Antibiotic Resistance Plasmids of *Staphylococcus aureus* and Their Clinical Importance

R. W. LACEY

Department of Bacteriology, University of Bristol, Bristol, United Kingdom

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INTRODUCTION

Since the discovery of transferable antibiotic resistance in 1959, a fairly clear picture of the process of conjugation in the Enterobacteriaceae has emerged, or at least of the process as it occurs in the laboratory. Although a huge amount of circumstantial evidence seems to establish beyond doubt that transfer of plasmids among these organisms has occurred in nature, it has often been impossible to calculate the frequency of transfer. This has resulted largely from the difficulty of identifying specific strains over many years.

It is well known that the introduction of each new antibiotic, initially effective against Staphylococcus aureus, has been followed by the appearance of strains resistant to that antibiotic (at present the only major antibiotic to which there is virtually no resistance is gentamicin). At the time of the introduction of each new antibiotic, including penicillin (see below), less than 1% of staphylococcal isolates have been resistant. Sooner or later resistant strains are encountered, particularly among those isolated from hospital sources. During the 1940's and 1950's, antibiotic resistance in bacteria, including S. aureus, was thought to arise by mutation and selection. This view was largely based on the readiness with which bacteria, in vitro, could develop resistance to antibiotics such as streptomycin. However, during the last 15 years, evidence has accrued that most antibiotic resistance in S. aureus, as in the Enterobacteriaceae, is plasmid determined. The demonstration of transfer of plasmids between strains of S. aureus in the laboratory by phage-mediated transduction, together with various sorts of epidemiological observations (discussed in later sections), raised the possibility that transfer of plasmids between strains of this species occurred also in nature. Research into staphylococcal plasmids and into the possibility of transferable antibiotic resistance has lagged some years behind that into the enteric organisms. But there is mounting evidence, at least in terms of plasmid transfer, that the genetics of the staphylococcus are analogous to those of the Enterobacteriaceae. Genetic analysis of S. aureus presents both advantages and disadvantages over that of other organisms. The disadvantages include the difficulty with which staphylococci grow on simple media, our inability (until recently) to extract intact deoxyribonucleic acid (DNA) molecules, and uncertainty over whether the chromosome is a single element or several. Furthermore, transduction (transfer of part of the cell DNA by bacteriophage) has been virtually the only means of transfer of genetic material between cells in vitro, and this process is severely limited as a genetic tool in that only a small fragment (30 x 10^4 daltons, which is only about 1% of the gene-set of S. aureus, and probably the upper limit) of DNA can be transferred at any one time, at least with the currently used transducing phages.

It is therefore not surprising that the elucidation of the detailed molecular properties of staphylococcal plasmids is difficult, and that rather few groups of workers have been engaged in this field in recent years. Despite these problems, some considerable insight into the structure and replication of the penicillinase plasmids has been achieved (146, 147).

The advantages of studying staphylococcal plasmids are concerned primarily with their epidemiological aspects. Any transfer of plasmids between cells that has occurred naturally has almost certainly been mediated by bacteriophages. (Conjugation is not known in this organism, and virtually all cultures contain high levels of nucleases [41] which can be expected to prevent transformation under natural conditions; it is true that transformation can be achieved in vitro, but the artificiality of the conditions under which it occurs, e.g., the need for 0.1 M calcium ions, makes its occurrence in vivo improbable). Infection of the bacterial cell by phages is generally species specific, and this is extremely true for the staphylococci; the phages of S. aureus are indistinguishable to other species with the exception of some cross-reactions with Staphylococcus albus (208, 222). Moreover, animal and human strains usually remain confined to their respective hosts. Epidemiological aspects of staphylococcal plasmids therefore involve a relatively small, and clearly defined, population of strains, and it should be possible to relate changes that have been observed in the plasmid or host bacterium to antimicrobial therapy that has been directed towards man. In gram-negative bacteria the possibility of interspecies transfer and the spread of pathogens between animals and man has complicated definitive epidemiological analysis of plasmids.

An important limitation in the epidemiological survey of plasmids in some members of the Enterobacteriaceae is the inability to identify adequately a particular bacterial strain. This is well illustrated by bacteriophage typing which, although a useful marker over the short term, is of less value in studies extending over many years. This shortcoming results from alterations in the phage-typing pattern of an organism due to loss or gain of prophages. There is strong evidence for the epidemic spread of certain
phages among populations of *S. aureus* (96); a frequent consequence of lysogenization by a "new" phage is alteration in phage typing pattern. Changes within an organism therefore can be due to alterations in (i) the chromosome, (ii) plasmids, (iii) prophage carriage, or a combination of them. In this review, some stress will be given to the usefulness of some phenotypic properties in clinical isolates of *S. aureus* which enable changes in both plasmids and its phage carriage to be monitored.

Because plasmids are nonessential to an organism under most conditions in vivo, they give the cell great potential for rapid evolution without endangering its viability as occurs with many chromosomal mutations. The importance of plasmid carriage from a therapeutic point of view is that change in the plasmids carried is one means by which a population of strains in a given habitat responds to the use (or lack of use) of antimicrobials. Some consideration will be given to such effects, with suggestions as to how the available drugs could be better exploited.

This review is thus intended primarily for the clinically oriented microbiologist; it will also discuss the evidence for, and the properties of plasmids in *S. aureus*, and the extent to which the various genetic manipulations of the plasmids that have been described in vitro may have also occurred in the organism in its natural environment. Details of plasmid replication and cell maintenance will not be considered specifically.

**DISTRIBUTION OF DNA IN THE STAPHYLOCOCCAL CELL**

Although the "chromosomal" (i.e., nonplasmid) DNA of several bacterial species exists as a single element (145), very little is known about the nature of the "chromosome" in *S. aureus*. In this organism as in other bacteria there is, of course, no nuclear membrane, so the existence of several genetic elements within the same cell permits a variety of interactions between them. Although there is some evidence that the staphylococcal chromosome may be composed of more than one element (5), further data are required before this view can be accepted generally for the species. The precise size of the staphylococcal chromosome may be composed of the chromosome of a single element or several, is still uncertain. Calculations of total DNA per cell have yielded figures that vary from $5.5 \times 10^{-14}$ to $12 \times 10^{-14}$ g/cell (i.e., equivalent to $2 \times 10^6$ to $4 \times 10^6$ daltons) (46, 76, 146). This variation reflects the difficulty in the definition of an individual cell. Disruption of the clumps of cell is probably never complete and may well vary from culture to culture. Even in cultures in "stationary phase," a proportion of the cells will be undergoing cell division; these, although appearing as a single coccol under the light microscope, may contain DNA equivalent to two or more cells.

A fundamental property of a plasmid is that it is physically distinct, at least at times, from the chromosome (48, 145). This definition is applied with difficulty to staphylococci since it is not known from what structure(s) a staphylococcal plasmid is (by definition) physically distinct. This is an important problem because several unstable characters seem to be determined by genes carried on elements that are not physically isolable as plasmids by techniques currently available (see below). A functional concept of plasmid inheritance is useful in this organism, i.e., genetic information that is dispensable under ordinary cultural conditions. Although subsequent work may establish that the genes coding for some of these unstable characters are in fact chromosomally located, it is their nonessential nature and instability that are important practically for two reasons: (i) such genes have the capacity to evolve rapidly, and (ii) a population of cells can have variation in its carriage of such elements and thus be better equipped to survive changing environments than a population uniform in its DNA content.

**EVIDENCE FOR PLASMID INHERITANCE IN S. AUREUS**

Spontaneous Loss of Characters After Growth in Ordinary Media

Instability of a phenotypic property (for details of detection by replica plating, see reference 118) has usually been the initial observation suggesting that the relevant genes may be carried on a plasmid. A frequency of loss of a property from about 1 in $10^8$ to $10^9$ per cell division is characteristic of many plasmid markers, although such a frequency can sometimes occur in unprovoked chromosomal mutations in other bacterial species. Two further features of spontaneous loss point to plasmid inheritance, although by no means conclusively. (i) Irreversibility of the change (in the absence of introduction of new genes by a transfer process) is the first feature; a point chromosomal mutation is often reversible, whereas loss of a relatively large piece of genetic material, such as occurs with loss of an entire plasmid, is always irreversible. (ii) The loss of two or more phenotypic characters simultaneously favors a plasmid location for the genes presumably concerned. It is, however, possible for a single mutation to alter several phenotypic properties simultaneously; for example, mutation (pre-
sumably chromosomal) of *S. aureus* to resistance to unsaturated fatty acids is accompanied by an increase in resistance to several aminoglycoside antibiotics (102).

In the calculation of the rate of plasmid loss, a correction may be necessary to compensate for differences in growth rates of the plasmid-negative and plasmid-positive cells; where there are differences in growth rates, the plasmid-negative cells overgrow the plasmid-positive cells (76, 114, 163). Thus, the true rate of plasmid loss may be less than it seems at first sight.

Although there is uncertainty about the location of genes for some characters (e.g., coagulase and hemolysin production) that are unstable, a plasmid inheritance for many such unstable characters of *S. aureus* has been confirmed by other methods.

**Acceleration of the Loss of Phenotypic Characters by Growth at High Temperatures or in the Presence of “Curing” Agents**

During growth at temperatures of 40 to 45°C, some of the plasmids that determine production of penicillinase (and also resistance to metal ions), or others determining tetracycline resistance may be lost at higher frequencies than when the culture is incubated at 37°C (11, 126). However, other plasmids that determine similar phenotypic characters are as stable during growth at 40 to 45°C as at 37°C (11, 112). The property of temperature instability seems, in general, to be a property of the plasmid itself, rather than the host cell in which it resides. This conclusion is based on experiments in which the plasmid is transduced to another host; resultant progeny are similar in temperature sensitivity in respect to the plasmid-determined trait of the original strain (112, 142).

Curing agents produce a specific effect resulting in loss of the plasmid from the cell, without exerting a mutagenic effect. Several such agents have been reported to accelerate the loss of plasmids from the staphylococcal cell (Table 1). In very few instances have the precise molecular events that the agent provokes been elucidated.

Controversy has centered around two of these substances: acridine dyes and rifampicin. Although in the experiments of Hashimoto, Kono, and Mitsuhashi (88) the loss of a penicillinase plasmid seemed to be accelerated by exposure of the culture to acriflavine, other authors failed to confirm such an effect with this plasmid, and Richmond (163) considered that the acridines do not cure the penicillinase genes; the discrepancy between the reports probably resulted from the absence of sufficient data from untreated control cultures. The ability to cure plasmids will be known by any worker with direct experience to be a chancy affair; the precise concentration of agent may be critical (or it may not), as may the host strain and the plasmid itself. Repeated subculture of an organism in the presence of a curing agent also seems to cause a progressive decline in any curing activity (R. W. Lacey, unpublished observations).

With these uncertainties in mind, the apparent discrepancy in the effect of rifampicin on the penicillinase plasmid becomes perhaps understandable. Johnston and Richmond (97) found a very high rate of plasmid loss—up to 60% of the cells of one strain had become penicillinase negative after growth for about 10 generations in the presence of rifampicin. In contrast, Zimmermann et al. found that the curing action of rifampicin for the same strain was generally less effective and unreliable (223).

It would seem, therefore, that caution should be used before generalizing about the curing activity of a particular substance. Although some reports of curing are convincing, there does not appear to be any agent that cures any one strain of *S. aureus* of all plasmids. Similarly, although a particular agent may eliminate a certain character from a strain, it may not do so in another isolate. In view of the unreliability of the activity of these agents, and the critical levels needed, it is difficult to anticipate any therapeutic application for curing agents. In any case, plasmid-positive populations of organisms tend to revert spontaneously to plasmid negative when the selecting agent for the plasmid (usually an antibiotic) is withdrawn (see below).

The usefulness of curing agents at present lies chiefly in the identification of plasmids, i.e., in those situations in which the phenotypic character is eliminated by the agent at very high frequency. Such studies may also throw some light onto biochemical events involved with plasmid maintenance and replication.

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**Table 1. Agents reported to accelerate the loss of plasmids from the staphylococcal cell**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Type of plasmid eliminated</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acridine dyes</td>
<td>pen</td>
<td>58, 88, but see 163</td>
</tr>
<tr>
<td>Ethidium bromide</td>
<td>pen</td>
<td>33</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>pen</td>
<td>97, 223</td>
</tr>
<tr>
<td>Sodium dodecyl sulfate</td>
<td>pen</td>
<td>194</td>
</tr>
<tr>
<td>Penicillins</td>
<td>str, neo, ero</td>
<td>110,113</td>
</tr>
</tbody>
</table>

*Abbreviations: pen, penicillinase; str, resistance to streptomycin; neo, resistance to neomycin; ero, resistance to erythromycin.*

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It must be stressed, however, that the inability to demonstrate instability of a marker, either spontaneous or after attempted "curing," in no way disproves plasmid inheritance. For example, a plasmid-determining tetracycline resistance (resistance established as plasmid mediated by observations, including physical isolation of the plasmid as covalently closed circular DNA) is exceptionally stable (112).

