***Staphylococcus aureus* Strain DU4916 – an Atypical Methicillin-resistant Isolate?**

By R. W. LACEY

Department of Bacteriology, University of Bristol, Bristol BS8 1TD

(Received 4 April 1974)

SUMMARY

Staphylococcus aureus strain DU4916 was examined for linkage of markers. No 'curing' action due to treatment with acridine dyes was demonstrated. The genes for penicillinase production and metal-ion resistance (borne by a plasmid of 20×10^6 daltons), tetracycline resistance (a plasmid of 2.9×10^6), streptomycin resistance (a chromosomal gene) and methicillin resistance (of uncertain locus) were separately transduced to recipients. No linkage of methicillin resistance with enterotoxin B, haemolysins, pigment, or a gene that suppresses inducible resistance to erythromycin was demonstrated. These data are consistent with the hypothesis that all methicillin-resistant strains of *S. aureus* have a common and relatively recent origin.

INTRODUCTION

Coelimination and cotransduction data suggest that a number of markers can be linked in *S. aureus* strain DU4916 (Dornbusch, Hallander & Löfquist, 1969; Dornbusch & Hallander, 1973; Dornbusch, 1973); these markers are penicillinase production, resistance to mercury and cadmium ions, resistance to methicillin, production of enterotoxin B, a factor that apparently suppresses phenotypic expression of erythromycin resistance, and production of β - and δ -haemolysin. In addition to these, alterations in phage-typing pattern and loss of pigment were shown by some derivatives of strain DU4916 after treatment with acriflavine.

In methicillin-resistant staphylococci isolated in the U.K., the genes that determine methicillin resistance are not linked to those specifying other antibiotic resistance, or to haemolysin or pigment production (Lacey, 1972). Methicillin resistance is also not linked to other markers in strains from several countries (Cohen & Sweeney, 1970; Kayser, Wüst & Corrodi, 1972). Thus methicillin-resistant staphylococci obtained from many sources possess very similar properties, and have probably evolved recently from a single clone (Lacey & Grinsted, 1973). If there is linkage of methicillin resistance to other markers in strain DU4916, then some modification of the single clone hypothesis for the origin of methicillin-resistant strains may be necessary. The linkage of so many markers, contributing to both antibiotic resistance and pathogenicity, in DU4916 could have important epidemiological significance. Therefore, in order to confirm or negate such linkage, strain DU4916 has been re-examined.

METHODS

Strains of S. aureus. Most of the experiments were performed on a clone of strain DU4916 supplied by Dr R. P. Novick. Strain DU4916 refers to this clone, unless otherwise stated. A thrice-plated single colony was maintained on nutrient agar slopes at 4 °C. A fresh slope was subcultured for each experiment and then discarded. Attempts to eliminate antibiotic

resistance with acriflavine treatment were also performed on another clone of strain DU4916 maintained in Stockholm. Strains DU4916 *pen*⁻ *met*-*r*, DU4916 *pen*⁺ *met*-*s*, and DU4916 *pen*⁻ *met*⁻ (Dornbusch & Hallander, 1973) were also examined. Strain 11164 *met*-*s* is a methicillin-sensitive derivative of strain 11164 wild (Lacey, 1972). Strain 649N is a derivative of strain 649 sensitive to antibiotics and metal ions and containing no detectable plasmid DNA (Grinsted & Lacey, 1973). Strain 13136 is a methicillin-resistant isolate (Lacey, 1972).

Sensitivity tests. These were performed by a plate dilution method using single cell inocula (Lacey & Grinsted, 1972). Results are expressed as minimum inhibitory concentration (m.i.c.). The nature of methicillin resistance was determined by performing Miles and Misra counts on nutrient agar containing doubling dilutions of methicillin with or without 5 % (w/v) NaCl. The inoculated plates were incubated for 48 h at 30, 37 and 42 °C. Penicillinase was detected by the starch-iodine method. Loss of antibiotic resistance was determined by replica plating, using media as described (Lacey, 1972).

Treatment with acriflavine and acridine orange. Cultures were inoculated to 10⁴ cocci/ml into nutrient broth containing doubling dilutions of either acridine, incubated overnight, and then the organisms from the tube containing the highest concentration of acridine permitting visible growth were examined for loss of resistance.

