Characterisation of chloramphenicol resistance plasmids of *Staphylococcus aureus* and *S. epidermidis* by restriction enzyme mapping techniques

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Summary. Chloramphenicol resistance (Cm^r) plasmids pSK2 and pSK5 from *Staphylococcus aureus* and pSK102 and pSK103 from *S. epidermidis* have been characterised and detailed restriction endonuclease cleavage maps constructed. *TaqI* digestion profiles illustrated the identity of pSK5 and pSK102 and also revealed a high degree of similarity between these four Cm^r plasmids from Australian staphylococci and three Cm^r plasmids from *S. aureus* strains of geographically unrelated origin. DNA-DNA hybridisation indicated that the chloramphenicol acetyltransferase determinant carried by pSK5/pSK102 could be found on other structurally-distinct Cm^r plasmids. The role of *S. epidermidis* as a reservoir for Cm^r plasmids found in *S. aureus* is discussed.

Introduction

Staphylococcal resistance to chloramphenicol is affected by the inducible enzyme chloramphenicol acetyltransferase (CAT) which converts the antibiotic to an inactive acetoxy-form in the presence of acetyl coenzyme A (Shaw, 1984). The *cat* genes of *Staphylococcus aureus* which encode this enzyme have previously been shown to exist on several small, multicopy plasmids of various incompatibility groups (Novick, 1976; Iordănescu *et al.*, 1978; Wilson and Baldwin, 1978). In agreement with these observations, chloramphenicol resistance amongst Australian isolates of multiresistant *S. aureus* isolated during the period 1979–1982, was found to be exclusively plasmid-mediated. Two Cm^r plasmids, pSK2 and pSK5, each *c.* 4.5 kilobase pairs (kb) in size but distinguishable on the basis of their restriction endonuclease profiles, have been identified in these strains by plasmid elimination studies and by transfer experiments (Lyon *et al.*, 1983, 1984a and unpublished data). Furthermore, we have described pSK102, a Cm^r plasmid isolated from a clinical strain of *S. epidermidis*, which closely resembled pSK5 (Tennent *et al.*, 1984). Preliminary analyses therefore suggested that common Cm^r plasmids may be present in Australian strains of *S.*

aureus and *S. epidermidis*. This paper reports the molecular characterisation of Cm^r plasmids isolated from both staphylococcal species and presents restriction endonuclease and DNA-DNA hybridisation data supporting the notion that the determinants for chloramphenicol resistance have spread amongst clinical isolates of *S. aureus* and *S. epidermidis*.

Materials and Methods

Bacterial strains and plasmids

Clinical isolates of *S. aureus* (SK52 and SK429) and *S. epidermidis* (SK99 and SK356) resistant to chloramphenicol were selected from a collection of multiresistant strains obtained from Australian hospitals during the period 1979–1982.

S. aureus strain RN1305 carrying the plasmid pC221 was kindly provided by R. P. Novick (Public Health Research Institute of the City of New York). *S. aureus* strains carrying plasmids pUB112 and pC223 were obtained from the *Bacillus* Genetic Stock Center (Columbus, OH, USA). A description of the plasmids, including their host strains, is presented in table I.

Recipient strains used in DNA transfer experiments were *S. aureus* strain SA113, received from Dr S. Iordănescu (Institute Cantucuzino, Bucharest, Romania), and strain SK982, a rifampicin- and novobiocin-resistant mutant of strain SA113 selected in this laboratory (Lyon *et al.*, 1984b).

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General procedures

The media used and the methods for the determination of antibiotic susceptibilities and minimum inhibitory concentrations (MICs), for transformation of plasmid DNA and for plasmid elimination were as described previously (Gillespie *et al.*, 1984; Lyon *et al.*, 1984a). Plasmid DNA was transferred to strain SK982 in mixed culture by the method of McDonnell *et al.* (1983). Overnight cultures of the donor and recipient strains were subcultured into fresh Brain Heart Infusion (BHI) broth (Oxoid) and incubated with aeration at 37°C. After 6 h, the cells were harvested, resuspended in nutrient broth and adjusted by optical density to $c. 10^7$ cfu/ml. One-ml volumes of donor and recipient suspensions were combined with 0.1 ml of a 0.2 M CaCl₂ solution and incubated at 37°C for 18 h with aeration. Samples of the mixed culture were plated onto BHI agar containing novobiocin 1 mg/L, rifampicin 10 mg/L and chloramphenicol 16 mg/L to select Cm^r transcipts. As controls in each experiment, the donor and recipient strains were each incubated singly and plated on to the selective media.