**Bacteriophages of S. Aureus and the Effect of UV Light on Transduction as a Means of Establishing Plasmid Inheritance**

Before considering the application of transduction techniques to plasmid identification, a brief comment on the relationship of bacteriophages to the staphylococcus is pertinent.

Almost all clinical isolates of *S. aureus* can be shown to harbor at least one temperate prophage, and frequently several; for example, strain 8325 is known to harbor three (148). There seems to be only one reported naturally occurring strain from which no phage could be obtained, i.e., strain 1030 (143). It is possible to cure strains of their prophages by, e.g., ultraviolet (UV) irradiation or treatment with mitomycin C. Strain RN 450 is a derivative of strain 8325 that has lost all three prophages (146).

The prophages are presumed to be integrated into the chromosome of *S. aureus*, although linkage to established chromosomal genes has not been shown; there is no evidence for their existence as plasmids, although rather few lysogenic strains have been examined for covalently closed circular DNA (76, 77, 146, 200). Information about the size, shape, and genetics of staphylococcal phages is still scanty.

The standard typing phages have been in use for about 30 years; as "hospital" strains of *S. aureus* acquire "new" prophages, their susceptibility to the typing phages is reduced. There is, therefore, a continual modification of the typing phages. Staphylococci are classified into phage groups I, II, III, or into a miscellaneous group. This division reflects not only a specificity of phage lysis but also has epidemiological significance. Group I strains characteristically produce localized primary skin sepsis such as boils, sties, and carbuncles. Group II strains produce a spreading infection of the skin (impetigo), and group III strains are frequently multiresistant and are associated with a variety of infections in hospital patients, other than primary skin sepsis (i.e., invasion of healthy skin). The serological classification of the typing phages resolves them into four groups (31), of which only those of serological group B are transducing.

Bülow has classified the phages obtained from phage group III staphylococci into α and β (36). The α phages produce small plaques on indicator strains and cannot transduce; β phages produce large plaques and can achieve transduction. There are also morphological and serological differences between them. All phages from *S. aureus* have either a relative or an absolute requirement for calcium ions (177).

The molecular weight of the DNA of one transducing phage (from strain 8325) has been calculated as about $28 \times 10^6$ (182). This figure is consistent with the finding that plasmids of molecular weight of about $20 \times 10^6$ or less are readily transducible, whereas a plasmid of $35 \times 10^6$ molecular weight can only be transduced at very low frequency, although the molecular weight of the phage used (typing phage 88) has not been determined (46). All the plasmids that have been physically isolated, and for which there is evidence for transfer in nature (see below), have molecular weights of about $20 \times 10^6$ or less.

In *S. aureus*, lysogenic conversion, specialized transduction, and generalized transduction all occur, at least in vitro.

**Lysogenic conversion.** In this process the acquisition of phage by the cell invariably alters a phenotypic property of the cell (other than the immunity to lysis by that particular phage that is also conferred). The mechanism of lysogenic conversion in *S. aureus* is presumably, as it is presumed to be in other bacterial species, the permanent incorporation of some "adventitious" genes into the phage genome. However, it is curious that two of the examples of lysogenic conversion that have been well documented in *S. aureus* involve suppression of certain cellular activities by lysogeny, rather than the acquisition of new activities as usually occurs in other species (89).

Many strains of *S. aureus* produce extracellular lipases, which hydrolyze egg-yolk lipid (3, 72), Tween compounds, or some triglycerides (187, 191). One of these lipases splits both egg-yolk lipid and the "higher" Tw eens, e.g., Tween 80, and it is this activity that is lost after lysogenization with the β phage (38, 175, 176). The other example of lysogenic conversion in staphylococci is the alteration of phenotype from beta-hemolysin positive, fibrinolytic negative to beta-hemolysin negative and fibrinolytic positive (221). The precise genetic changes responsible for these phenomena are not known, and would merit further study, particularly the blocking of lipase activity as it could have important epidemiological significance (see below).

**Specialized transducing agents.** Special transducing variants of generalized transducing...
phages arise by incorporation of particular chromosomal or plasmid genes into the phage gene-set. Novick has constructed an agent of this type in *S. aureus* by a recombination event between a phage and a penicillinase plasmid (143). The resultant element is a plasmid since it is isolable as covalently closed circular DNA (182), and, although it has lost regions of both the phage and plasmid, some phage and plasmid functions are retained (143). Novick and Morse (148) injected it that likely in experimentally resistance (in this case resistance to erythromycin) could be transferred between staphylococci experimentally in vivo. However, such specialized transducing agents seem to occur rarely in nature; any plasmid transfer in nature is therefore likely to be promoted by generalized transduction. (Novick and Morse [148] did mention that such transfer could occur by generalized transducing agents, but gave no data). The principal value of this specialized transducing agent has been in the study of replication of penicillinase plasmids (147).

**Generalized transduction.** All of the naturally occurring phages of *S. aureus* that can transduce are general transducing phages. As with such phages of other species, almost any character can be transduced, at frequencies from about $10^{-4}$ to $10^{-18}$ per plaque-forming unit (PFU) of phage. However, some characters in some strains, e.g., resistance to methicillin or streptomycin, cannot be transduced (60, 77). Whether this failure is a matter of frequency, or whether the genes in question never become incorporated into the phage vector, or never become established in the recipient is uncertain. At present, although the data are inconclusive, the inability to transduce a marker probably favors an extrachromosomal rather than a chromosomal locus for the genes. Virtually all chromosomal genes in other organisms seem capable of transduction (89). All the staphylococcal phages that can effect transduction are of serological group B, an empirical definition based on cross-inactivation by antisera (31), and the phages most commonly employed in experiments are typing phages 29 and 80 (of group I), and 53 and 88 (of group III). Characters can be transduced between phage group II strains and strains of other groups only with difficulty; there have also been few reports of transduction within group II. It is interesting that phage group II strains are still predominantly sensitive to some antibiotics, such as tetracycline, although many produce penicillinase.

Transduction procedures used for plasmid identification employ a high-titer phage lysate. This is obtained either by propagation of an external phage on the donor or by induction of the donor's own prophage, for instance by treatment with UV light, mitomycin C, or by growth at elevated temperature. Propagation of external phage can be performed by a variety of methods, e.g., growth in broth, soft agar, or on the surface of agar. Several propagatory cycles may be needed to obtain a lysate of adequate titer (preferably $>10^8$ PFU per ml).

Modification and restriction of staphylococcal phages is extremely common after propagation on strains, perhaps inevitable (31), so that a phage can be completely changed in its host specificity by one passage. A point of practical value is that the phage susceptibility spectrum of a culture can be dramatically widened by prior heating of the culture (to about 56 C for 2 min). Loss of prophage can also widen the spectrum.

In the location of genes as either plasmid or chromosomal, the transduction procedures and interpretations initially established in *Escherichia coli* (9, 69) are, in general, valid for *S. aureus* (10, 12, 14). In this “Arber” type of experiment, UV irradiation of the transducing lysate before addition to the recipient has generally one of two effects on the frequency of complete transduction. Sometimes small doses of irradiation increase the frequency (often by more than 10-fold), but larger doses cause a decline. In other experiments increasing doses of irradiation causes a progressive decline in both the transduction frequency and the viability (proportion of particles surviving as PFU) of the phage. The first type of response (stimulation of frequency) is characteristic of transduction of chromosomal genes, and the second is typical of transduction of plasmids. Explanations for these findings are still uncertain. The increased transduction frequency of chromosomal genes may be the result of increased probability of incorporation, by recombination affecting homologous DNA segments, perhaps because of UV stimulation of frequency of crossing-over. The loss of phage viability and the reduction in transduction frequency of plasmid genes is probably simply due to point damage to the DNA.

In the interpretation of the effect of UV light on transduction, it has usually been assumed that the gene(s) in question has the same location in both the donor and transductant and that plasmids are transduced in toto and do not become integrated into the chromosome of the recipient. The general validity of this assump-
tion can be questioned, since on rare occasions plasmid genes can become integrated into the chromosome (144) or become incorporated into an existing plasmid (181); the latter event was stimulated by UV light. It is therefore possible for a gene of plasmid origin to give a "chromosomal type" UV-transduction effect. There is as yet no evidence that genes which are chromosomal in the donor can become established as a plasmid in the recipient. Interpretation of these transduction experiments must be made with caution: UV stimulation probably indicates a chromosomal locus, but not necessarily so; an exponential decline in the frequency probably denotes a plasmid gene.

Despite these uncertainties, the UV-transduction effect still has an important role in the identification of plasmids. Several elements which are presumably plasmids as judged by other criteria are not isolable physically (see below). UV-transduction experiments are of particular value in deciding the nature of such elements.

**Isolation of Plasmid DNA**

In several bacterial genera, plasmid DNA can be separated from chromosomal DNA because of differences in the physico-chemical properties of the two types (48). Until recent years, few staphylococcal plasmids had been so isolated, mainly because of the difficulty in lysing the organism without denaturing its DNA. This was chiefly because staphylococci, in contrast to many other species, are relatively resistant to the lytic action of lysozyme. The discovery of lysostaphin (184), an enzyme which rapidly lyses the staphylococcal cell wall, has permitted the isolation of plasmids from this organism.

Apart from the use of lysostaphin, rather than lysozyme, procedures for the isolation and characterization of staphylococcal plasmids are similar to those for plasmids of other genera (48). Freshly isolated plasmid DNA from staphylococci exists chiefly in the covalently closed circular form and tends to change progressively to the open circular form on storage, or by treatment with minute amounts of deoxyribonuclease.

The correlation of presence or absence of plasmid DNA with presence or absence of a certain phenotypic character gives strong evidence for plasmid inheritance of the character. The isolation of plasmid DNA should be made after transduction of the marker into a host which does not itself yield plasmid-type DNA.

Before correlation of the isolated plasmid DNA with a marker can be established, the following two sources of spurious association should be excluded. (i) During transduction to the host, the possibility that transduction of another marker has simultaneously occurred must be eliminated. The use of a low phage-to-cell ratio is thus advisable. (ii) If the marker in question cannot be transduced into another host, then examination of a "cured" derivative is the next best test, but may give misleading information. The cured derivative may have lost two plasmids, and an incorrect correlation may be made for plasmid and marker. This error has indeed occurred. Strain 649 wild is resistant to streptomycin (77) but to no other antibiotics. Since streptomycin resistance was not transducible from strain 649 wild, this culture and a derivative (649 str-s) that had lost the resistance spontaneously on storage were analyzed for plasmid DNA. A plasmid of 35 × 10^4 daltons was isolated from the wild strain, but no plasmid was present in 649 str-s. It was inferred that the genes for streptomycin resistance were carried by the 35 × 10^4 plasmid (77). In subsequent experiments, this conclusion was found to be erroneous: strain 649 wild is also resistant to cadmium, mercury, and arsenate ions, and the cured derivative (649 str-s) is sensitive to these ions. Resistance to the metal ions was transducible (without resistance to streptomycin) to another host from which a plasmid of 35 × 10^4 could thereafter be isolated. Thus this plasmid carries the genes for metal ion resistance and not that for streptomycin resistance (110).

One final point about the isolation of plasmid DNA: although the presence of a marker in an organism may correspond exactly with the presence of plasmid DNA, the precise functional significance of this DNA has rarely been established, particularly in *S. aureus*. The possibility that this DNA is an incidental product of an as-yet-unidentified linkage group would seem to merit some consideration. Perhaps the failure to isolate some plasmids physically is due to the absence of such a by-product.

**Use of Recombination-Deficient Mutants**

Recombination-deficient mutants (usually denoted *rec -*) are mutant strains isolated in vitro which are unable to effect the integration of incoming DNA into their chromosome, presumably on account of abnormality in some relevant enzyme. However, plasmids can become established in these cells. *rec -* mutants have been extensively studied in *Escherichia coli* but much less so in *S. aureus*, probably because of the difficulty in isolating them. One such mutant has been used in attempting to locate the genes for erythromycin resistance
Thus, although erythromycin resistance can be transduced at high frequency and UV irradiation does not stimulate the frequency (105), i.e., suggestive of a plasmid location, such a plasmid is not physically isolable (146). The use of a rec" mutant, to which the resistance is transducible (146), lends further support to the hypothesis of plasmid location.

**Plasmid-Specific, DNA-Mediated Transformation in S. aureus**

Lindberg et al. have established an effective transforming system in *S. aureus* (122). Recipients can be transformed for plasmid characters (penicillinase production or tetracycline resistance), but not for chromosomal genes, with circular duplex DNA isolated from the donor (121). This technique could provide useful additional evidence for plasmid inheritance, but will be of less value in locating the genes for some unstable characters that are apparently not present in the circular duplex form (see below).