Media and bacteriophage typing. These were described previously (Lacey, 1972).

Mutagenesis. Mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) was by the method of Lacey & Grinsted (1972).

Preparation of transducing lysate. Typing phages 29 and 88 were propagated on the donor in three successive cycles by the freeze-thawing method of Williams and Rippon (Lacey, 1972). The lysates were sterilized by two successive filtrations through 0.45 µm Millipore filters and 1 ml samples were then checked for sterility by inoculating nutrient agar plates. The lysates were stored at 4 °C and before each transduction experiment were filtered once more. These lysates contained 10⁸ to 10¹⁰ p.f.u./ml (tested on strain 649).

Transduction and irradiation of lysates by u.v. radiation. These were essentially as described previously (Lacey, 1972) with a phage-to-cell ratio of about 0.1. To test for cotransduction of resistance markers, transductants were selected on media containing single agents and the resultant colonies replica plated onto media containing other agents.

Isolation and characterization of plasmid DNA. Covalently closed circular (c.c.c.) plasmid DNA was isolated by ethidium bromide/CsCl gradient centrifugation and analysed on sucrose gradients as described previously (Lacey & Grinsted, 1972).

Identification of derivatives. A proportion of the recipients in each transduction experiment were phage-typed and examined for other properties [pigment, Tween 80 reaction (Lacey, 1972); haemolysin production and antibiogram] to ascertain that these colonies were true transductants, and not contaminants. Detection of α-, β- and δ-haemolysins and recording of pigment were as described previously (Lacey, 1972). Enterotoxin production was assayed on culture filtrates, some of which were concentrated, by the method of Šimkovičová & Gilbert (1971).

RESULTS

Phenotypic properties of strain DU4916 and derivatives

The isolate of strain DU4916 examined resembled that of Dornbusch & Hallander (1973), giving a phage typing pattern of 29/88 with group inhibition reactions, and resistant to streptomycin (m.i.c. > 10 mg/ml), tetracycline (m.i.c. 200 µg/ml), cadmium ions (m.i.c. > 10⁻⁴ M) and mercury ions (m.i.c. 100 µg/ml). Strain DU4916 produced penicillinase, but was sensitive to erythromycin, neomycin and novobiocin; it was resistant to all penicillins

and cephalosporins. However, the phenotypic nature of the methicillin resistance in strain DU4916 was different from that of other methicillin-resistant strains which show heterogeneous resistance; that is, only a minority of the cells are resistant at 37 °C, but at 30 °C or below, or in the presence of added NaCl, the entire cell population is resistant to high levels of methicillin (Dyke, 1969). Thus every cell of strain DU4916 was resistant to methicillin at 30, 37, and 42 °C, and at 37 °C in the presence of 5 % (w/v) NaCl. The m.i.c. of methicillin varied from 2000 µg/ml at 30 °C to 400 µg/ml at 42 °C. Since such homogeneously resistant variants can be obtained from heterogeneously resistant populations (Dyke, 1969), the clone examined here may be such a derivative. The mean doubling time of strain DU4916 was increased in the presence of methicillin (> 60 min in nutrient broth at 37 °C containing 50 µg methicillin/ml) and therefore DU4916 resembles other methicillin-resistant isolates which also have reduced growth rates in the presence of methicillin (Sutherland & Rolinson, 1964).

Strain DU4916 *pen*⁻ *met-r* resembled strain DU4916 wild except for the absence of phenotypic properties associated with a penicillinase plasmid, and thus seems a *bona fide* derivative of strain DU4916 as proposed by Dornbusch & Hallander (1973).

However, strains described as DU4916 *pen*⁺ *met-s* and DU4916 *pen*⁻ *met-s* differed from strain DU4916 wild-type in their sensitivity to streptomycin, in addition to their reaction to erythromycin (Dornbusch & Hallander, 1973). Methicillin-resistant staphylococci are also characterized by a ribosomal resistance to streptomycin (Lacey & Grinstead, 1973), which enables the cell to withstand 10 mg streptomycin/ml. Both strain DU4916 wild-type and DU4916 *pen*⁻ *met-r* were, as expected, resistant to 10 mg streptomycin/ml. But strains 'DU4916 *pen*⁺ *met-s*' and 'DU4916 *pen*⁻ *met-s*' were resistant to only 100 µg streptomycin/ml and do not seem to be so closely related to DU4916 wild-type.

