Comparative sensitivity and resistance of some strains of *Pseudomonas aeruginosa* and *Pseudomonas stutzeri* to antibacterial agents

A. D. RUSSELL AND A. P. MILLS

From the Welsh School of Pharmacy, University of Wales Institute of Science and Technology, Cardiff

SYNOPSIS A comparison has been made of the sensitivities to various antibiotic and non-antibiotic substances of some strains of *Pseudomonas aeruginosa* and *P. stutzeri*, the latter including strains isolated from eye and other cosmetic products and from other sources. Whereas *P. aeruginosa* strains showed a high resistance to cetrимide and to benzalkonium chloride, the *P. stutzeri* strains were generally more sensitive to these and to chlorhexidine. The *P. stutzeri* strains were also more sensitive to the various antibiotics tested. The loss of the ability to transfer an R factor by two strains of *P. aeruginosa* caused no significant change in their drug sensitivity pattern.

The resistance of *Pseudomonas aeruginosa* to many antibacterial agents has long been a problem (see Brown, 1971, for a comprehensive review). The comparative resistance of other pseudomonads has, in contrast, been little studied. *P. stutzeri* has been reported to be a frequent contaminant of eye cosmetics (Bruch, 1971) and it was thus decided to compare the sensitivity to various substances of some NCTC and ATCC strains and of some strains isolated from eye cosmetics and other sources with some previously studied *P. aeruginosa* strains (Russell, 1972a) and with some recent hospital isolates of the latter organism.

Materials and Methods

SUBSTANCES USED

These were chlorhexidine diacetate, cetrимide, benzalkonium chloride (BZK), tobramycin, gentamicin, polymyxin B sulphate, ampicillin, carbenicillin, cephaloridine and a new cephalosporin, cephaclor (the sodium salt of 7-cyanacetamidocephalosporanic acid).

ORGANISMS

Hospital isolates of *P. aeruginosa*, obtained from hospitals in Cardiff and Dublin, were kindly provided by Dr D. J. Stickler. Five NCTC strains of *P. aeruginosa* (10662, 6750, 8203, 10701, and 10490) and two strains bearing R factors (R⁺1822 and R⁺3425), kindly supplied by Dr E. J. L. Lowbury, were also used.

Three NCTC strains of *P. stutzeri* (10450, 10473 and 10475), two ATCC strains, and some strains isolated from eye and other cosmetic products and from other sources were also studied. The *P. stutzeri* strains are listed in table I. All strains were grown overnight at 37°C in 10 ml of nutrient broth (Oxoid no. 2) in McCartney bottles.

<i>P. stutzeri</i> Strain	Source
10450, 10473, 10475	NCTC
17592, 17685	ATCC
1159 ¹	Treatment room floor
1195 ¹	Scalp wound
1290 ¹	Patient's urine
2771 ¹	Sink drain
3197 ¹	Cutting oil
3282 ¹	Sink drain
1512 ²	Brown cosmetic colour material
1514 ²	Eye shadow
1520 ²	Eye shadow
1527 ²	Lemon lotion
1531 ²	Body cream

Table I Source of *P. stutzeri* strains

¹Obtained from Dr Marcia A. Moody, Baltimore, Maryland, USA

²Obtained from Dr C. W. Bruch and Dr J. R. Evans, Washington, DC, USA

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATIONS (MICS)

Overnight cultures of the organisms were diluted 1/100 in nutrient broth in McCartney bottles, and these were incubated for two hours at 37°C to allow the organisms to be in the logarithmic growth phase. Volumes, each of 0.05 ml (containing about 10⁶

viable cells) were then dropped on to the surface of nutrient agar (Oxoid) plates containing the desired concentration of the required drug, the plates having previously been dried for three hours at 37°C. Plates were incubated for 24 hours at 37°C, and the lowest concentration of a drug which prevented colony formation was taken as the MIC.