Plasmid DNA isolation, agarose gel electrophoresis and restriction endonuclease analysis

Plasmid DNA prepared from cultures of *S. aureus* and *S. epidermidis* was purified in caesium chloride-ethidium bromide density gradients as previously described (Tennent *et al.*, 1984). Restriction endonuclease analysis of plasmid DNA with each of the restriction endonucleases (New England Biolabs, Beverly, MA 01915, USA) listed in table II was as described by Lyon *et al.* (1983); DNA samples were electrophoresed through Tris-acetate-EDTA buffered agarose gels or Tris-borate-EDTA buffered polyacrylamide gels with either the *Hind*III-*Eco*RI fragments of bacteriophage lambda DNA or the *Hae*III fragments of pBR322 DNA, respectively, as standards (Sutcliffe, 1978; Daniels *et al.*, 1983). DNA from either gel system was transferred to nitrocellulose for hybridisation analysis by the bidirectional procedure of Smith and Summers (1980). The electroelution technique of Smith (1980) was employed to separate whole plasmids or restriction endonuclease fragments from a heterogeneous sample of DNA.

Hybridisation analysis of plasmid DNA

A DNA fragment containing part of the *cat* gene was prepared by electroelution after double digestion of pC221 with *Acl*I and *Mbo*I. The fragment was radiolabelled with 10 µCi of [α -³²P] ATP (1800 Ci/mmol) by nick translation and hybridised with nitrocellulose filters carrying the target DNA as described by Maniatis *et al.* (1982). Washed and dried filters were then exposed to Fuji RX film at -70°C for sufficient time to produce an acceptable autoradiograph. The hybridisation experiments reported in this paper were repeated on several occasions and produced identical results.

Table I. Characteristics of chloramphenicol resistance plasmids

Plasmid	Size (kb)	Isolated from	Reference
pSK2	4.5	<i>S. aureus</i> SK52	This paper
pSK5	4.55	<i>S. aureus</i> SK429	Lyon <i>et al.</i> , 1983
pSK102	4.55	<i>S. epidermidis</i> SK99	Tennent <i>et al.</i> , 1984
pSK103	4.6	<i>S. epidermidis</i> SK356	This paper
pC221	4.55	<i>S. aureus</i>	Projan <i>et al.</i> , 1985
pC223	4.6	<i>S. aureus</i>	Novick, 1976
pUB112	4.05	<i>S. aureus</i>	Brückner <i>et al.</i> , 1984

Results

Plasmid-mediated chloramphenicol resistance

Purified plasmid DNA from the clinical *S. aureus* strain SK52 (table I) was used to transform the plasmid-free recipient SA113 to chloramphenicol resistance. All Cm^r transformants were found to contain plasmid DNA equivalent in size and restriction profile to pSK2, confirming that Cm^r was mediated by this plasmid.

Plasmids of $c. 4.5$ kb were also found to mediate Cm^r in clinical isolates of *S. epidermidis* such as SK99 and SK356 (table I). Plasmid pSK102 was found to mediate this phenotype in strain SK99 as demonstrated by curing studies (Tennent *et al.*, 1984) and by transformation to strain SA113. Another, slightly larger, Cm^r plasmid, pSK103, was transferred from strain SK356 to recipient SK982 in mixed culture. Once again, when plasmid DNA was isolated from the Cm^r transformants and transcipts they were found to contain plasmid DNA electrophoretically indistinguishable from either pSK102 or pSK103, respectively.

The MIC of chloramphenicol for the Cm^r clinical strains of *S. aureus* and *S. epidermidis* (table I), and for transformants and transcipts which carried the plasmids pSK2, pSK102 or pSK103, was 60 mg/L.