Transduction of antibiotic resistance from strain DU4916

In preliminary experiments, higher transduction frequencies were obtained with phage 88 propagated on strain DU4916 than with phage 29. In subsequent experiments markers were therefore transduced by phage 88.

Resistance to cadmium ions was transduced to strain 649N at a frequency of 6×10^{-7} . Seventy transductants were tested for resistance to mercuric and arsenate ions and penicillinase production: all had become resistant to the metal ions and produced penicillinase. Six hundred transductants selected on cadmium agar were examined by replica plating for penicillinase production and resistance to methicillin, tetracycline, erythromycin and streptomycin. All produced penicillinase but no colony was resistant to the other antibiotics. Thus strain DU4916 harbours linked genes for penicillinase production and resistance to mercury, cadmium and arsenate ions. Exposure of the phage preparation to u.v. radiation before addition to the recipient produced a progressive decline in the transduction frequency (Fig. 1), which supports the suggestion that these genes are borne by a plasmid (Dornbusch, 1973).

Tetracycline resistance was transduced from strain DU4916 to 649N at a frequency of 3×10^{-6} and there was also no cotransduction of other markers (resistance to methicillin, streptomycin, cadmium ions and erythromycin) in about 650 transductants that were tested. Streptomycin resistance was also transduced singly to strain 649N at a frequency of 3×10^{-8} . Exposure of the phage preparation to u.v. light caused a rise in the transduction frequency of streptomycin resistance and a progressive fall in that of tetracycline resistance (Fig. 1), suggesting a chromosomal locus for the genes coding for streptomycin resistance and a plasmid locus for those determining tetracycline resistance in strain DU4916.

Fig. 1. Transduction of resistance to tetracycline, cadmium ions and streptomycin by propagation of phage 88 on strain DU4916. The lysate was exposed to u.v. light for up to 6 min. Recipient was strain 649N. ●, Tetracycline resistance; ○, cadmium ion resistance; ×, streptomycin resistance.

Methicillin resistance was not transduced to strain 649N (frequency $< 10^{-10}$). Several derivatives of strain 649 were then examined for their ability to be converted to methicillin resistance by phage 88; these were 649 *tet-r* which contains a plasmid coding for tetracycline resistance transduced from strain DU4916 (see below), strain 649 *pen*⁺ which contains the penicillinase plasmid from strain DU4916 (see below), strain 649 *pen*⁺ → *pen*⁻, a derivative of strain 649 *pen*⁺ which had lost this plasmid, and strain 649MR which harbours at least six plasmids including one coding for penicillinase production and another for tetracycline resistance (Lacey & Chopra, 1974). Methicillin resistance was transduced from unirradiated phage preparations to strains 649 *pen*⁺, 649 *pen*⁺ → *pen*⁻, and 649MR at frequencies between 1.5×10^{-9} and 6×10^{-9} . But methicillin resistance could not be transduced to strain 649 *tet-r*. The failure to transduce methicillin resistance to 649 *tet-r* was probably not due to incompatibility between the *tet-r* and *met-r* genes, because the latter were stable in 649MR which contained the *tet-r* plasmid. Thus the previous presence of the penicillinase plasmid in the recipient seems essential for the detection of transductants resistant to methicillin, although the presence of the penicillinase plasmid itself is not necessary. Strain 649 *pen*⁺ → *pen*⁻ was examined for c.c.c. DNA (see below) and none was found. These results are similar to those of Cohen & Sweeney (1970) for another strain from which methicillin resistance could only be transduced to a recipient that had previously harboured a penicillinase plasmid. The possibility that recombination occurs between the penicillinase plasmid and genes for methicillin resistance as suggested by Dornbusch & Hallander (1973) seems to be excluded by the inability to demonstrate cotransduction of methicillin resistance and penicillinase, and by the transduction of methicillin resistance into strain 649 *pen*⁺ → *pen*⁻.

