottingham</td>
<td>O:1</td>
<td>&gt;2048</td>
<td>7</td>
</tr>
<tr>
<td>M1499</td>
<td>Nottingham</td>
<td>O:11</td>
<td>&gt;2048</td>
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<td>M2566</td>
<td>Nottingham</td>
<td>P.A.</td>
<td>2048</td>
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<td>London</td>
<td>O:3</td>
<td>1024</td>
<td>7, 21, 24, 44, 68, F8, 109, 352, 1214, Col.11</td>
</tr>
</tbody>
</table>

Figure. Electrophoretic profiles of LPS from carbenicillin-sensitive and -resistant *P. aeruginosa*. Tracks 1–6 contain LPS from serotype O:11 strains with carbenicillin MICs as follows: (1) strain M825, MIC 32 mg/L; (2) strain M921, MIC 32 mg/L; (3) strain M346, MIC 256 mg/L; (4) strain M307, MIC 512 mg/L; (5) strain M1499, MIC >2048 mg/L; (6) strain M76, MIC 2048 mg/L. Tracks 7–10 contain LPS from serotype O:1 strains as follows: (7) strain M1413, MIC <16 mg/L; (8) strain M918, MIC 32 mg/L; (9) strain M804, MIC 256 mg/L; (10) strain M1426, MIC >2048 mg/L. Tracks 11–15 contain LPS from serotype O:3 strains as follows: (11) strain M1718, MIC 64 mg/L; (12) strain S12, MIC 64 mg/L; (13) strain 654, MIC 256 mg/L; (14) strain M85, MIC 512 mg/L; (15) strain M2102, MIC 1024 mg/L. The intermediate mol. wt bands typical of serotype O:3 are arrowed.
method of Hitchcock and Brown (1983) are iden-
tical to those for LPS purified from the same strain by
the procedure of Darveau and Hancock (1983). The
profiles revealed a large low-mol. wt band, which
sometimes appeared as a doublet, above which was
a more or less extensive ladder of higher-mol. wt
bands (figure). Results of this type are interpreted
usually as indicating that the LPS within the strain
is micro-heterogeneous in respect of the number of
repeating O-antigenic sugar subunits attached to
the core oligosaccharide-lipid A component (Palva
and Mansera, 1980; Hitchcock and Brown). The
low-mol. wt band corresponds to rough LPS,
composed of core oligosaccharide-lipid A but
devoid of the repeating O-antigenic subunits, wher-
as the ladder is given by smooth LPS forms with
various numbers of O-antigenic subunits attached
to the lipid A-core oligosaccharide component. The
smooth forms with the greatest number of subunits
migrate the shortest distance and form the top-most
bands of the ladder.

The prominence of the ladder varied between
strains, both quantitively and in terms of the
positions of individual bands. These results fre-
cently could be related to serotype, for example,
serotype O:11 strains (tracks 1–6) usually had a
very large amount of smooth LPS material (figure
3), and O:3 strains (tracks 11–15) gave a distinctive
pattern of bands of intermediate mol. wt (arrow).
Smooth LPS was not detected in a few organisms
and these invariably gave a very diffuse rough LPS
band. These organisms usually were not serotyp-
able and were not sensitive to phages. However, not
all the non-typable strains gave such profiles and we
deduce that the lack of reaction with O-antisera did
not invariably reflect the lack of smooth LPS.

Very little correlation was apparent between LPS
profile of strains and resistance to antibiotics. The
only noteworthy association was that the two
carbenicillin-highly-resistant (MIC > 1024mg/L)
serotype O:11 strains (M1499 and M76; figure,
tracks 5 and 6 respectively) lacked the highest mol.
wt smooth LPS forms that were usually so charac-
teristic of this serotype (figure 3). Carbenicillin-
highly resistant P. aeruginosa strains of other
serotypes gave LPS profiles similar to those of
carbenicillin-sensitive strains of the same serotype
as shown by serotype O:1 strains (tracks 7–10) and
by O:3 strains (tracks 11–15).

Discussion

Williams et al. (1984a and b) found that carbeni-
cillin resistance in P. aeruginosa was determined
most frequently by intrinsic factors and not by β-
lactamase. Representative intrinsically-resistant
strains later were shown to have penicillin-binding
proteins with unaltered affinity for β lactams, and it
was suggested that their insensitivity may have been
due to reduced permeability. This was not deter-
mined by decreased porin expression (Livermore,
1984) but may have reflected porin regulation by
other outer-membrane components such as the
LPS. We therefore examined LPS-related surface
properties in large groups of distinct carbenicillin-
resistant and -sensitive P. aeruginosa strains for
possible relationships to antibiogram. Intrinsically-
resistant strains almost invariably had unusually
high levels of resistance to tetracycline, chloram-
phenicol and nalidixic acid, as well as reduced
susceptibility to other antipseudomonal β-lactam
compounds, and this was taken to suggest a non-
specific, impermeability-based mechanism. In-
volve ment of β lactamase in their resistance had
been discounted previously (Williams et al., 1984b).
A similar cross-resistance profile is observed in naf/B
laboratory mutants obtained under selection press
ure with nalidixic acid (Rella and Haas, 1982) and
a cross-hypersusceptibility to tetracyclines, chlor-
amphenicol and quinolones is observed in some
carbenicillin-hypersusceptible strains (Noguchi
et al., 1980; Livermore, 1984). It seems unlikely that
any specific target modification simultaneously
could modulate susceptibility to such a range of
diverse chemicals. The continued full activity of
gentamicin against the carbenicillin-resistant
strains could be accommodated by the hypothesis
that aminoglycosides permeate the P. aeruginosa
outer membrane by a self-promoting pathway
different from that taken by most other hydrophilic
molecules (Hancock, 1984), or by the hypothesis
that cytoplasmic-membrane transport is the rate-
limiting step in the uptake of gentamicin.