In conclusion, none of the above methods for testing plasmid inheritance should be used singly. With the use of several of them, some markers can be definitely assigned to a plasmid inheritance, but others fail to give clear-cut properties of either plasmid or chromosomal determination. Some of this ambiguity may be resolved by information about the nature of the staphylococcal chromosome.

The list of plasmid-determined characters in the next section is provisional: one character may well be plasmid determined in some strains, but determined by chromosomal genes in other strains. Such variation might perhaps even occur in different cells of the "same" culture.

**Phenotypic Characters for Which a Plasmid Inheritance Seems Certain or Very Probable (Table 2)**

**Penicillinase plasmids.** Although a preliminary report of the instability of penicillinase production was made as long ago as 1948 (209), and documented more fully by Barber the following year (16), it was not until transduction techniques were developed for staphylococci (132, 151, 152, 169) that substantial evidence for a plasmid inheritance of penicillinase production was presented. In an important paper, Novick (141) showed that both the genes for determining the synthesis of penicillinase and for the control of its production were very probably carried by one plasmid. Some years later, plasmid DNA corresponding to the phenotypic properties of penicillinase production and metal-ion resistance was isolated (182), and this established beyond any doubt that the genes formed part of a plasmid. In subsequent surveys, penicillinase production has been found to be plasmid determined in the great majority of penicillin-resistant strains (61). There are, however, four reported penicillin-resistant strains in which the genes for penicillinase are chromosomal (10, 86, 157, 202). Evidence for a chromosomal locus includes stability of enzyme production after growth at 43 C, the demonstration of linkage of the genes in question with known chromosomal genes, and transduction data.

Penicillinase plasmids determine a variety of other traits; the proof of linkage (i.e., determination by the same plasmid) of some genes is conclusive for some characters, e.g., resistance to cadmium ions, but for others the evidence is less impressive. Thus, resistance to erythromycin (129), kanamycin (7), fusidic acid (discussed below) (111), ethidium bromide (98), cadmium, arsenate, arsenite, bismuth, lead, and mercury ions (155) may be determined by genes present on penicillinase plasmids.

Four different serotypes—A, B, C, and D—of penicillinase have been identified (161, 174). An explanatory note here on the nature of these serotypes may be helpful. Richmond (161) found that the injection of one batch (other batches did not have the same effect) of purified A-type penicillinase into two rabbits led to the production of antibodies to penicillinase in each. Although these antibodies bound to penicillinase, they did not inactivate the enzyme, but, surprisingly, often increased its rate of

<table>
<thead>
<tr>
<th>Character*</th>
<th>Mol wt (x10^6)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>tet</td>
<td>2.9</td>
<td>114</td>
</tr>
<tr>
<td>tet</td>
<td>2.66</td>
<td>114</td>
</tr>
<tr>
<td>chm</td>
<td>2.9</td>
<td>46</td>
</tr>
<tr>
<td>chm</td>
<td>3.1</td>
<td>146</td>
</tr>
<tr>
<td>neo</td>
<td>5.9</td>
<td>46</td>
</tr>
<tr>
<td>pen,cad,fus</td>
<td>12-16</td>
<td>46, 111, 114</td>
</tr>
<tr>
<td>pen,cad,mer,asa,ero (Pl18a)</td>
<td>18</td>
<td>146</td>
</tr>
<tr>
<td>pen,cad,mer,asa, Pl18a, and unclassified</td>
<td>20</td>
<td>K. P. Novick, personal communication, 112</td>
</tr>
<tr>
<td>pen,cad,mer,asa (PlIIa)</td>
<td>21</td>
<td>192</td>
</tr>
<tr>
<td>cad,mer,asa</td>
<td>35</td>
<td>46</td>
</tr>
</tbody>
</table>

* tet determines resistance to tetracycline, chm to chloramphenicol, neo to neomycin, cad to cadmium ions, mer to mercuric ions, asa to arsenate ions, fus to fusidic acid, ero to erythromycin. pen indicates penicillinase determinant.

* For terminology see reference 145.

**Table 2. Physically isolated plasmids of S. aureus**

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penicillin hydrolysis. The degree of this stimulation forms the basis of serotyping the various penicillinas. A-type penicillinase was stimulated by the anti-A-type penicillinase antiserum up to 4-fold, B-type was stimulated 1.5-fold, C-type was stimulated 1.0-fold (i.e., no stimulation), and D-type was stimulated about 2.2-fold (161, 174). Although data is not available for type D enzyme, the properties of each of the types A, B, and C are extremely similar in (i) sedimentation coefficient, (ii) amino acid analysis, and (iii) kinetics of hydrolysis of several penicillinas (161). Furthermore, peptide maps obtained from enzyme types A and C suggest that these two variants differ in only a very few amino acid residues (161). These data indicate that the enzymes are indeed very closely related; perhaps differences in their immunological activities exaggerate the small variation between the molecules. Clinical strains of penicillin-resistant S. aureus show at least three types of enzyme control—macroinducible, macroconstitutive, and microconstitutive (116, 174). This variation in enzyme control results from at least two sets of "control" genes carried by the plasmid (162).

The size of the penicillinase plasmids varies from about 12 x 10^6 to 21 x 10^6 daltons (112, 114, 116, 146, 182, 192).

Estimates of the numbers of copies of the penicillinase plasmid per cell or chromosome have varied from 2 to 3 (146) and 4 to 5 (200) in strain 8325 to up to 8 in strain 649 (46). Evidence is therefore inconclusive as to whether the replication of these plasmids should in general be considered "relaxed" or "stringent." It is relevant to this problem that in the Enterobacteriaceae, plasmids similar in size to the penicillinase plasmids (molecular weight 20 x 10^6) or smaller have relaxed replication (48).

Two incompatibility groups have been described among the penicillinase plasmids (149), although little is known about the incompatibility of some of the more recently described plasmids (e.g., those carrying the genes, pen, fus, cad, asa, and mer; Table 2). The penicillinase plasmids do not appear to show any incompatibility with any of the other plasmids listed in Table 2. Since multiresistant strains of staphylococci probably contain several plasmids, each often coding for resistance to one antibiotic (see below), and no antibiotic resistance traits are apparently "incompatible," incompatibility is probably not common among staphylococcal plasmids in general.

In view of the large numbers of different characters that the penicillinase plasmids may determine, and the relative instability of some of them (see above), a simple classification of these elements seems impossible.

Tetracycline resistance. In most tetracycline-resistant strains of S. aureus, there is clear-cut evidence for plasmid inheritance of tetracycline resistance (11, 112, 126, 146); it is possible that there is in reality only one "tetracycline plasmid," i.e., that all those now observed have a recent common origin. Consistent with this is the uniform level of resistance to tetracycline (minimal inhibitory concentration [MIC] 100 μg/ml) that tetracycline-resistant strains exhibit. The plasmid is 2.66 x 10^6 to 2.9 x 10^6 daltons in size (112, 146), and is present in about 30 to 50 copies per cell (46, 146). This number of copies would seem to represent relaxed replication, although attempts to alter the numbers of copies per cell by incubation in the presence of tetracycline have not been successful (110). However, in two strains, Kayser et al. (99, 100) found that tetracycline resistance is chromosomal; this has been confirmed for one of these strains (I. Chopra, and R. W. Lacey; unpublished observations). In this strain, there is no covalently closed circular DNA equivalent to tetracycline resistance, the resistance is stable after growth at 43 C, and the frequency of transduction of the resistance was stimulated >10-fold by UV light.

Neomycin resistance. Genes that determine neomycin resistance may be present in plasmids conferring resistance to streptomycin and other antibiotics (see below). In many resistant strains, however, neomycin resistance is not accompanied by resistance to streptomycin and is plasmid mediated (13, 14, 37, 45, 99, 100, 103, 112). Neomycin resistance is unstable under most cultural conditions. Strains that are resistant to neomycin are also resistant to kanamycin and paromomycin (42, 103). One neomycin-resistance plasmid has been isolated and is 5.9 x 10^6 daltons in size and is present in multiple copies per cell (46).

Chloramphenicol resistance. All the evidence points to a plasmid locus for the genes determining chloramphenicol resistance in all chloramphenicol-resistant strains investigated (45, 99, 100, 112). The plasmids that have been isolated vary from 2.9 x 10^6 to 3.1 x 10^6 daltons and are present in multiple copies (110, 146).

Phenotypic Characters for Which a Plasmid Inheritance Seems Likely but the Evidence is Inconclusive (Table 3)

Methicillin resistance. Not only the genetic basis, but also the mechanism, clinical importance (does the resistance spell therapeutic failure with methicillin?), and the reason for the
uneven geographical incidence of methicillin resistance are in dispute.

Methicillin resistance is shown by a rather few staphylococcal overall and is characterized by a degree of resistance (see below) to all of the penicillins and cephalosporins in vitro (150); the mechanism of this resistance is not by enzymatic inactivation (60) and is quite distinct from resistance by production of penicillinase, even though most methicillin-resistant strains also produce penicillinase (60). In methicillin-resistant cultures grown at 37 C on ordinary media, only about 1 cell in 10^5 is resistant as indicated by the ability to give rise to a colony on medium containing levels of methicillin 10-fold greater than that inhibiting the growth of methicillin-sensitive cocci (60, 150). These are not mutants in the usual sense as subculture of such a clone yields a population with variable resistance (60, 185). At temperatures of 30 C or below, or in the presence of 5% (wt/vol) NaCl at 37 C, every cell of a culture is resistant by the same criterion (6, 60). This type of resistance is referred to as "heterogeneous" and all, or nearly all, clinical strains, if methicillin resistant, show this type of resistance.

Experiments aimed at defining the location of the genes that determine methicillin resistance have resulted in the following findings. (i) Repeated subculture or storage of resistant strains in vitro yields methicillin-sensitive derivatives (i.e., every cell of the clone is sensitive to methicillin under all conditions; penicillinase productin may or may not be retained) (8, 78, 105). (ii) Once lost, methicillin resistance cannot be restored (105), nor has mutation to this type of resistance been described in methicillin-sensitive cocci. (iii) Plasmid DNA equivalent to the resistance has not been isolated (112, 200). (iv) The resistance is not transducible to some antibiotic-sensitive wild strains (49, 60, 105), but is transducible at frequencies of about 10^-7 to 10^-4 to recipients that have previously either (a) harbored a penicillinase plasmid (49), or (b) lost the genes for methicillin resistance (105), or (c) been lysogenized with a certain prophage (50). Thus the specificity of the recipient is striking. (v) The frequency of transduction of methicillin resistance is increased to a moderate extent by UV light irradiation (49, 99, 100). This presumably indicates that the transduced genes have become integrated into recipient genetic material, which may or may not be the chromosome.

The above data seem to indicate some unique type of inheritance. Further research might yield important information about the organization of the staphylococcal genome in general.

**Erythromycin resistance.** Although there is no doubt that the genes coding for erythromycin resistance are plasmid borne when they form part of a penicillinase plasmid (Table 2), in other instances the criteria for plasmid inheritance are conflicting. Thus the observations that erythromycin resistance can be lost on storage (105), that the UV transduction results are "plasmid" type (105), and that the genes can be expressed after transduction to a rec^- cell (146), all argue for plasmid inheritance. But no circular duplex DNA corresponding to the resistance has been isolated (146). A single plasmid often carries the genes for erythromycin resistance and those for following characters: spectinomycin resistance (77), lincomycin and spectinomycin resistance (77), lincomycin resistance, beta-hemolysin production and aminoglycoside resistance (77), or the penicillinase plasmid (182).

**Streptomycin resistance.** About 70% of clinical isolates of *S. aureus* resistant to streptomycin show the high-level resistance to streptomycin (MIC > 10 mg/ml) which is characteristic of chromosomal/ribosomal type of resistance in other bacteria (87, 205). In a few strains of *S. aureus*, the resistance has been shown to be of this ribosomal type (109). In other staphylococci, the resistance is of the low level type (MIC ~100 μg/ml) which is typical of plasmid inheritance (77). In several strains, low-level streptomycin resistance is unstable, which suggests a plasmid determination (13, 77, 79, 80). However, strains of *S. aureus* with unstable, low-level streptomycin resistance do not show the spectinomycin resistance seen in *E. coli* with one type of plasmid-determined streptomycin resistance (77).