The effect of u.v. exposure of the phage preparation on the transduction frequency of methicillin resistance was investigated with strains 649MR and 649 *pen*⁺ as recipients. The maximum increases in transduction frequency were approximately six- and sevenfold for recipients 649MR and 649 *pen*⁺ respectively. One resistant recipient (649MR, *met-r*) obtained

by the addition of unirradiated phage to strain 649MR was itself used as donor in transduction of methicillin resistance to strain 649MR, *met-s*. The frequency was 3×10^{-9} from unirradiated lysate, rising to a maximum of 1.3×10^{-8} after exposure to u.v. irradiation for 1 min. Thus, as in other methicillin-resistant strains (Cohen & Sweeney, 1970; Lacey, 1972) the genes for methicillin resistance were transduced singly at low frequency from strain DU4916, and the slight increase in transduction frequency after u.v. irradiation to the phage is not characteristic of either plasmid or chromosomal genes.

In other experiments methicillin resistance was transduced from strain DU4916 by unirradiated phage to strain 11164 *met-s* at a frequency of 5×10^{-9} .

Properties of methicillin-resistant transductants

Twenty colonies of strains 649MR, *met-r* and 11164 *met-r* together with the strains from which they were derived (649MR, *met-s* and 11164 *met-s*) were plated on antibiotic-free medium and then examined quantitatively for antibiotic resistance. Strain 649MR, *met-s* and the 649MR, *met-r* derivatives all showed the same level of resistance to streptomycin, tetracycline, erythromycin, chloramphenicol and penicillin. Similarly strains 11164 *met-s* and the derivatives 11164 *met-r* were resistant to streptomycin, tetracycline, erythromycin, neomycin and novobiocin. These findings exclude the possibility that the derivatives 649MR, *met-r* and 11164 *met-r* are residual whole cells of strain DU4916 contaminating the phage preparation (strain DU4916 is sensitive to erythromycin, neomycin, chloramphenicol and novobiocin).

Strain 11164 *met-s* produces no β - or δ -lysin and little α -lysin, is non-pigmented, and gives a phage typing pattern of 84/85/88. Each of the transductant 11164 *met-r* isolates retained these properties. Similarly both strain 649MR, *met-s* and the *met-r* derivatives of 649MR produced α - and δ -, but not β -lysin, were orange pigmented and gave a phage typing pattern of 88.

Although every organism of cultures of strain DU4916 showed homogeneous resistance to methicillin, all ten *met-r* transductants of strains 649MR and 11164 *met-s* tested were heterogeneously resistant to methicillin; i.e. at 30 °C or in the presence of 5 % (w/v) NaCl every organism of each culture was resistant (m.i.c. 400 μ g methicillin/ml), but at 37 and 42 °C only about one in 10^5 cocci produced a colony on media containing moderate to high levels (25 to 200 μ g/ml) of methicillin. The reason for the expression of methicillin resistance homogeneously in DU4916 but heterogeneously in the transductants is not clear. The possibility that two sets of genes contributed to methicillin resistance in DU4916 could not be investigated because of the inability to transduce homogeneous resistance.

Strain 649MR does not produce enterotoxins A, B, C or E, while strain 11164 *met-s* produces enterotoxin B although not A, C or E. Three transductants of 649MR, *met-r* were tested and all were enterotoxin B negative, and each of the three transductants of 11164 *met-r* tested retained the ability to produce enterotoxin B. Thus although many methicillin-resistant staphylococci produce enterotoxin B (Dornbusch *et al.* 1969), there does not appear to be linkage between the genes coding for methicillin resistance and production of enterotoxin B. Strain 11164 *met-s* is expected to be enterotoxin B positive as it was derived from strain 11164 wild-type, a methicillin-resistant isolate (Lacey, 1972).