We used serotyping and bacteriophage-suscepti-
bility tests as indirect probes of cell-surface architec-
ture and LPS structure. Serotype specificity in P.
aeruginosa relates to the sugar composition of the
O-antigenic side chains of the LPS (Chester et al.,
1973), and many bacteriophages have receptor sites
which depend, at least partly, on the LPS (Koval
and Meadow, 1977; Mutharia et al., 1982; Meadow
and Wells, 1985). Polyalanine gel electrophore-
sis was used to obtain direct profiles of the LPS and
to examine the relative amounts of rough and
smooth material in each strain.

Little correlation was detected between the sur-
face architecture, as elucidated by these methods,
and intrinsic resistance to antibiotics, even when
only the most highly resistant organisms were
considered. A wide range of serotypes was observed
amongst the resistant strains and the prevalence of individual types did not vary significantly between the carbenicillin-resistant and -sensitive groups. Similarly, no significant differences were observed between the susceptibility of carbenicillin-resistant and -sensitive strains to lysis by various bacteriophages except possibly by Col. 21. Nor, in general, did the relative amounts of smooth and rough LPS present in the cells differ between the resistant and sensitive strains. We do not afford great significance to the absence of high mol. wt smooth LPS forms from the two highly resistant O:11 strains M76 and M1499. Highly resistant strains of other serotypes had LPS which was indistinguishable from that of sensitive strains of the same serotype, and other investigators have associated lack of high mol. wt LPS forms with hypersusceptibility to \( \beta \)-lactam and other antibiotics (Koval and Meadow, 1977; Darveau and Hancock, 1983).

Our findings contrast with those obtained in a six-centre study undertaken in Greece, where it was observed that carbenicillin-resistant \( P. \) aeruginosa strains that did not produce carbenicillinase generally were agglutinated by pooled antisera to serotypes O:2, O:5, O:15 and O:16 but not by any individual antiserum (Legakis et al., 1982). Carbenicillin-sensitive isolates from the same hospitals gave widely diverse serotype reactions. Intrinsically carbenicillin-resistant isolates from the Greek hospitals also were reported by Shearer and Legakis (1985) not to be lysed by bacteriophages 16, 44, 109 and F8, which these authors considered to be specific for smooth LPS. Other groups, e.g., Meadow and Wells (1985), believe that phage 109 has a receptor site determined by the core oligosaccharide. These findings may indicate a basic difference in the types of intrinsic carbenicillin resistance prevalent in \( P. \) aeruginosa from Britain and Greece. It is noted, in this context, that the carbenicillin-resistant isolates from Greece were often cross-resistant to gentamicin.

It is also interesting to compare our findings regarding the surface architecture of naturally-occurring carbenicillin-resistant \( P. \) aeruginosa with the results of studies performed on strains which developed \( \beta \)-lactam resistance in the laboratory. Loss of O-serotype determinants and phage receptors during the selection of cefsulodin resistance in \( P. \) aeruginosa was described by Slack and Pitt (1982) and increased carbenicillin resistance was observed in several \( P. \) aeruginosa mutants selected for resistance to phage 16 by Shearer and Legakis (1985). Neither of these associations was observed in the naturally-occurring resistant strains, where nontypability was as rare as in sensitive isolates and where bacteriophage 16 lysed similarly small numbers of resistant (12 out of 122) and sensitive (16 out of 127) strains. Using ethyl methane sulphonate mutagenesis, Godfrey et al. (1984) obtained four different types of \( P. \) aeruginosa mutants that had altered LPS sugar composition in association with impermeability-mediated \( \beta \)-lactam resistance. However, none of these mutants showed the increased resistance to chloramphenicol and tetracycline that was typical of the naturally-occurring carbenicillin-resistant strains.

Our findings do not disprove the involvement of the LPS in regulating antibiotic uptake in \( P. \) aeruginosa. Changes in the unexposed core oligosaccharide-lipid A region of the LPS could be envisaged that might be significant in regulating porin function without changing those surface properties of the cell that were examined in the present study. Our results do, however, reveal the great diversity of surface properties and LPS types present in the naturally-occurring resistant strains and caution against affording general significance to minor changes in LPS that may be observed concommittantly with the development of resistance in vitro.

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