**Pigment production.** The instability of staphylococcal pigment production has been known for more than 30 years (59, 156). Some-

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**Table 3. Characters for which a plasmid inheritance seems probable, but no equivalent plasmid DNA has been isolated**

<table>
<thead>
<tr>
<th>Character*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>mtc</td>
<td>8, 50, 58, 105, 112, 200</td>
</tr>
<tr>
<td>ero, spc</td>
<td>77, 146</td>
</tr>
<tr>
<td>ero, spc, neo, str, hem</td>
<td>77, 114</td>
</tr>
<tr>
<td>str</td>
<td>80, 110</td>
</tr>
<tr>
<td>str, neo</td>
<td>13, 77, 79</td>
</tr>
<tr>
<td>pig</td>
<td>76, 186</td>
</tr>
</tbody>
</table>

* Abbreviations: mtc, determinant for methicillin resistance; ero, for erythromycin; spc, for spectinomycin; neo, for neomycin; str, for streptomycin; hem, determinant of beta-hemolysin production; pig, determinant of pigment production.
times the instability is so marked that colonies appear sectored (186). In our attempts to locate the genes for pigment, inconclusive results were obtained (76). Although two genes, at least, probably contributed to the formation of pigment (mutagenesis of wild strains resulted in two pigment types), and both were lost simultaneously and irreversibly, no plasmid DNA was isolated from cultures capable of forming pigment. A very large linkage group was suggested by our finding that the nonpigmented cells appeared to possess about 30% less DNA than the pigmented. This may be less significant than it might appear because of the difficulty in defining a single cell. Moreover, DNA/DNA hybridization could not detect the loss of plasmid genome of this postulated size. However, there is general agreement that the production of pigment is unstable in vitro (probably the reason why so many clinical strains produce pigment is the fact that production of pigment protects the cell, chiefly against desiccation [76]). Further work is needed to establish whether these genes are borne on a plasmid, or a "chromosomal" element.

Mechanism of Antibiotic Resistance in S. aureus

The biochemical mechanisms, where known, of plasmid-mediated and of chromosomal resistances to antibiotics are shown in Table 4. Apart from penicillinase, which has been detected in many cultures, most of these reports are culled from the analysis of one or a few strains. It is certainly possible that each resistance results from more than one mechanism. However, where the mechanism of plasmid-mediated resistance is known, there is a striking similarity to that found in the Enterobacteriaceae. Resistance to chloramphenicol, neomycin/kanamycin, and streptomycin are due to inactivating enzymes and that to tetracycline is due to decreased uptake. It is curious that although resistance to erythromycin is probably plasmid-determined in S. aureus (see above), the mechanism involves ribosomal modification (213). This seems an important exception to the general rule that plasmid-determined resistance involves alterations in the cell surface.

Manipulation of Staphylococcal Genes In Vitro and Possible Relevance to the In Vivo Situation

The formation of a specialized transducing element by recombination of plasmid and phage has already been mentioned (see above). The following section describes other experiments involving staphylococcal plasmids; some indication is given as to the extent that the phenomena demonstrated may have occurred in S. aureus in its natural habitat.

Reversible Integration of Plasmids into the Chromosome

There is no proof of an entire plasmid becoming integrated into the chromosome, in the laboratory, or in nature. Genes for erythromycin resistance, initially part of a penicillinase plasmid, can become integrated into the chromosome after prolonged UV irradiation to the transducing lysate (144). Evidence for integration was derived from stability of the resistance after growth at 43 C and transduction data. The genes for penicillinase production can also be integrated into the chromosome, at a situation

<table>
<thead>
<tr>
<th>Resistance</th>
<th>Locus of gene determining resistance*</th>
<th>Mechanism of resistance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin (penicillinase)</td>
<td>P</td>
<td>Hydrolysis of β-lactam bond</td>
<td>Many</td>
</tr>
<tr>
<td>Streptomycin, high level</td>
<td>C</td>
<td>(1) Ribosomal modification</td>
<td>109</td>
</tr>
<tr>
<td>Streptomycin, low level</td>
<td>P</td>
<td>(2) Adenylation of streptomycin</td>
<td>130</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>P</td>
<td>Probably reduced permeability</td>
<td>193</td>
</tr>
<tr>
<td>Neomycin/kanamycin</td>
<td>P</td>
<td>Phosphorylation of the antibiotic</td>
<td>55</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>?P</td>
<td>Acetylation of chloramphenicol</td>
<td>190</td>
</tr>
<tr>
<td>Erythromycin/lincomycin</td>
<td>?P</td>
<td>Ribosomal modification</td>
<td>213</td>
</tr>
<tr>
<td>Methicillin</td>
<td>?P,C</td>
<td>Not known; not enzymatic hydrolysis</td>
<td>60</td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>P,C</td>
<td>Not known</td>
<td></td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>C</td>
<td>Not known</td>
<td></td>
</tr>
<tr>
<td>Sulfonamide</td>
<td>?</td>
<td>Not known</td>
<td></td>
</tr>
<tr>
<td>Novobiocin</td>
<td>C</td>
<td>Not known</td>
<td></td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>?P</td>
<td>Not known</td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td></td>
<td>No resistance</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: P, plasmid locus; C, chromosomal locus.
distinct from that for the integration of erythromycin resistance (146). Using the above-mentioned strain of Novick which contained chromosomally located genes for erythromycin resistance, Richmond and Johnson (166) have probably reversed this process, presumably by recombination of these genes with a penicillinase plasmid newly introduced into the cell. After introduction of the penicillinase plasmid into the cell by transduction, followed by purification and growth at 43°C, clones which were resistant neither to penicillin nor to erythromycin were isolated. The most likely explanation for this phenomenon was the occurrence of recombination between the introduced penicillinase plasmid and the chromosomal gene for erythromycin resistance, resulting in production of a bacterium carrying a plasmid determining both penicillinase production and erythromycin resistance, and lacking chromosomal genes for erythromycin resistance, followed by loss of the whole plasmid. The case for reversible integration would have been strengthened by isolation of the postulated intermediate derivatives, i.e., cells harboring the initially chromosomal genes for erythromycin resistance as part of the penicillinase plasmid newly introduced. However, an exceptionally tedious search would be required. The extent to which these phenomena occur naturally is difficult to assess. Since the great majority of penicillinase-producing strains of *S. aureus* harbor the genes for penicillinase on a plasmid, transition of the plasmid genes to the chromosome does not seem common in strains under natural conditions.

There is evidence that the plasmid coding for tetracycline resistance (or part of it, at least) may have become integrated into the chromosome in one strain. This comes from the study of methicillin-resistant strains which probably all evolved from one clone (see below which is characterized by, among other properties, plasmid-mediated tetracycline resistance. In the early isolates, from 1960 to 1969, the tetracycline resistance appeared to be always determined by plasmid genes (105, 112). Recently Kayser et al. isolated two methicillin-resistant variants with chromosomal resistance to tetracycline (see above), although in other properties they are typical methicillin-resistant strains (99, 100). The genes for tetracycline resistance have probably become integrated into the chromosome in these cultures. The possibility that a mutation to tetracycline resistance has occurred de novo is unlikely because (i) the plasmid specifying tetracycline resistance in the earlier methicillin-resistant strains is relatively stable (112) and would be expected to be retained by the cell, and (ii) mutation of tetracycline-sensitive strains of *S. aureus* to tetracycline resistance of the level (MIC 100 μg/ml) found in the clinical strains does not occur in vitro (104).

**Recombination of Chromosomal with Plasmid Genes**

Experiments by Asheshov have demonstrated the transition of chromosomal genes into a plasmid. Propagating strain 80 (PS 80) contains chromosomal genes for penicillinase production (evidence for this includes stability after growth at 43°C and transduction data) and a plasmid containing the genes for resistance to cadmium, arsenic, and mercury ions (how or if these resistance genes benefit the cell is unknown); on storage of the culture, a reduplication of the chromosomal penicillinase genes seems to occur, one copy becoming integrated into the plasmid (10, 12). These findings are particularly significant as they occurred spontaneously and at high frequency. The penicillinase determined by the chromosomal gene in PS 80 is extremely similar, perhaps identical, to that produced by another strain in which the gene is plasmid borne (165). It is reasonable to assume that the two sets of genes are similar. This observation is certainly compatible with the natural occurrence of chromosome-plasmid transition.

**Recombination Between Staphylococcal Plasmids**

Recombination between plasmids (in conjunction with transfer between cells) certainly provides theoretical potential for gene reassortment (164). It will be difficult to detect the natural occurrence of this process. For example, the plasmid that determines production of penicillinase and resistance to fusidic acid and cadmium ions (see below) may have resulted from such a recombination event, e.g., between a penicillinase plasmid and chromosomal genes coding for fusidic acid (111). This plasmid seems to have gained a gene(s), i.e., resistance to fusidic acid, and lost at least one, i.e., resistance to arsenate ions (other penicillinase plasmids conferring resistance to cadmium ions also confer resistance to arsenate ions [61]). Thus the inferred changes in this plasmid could have resulted from recombination, although they could also have occurred by successive mutations.

**Loss of Fragments of DNA from Staphylococcal Plasmids**

In vitro, markers have been lost from several staphylococcal plasmids (111, 112, 149); the
genes for fusidic acid resistance can be lost from the PF plasmid (see above) after growth at 42°C (111). The loss of fragments from this plasmid probably also occurs naturally (114, 116). Whether this type of fragmentation occurs in other plasmids under natural conditions is unknown; if it did, it could be an important factor in the spread of antibiotic resistance between strains in nature since the smaller the plasmid the greater its frequency of transfer between cells in mixed cultures in vitro (46).

**TRANSFER OF ANTIBIOTIC RESISTANCE IN S. AUREUS IN MIXED CULTURES**

**Transfer of Plasmid Genes Between Cultures In Vitro**

The transduction procedures described above have been of value in the location of specific genes, and in the definition of interactions between the genetic elements. However, these methods always involve the artificial formation of a cell-free lysate containing a high concentration of phage particles. This procedure obviously differs dramatically from the natural environment of the staphylococcus where the numbers of free phage particles are probably few. Even in supernatants of broth cultures of staphylococci, the number of phage particles is often no more than $10^4$/ml (104, 177). Since transduction of a marker may occur at frequencies of less than $10^{-6}$ PFU, doubt has been expressed at the potential of generalized transducing agents to affect transfer in nature (164).

However, after incubation of strains of staphylococci together in nutrient broth, without any attempt to create a specialized transducing agent or to extract a phage, transfer of markers between the cultures can certainly occur, and sometimes at high frequency (103).

Several plasmids can be transferred in mixed culture, and successively through several strains (46, 103, 104, 105). The plasmids that have been thus transferred are those determining resistance to neomycin, tetracycline, erythromycin (and spectinomycin), or to chloramphenicol, and also the penicillinase plasmids (Table 5).

In general, the rate of transfer in mixed culture correlates fairly well with that of transfer by transduction by cell-free lysates (46). This suggests that both processes are mediated by the same vector (a phage). Further evidence for a phage vector in mixed culture transfer is the requirement of calcium ions for transfer (104) and the absence of transfer either from nonlysogenic donors or to phage-resistant recipients (104). Thus, strain 1030 which is nonlysogenic (see above) cannot act as a donor unless it is lysogenized with a transducing phage—P609, a β phage (104). The use of anti-phage sera has given inconclusive results; i.e., transfer has not been inhibited by the presence of the antisera (148; R. W. Lacey, unpublished observations).

The destruction of such antibodies by proteolytic enzymes is difficult to prevent, particularly as incubation for some hours is often necessary to detect significant transfer.

However, cell-to-cell contact favors transfer, as the transfer frequency (expressed as the number of resistant recipients per either donor or recipient) increases disproportionately with the number of cocci present (R. W. Lacey and P. S. Ward, unpublished observations). Transfer does not occur between strains separated by a bacterial filter as in the Davis U-tube experiment (104). The frequency of transfer is also high when the donor and recipient are grown together on the surface of agar or in well aerated broth (104), i.e., in situations where the cell density is high.

Another curious feature of this transfer process is that virtually no transducing particles are detectable in supernatants from donors (104). The transfer vector is probably a defective phage and presumably some of the phage genome has been replaced by the plasmid. This is supported by the observation that recipients that have acquired the plasmid do not become lysogenic for the phage, although “normal” phages plaque on the recipient (104). The absence of such agents in the culture supernatant must be explained. The reason for this could be that (i) the transferring particle is unstable, or (ii) it remains cell-bound throughout the transfer process, possibly forming a bridge between the donor and recipient. In favor of the cell binding is the demonstration that the addition of a high-titer phage preparation to a culture of

**Table 5. Plasmids of S. aureus that can be transferred between strains in mixed cultures**

<table>
<thead>
<tr>
<th>Plasmid determinant*</th>
<th>Maximum frequency of transfer after 18-h incubation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>tet</td>
<td>$10^{-1}$</td>
<td>R. W. Lacey and P. S. Ward, unpublished observations</td>
</tr>
<tr>
<td>neo</td>
<td>$10^{-4}$</td>
<td>104</td>
</tr>
<tr>
<td>pen, cad, mer, asa</td>
<td>$10^{-4}$</td>
<td>103</td>
</tr>
<tr>
<td>pen, cad, fus</td>
<td>$10^{-5}$</td>
<td>105</td>
</tr>
<tr>
<td>chm</td>
<td>$10^{-4}$</td>
<td>46</td>
</tr>
<tr>
<td>ero</td>
<td>$10^{-5}$</td>
<td>R. W. Lacey, unpublished observations</td>
</tr>
</tbody>
</table>

*For abbreviations see Table 2.*
a nonlysogenic strain is followed by > 99% adsorption of phage to cell within 10 min (R. W. Lacey, unpublished observations). This implies that the true number of phage particles in a culture may be more than 100-fold greater than the number demonstrated in the culture supernatant, the majority of phage particles, both normal and transducing, having been readSORBED.