Plasmid (c.c.c.) DNA from strain DU4916

Strain DU4916 wild-type was grown in [³H]thymidine, lysed and the lysate analysed by ethidium bromide/CsCl-gradient centrifugation. A satellite band of c.c.c. DNA occurred on the dense side of the chromosomal band and was analysed on sucrose gradients. The

Fig. 2. Sucrose-gradient analysis of c.c.c. DNA from strain DU4916 labelled with [^3H]thymidine and from strain 13136 labelled with [^{14}C]thymidine. ●, ^3H (DU4916); ○, ^{14}C (13136).

plasmid DNA was resolved into two major bands and one minor band. Plasmid DNA from another methicillin-resistant isolate (strain 13136) was labelled with [^{14}C]thymidine and used as an internal marker. The plasmid DNA from strain 13136 also sediments as three bands (Lacey & Grinsted, 1973); these bands co-sedimented with each of those bands obtained from strain DU4916 (Fig. 2); the major bands were at 25 and 50 S and the minor at 36 S. The 50 and 36 S components from strain 13136 represent the penicillinase plasmid, and the 25 S the tetracycline plasmid (Lacey & Grinsted, 1973). Thus strain DU4916 probably contains plasmids for penicillinase production and tetracycline resistance which are the same size as those in strain 13136. This was confirmed by analysis of plasmid DNA after transduction of genes determining metal ion resistance and penicillinase production, or tetracycline resistance to strain 649N. The transductants (649 *pen*⁺ and 649 *tet*-*r*) were checked for phage-typing pattern and then labelled with [^{14}C]thymidine. The plasmid DNA isolated was mixed with ^3H -labelled plasmid DNA from strain DU4916 and analysed on sucrose gradients. The plasmid DNA from strain 649 *tet*-*r* sedimented as a single band corresponding to the 25 S component from strain DU4916 (Fig. 3), and that from strain 649 *pen*⁺ sedimented as two bands corresponding to the 36 S and 50 S bands from strain DU4916 (Fig. 4). Thus the plasmid DNA from strain DU4916 consists of a single band (25 S) which presumably represents the c.c.c. form of the 'tetracycline plasmid' and two bands (50 and 36 S) which represent respectively the c.c.c. and open circular form of a penicillinase plasmid. Since the molecular weights of the plasmids in strain 13136 have been established by contour length measurement (Lacey & Grinsted, 1973), similar molecular weights can be assigned to the plasmids from strain DU4916, i.e. 2.9×10^6 for the plasmid coding for tetracycline resistance and 2.0×10^7 for that determining penicillinase production and metal ion resistance.

Does strain DU4916 carry a gene(s) that blocks expression of erythromycin resistance?

It seemed possible that strain DU4916 carries a gene(s) that blocks expression of erythromycin resistance, since some derivatives of strain DU4916 had apparently acquired erythro-

Fig. 3. Sucrose-gradient analysis of c.c.c. DNA from strain DU4916 labelled with [^3H]thymidine and from strain 649 *tet-r* labelled with [^{14}C]thymidine. $\circ$, ^3H (DU4916); $\bullet$, ^{14}C (649 *tet-r*).

Fig. 4. Sucrose-gradient analysis of c.c.c. DNA from strain DU4916 labelled with [^3H]thymidine and from strain 649 *pen*⁺ labelled with [^{14}C]thymidine. $\circ$, ^3H (DU4916); $\bullet$, ^{14}C (649 *pen*⁺).

mycin resistance after treatment with acriflavine (Dornbusch & Hallander, 1973). The following experiments were undertaken in an attempt to verify this assertion.

(i) Treatment of strain DU4916 with MNNG. Strain DU4916, harvested from broth, was incubated with 100 μg MNNG/ml for sufficient time to kill about 95 % of the bacteria. The organisms were then resuspended in broth containing 0.02 μg erythromycin/ml (to induce erythromycin resistance), incubated overnight and then plated on agar containing 50 μg erythromycin/ml. From about 2×10^{10} cells treated with MNNG, no derivative resistant to erythromycin was obtained.

Table 1. *Stability of plasmids and markers derived from strain DU4916*

The plasmids were tested in strains DU4916 and 649. They determined (i) penicillinase production and metal ion resistance (designated *pen*⁺), and (ii) tetracycline resistance (designated *tet-r*).