Restriction endonuclease analysis

To examine the relatedness of the four phenotypically-similar plasmids from *S. aureus* and *S. epidermidis*, purified plasmid DNA was cleaved with various restriction endonucleases and the restriction profiles analysed by agarose gel electrophoresis; purified pSK2, pSK102 and pSK103 DNA was prepared from the transformants and transcipts described above, but pSK5 DNA was obtained by electroelution from a sample of SK429 DNA which contained two other plasmids (Lyon *et al.*, 1983). Each of the plasmids pSK2, pSK5, pSK102 and

Table II. Restriction sites on *Cm^r* plasmids

Plasmid	Numbers of sites for restriction endonucleases*												
	<i>AccI</i>	<i>BglII</i>	<i>BstEII</i>	<i>ClaI</i>	<i>EcoRI</i>	<i>HindIII</i>	<i>HpaII</i>	<i>MboI</i>	<i>PvuII</i>	<i>SalI</i>	<i>SacI</i>	<i>TaqI</i>	<i>XbaI</i>
pSK2	1	0	1	1	0	1	3	3	1	1	0	11	1
pSK5	2	0	1	1	1	1	3	3	0	0	1	12	2
pSK102	2	0	1	1	1	1	3	3	0	0	1	12	2
pC221	2	0	1	1	1	1	3	3	0	0	1	12	2
pUB112	1	0	1	0	0	1	2	4	0	0	0	7	0
pSK103	0	1	1	1	0	1	1	4	0	0	0	11	0
pC223	0	1	1	1	0	1	1	4	0	0	0	12	0

* No sites were detected on any of the plasmids for the restriction endonucleases *Bam*HI, *Bgl*II, *Eco*RV, *Hae*III, *Hpa*I and *Kpn*I.

pSK103 possesses a single recognition site for *Hind*III (table II); however, *Hind*III-*Hpa*II double digestion easily differentiated pSK2 from pSK103; the digestion products of pSK2 were 2.1, 1.85 and 0.55 kb (fig. 1, lane a) whereas those of pSK103 were 4.2 and 0.4 kb (fig. 1, lane d). In contrast, pSK5 and

pSK102 were indistinguishable after double digestion with these endonucleases; both plasmids generated three fragments of 1.85, 1.45 and 1.25 kb (fig. 1, lanes b and c). They were distinct from pSK2 and pSK103.

Digestion of these plasmids with *Taq*I (fig. 2A) further substantiated the close relatedness of pSK5 and pSK102, and also revealed a relationship between these two plasmids and pSK2. Eight of the eleven *Taq*I fragments of pSK2 (table II) were clearly resolved in fig. 2A (lane a); the remaining three fragments were detected by polyacrylamide gels electrophoresed under conditions better suited to the resolution of fragments of less than 0.1 kb. *Taq*I digestion of pSK5 and pSK102 (fig. 2A, lanes b and c) indicated that the two plasmids were identical. Ten of the twelve *Taq*I fragments of pSK5 and pSK102 (table II) have been resolved; it should be noted that four fragments of 1.16, 0.47, 0.28 and 0.11 kb were equivalent in size to fragments generated from pSK2. The *Taq*I profile of pSK103 (fig. 2A, lane f) revealed that this plasmid, while possessing eleven *Taq*I sites (table II), was not related to pSK2, pSK5 or pSK102.

*Taq*I restriction profiles were also used to compare the four *Cm^r* plasmids isolated from Australian strains of *S. aureus* and *S. epidermidis* with three *S. aureus* *Cm^r* plasmids (table I) isolated elsewhere. Plasmids pSK5 and pSK102 had *Taq*I fragments of electrophoretic mobility identical to those fragments derived from pC221 (fig. 2A, lane d) and each of these plasmids, together with pSK2, shared several fragments of similar size with pUB112 (fig. 2A, lane e). The *S. epidermidis* *Cm^r* plasmid pSK103 was found to have nine *Taq*I restriction fragments in common with pC223 (fig. 2A, lane g), two of which form a doublet of c. 0.25 kb in size.