Another factor that may explain some of the unexpectedly high rate of transfer in mixed culture is that this process is continuous, whereas the traditional transduction experiments are a "once and for all" reaction.

The frequency that can be obtained in mixed culture experiments seems to vary with (i) the plasmid in question, (ii) the donor, (iii) the prophage in the donor (or possibly in the recipient initially), (iv) the recipient, or (v) cultural conditions, particularly the proportion of donors to recipients (104, 110). If pairs of clinical strains taken at random are used as donor or recipient, then transfer frequency of plasmids is typically from 10^-4 to 10^-6 per donor or recipient after overnight incubation (105).

Those clinical strains that do not harbor a transducing phage obviously cannot act as donors. Under optimal conditions, transfer can be as high as 10^-3 to 10^-4 within 4 h of mixing the cultures and 10^-1 after overnight incubation (R. W. Lacey and P. S. Ward, unpublished observations).

Such experiments demonstrate the feasibility of transfer of plasmid genes (chromosomal genes can also be transferred, but at lower frequencies; 106) between staphylococci growing together under natural conditions; the process in many ways comparable to conjugation in the Enterobacteriaceae, although it is phage mediated in staphylococci.

The precise molecular events that bring about this transfer are not known, and would seem a profitable topic for further study. However, the techniques available may not altogether measure up to this problem.

Transfer of Plasmids Between Staphylococci Experimentally Seeded onto the Skin Surface

The transfer of several plasmids (determining resistance to neomycin, tetracycline, or erythromycin or the production of penicillinase) can occur experimentally on the surface of the healthy skin of volunteers (103, 115). For transfer to occur, the organisms must be able to grow, and as exposed dry skin is endowed with good antibacterial activity (108, 167) this must first be neutralized by providing a moist environment and reducing the antibacterial effect of unsaturated fatty acids. This is easily achieved by suspending the donor and recipient organisms in human serum. The resultant conditions on the skin surface resemble reasonably well those in superficial wound and other infections, which are often associated with serous exudate and are covered.

The natural habitat of the staphylococcus is the body surface (see below), so transfer of antibiotic resistance between strains on the skin surface is therefore comparable to transfer of resistance between members of the Enterobacteriaceae experimentally in the gut.

In the following section, an attempt is made to ascertain to what extent, if at all, transfer of antibiotic resistance occurs between staphylococci in their natural environment (it is, of course, impossible to define what is "natural;" the hospital environment is in many ways artificial, although the relationship of man with his ectoparasites can still be considered natural).

Epidemiology of Penicillinase Plasmids

Study of the possible epidemiology of the penicillinase plasmids is complicated by the large variety of plasmid types that have been identified (see above). The absence of a good baseline (the incidence of penicillinase-producing strains before the introduction of penicillin is poorly documented) has also produced difficulties. Although a few strains produced penicillinase at the time of, and before the introduction of penicillin (47), they were probably rare.

Spink found that none out of 67 strains (196), and Rammelkamp and Moxon found that none out of 27 strains isolated before 1942 were resistant to penicillin (160). North and Christie found that none out of 128 strains isolated in 1944 were resistant (139). However, during the late 1940's, the incidence of penicillin resistance in strains of phage groups I, II, and III rapidly increased (e.g., 15, 20, 21, 23).

The majority of both hospital and nonhospital staphylococci of every phage-typing pattern now produce penicillinase (36, 37, 105, 150). It is well known that the capacity to form penicillinase does not arise by mutation in vitro (mutants resistant to penicillin do not inactivate the drug and are unstable; see references 30, 140, 197).

It seems impossible now to come to any certain conclusion about the mechanism for the sudden increase in penicillin resistance in the late 1940's. The part played by (i) selection of rare existing resistant strains, (ii) transfer of the genes between strains, and (iii) evolution of the genes de novo cannot be assessed with accuracy. Most of the cultures isolated at this time have
been lost and the genetic basis of the resistance in many of these strains will never be known.

We have attempted recently to study penicillinase plasmids epidemiologically, by considering a rare kind of penicillinase plasmid (111) which probably comprises less than 1% of the penicillinase plasmids overall. This plasmid is designated PF because it also carries the genes for fusidic acid resistance (it also carries the genes for cadmium ion resistance). The first possible record of this plasmid was in 1965 (65), since in one strain (plasmid type 7/47/54/75) resistance to fusidic acid and production of penicillinase were co-eliminated. Unfortunately, this strain has been lost.

It was not until 1971 that strains carrying the PF plasmid were reported again (111). The combination of characters determined by this plasmid is sufficiently distinctive to enable its fairly certain identification. These markers are (i) production of penicillinase of D-serotype, (ii) resistance to fusidic acid, (iii) resistance to cadmium ions, but not resistance to mercury or to arsenate ions. In extensive surveys which have characterized a total of about 200 penicillinase plasmids between 1963 and 1970, apart from the one possible example mentioned above, no other PF plasmid has been described (61, 155, 161).

In strains isolated in 1971 and 1972, the PF plasmid has been identified in 18 strains of staphylococci, all judged to be different because of variation in other properties (116). The staphylococci belonged to phage groups I, II, and III or were nontypable (Table 6). This plasmid is considered to be the same in each strain; in addition to the features listed above, it is characterized by a molecular weight of $15 \times 10^6$ to $16 \times 10^6$ (determined in 7 out of the 18 isolates), and is compatible with another penicillinase plasmid of compatibility group I (116). Test of a single identity of this plasmid could be made by DNA/DNA hybridization and heteroduplex formation. However, both of these techniques require experience and are performed on cultures which, although originating from a single cell, could well contain variant plasmids (the PF plasmid seems capable of rapid evolution; see below). It seems reasonable to conclude that this plasmid is the same in these strains.

The presence of this unusual plasmid in a variety of strains isolated during 1971 and 1972 from patients in Bristol, London, and Birmingham, United Kingdom had the following three possible explanations, or combinations of them.

(i) The plasmid has evolved de novo (i.e., a PF plasmid has originated by an unspecified mechanism, in several strains of different phage group) in most, if not all, the strains. (ii) A single clone harboring this plasmid has differentiated, so as to produce the variation in host

---

**Table 6. Pigment, antibiotic sensitivity, and phage patterns of strains of S. aureus harboring a plasmid determining resistance to fusidic acid, cadmium ions, and penicillin (penicillinase)**

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>Color of pigment*</th>
<th>Sensitivity or resistance to:</th>
<th>Phage sensitivity pattern at RTD*</th>
<th>Phage group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S</td>
<td>T</td>
<td>E</td>
</tr>
<tr>
<td>FAR 1</td>
<td>O</td>
<td>(R)</td>
<td>(R)</td>
<td>(R)</td>
</tr>
<tr>
<td>FAR 2</td>
<td>B</td>
<td>(R)</td>
<td>(R)</td>
<td>(R)</td>
</tr>
<tr>
<td>FAR 4</td>
<td>O</td>
<td>(R)</td>
<td>(R)</td>
<td>S</td>
</tr>
<tr>
<td>FAR 5</td>
<td>O</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>FAR 6</td>
<td>O</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>FAR 7</td>
<td>W</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>FAR 8</td>
<td>B</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>FAR 9</td>
<td>B</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>FAR 10</td>
<td>B</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>FAR 11</td>
<td>B</td>
<td>S</td>
<td>(R)</td>
<td>S</td>
</tr>
<tr>
<td>FAR 12</td>
<td>B</td>
<td>S</td>
<td>(R)</td>
<td>S</td>
</tr>
<tr>
<td>FAR 13</td>
<td>B</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>FAR 14</td>
<td>Y</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>FAR 15</td>
<td>B</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>FAR 16</td>
<td>B</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>FAR 17</td>
<td>O</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>FAR 18</td>
<td>W</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>FAR 19</td>
<td>Y</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

*Strains isolated in 1971 and 1972 were from patients in Bristol, Birmingham, and London, United Kingdom.

*O, orange; B, buff; W, white; Y, yellow.

*S, streptomycin; T, tetracycline; E, erythromycin; N, neomycin; M, methicillin.

*RTD, Routine test dilution; NT, nontypable.
properties of strains that now contain the plasmid. (iii) The plasmid has spread among the staphylococcal population by cell-to-cell transfer.

The possibility (i) of evolution de novo seems extremely unlikely and would represent convergent evolution to a remarkable degree. Available evidence suggests that the formation of new plasmids is a rare event indeed.

The possibility that the host cell has evolved is very unlikely because the properties of the cells that now harbor the plasmid seem to be too variable to be explained by evolution from one clone (Table 6). This is particularly so for the variation in phage typing pattern. Although the susceptibility of staphylococci to phage lysis can be altered by changes in prophage carriage in vitro (28, 93, 95, 96, 175, 176) these changes occur only within the various phage groups. They involve either a restriction or a widening of sensitivity to typing phages in group I or a progressive restriction in group III. Thus, there is, for example, no evidence for conversion between phage group III and II, or between II and I. The changes in lysogeny that occur in vitro have probably also occurred naturally (37, 38, 93, 95, 96), but there is also no indication that other changes have occurred.

Thus, the most likely explanation for the epidemiology of the PF plasmid is that the ancestral plasmid was formed in one strain or perhaps a few and spread to others.

If this reasoning is correct, it is probable that other penicillinase plasmids have also spread between strains. The epidemiology of the penicillinase plasmids may well have resulted from a very economical evolutionary process. It is possible to account for the fact that most staphylococci now produce penicillinase and that the plasmids determining this enzyme code for very variable properties, by the following postulates: (i) the formation of a few, or even just one primordial penicillinase plasmid; (ii) evolution of this (these) plasmid(s) by mutation and recombination with other genetic elements; (iii) interstrain transfer of the resultant elements by phage transduction; (iv) further evolution after transfer; (v) selection pressure by antibiotics.

Understanding of the original formation of these (or other) plasmids is minimal. The phenomenon reported by Asheshov (10, 12), in which chromosomal penicillinase genes became incorporated into an existing plasmid containing genes for metal ion resistance, could have been a step in the formation of the penicillinase plasmid. However, the origin of the plasmid coding for metal ion resistance is quite obscure.

**Epidemiology of Neomycin/Kanamycin Resistance**

Clinical strains of *S. aureus* that are neomycin resistant always shown resistant to kanamycin (42, 103). Because of its toxicity, neomycin has been used in clinical medicine only for topical application or locally in the gut (from which it is little absorbed). Kanamycin is used chiefly for treatment of systemic infections. Because of the importance of the interaction of antibiotics with the normal flora (see below), it is likely to be the use of neomycin locally rather than use of kanamycin systemically that has selected resistant strains. The cross-resistance between neomycin and kanamycin is considered to reflect the inactivation of both antibiotics by a single phosphotransferase enzyme (55), although the mechanism of resistance has been elucidated in rather few strains.

The epidemiological pattern of neomycin resistance is remarkable. Despite the widespread use of neomycin on the skin and in the nose, resistance of *S. aureus* to it was virtually unknown from 1951 to 1959 (29, 39, 40, 67, 123, 154, 180, 210). Enormous populations of staphylococci must have been exposed to the drug. It is relevant here to note that the sort of resistance to neomycin seen in clinical strains cannot be developed in vitro by mutation from sensitive cultures; any resistant mutants are slow growing and often have specific substrate requirements, and are also gentamicin resistant (102); clinical resistant isolates are normal growing and gentamicin sensitive (103).

Suddenly, in late 1959 and 1960, neomycin-resistant strains were reported from several centers in the United States (66, 75, 159, 204). These strains were resistant to several other antibiotics and all appeared closely related on the basis of phage typing pattern—type 54 or nontypable by the phages then in use. During the next few years, similar strains were isolated widely, being particularly prevalent in the United Kingdom (90, 91, 95, 170). They were isolated from patients, particularly after the use of neomycin topically (2, 124, 178).

However, in 1967 and 1968, neomycin resistance appeared in several countries, including the United Kingdom, Switzerland, and Denmark in a variety of "new" strains as judged by marked variations in their phage-typing and antibiotic sensitivity patterns (Table 7; 37, 99, 100, 103, 112, 150).