Plasmid or marker	Host	Conditions of test	No. colonies examined	No. colonies negative
<i>pen</i> ⁺	DU4916	Growth at 43 °C	5900	0
<i>pen</i> ⁺	DU4916	Growth in the presence of 10 µg acriflavine/ml	4000	0
<i>pen</i> ⁺	DU4916	Growth in the presence of 10 µg acridine orange/ml	2500	0
<i>pen</i> ⁺	649	Growth at 43 °C	3500	1
<i>tet-r</i>	DU4916	Growth at 43 °C	4000	0
<i>tet-r</i>	649	Growth at 43 °C	3500	0
δ-Haemolysin	DU4916	Growth in the presence of 10 µg acriflavine/ml	3000	0
Methicillin resistance	DU4916	Growth in the presence of 10 µg acriflavine/ml	3750	0

(ii) Strain DU4916 was plated onto nutrient agar containing 0.02 µg erythromycin/ml, incubated overnight and then samples of about 10¹¹ colonies were transferred to media containing 10, 25 or 50 µg erythromycin/ml. No resistant colonies appeared after 48 h incubation at 37 °C.

(iii) Treatment with acriflavine. Strain DU4916 was incubated overnight in 9 ml volumes of broth containing doubling dilutions of acriflavine. The cells in the tubes containing the maximum level of acriflavine permitting visible growth were then resuspended in 100 ml fresh nutrient broth containing 0.02 µg erythromycin/ml. After further overnight incubation the cells were inoculated onto the surface of nutrient agar containing 50 µg erythromycin/ml. From about 3 × 10⁹ cells treated with acriflavine, no derivatives resistant to erythromycin were obtained.

If strain DU4916 does contain gene(s) that block expression of resistance to erythromycin, derivatives deficient in such genes should have been detected by at least one of the above procedures. In particular, it has not been possible to repeat the isolation of such derivatives after acriflavine exposure as were described by Dornbusch & Hallander (1973).

Stability of plasmids in strain DU4916

Cells of strain DU4916 were inoculated to a final density of about 10⁴ cocci/ml into broth which was incubated at 43 °C or into broth containing doubling dilutions of acriflavine or acridine orange and incubated at 37 °C. After overnight incubation, cells from the broths at 43 °C and those in the broths containing the maximum concentration of acridines permitting visible growth were diluted and inoculated onto the surface of nutrient agar plates some of which contained 5 % (v/v) human red cells (to detect loss of δ-haemolysin production). Loss of resistance to cadmium ions, tetracycline or methicillin was examined by replica plating. Both production of δ-haemolysin and resistance to each agent were stable (Table 1). Strains 649 *tet-r* and 649 *pen*⁺, which contained respectively the plasmids for tetracycline resistance and penicillinase production transduced from strain DU4916, were also examined for the stability of their resistances. Tetracycline resistance was stable in strain 649 *tet-r*, but with strains 649 *pen*⁺ one colony, out of 3500 tested, became *pen*⁻ and sensitive to metal ions after growth overnight at 43 °C (Table 1). Thus the stability of the plasmids for tetracycline resistance and penicillinase production seem to be a property of the plasmid rather than the host. [Other plasmids, including another penicillinase plasmid

and another coding for tetracycline resistance, are unstable in strain 649 (Lacey & Chopra, 1974).]

The clone of strain DU4916 maintained in Stockholm was also treated with acriflavine. On one occasion 5 µg acriflavine/ml was the maximum concentration allowing visible growth and on another 10 µg/ml. A total of 3000 colonies were examined for loss of (i) resistance to methicillin, (ii) resistance to cadmium ions, and (iii) production of δ-haemolysin. No loss was detected.

DISCUSSION

The findings presented here differ in several ways from those of Dornbusch & Hallander (1973). The wild strains DU4916 are almost certainly identical, since they both possess the same phenotypic properties, including phage typing pattern. In this study there is no evidence for linkage of methicillin resistance with other resistances, or with the production of δ-haemolysin or enterotoxin B. The suggestion that recombination between the penicillinase plasmid and the genes for methicillin resistance has occurred (Dornbusch & Hallander, 1973) has not been confirmed. The association of certain genes as suggested by Dornbusch & Hallander (1973) could have resulted from cotransduction of unlinked markers, because in their experiments the phage-to-cell ratio was relatively high. Thus, the genes for penicillinase production and methicillin resistance could have been successively transduced to one recipient cell.

