Factors Involved in Beta-Lactam Antibiotic Resistance in
Pseudomonas aeruginosa

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A temperature-sensitive mutant, strain SS, which is assumed to be devoid of
beta-lactamase activity and deficient in a permeability barrier to antibiotics, was
isolated from a beta-lactamase-less mutant, strain L-2, derived from Pseudomonas
aeruginosa IFO 3080. By comparing the beta-lactam antibiotic susceptibility be-
tween strains IFO 3080, L-2, and SS, the involvement of both beta-lactamase and
a permeability barrier in determining the beta-lactam antibiotic resistance was
estimated.

In gram-negative bacteria, it has been indi-
cated that not only beta-lactamase but also the
outer membrane appears to be involved in the
beta-lactam antibiotic resistance, the latter as a
permeability barrier to the drugs (6). This is
considered to be the case in Pseudomonas
aeruginosa (8).

We have attempted to estimate the participa-
tion of both beta-lactamase and a permeability
barrier in resistance to individual beta-lactam antibi-
tsics in P. aeruginosa. P. aeruginosa IFO 3080 was found to possess an inducible beta-lacta-
mande (type 1d) as repressed in other strains of
this organism (2, 6, 7). A penicillinase plasmid
was not detected in this strain. To elucidate the
role of the inducible beta-lactamase, a beta-lacta-
mande-less mutant, strain L-2, was obtained from
P. aeruginosa IFO 3080 as follows. The parent-
ral strain, IFO 3080, was first converted to a beta-
lactamase-constitutive mutant that was fur-
ther mutated to a beta-lactamase-less strain, L-2,
as reported earlier (4). The beta-lactamase-consti-
tutive revertants that recovered enzyme activity
simultaneously with resistance to beta-lactam antibi-
tsics were obtained with a frequency of
about 10^{-6}, thus indicating that strain L-2 is a
beta-lactamase-less rather than a noninducible
mutant.

Table 1 indicates that among the beta-lactam
antibiotics tested the resistance to penicillin
G, ampicillin, and cephaloridine was dramati-
cally reduced in strain L-2. Thus, it appears that
the inducible beta-lactamase plays a major role
in resistance to these antibiotics. However,
the lack of beta-lactamase seems to have no sig-
nificant effect on the sensitivity to non-beta-lac-
tam antibiotics. On the other hand, strain L-2
remained resistant to methicillin, cephalothin,
cefazolin, and cephalaxin.

Methicillin was not inactivated by the beta-lac-
tamase of strain IFO 3080. It seems curious,
however, that the beta-lactamase-less mutant,
strain L-2, did not become sensitive to cephalo-
thin, cefazolin, and cephalaxin, which are all
readily hydrolyzed by the enzyme of IFO 3080.
The possibility that some inactivation mech-
nisms other than beta-lactamase are operative in
the resistance to cephalothin, cefazolin, and
cephalaxin was eliminated when we observed
that contact of intact cells of strain L-2 with
these drugs did not result in a significant de-
crease in their antibiotic activities.

Thus, it is anticipated that the high resis-
tance to methicillin, cephalothin, cefazolin, and
cephalaxin in strain L-2 might be due to their
poor permeability through the outer mem-
brane, or to their weak inhibitory effects on
target enzymes.

To clarify the mechanism of this resistance, a
cefazolin-sensitive mutant was isolated from
strain L-2 after treatment with N-methyl-N'-
nitro-N-nitrosoguanidine. The sensitive mu-
tant, strain SS, which was unable to grow in
the presence of 10 μg of cefazolin per ml, was
selected by replica plating. The minimal inhibi-
tory concentrations of various antibiotics were
drastically and nonspecifically lowered in
strain SS, as indicated in Table 1. In particular,
strain SS became sensitive to actinomycin D,
which is known to be less permeable through the
outer membrane of Escherichia coli (3, 9).
It therefore appears that strain SS is a perme-
ability mutant in which various drugs pene-
trate the cells more readily.

Furthermore, strain SS was a temperature-
sensitive mutant; growth was rather good at
28°C, although slower than the parent, was
suppressed significantly at 37°C, and was negli-
gible at 42°C. The growth at 37°C was stimu-
lated by 0.05 to 0.1 M inorganic salts or
sucrose. A 0.05 M sodium phosphate buffer (pH 7.0) was the most effective and was usually added to the medium for minimal inhibitory concentration determinations. These results suggest that strain SS may have a defect in peptidoglycan synthesis and, therefore, is osmotically labile. It was reported that bacterial cells in which peptidoglycan synthesis was inhibited by penicillins lost their permeability barrier drastically (1). Preliminary experiments showed that strain SS was more susceptible to the treatment by lysozyme in the absence of ethylenediaminetetraacetic acid or by deoxycholate than strain L-2. Therefore, strain SS could be regarded as a permeability barrier-deficient mutant.

By comparing \( \beta \)-lactam antibiotic susceptibility between strains IFO 3080, L-2, and SS, the involvement of \( \beta \)-lactamase and a permeability barrier in individual \( \beta \)-lactam antibiotic resistance could be estimated separately.

It therefore appears that \( \beta \)-lactamase is a major factor in the resistance to penicillin G, ampicillin, and cephalexin. On the other hand, a permeability barrier rather than \( \beta \)-lactamase appears to be mainly responsible for the resistance to methicillin, cephalothin, cefazolin, and cephalaxin because the resistance to these antibiotics was retained in strain L-2 but completely lost in strain SS.

The situation seems to be different in \( E. \) coli, in which cephalosporins penetrate more readily into the cells than penicillins, as recently reported (5). A permeability barrier-deficient mutant of \( P. \) aeruginosa was isolated (8), but it still possessed an inducible \( \beta \)-lactamase.

The mutant strains described here would be useful in estimating separately the role of \( \beta \)-lactamase and a permeability barrier in \( \beta \)-lactam antibiotic resistance in \( P. \) aeruginosa.

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**LITERATURE CITED**


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