Although the genes for neomycin resistance have been shown to be plasmid borne in several cultures of the more recently isolated resistant strains (see above), the precise genetic and bio-
Table 7. Variations on properties of neomycin-resistant staphylococci isolated from Bristol hospitals, 1967 and 1968

<table>
<thead>
<tr>
<th>Anti-biogram</th>
<th>Phage-typing pattern at RTD</th>
<th>Phage-group</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSTEN</td>
<td>54</td>
<td>III</td>
</tr>
<tr>
<td>N</td>
<td>29/52</td>
<td>I</td>
</tr>
<tr>
<td>N Untypable</td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>PN</td>
<td>6/47/53/54/75/85</td>
<td>III</td>
</tr>
<tr>
<td>TN</td>
<td>52</td>
<td>I</td>
</tr>
<tr>
<td>TN</td>
<td>29/52A/80/6/42E/47/53/54/75/77/83A/84/85</td>
<td>I, III</td>
</tr>
<tr>
<td>PTN</td>
<td>29/52</td>
<td>I</td>
</tr>
<tr>
<td>PSEN</td>
<td>6/42/47/64/85/81</td>
<td>III</td>
</tr>
<tr>
<td>PSTN</td>
<td>85</td>
<td>III</td>
</tr>
<tr>
<td>PSTN</td>
<td>75/77/84/85</td>
<td>III</td>
</tr>
<tr>
<td>PSTN</td>
<td>84/85</td>
<td>III</td>
</tr>
<tr>
<td>PSTEN</td>
<td>84/85</td>
<td>III</td>
</tr>
<tr>
<td>PSTEN</td>
<td>81/6/42E/47/54/85</td>
<td>III</td>
</tr>
<tr>
<td>PSTEN</td>
<td>Untypable</td>
<td></td>
</tr>
<tr>
<td>PSTEN</td>
<td>77/84/85</td>
<td>III</td>
</tr>
<tr>
<td>PSTEN</td>
<td>52/79/6/47/53/54/83A/85</td>
<td>I, III</td>
</tr>
</tbody>
</table>

* P. Penicillin (penicillinum); S, resistance to streptomycin; T, to tetracycline; E, to erythromycin; N, to neomycin.
* RTD, Routine test dilution.
* "Original" neomycin resistant strain.

chemical characterization of the resistance is still scanty. Nevertheless, the following could explain the curious epidemiology of the resistance.

The long interval in which the resistance was not reported (many laboratories were testing for this resistance during this time) must indicate the extreme rarity of the resistance (inactivation) genes in the population of staphylococcal strains. When the resistance first appeared, the wide dissemination of a single strain was responsible. The subsequent appearance of the resistance in many different phage types over a short period is likely to have resulted from transfer of the genes from the original strain.

For transfer to occur, the following conditions must have been met. (i) The gene for neomycin resistance must be present as part of a plasmid sufficiently small for inter-cell transfer. Staphylococcal plasmids can decrease in size in vitro (111, 112), and in vivo (113, 114, 116), and such a change could have occurred in the original neomycin-resistant cell. (ii) A transducing phage must be present in or accessible to that cell. There is good evidence that such phages have spread epidemically among staphylococci within phage groups I or III (37, 95). Once these two criteria have been fulfilled and the organism resides in a situation from which resistance can be transferred to recipients, transfer of resistance to other strains could occur.

It will probably be impossible to prove this hypothesis, but further study on the genetic and biochemical mechanism of the resistance should furnish additional evidence.

Epidemiology of Tetracycline Resistance

The epidemiology of tetracycline resistance presents a similar pattern as that for neomycin resistance, although the baseline is less clear-cut.

For a year or two after the introduction of the tetracyclines from 1947 to 1949, resistance to them was rare (28, 63). This was soon followed by the appearance of resistance in many strains (125, 131). Since tetracycline resistance (of the type found in clinical strains, i.e., a MIC of ~100 μg/ml with normal growth characteristics) does not arise by mutation in vitro, and tetracycline resistance is plasmid borne in a wide variety of strains (see above), it is tempting to postulate that one plasmid has spread through the staphylococcal population. Consistent with this is the finding that all the plasmids specifying tetracycline resistance are similar in that the level of resistance is constant, the only marker expressed is tetracycline resistance, and that the size shows little variation (2.66 x 10⁴ to 2.9 x 10⁴ daltons) (46, 104, 112, 146).

Further research into the epidemiology of the plasmids coding for tetracycline or for neomycin resistance should be able to utilize profitably techniques of DNA/DNA hybridization and heteroduplex formation which have been established in the Enterobacteriaceae.

Epidemiology of Other Resistances

Examination of methicillin-resistant staphylococci suggests that plasmid-mediated resistance to erythromycin/lincomycin or chloramphenicol may have also spread between staphylococci (see below). But for resistance to streptomycin, novobiocin, trimethoprim, and sulfonamides, there is no evidence for interstrain transfer, and some of these resistances have probably arisen by mutation in vivo, as one-step resistance to these can occur in vitro (Table 8). There is also good evidence that methicillin resistance has not spread epidemically (see below).

METHICILLIN/RESISTANT STRAINS OF S. AUREUS—THE SINGLE-CLONE HYPOTHESIS

Methicillin-resistant cultures were first isolated in 1960 (94, 101); numerous reports of
these organisms have since appeared, particularly from European sources. The similarities in their properties is pronounced, particularly in their resistance to several other antibiotics which seems to be universal (99, 100, 112, 150). A single-clone origin for all these cultures seems probable (99, 100, 105, 112); i.e., there is in reality only “one” methicillin-resistant strain.

The evidence for this is as follows. (i) As to the nature of methicillin resistance, virtually all strains that have been examined show pheno-
typic heterogeneous resistance. In an extensive survey, Dyke confirmed that all naturally occurring strains showed this type of resistance, and that none of the cultures produced a “methicillinase” (60). Although the genetic basis of the resistance is uncertain, the location of the genes in almost all strains that have been investigated could be the same (see above). (The one strain that may be exceptional is strain DU 4916 [56–58]—the genes coding methicillin resistance may be carried on a penicillin-
ase plasmid; this possible exception need not invalidate the general hypothesis.)

(ii) All of approximately 800 methicillin-resistant cultures were resistant to streptomycin, and all except one were resistant to tetracycline (Table 9; 17, 22, 24, 32, 43, 51, 64, 84, 93, 99, 100, 150, 199). This contrasts with methicillin-sensitive staphylococci most of which are sensitive to streptomycin and/or tetracycline (Table 9).

Table 8. Summary of loci of various resistance genes in clinical isolates of S. aureus, and evidence for transfer in nature

<table>
<thead>
<tr>
<th>Marker*</th>
<th>Locus (also see text)</th>
<th>Evidence for transfer between strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>pen</td>
<td>Plasmid in &gt;95% of resistant strains</td>
<td>+++</td>
</tr>
<tr>
<td>str</td>
<td>Usually chromosomal, sometimes plasmid</td>
<td>–</td>
</tr>
<tr>
<td>tet</td>
<td>Nearly always plasmid</td>
<td>++</td>
</tr>
<tr>
<td>neo</td>
<td>Plasmid</td>
<td>++</td>
</tr>
<tr>
<td>che</td>
<td>Plasmid</td>
<td>+</td>
</tr>
<tr>
<td>ero</td>
<td>Probably plasmid</td>
<td>+</td>
</tr>
<tr>
<td>mtc</td>
<td>Probably unusual plasmid</td>
<td>–</td>
</tr>
<tr>
<td>fus</td>
<td>Usually plasmid, sometimes chromosomal (116)</td>
<td>+ + +</td>
</tr>
<tr>
<td>tmp</td>
<td>Chromosomal (120)</td>
<td>–</td>
</tr>
<tr>
<td>sul</td>
<td>?</td>
<td>–</td>
</tr>
<tr>
<td>nov</td>
<td>Probably chromosomal (112)</td>
<td>–</td>
</tr>
</tbody>
</table>

*See Table 2; tmp, resistance to trimethoprim; sul, resistance to sulfonamides; nov, resistance to novobiocin.

The strongest epidemiological evidence for transfer is denoted by ++ +, the absence if denoted by –; intermediate evidence is shown by + or ++ (see text).

(iii) Resistance to streptomycin is of a high level (MIC > 10 mg/ml) characteristic of the chromosomal/ribosomal type (109, 112).

(iv) Methicillin-resistant strains are resistant to sulfonamides (99). Other staphylococci are frequently sensitive to sulfonamides (135).

(v) Methicillin-resistant staphylococci usually produce orange pigment on suitable media and have a characteristic rate of destruction by drying (112). The genes that govern these properties are probably carried by the same plasmid (76).

(vi) Most of the cultures produce large amounts of penicillinase of A serotype (60).

(vii) The cultures produce enterotoxin B (58).

(viii) The bacteriophage typing pattern of methicillin-resistant staphylococci is consistent with a single-clone origin for these organisms. The phage pattern of strains isolated from 1960 to 1969 is group III with variable reactions; as with other staphylococci, the overall trend has been a progressive restriction in their spectrum of phage susceptibility (150). This has presumably resulted from alterations in prophage carriage (see above).

The properties of methicillin-resistant staphylococci are summarized in Table 10. If these organisms have indeed evolved from a single clone, then methicillin resistance should be expected to be “nontransferable” to other strains. This would appear to be so. Thus, methicillin resistance cannot be transferred to recipients in mixed culture (105). Furthermore, although the resistance can be transduced with cell-free lysates at low frequencies to a few recipients (these must have specific properties

Table 9. Association of resistance to streptomycin and tetracycline with methicillin resistance

<table>
<thead>
<tr>
<th>Determination</th>
<th>No. of isolates resistant to</th>
<th>Neither streptomycin nor tetracycline</th>
<th>Streptomycin alone</th>
<th>Tetracycline alone</th>
<th>Both streptomycin and tetracycline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methicillin-resistant organisms from many sources, 1960–1973 (see text).</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>~800</td>
<td></td>
</tr>
<tr>
<td>Methicillin-sensitive staphylococci isolated from miscellaneous sources in Bristol, U.K., from 1967–1972.</td>
<td>~1,400</td>
<td>32</td>
<td>112</td>
<td>409</td>
<td></td>
</tr>
</tbody>
</table>
to act as recipients; see above), there are several staphylococci to which methicillin resistance cannot be transduced (49, 60, 105). Thus, methicillin resistance should be considered, at least from an epidemiological basis, as essentially nontransferable. The absence of methicillin resistance in strains sensitive to streptomycin and tetracycline is most striking, and is consistent with the proposal that the resistance is nontransferable.

Final evidence for a single-clone origin is that the heterogeneous type of resistance of clinical strains has not been reported to arise in sensitive cultures in vitro by mutation.

Although the genetic basis of methicillin resistance may or may not be a plasmid, it seems sufficiently stable (49, 99, 100) to provide a suitable marker for detecting this postulated clone. If this single-clone origin is correct, and the evidence appears very strong, then considerable evolutionary changes must have occurred in this clone.

Most methicillin-resistant staphylococci isolated between 1960 and 1965 were resistant only to the antibiotics listed in Table 10 (see above), although a few were resistant to erythromycin or chloramphenicol (17, 24).

Since 1965, methicillin-resistant strains have been isolated that were also resistant to other antibiotics. In some of these isolates, resistance to neomycin, erythromycin/lincomycin, chloramphenicol, or fusidic acid (as part of a penicillinase plasmid) has been found to be plasmid mediated (37, 99, 100, 112). The probability is high that these four plasmids have been transferred to this clone from other staphylococci (as with the PF plasmid, the possibility that each plasmid has formed de novo in the cell cannot be excluded formally, but seems remote). This provides further circumstantial evidence that plasmid transfer occurs between staphylococci in nature at fairly high frequencies.

The inferred locations of several resistance genes in methicillin-resistant staphylococci are listed in Table 11.

### Table 10. Properties common to methicillin-resistant strains of S. aureus

<table>
<thead>
<tr>
<th>Property</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Heterogeneous resistance to methicillin and all other penicillins and cephalosporins, including penicillin-resistant penicillins</td>
<td>60, 150</td>
</tr>
<tr>
<td>2. High-level resistance to streptomycin</td>
<td>105, 112</td>
</tr>
<tr>
<td>3. Resistance to tetracycline (MIC 100 μg/ml)</td>
<td>99, 100, 105, 112</td>
</tr>
<tr>
<td>4. Resistance to sulfonamides</td>
<td>99, 100</td>
</tr>
<tr>
<td>5. Orange pigment on suitable media</td>
<td>112</td>
</tr>
<tr>
<td>6. Producers of enterotoxin B</td>
<td>58</td>
</tr>
<tr>
<td>7. Well-defined phage patterns—essentially phage group III with evolutionary changes</td>
<td>99, 150</td>
</tr>
<tr>
<td>8. Nearly always product penicillinase</td>
<td>60, 150</td>
</tr>
</tbody>
</table>

### Table 11. Location of genes in methicillin-resistant strains of S. aureus

<table>
<thead>
<tr>
<th>Index no. of strain</th>
<th>Year of isolation</th>
<th>Location of gene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>str&lt;sup&gt;c&lt;/sup&gt;</td>
<td>tet&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>1316</td>
<td>1960</td>
<td>C</td>
</tr>
<tr>
<td>13137</td>
<td>1960</td>
<td>C</td>
</tr>
<tr>
<td>2273</td>
<td>1965</td>
<td>C</td>
</tr>
<tr>
<td>9463</td>
<td>1967</td>
<td>?</td>
</tr>
<tr>
<td>11164</td>
<td>1967</td>
<td>C</td>
</tr>
<tr>
<td>B 109</td>
<td>1969</td>
<td>C</td>
</tr>
<tr>
<td>8657</td>
<td>1970</td>
<td>C</td>
</tr>
<tr>
<td>FAR 1</td>
<td>1971</td>
<td>C</td>
</tr>
<tr>
<td>FAR 2</td>
<td>1971</td>
<td>C</td>
</tr>
<tr>
<td>B 262</td>
<td>1971</td>
<td>C</td>
</tr>
</tbody>
</table>

<sup>a</sup> Isolated from sources in the United Kingdom from 1960–1971 (105, 112).