A further discrepancy between the results described here and those obtained by Dornbusch & Hallander (1973) is the effect of acridine dyes on the stability of several markers. One explanation may be that the clones tested in the present report have acquired an 'immunity' to the action of acridines. However, few, if any, other staphylococcal plasmids have been shown convincingly to be 'cured' by acridines. The cured variants of DU4916 isolated by Dornbusch & Hallander (1973) differed in a wide range of properties from strain DU4916 wild-type. These properties consisted of phage-typing pattern, production of haemolysins, penicillinase and pigment, and resistance to erythromycin, metal ions, methicillin and streptomycin (Dornbusch & Hallander, 1973; this paper). The reasons for this are obscure.

Strain DU4916 possesses several properties common to other methicillin-resistant staphylococci (Lacey, 1972; Lacey & Grinsted, 1973). These are: a plasmid determining tetracycline resistance of molecular weight 2.9×10^6 daltons which is stable at 43 °C; high-level resistance to streptomycin, chromosomally determined; orange pigment; genes determining heterogeneous resistance to methicillin that are expressed in a few recipients.

Thus strain DU4916 does resemble other methicillin-resistant staphylococci in many properties and these findings are certainly consistent with the hypothesis of a single clone origin for these strains.

I thank Mrs E. Lewis for expert technical assistance, Dr V. G. Alder for phage typing, Miss Antonette Wieneke, Food Hygiene Laboratory, Central Public Health Laboratory, Colindale, for performing the assays for enterotoxin production, and Dr K. Dornbusch and Dr R. P. Novick for providing strain DU4916. This work was financed by a grant from the Medical Research Council to Professor M. H. Richmond.

REFERENCES

- COHEN, S. & SWEENEY, H. M. (1970). Transduction of methicillin resistance in *Staphylococcus aureus* dependent on an unusual specificity of the recipient strain. *Journal of Bacteriology* **104**, 1158-1167.
- DORNBUSCH, K. (1973). Compatibility tests between genetic linkage groups in a strain of *Staphylococcus aureus*. *Journal of General Microbiology* **76**, 13-19.
- DORNBUSCH, K. & HALLANDER, H. O. (1973). Transduction of penicillinase production and methicillin resistance-enterotoxin B production in strains of *Staphylococcus aureus*. *Journal of General Microbiology* **76**, 1-11.
- DORNBUSCH, K., HALLANDER, H. O. & LÖFQUIST, F. (1969). Extrachromosomal control of methicillin resistance and toxin production in *Staphylococcus aureus*. *Journal of Bacteriology* **98**, 351-358.
- DYKE, K. G. H. (1969). Penicillinase production and intrinsic resistance to penicillins in methicillin-resistant cultures of *Staphylococcus aureus*. *Journal of Medical Microbiology* **2**, 261-278.
- GRINSTED, J. & LACEY, R. W. (1973). Genetic variation of streptomycin resistance in clinical strains of *Staphylococcus aureus*. *Journal of Medical Microbiology* **6**, 351-362.
- KAYSER, F. H., WÜST, J. & CORRODI, P. (1972). Transduction and elimination of resistance determinants in methicillin-resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* **2**, 217-223.
- LACEY, R. W. (1972). Genetic control in methicillin-resistant strains of *Staphylococcus aureus*. *Journal of Medical Microbiology* **5**, 497-508.
- LACEY, R. W. & CHOPRA, I. (1974). Genetic studies of a multiresistant strain of *Staphylococcus aureus*. *Journal of Medical Microbiology* **7**, 285-297.
- LACEY, R. W. & GRINSTED, J. (1972). Linkage of fusidic acid resistance to the penicillinase plasmid in *Staphylococcus aureus*. *Journal of General Microbiology* **73**, 501-508.
- LACEY, R. W. & GRINSTED, J. (1973). Genetic analysis of methicillin-resistant strains of *Staphylococcus aureus*; evidence for their evolution from a single clone. *Journal of Medical Microbiology* **6**, 511-526.
- ŠIMKOVIČOVÁ, M. & GILBERT, R. J. (1971). Serological detection of enterotoxin from food poisoning strains of *Staphylococcus aureus*. *Journal of Medical Microbiology* **4**, 19-28.
- SUTHERLAND, R. & ROLINSON, G. N. (1964). Characteristics of methicillin-resistant staphylococci. *Journal of Bacteriology* **87**, 887-889.