The analysis of the seven *Cm^r* plasmids was

Fig. 1. Agarose gel (1.6% w/v) electrophoresis of purified staphylococcal DNA cleaved with the restriction endonucleases *Hind*III and *Hpa*II: Lanes (a) pSK2; (b) pSK5; (c) pSK102; (d) pSK103; (e) lambda phage DNA cleaved with *Hind*III and *Eco*RI as standards. Fragment sizes (in kb) are shown on the right. The faint bands present in lanes (a) and (d) are incomplete digestion products.

Fig. 2. (A). Polyacrylamide gel (6% w/v) electrophoresis of purified staphylococcal DNA cleaved with the restriction endonuclease *TaqI*: Lanes (a) pSK2; (b) pSK5; (c) pSK102; (d) pC221; (e) pUB112; (f) pSK103; (g) pC223; (h) pBR322 cleaved with *HaeIII* as standards. Sizes of selected fragments (in base pairs) are shown on the right. The sixth band from the well in lanes (a) and (f) and the seventh band from the well in lane (g) are doublets; these fragments, together with *TaqI* digestion products <0.1 kb, have not been clearly resolved in this gel. (B) Autoradiograph of gel shown in (A) after hybridisation with a radiolabelled *cat* probe derived from pC221 as described in the text. No homologous sequences were detected in lane (h).

extended by restriction site mapping with *TaqI* and a further twelve endonucleases (table II). Double digestion with appropriate combinations of these endonucleases enabled the order and position of recognition sites within a particular plasmid to be established (fig. 3). The restriction endonuclease maps determined for plasmids pSK5 and pSK102 were indistinguishable from each other and from that of pC221 and we conclude that these three plasmids are identical. Although some uncertainty remains as to the relative positions of seven identically-sized *TaqI* fragments of pSK103 and pC223 (fig. 3), the restriction maps of these plasmids were identical, with the exception of an additional *TaqI* site on pC223.

Hybridisation analysis of the staphylococcal Cm^r plasmids

Restriction endonuclease mapping suggested that a region of homology was shared by the *Cm^r*

plasmids pSK2, pSK5/pSK102, pC221 and pUB112 (fig. 3). The common DNA sequences, located approximately between the *TaqI* sites at coordinates 0.5 and 2.53 on the pSK2 map, specify the replication functions (REP D) and an inducible chloramphenicol acetyltransferase (CAT) in pC221 (Brenner and Shaw, 1985) (fig. 3).

The possibility that the *Cm^r* plasmids pSK2, pSK5/pSK102, and pUB112 carried the same *cat* gene as pC221 was confirmed by hybridisation experiments. The *TaqI* digests of the *Cm^r* plasmids (fig. 2A) were hybridised with a ³²P-labelled probe consisting of the 0.43-kb *AccI-MboI* fragment of pC221 which comprised a large portion of the *cat* gene (fig. 2B). As could be predicted from the restriction endonuclease maps (fig. 3), the largest *TaqI* fragment of each of pSK2, pSK5/pSK102, pC221 and pUB112—all of which contain the sequence encompassing the common *AccI*, *BstEII* and *MboI* recognition sites—hybridised with the probe (fig. 2B, lanes a–e). Unexpectedly, a 0.5-kb

Fig. 3. Restriction endonuclease maps of *Cm^r* plasmids from *S. aureus* (pSK2, pSK5, pC221, pUB112 and pC223) and *S. epidermidis* (pSK102 and pSK103). The unique *Hind*III site was chosen as the reference point (0 kb) for all plasmid maps which have been aligned by reference to the single *Bst*EII site present on each. Areas of restriction identity between pSK2, pSK5/pSK102, pC221 and pUB112, as referred to in the text, are indicated by underlining; hatching denotes the identical 2.11-kb regions of pSK103 and pC223 that contain six unmapped *Taq*I sites. Regions of pC221 which specify the protein required for replication (REP D) and the inducible chloramphenicol acetyltransferase (CAT), as described by Brenner and Shaw (1985) and Projan *et al.* (1985), are shown; the direction of transcription of these regions is indicated by the arrowheads. Restriction endonuclease sites are designated: A, *Acc*I; Bg, *Bgl*III; Bs, *Bst*EII; C, *Cl*aI; E, *Eco*RI; H, *Hind*III; Hp, *Hpa*II; M, *Mbo*I; Pv, *Pvu*II; S, *Sal*I; Sc, *Sac*I; T, *Taq*I; X, *Xba*I. Map coordinates are in kilobase pairs. Only those *Taq*I sites of pSK2 referred to in the text have been assigned coordinates.