<sup>b</sup> Abbreviations: C, chromosomal locus; P, plasmid locus; —, organism is sensitive to corresponding antibiotic; ?, location is uncertain.

<sup>c</sup> For abbreviations see footnotes to Tables 2, 3, and 8.
organisms, and insignificant amounts of the enzyme would probably be produced by a few cocci).

Much of the resistance in microorganisms has probably resulted from an interaction with antibiotic with the microbe in its natural habitat (closed infections represent a blind alley, epidemiologically, and even if resistance does emerge in them, it is unlikely to spread further). Thus, the appearance of resistance may not necessarily be correlated with therapeutic failure of the drug, but could merely indicate the exposure of commensal bacteria to it.

Methicillin resistance could well be such an example, and the following features suggest that "methicillin-resistant" staphylococci can be eliminated from deep infections by semisynthetic penicillins (e.g., methicillin, cloxacillin, oxacillin, flucloxacillin) as efficiently as "methicillin-sensitive" staphylococci. It must be emphasized that there is no situation in which an antibiotic can be guaranteed to eliminate an organism, since the interplay of host factors is always significant in determining the outcome of therapy.

The following are the properties of methicillin-resistant staphylococci that suggest that resistance per se may not be clinically important: (i) At normal body temperatures (skin surface apart), very few cells, from about 1 in $10^4$ to $10^7$, of methicillin-resistant culture produce colonies in the absence of high levels of methicillin (6, 60, 150), and even these grow abnormally slowly in the presence of methicillin (201). Apart from growth at 30 C or below, the only other environment in which the resistance is fully expressed is in hypertonic saline (5%, wt/vol) (150). Such conditions do not exist in vivo.

(ii) The correlation of the incidence of methicillin-resistant isolates with the use of the penicillin-resistant penicillins is poor (150).

(iii) The incidence of resistant strains is still extremely low (about 3% of hospital strains in the United Kingdom are currently resistant; M. T. Parker, personal communication), particularly in the United States. Since "methicillin resistance" denotes a resistance to all the penicillins and cephalosporins in vitro (150), this low incidence is surprising, particularly as such strains have been isolated since 1960 (94, 101). The low incidence theoretically may be due to associated defects in the organism that have given the organism a disadvantage. But these isolates produce a variety of infections in hospital patients (25, 37, 81), although, as with other "hospital" staphylococci, they rarely cause primary cutaneous sepseis. They also produce a full complement of virulence factors in vitro (68, 81, 105) and have average capacity to survive in air (107, 112). It is therefore not possible to explain the overall low incidence by the supposition that these strains possess associated defects. Their small numbers may point to the absence of benefit conferred by their methicillin resistance.

(iv) Almost all (>95%) methicillin-resistant staphylococci produce penicillinase in large amounts (60, 150), but lose this property at high frequency in vitro (60, 105). Penicillinase production also tends to be lost from methicillin-sensitive staphylococci both in vitro and in vivo (82, 83, 137); the extensive use of some penicillins (e.g., ampicillin) probably accounts for the large number of methicillin-sensitive staphylococci that produce penicillinase. But why do so many methicillin-resistant strains produce penicillinase? If methicillin resistance effectively enabled the bacteria to withstand all the penicillins in vivo, the capacity to produce penicillinase would be superfluous and should therefore have been lost. Thus, the lavish production of penicillinase by methicillin-resistant strains argues against the efficacy of methicillin resistance in vivo. Methicillin resistance and penicillinase production might act additively (150) or even synergistically in vivo (there is little evidence for this in vitro); if so the possession of these two mechanisms simultaneously should have given the organism a decisive advantage over other staphylococci.

(v) In rats experimentally infected with methicillin-resistant staphylococci, cephalothin (to which methicillin-resistant strains are resistant in vitro) has been found to be effective in treating the infection (34). Although methicillin did not significantly reduce the numbers of cocci, this is less conclusive than the results with other antibiotics because of the low potency of methicillin and the absence of controls using methicillin-sensitive cocci.

The above considerations certainly raise the possibility that the methicillin resistance of these strains may not prevent the eradication of the organism by the use of the penicillinase-resistant penicillins in practice. If so, how can the presence of these organisms at all be explained? Two factors may be relevant: (i) their almost inevitable resistance to several other antibiotics (see above); and (ii) the possibility (150) that their methicillin resistance protects the cocci from penicillins and cephalosporins on the body surface (i.e., in the natural habitat of the organism) where the temperature is lower (30 to 33 C); at this temperature the majority of a methicillin-resistant culture may express resistance.

Evidence that the resistance is important
clinically comes from the following. (i) Methicillin-resistant staphylococci have been isolated from patients dying of septicemia (25). But many of these infections were in debilitated patients, and other antibiotics (even those to which the organism might appear fully sensitive in vitro) might also have failed. (ii) In two clinical trials, Chabbert and his colleagues found that methicillin or cephalothin was less effective than other antibiotics in eliminating methicillin-resistant strains (1, 44). The numbers of patients were, however, probably too small to compensate adequately for the variables in the hosts. It is nevertheless fully understandable that such trials were limited; once a trend was established, ethical considerations would necessitate an alternative drug.

Thus, the evidence that methicillin resistance is clinically significant is by no means conclusive. To resolve this question, multicenter trials seem desirable; ethical objections to such trials could be overcome by initially treating patients with infections that were not immediately dangerous.

LOSS OF PLASMIDS FROM S. AUREUS IN VIVO

Although many plasmid markers are highly unstable in vitro, there are relatively few well documented reports of loss of these elements from the cell in vivo. Two factors have caused difficulty in this type of study, and are as follows. (i) Although plasmid-positive and plasmid-negative variants of the same strains may be isolated simultaneously (7, 14, 82, 83, 113, 137), this variation could in some instances be due to either gain or loss of the plasmid, or to a combination of these processes. (ii) The loss of the plasmid could have occurred during the laboratory procedures rather than in the patient.

Surveys of the overall incidence of resistance may throw some light on this problem. The numbers of strains resistant to a particular antibiotic progressively decline in the absence of use of that drug (35, 68, 119, 168). This has been demonstrated most impressively by Bulger and Sherris (35) who showed that a policy of withholding the use of most antibiotics except the penicillins over a period of several years was followed by a progressive fall in the incidence of resistance, except in the numbers of strains that produced penicillinase. Such a decline could well be due to loss of plasmids from cells. However, two other factors may also have contributed: (i) the proliferation of the plasmid-negative derivatives at the expense of plasmid-positive derivatives (i.e., the possession of the plasmid confers a disadvantage to the cell [76, 163]); or (ii) the replacement of the resistant strains by other strains.

Loss of plasmids would certainly be expected to occur from staphylococci in vivo. This is because plasmid gain probably occurs (see above), and that there is probably a limit to the number of plasmids that a cell can maintain stably (110). Presumably an equilibrium exists in the cell between the acquisition and loss of plasmids. Acquisition is favored by the use of an antibiotic; disposal occurs spontaneously.

In the hope of gaining some quantitative data on the loss of plasmid genes in vivo, we have examined changes in plasmid carriage in a strain of S. aureus. This strain has been isolated at frequent intervals over 2 years from the sputum of a patient with cystic fibrosis (113, 114). Many colonies from each specimen were tested individually for antibiotic sensitivity and other properties. During the months when a variety of antibiotics were administered, the majority of the cocci were resistant to several antibiotics, including penicillin and fusidic acid (113, 114). But during a 6-month period when no antibiotics were given, the loss of several resistant traits determined by plasmid genes was striking (Table 12). Although there was a progressive decline in the incidence of resistant isolates, there was variation in the actual trait shown between individual cocci (see Table 13). There is no doubt that this was the same strain isolated repeatedly on account of its constant phage typing pattern (3A), and DNA/DNA hybridization studies (113).

The loss of resistance was considered to occur in vivo because of the extremely good baseline when, during antibiotic therapy, every colony tested was fully resistant to the antibiotics, and that under most cultural conditions plasmid carriage was relatively stable (113). However, even in such a study, it is impossible to calculate the part played by loss of plasmid genome from the cell and overgrowth of plasmid-positive cells by plasmid-negative ones.

Study of this strain has also illustrated the rapidity with which evolution can occur in this organism. The single wild-type culture has given rise to 29 derivatives with different phenotypic properties over 2 years (Table 12). Constant features of this strain comprised a phage type 3A (this is in itself a valuable marker, as this phage type is rarely incriminated in pulmonary infections), a deep orange pigment, and sensitivity to tetracycline, chloramphenicol, and gentamicin. The unstable characters comprised resistance to fusidic acid and cadmium ions, and production of penicillinase—i.e., a PF
plasmid. The other unstable characters were resistance to streptomycin, erythromycin, lincomycin, neomycin, and to spectinomycin, and also production of beta-hemolysin. These characters are probably all borne by a second plasmid (77). One feature shown by this strain is that many of the changes are due to loss of fragments of DNA from plasmids. Thus, the PF plasmid initially of size $16 \times 10^6$ gave rise to plasmids of $15 \times 10^4$, $13 \times 10^4$, and $12 \times 10^4$ daltons (114).

The overall impression, therefore, is that loss of plasmid genes occurs frequently in staphylococci in nature, and is an important aspect of the genetic flexibility that plasmid carriage bestows on cell populations.

**RELATIONSHIP OF ANTIBIOTIC RESISTANCE TO VIRULENCE IN S. AUREUS**

Organisms trained to antibiotic resistance in vitro often have reduced growth rates and diminished virulence for animals (70). But are naturally occurring resistant strains more or less virulent than sensitive strains? Some resistant staphylococci have been thought to show exceptional virulence compared to other hospital strains (179, 203). But virulence, particularly to humans, seems impossible to quantify, chiefly because of its multifactorial nature; virulence of staphylococci is thought to result from interactions between many properties (and some of these may still not be defined) of the parasite and host (63). Animal models for assessing virulence may not necessarily be applicable to humans.

Although there have been reports (e.g., reference 211) of an increase in the incidence of staphylococcal bacteremia, this could have resulted from changes in the type of hospital patient rather than in the pathogen, since deaths from this cause were confined to patients with severe underlying disease (211). Measures aimed at preventing staphylococcal cross-infection must also alter the incidence and type of staphylococcal disease. It is therefore impossible to monitor changes in virulence by the study only of the incidence, morbidity, and mortality of staphylococcal infections of man.

**Table 12. Phenotypic variation in a strain of S. aureus\(^6\) isolated repeatedly over two years from a patient (113, 114)**

<table>
<thead>
<tr>
<th>S. aureus</th>
<th>Markers</th>
<th>Phage pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild strain</td>
<td>pen</td>
<td>cad</td>
</tr>
<tr>
<td>Variants (in order of isolation)</td>
<td>+</td>
<td>R</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>S</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>R</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>S</td>
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<tr>
<td>4</td>
<td>-</td>
<td>S</td>
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<tr>
<td>5</td>
<td>+</td>
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<td>R</td>
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<td>R</td>
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<tr>
<td>29</td>
<td>(+)</td>
<td>R</td>
</tr>
</tbody>
</table>

\(^{6}\) Isolated repeatedly over 2 years from a patient (113, 114).

\(^{b}\) See footnote to Tables 2 and 3. Abbreviations: lin, lincomycin resistance; +, production of penicillinase macroconstitutively; (+), microconstitutively; −, no detectable enzyme; R, resistant; S, sensitive.