Results and Discussion

The MICs of non-antibiotic substances against the various strains are listed in table II. The hospital isolates of *P. aeruginosa* show a similar response to the NCTC strains, including NCTC 10662 which is used as a 'standard' strain (National Collection of Type Cultures, 1972). Minimum inhibitory concentration values may be slightly different from those described earlier (Russell, 1972a) because of a slight modification in technique. The rather high resistance of *P. aeruginosa* to cetrime and to BZK is to be noted, although three *P. stutzeri* strains (1290, 2771, and 3197) are much less susceptible to these agents than the other strains. Whereas most of the *P. stutzeri* strains are inhibited by < 10 µg/ml of chlorhexidine, a few strains are only inhibited at levels corresponding to the MIC values of this compound against *P. aeruginosa*.

Table III gives the MICs of some antibiotics

against the various strains. As before, *P. aeruginosa* (NCTC 10662) is used as a 'standard' strain. All strains of *P. aeruginosa* grew in agar containing 500 µg/ml of cephaloridine, ampicillin, and a new cephalosporin, cephacetrile (Russell, 1972b). Strain NCTC 10701 is extremely sensitive to carbenicillin and is the test organism in its assay. Strains of *P. aeruginosa* were fairly sensitive to tobramycin, polymyxin, and gentamicin, although no allowance was made for the magnesium content of the agar medium (Garrod and Waterworth, 1969). Most of the *P. stutzeri* strains were inhibited by 20 to 25 µg/ml of carbenicillin and were highly sensitive to polymyxin, tobramycin, and gentamicin. They were generally fairly sensitive to cephaloridine and to ampicillin, but were generally not inhibited even by high concentrations of cephacetrile; in this context, however, two strains (1159 and 1520) were abnormally sensitive to carbenicillin, ampicillin, cephacetrile, and cephaloridine and two other strains of *P. stutzeri* (1195 and 3282) showed an above-average resistance to carbenicillin, ampicillin, and cephaloridine. The possibility that the presence or absence of a β-lactamase is involved has yet to be considered.

Moody, Young, and Kenton (1972) have found that *P. stutzeri* strains were susceptible to most of the antibiotics which they tested.

<i>Pseudomonas</i> Strains	MIC (µg/ml) after 24 Hours at 37°C of		
	BZK ¹	CA ¹	CET ¹
<i>P. aeruginosa</i>			
16 hospital strains	100- >250	25-50	500-750
NCTC 10662	150	50	750
NCTC 6750	256	20	> 1000
NCTC 8203	256	20	> 1000
NCTC 10701	256	20	512
NCTC 10490	512	40	1024
R+ 3425	512	20	1024
3425	256	20	512
R+ 1822	256	20	512
1822	256	20	512
<i>P. stutzeri</i>			
NCTC 10450	25	<10	50
NCTC 10473	10	<10	50
NCTC 10475	10	<10	50
ATCC 17592	50	<10	25
ATCC 17685	25	<10	50
1159	5	<10	> 10
1195	50	<10	50
1290	> 100	<10	250
2771	> 100	<10	250
3197	100	> 10	250
3282	50	<10	75
1512	100	20	75
1514	75	20	75
1520	10	20	> 10
1527	100	50	100
1531	75	50	100