*Taq*I fragment and, at a very low level, a 0.25 kb *Taq*I fragment, of both pSK103 and pC223 were found to hybridise with the pC221-derived probe (fig. 2B, lanes f and g). Only the position of the larger of these has been mapped on pSK103 and pC223; it contained the recognition sites for *Bst*EII and *Bgl*III/*Mbo*I which are approximately 50 base pairs apart (fig. 3).

The epidemiology of *Cm^r* plasmids was assessed in more than 50 nosocomial isolates of *Cm^r* coagulase-negative staphylococci. Hybridisation analysis of these strains indicated that approximately half carried a 4.5-kb plasmid with a pC221-like *cat* gene, whereas the remainder harboured a plasmid of similar size which resembled pSK103 in its response to the pC221-derived *cat* probe.

Discussion

The *Cm^r* plasmids characterised in this study were divided into two groups on the basis of restriction endonuclease fingerprinting and DNA-DNA hybridisation. The first group comprised the identical plasmids pSK5 and pSK102, from *S. aureus* and *S. epidermidis*, respectively, together with the *S. aureus* plasmid pSK2. Each possessed DNA sequences homologous with those that encode CAT on the *S. aureus* *Cm^r* plasmids pC221 and

pUB112. DNA-DNA heteroduplex analyses support the absolute identity of pSK2 and pC221 over the *cat* sequences and, in agreement with the restriction mapping data presented, extend the homology between these two plasmids to include the DNA sequences that encode replication functions (Tennent, May and Skurray, unpublished data).

Subsequent to this study, the complete nucleotide sequence of pC221 was reported (Brenner and Shaw, 1985; Projan *et al.*, 1985), as was a restriction map of pUB112 (Brückner *et al.*, 1984). With the exception of an undetected 16 base pair *Hpa*II fragment, the presence of which was inferred on pSK2, pSK5, pSK102 and pUB112 from the pC221 sequence (table II; fig. 3), our data were in total accord with this published information.

The remaining *Cm^r* plasmid, pSK103, detected in *S. epidermidis*, closely resembled the *S. aureus* plasmid pC223. With the exception of a single *Bst*EII site, which was present on all of the *Cm^r* plasmids studied, the restriction maps of pSK103 and pC223 were dissimilar from those constructed for the pC221-like plasmids. Nevertheless, heteroduplex studies and DNA sequence data will ultimately be required to determine the relatedness of members of the two groups because disparate restriction maps may arise from closely related

plasmids via the effects of minor alterations to the DNA sequences.

The presence of common antibiotic resistance plasmids has previously been reported for strains of *S. aureus* and *S. epidermidis* resistant to penicillin (Totten *et al.*, 1981), tetracycline (Groves, 1979; Cooksey and Baldwin, 1985) or gentamicin (Cohen *et al.*, 1982; Jaffe *et al.*, 1982; Archer and Johnston, 1983). Our results show that similar, and in some cases identical, Cm^r plasmids are associated with multiresistant *S. aureus* and *S. epidermidis* strains isolated from Australian hospitals. Taken together, these observations strengthen the role of *S. epidermidis* as a possible reservoir of resistance determinants which are available for transfer to *S. aureus*.

Likened to conjugation, the exact mechanism by which interspecific transfer occurs amongst the staphylococci remains unclear. However, the transfer of resistance plasmids from *S. epidermidis* to *S. aureus* has been demonstrated *in vivo* (Naidoo, 1984), thus implicating the natural habitat of these organisms as a factor involved in genetic transfer.

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