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**Table 13. Loss of phenotypic markers from a strain of S. aureus\(^a\)**

<table>
<thead>
<tr>
<th>Date of isolation</th>
<th>Colonies (%) resistant by(^b):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pen</td>
</tr>
<tr>
<td>4/17/73</td>
<td>83</td>
</tr>
<tr>
<td>5/18/73</td>
<td>52</td>
</tr>
<tr>
<td>7/10/73</td>
<td>34</td>
</tr>
<tr>
<td>7/17/73</td>
<td>28</td>
</tr>
<tr>
<td>9/1/73</td>
<td>0</td>
</tr>
<tr>
<td>9/14/73</td>
<td>0</td>
</tr>
<tr>
<td>9/25/73</td>
<td>0</td>
</tr>
<tr>
<td>10/20/73</td>
<td>0</td>
</tr>
<tr>
<td>11/12/73</td>
<td>6</td>
</tr>
</tbody>
</table>

\(^{a}\) Isolated at intervals over 6 months from a patient not receiving antibiotics (114; and R. W. Lacey, unpublished observations).

\(^{b}\) Abbreviations: pen, production of penicillinase in large amounts under induced and basal conditions (macroconstitutive); (pen), production of penicillinase in small amounts under both induced and basal conditions (microconstitutive); cad, resistance to cadmium ions; fus, resistance to fusidic acid.
However, some indication that the effect of antibiotic resistance, and in particular plasmid carriage, has on the virulence of the cell can be obtained by study of the organism. (i) In the absence of antibiotics, resistant strains tend to be replaced by sensitive (see above). Advantages enjoyed by the plasmid-negative cell might include enhanced virulence.

(ii) Individual plasmids comprise from about 2 to 6% of the total cellular DNA (46, 146); combinations of plasmids comprise up to 10% (110). It is a reasonable supposition that the cell “machinery” needed to maintain and replicate these plasmids might put other functions of the cell in jeopardy. This may be manifested by decreased virulence.

(iii) Few defined “virulence factors” are carried by the same plasmid as that carrying the genes for antibiotic resistance. An exception to this may be the association of methicillin resistance with enterotoxin B production in some strains (56, 57, 58). However, the association of these properties may be of little epidemiological importance because (a) methicillin resistance may not be clinically important and (b) there is no evidence for the interstrain spread of this resistance (see above).

(iv) Antibiotic-resistant cells survive on glass as well as their isogenic antibiotic-sensitive derivatives (107). This type of survival is essentially a test of the ability of the organism to resist desiccation which is important in the elimination of staphylococci from the skin (108) and presumably in the air. Any decrease in the incidence or in the severity of staphylococcal infections is therefore probably not due to the failure of the organism to survive in the environment.

In summary, although virulence cannot be quantified, the above considerations suggest that carriage of plasmids may tend to make the cell less, rather than more virulent.

During the last 20 to 30 years there has almost certainly been a decrease in the virulence of the “hospital” staphylococcus (218). This has been most evident in the decline in the incidence of primary sepsis in the skin of healthy individuals such as nurses. Acquisition of plasmids by the cell could be a factor in this trend, as could improved prophylaxis of infection and the use of new antibiotics.

These explanations do not seem sufficient, and another factor could be the spread of certain bacteriophages in S. aureus. The evidence for this is as follows. Most strains of S. aureus isolated about 20 years ago produced large quantities of an extracellular lipase—the egg-yolk or Tween 80 lipase (3, 72). During subsequent years, the proportions of strains that produced this enzyme has progressively decreased, and this decrease has apparently resulted from the presence of a prophage that blocks the production of this enzyme (37, 95). This prophage is also capable of transducing antibiotic resistance (37, 104) and has probably been the vector for transfer of such resistance in nature (37). The loss of extracellular lipase could well contribute to a reduction in the virulence of the organism since the production of lipase seems essential for the organism to produce invasion of subcutaneous tissues, at least in pigs (92). In strains isolated from humans, there is a close correlation between the production of lipase in vitro and the ability to produce boils (3, 72), although this has not been demonstrated experimentally in man. Most coagulase-negative staphylococci from normal human skin also produce lipase (4). All this evidence does suggest that the production of lipase is essential for the staphylococcus to invade healthy cutaneous and subcutaneous tissues.

The spread of this transducing phage—i.e., the Tween 80 converting one just described—in the staphylococcal population has therefore probably had two consequences: (i) transfer of antibiotic resistance between cells, and (ii) decrease in virulence of the cell. Such association of properties might seem highly unlikely. However, if infection, particularly if severe, is considered to be an accident that has resulted from a chance reaction between parasite and host, without benefit to either, then the association of these properties becomes less improbable; i.e., such a phage could benefit the species by causing both resistance transfer and a reduction in virulence.

ECOLOGICAL RELATIONSHIP OF S. AUREUS TO MAN

Before considering the future deployment of antimicrobials against the staphylococcus, it is necessary to consider briefly the ecology of the organism in man.

The surface of the body provides the main natural reservoir of S. aureus; up to 50% of individuals harbor the organism in their nose at any one time (216, 217) and although skin carriage is less common, over a period of a year most individuals will carry the organism from time to time (216). It is probable that the skin carriage strains fall into Price’s categories of transient and resident flora (158). “Resident” flora implies actual multiplication of the organism rather than temporary contamination as in “transient” flora. Multiplication is likely only in moist areas, such as the axilla and perineum. Exposed, dry skin provides a hostile
environment for the staphylococcus (108, 167). This may be why the nose and moist skin are the important reservoirs.

Nasal carriage of *S. aureus* is a well established source of infection in general (73, 85, 134, 207, 212, 219) and of skin carriage (71, 171, 198, 206, 215). The dissemination of staphylococci from the nose is greater where the nasal mucosa is heavily colonized by the organism (214). Cutaneous lesions are thought to have resulted from nasal carriage (212, 219, 220).

In instances where both the nose and the skin of a person harbor the same staphylococcal strain (as judged by phage-typing pattern), the relative importance of the two sites in the dispersion of that strain is uncertain (133, 188).

Staphylococcal skin carriage, particularly in superficial lesions, has been incriminated in many outbreaks of infection (18, 19, 74, 117, 128, 133, 172, 173, 189). Some phage types have been particularly prone to spread from skin to skin (14, 128, 133, 153).

Among skin carriers, relatively few individuals release large numbers of staphylococci into the atmosphere (136, 138, 189). The organisms are shed on epithelial fragments (54). Men disseminate more organisms than women, and dispersal from both sexes is more profuse from the skin below the waist, than from skin above it (27). The number of staphylococci dispersed rises immediately after shower-baths (195), but this increase may be abolished by disinfection of the skin (26).

A few neonates also disseminate large numbers of staphylococci; the intensity of atmospheric contamination has caused these infants to be described as "cloud babies" (62).

Although some individuals carry the staphylococcus in the alimentary tract, this carriage is probably a result of nasal carriage (216) and is probably not very important epidemiologically.

Thus, in summary, both the skin and nose form important reservoirs of *S. aureus*; spread to other individuals is common. Disease, as a relatively rare complication of this carriage, has been summarized succinctly by Williams (216): "The excision of the staphylococcus into disease production seems to be aberrant activities outside the main stream of its existence."

An important feature of the epidemiology of *S. aureus* is that mixtures of strains commonly occur in carriage sites and superficial lesions (137, 148). This situation presumably permits transfer of resistance between the strains.

Finally, the relationship of *S. aureus* to *S. albus* deserves comment. The carriage of *S. albus* (epidermidis) is more universal than that of *S. aureus*. It is possible that a reservoir of plasmids exists in *S. albus* and that some of them have spread to *S. aureus*. The following observations have raised this possibility. (i) *S. albus* is often antibiotic resistant (52). (ii) Plasmids exist in *S. albus* (127, 183). (iii) The plasmid coding for tetracycline resistance in a strain of *S. albus* has similar properties to those of *S. aureus* (127). (iv) Some prophages carried by *S. albus* are transducing (222). (v) There is some degree (albeit rather small) of cross-reaction between the prophages of *S. albus* and *S. aureus* (208, 225).

If transfer of plasmids between *S. albus* and *S. aureus* had occurred, then the site has probably been the body surface. If so, the advisability of withholding certain topical antibiotics (see below) would be extremely pertinent.

**FUTURE ANTIBIOTIC STRATEGY AGAINST S. AUREUS**

The overall impact of the plasmid on a population of cells is that it confers greater flexibility on the microbe. In *S. aureus* this flexibility is manifested under natural conditions by gain or loss of the plasmid from the cell, in addition to changes in the plasmid itself. Although the potential for further gene reassortment by recombination of plasmid with chromosome, or of plasmid with plasmid, coupled with intercell transfer certainly exists, recombination has not been detected naturally. (It will be very difficult to prove that these changes have occurred in vivo rather than during plating.)

The use of antibiotics has undoubtedly increased the number of plasmid-positive cells and probably also changes in plasmids. Future chemotherapeutic strategy should be aimed at reducing the incidence of plasmid carriage in this organism, not only to retain the usefulness of a particular antibiotic, but to remove the organisms' most valuable evolutionary weapon.

The epidemiology of neomycin resistance illustrates that changes in the organism do not occur uniformly, and that a long period during which only sensitive strains are isolated, despite extensive use of the agent, in no way guarantees subsequent freedom from resistance to it. Thus, although resistance to *S. aureus* to gentamicin is still exceedingly rare despite the use of the antibiotic for a decade, it is probably only a matter of time before the resistance becomes widespread. It is probable that the epidemiology of gentamicin resistance will follow that of neomycin resistance with the interaction of antibiotic with *S. aureus* residing on the body surface as the key in selecting the resistance.

Prevention of the appearance of gentamicin resistance is certainly feasible, and could well
be achieved by the limitation in topical use of the antibiotic. In general, topical antibiotics can and should be withheld or replaced by disinfectants. One of the chief reasons for the administration of topical antibiotics has been that many commercially available preparations contain both antibiotic(s) and corticosteroids. Although the use of such preparations may enable successful treatment of both infectious and noninfectious dermatoses without establishing a diagnosis, there must be a frequent and unnecessary exposure of the natural populations of *S. aureus* and *S. albus* to antibiotics.

The prohibition of the use of these proprietary mixtures should cause few therapeutic problems; any dangerously invasive skin infection is treated anyway with systemic therapy, and many minor infections either do not require antibacterial therapy, or can be treated with a disinfectant. Apart from restriction of the use of these mixtures, a general reduction in the use of topical antibiotics is desirable. Such policies should be implemented on as wide a scale as possible.

The deployment of antibiotics in general against the staphylococcus should also follow a more rational program than is often followed at present. The incidence of resistance is generally related to the overall use of that antibiotic. Thus, in the absence of antibiotics, the population of bacteria strains tend to revert to sensitivity. This strongly argues for some sort of rotation in the use of antibiotics.

Rather few important new antibiotics have been introduced in recent years, although many variants of existing agents have been described. Bacteria often show partial or complete cross-resistance between the variants and their parent compounds. The possibility that the number of distinct antibiotics is finite makes the optimal deployment of the existing agents a matter of urgency.

**CONCLUSIONS**

Genetic analysis of *S. aureus* has been handicapped by ignorance about the distribution of the bulk of the cellular DNA. Despite this, a variety of plasmids have been isolated physically, and most antibiotic resistance is thought to be plasmid mediated.

A number of characters (e.g., resistance to erythromycin or methicillin, and production of pigment) are determined by genes that do not give clear indications of either plasmid or chromosomal location. Such elements seem common and their further characterization will be closely related to that of the chromosome itself.

From the practical standpoint, the decisive feature of the plasmid is its dispensability which permits rapid evolution of the element under most environments, without endangering the viability of the cell. The rapid diversification in the properties of the staphylococcus over the last 20 years has resulted chiefly from alterations in plasmid carriage, by transfer of plasmids and phages between cells; gain of these elements has been balanced by loss of them from some cultures.

The overall effect of these changes is to produce a varied population of strains with a capacity for rapid change, for example, when antibiotic use is altered. Associated with this change has been a concomitant decline in virulence. However, organisms may appear in the future that are both fully virulent and multiresistant.

Although the formation of a particular plasmid is probably, even in bacterial terms, a very rare event, once formed such an element can spread rapidly among the bacterial population. The spectacular increase in the incidence of penicillinase-producing hospital strains in the late 1940's could have been due in part to this process. Evidence is stronger, however, for the intercell transfer of recently isolated plasmids coding for resistance to fusidic acid (and penicillinase production), or for neomycin, or for tetracycline resistance.

Study of bacterial plasmids can resolve fundamental biochemical problems, and give some insight into the life of the cell at the molecular level. But the immediate application of the study of staphylococcal plasmids may be directed towards improving the effectiveness of antibiotic therapy. Of critical importance is the relationship of the antibiotic to the organism in its natural habitat (the body surface). In this situation, not only may resistant strains be selected, but also the genes may then be transferred to other strains, and any resistant progeny may be disseminated to new hosts.

The most important aspect of future anti-staphylococcal chemotherapy should thus be the limitation of the use of antibiotics, particularly for application to the skin and nose.

**ACKNOWLEDGMENTS**

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