Table II MIC values for different non-antibiotic antibacterial agents

¹BZK = benzalkonium chloride, CA = chlorhexidine diacetate, cet = cetrime

<i>Pseudomonas</i> Strains	MIC (μ g/ml) after 24 Hours at 37°C of						
	Carb	Amp	CAA	Cer	PM	GM	Tob
<i>P. aeruginosa</i>							
16 Hospital strains	100-250	> 500	> 500	> 500	5-15	1.5-6	
NCTC 10662	125	> 500	> 500	> 500	2.5-5	2.5	2
NCTC 6750	62.5	2000	> 4000	> 4000	32	10	1.5
NCTC 8203	62.5	2000	> 4000	4000	16	2.5	1.5
NCTC 10701	0.8	< 500	> 4000	2000	16	2.5	1
NCTC 10490	7.75	1000	> 4000	4000	16	10	2
R+3425	> 4000	> 4000	> 4000	> 4000	32	5	5-10
3425	> 4000	> 4000	> 4000	> 4000	8	5	
R+1822	> 4000	> 4000	> 4000	> 4000	32	10	5
1822	> 4000	> 4000	> 4000	> 4000	32	10	
<i>P. stutzeri</i>							
NCTC 10450	5	5		25	< 2.5	< 1.5	
NCTC 10473	250	> 25	> 500	> 25	< 2.5	< 1.5	
NCTC 10475	50	5	> 500	25	< 2.5	< 1.5	
ATCC 17592	25	25	250	10	1.25	1	
ATCC 17685	25	25	> 250	25	1.25	2	
1159	< 0.5	1	15	10	2.5	< 0.5	0.5
1195	> 40	> 40	> 500	100	2.5	1.5	1.5
1290	5	25	100	25	5	1.5	1
2771	2.5	25	> 500	50	5	1.5	1
3197	10	5	> 500	10	2.5	1.5	1
3282	> 40	> 40	> 500	100	2.5	1.5	1.5
1512	20	25	> 500	100	5	1.5	1
1514	20	10	> 500	2.5	2.5	5	
1520	< 0.5	1	15	5	2.5	< 0.5	< 0.5
1527	20	25	> 500	25	2.5	1.5	1
1531	20	10	> 500	25	5	1.5	1

Table III MIC values for different antibiotics

Carb = carbenicillin, Amp = ampicillin, CAA = cephradine, Cer = cephaloridine, PM = polymyxin B sulphate, Gm = gentamicin, Tob = tobramycin.

Old subcultures, stored on nutrient agar slopes at 4°C, of *P. aeruginosa* R+1822 and R+3425 appeared to have lost their ability to transfer their R factor to an *E. coli* acceptor strain, and accordingly a brief study was made of the sensitivity of these latter strains (designated simply 1822 and 3425) to some of the antibacterial agents. The results (tables II and III) indicate that 3425 and 1822 show a similar sensitivity to antibiotic drugs and to non-antibiotic antibacterial agents as do R+3425 and R+1822, respectively. In particular, the very high resistance of all four strains to the 4 β -lactam antibiotics is to be noted. Preliminary experiments suggest that, although 3425 and 1822 have lost the ability to transfer the R-factor, they still produce β -lactamase, and this could obviously contribute to their resistance to these drugs.

Of the pseudomonads (reviewed by Stanier, Palleroni, and Doudoroff, 1966), *P. stutzeri* NCIB 9040 (NCTC 10473) was found by Wilkinson (1967, 1970) to be even more sensitive than *P. aeruginosa* to the chelating agent, ethylenediamine tetraacetic acid (EDTA), and, with *P. synchyanea*, to be remarkably sensitive to mechanical disruption (Wilkinson, 1970). Differences in cell wall chemistry (Wilkinson and Carby, 1971) and, more especially in the arrangement of wall layers, could be re-

sponsible for the comparative sensitivity of *P. stutzeri* to EDTA and to the compounds contained in table I. Both *P. stutzeri* and *P. synchyanea* contain less wall muramic acid than other strains of pseudomonads, and appear to stand apart from other species and from each other with regard to their cell wall structure according to Wilkinson (1970), who also suggests that the chemical basis for sensitivity of *P. stutzeri* to EDTA may not be strictly comparable with that of the other *Pseudomonas* sp investigated. However, most of the strains of *P. stutzeri* are sensitive to ampicillin and are more sensitive to carbenicillin than the hospital isolates of *P. aeruginosa* (table II).

The pathogenicity of *P. stutzeri* strains is not clear (Bruch, 1971). However, their occurrence as contaminants in eye and other cosmetics and in other sources suggests that more knowledge is needed of their sensitivity to antimicrobial agents. The results presented here indicate that they are generally more sensitive than *P. aeruginosa* to various types of antibiotics, and to non-antibiotic antibacterial agents of the type used as disinfectants and/or as preservatives in a wide variety of pharmaceutical and cosmetic products.

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