CHARACTERIZATION OF THE TOBRAMYCIN-KANAMYCIN-NEOMYCIN RESISTANCE PLASMID IN *STAPHYLOCOCCUS EPIDERMIDIS*

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A strain of *Staphylococcus epidermidis* was transduced to tobramycin resistance and all transductants were also resistant to kanamycin and neomycin. Results of curing studies also indicated that these resistances were controlled by a single determinant on a plasmid. Agarose gel electrophoresis of the plasmid DNA from parent, cured, and transduced strains showed a single plasmid was responsible and its molecular weight was calculated to be 2.85×10^6. Attempts to determine other properties of the organism controlled by this plasmid were unsuccessful.

Staphylococcus epidermidis can be considered a pathogen in certain instances and generally is resistant to many antibiotics[1]. The presence in this organism of resistance plasmids which might be transferred to *Staphylococcus aureus* emphasizes the need to characterize its resistance plasmids more fully. In this communication, we describe a strain of *S. epidermidis* resistant to tobramycin, kanamycin, and neomycin. Transduction of all three markers concomitantly indicated that resistance was coded for by a single genetic determinant. Results of curing studies also suggested this. Agarose gel electrophoresis of its plasmid DNA provided a quick easy method of determining the nature of this resistance and the molecular weight of the plasmid containing the resistance determinant.

Materials and Methods

S. epidermidis strain 870 was an isolate from the British Isles obtained during an international collaborative study on phage typing of coagulase-negative staphylococci. *S. epidermidis* strain 158 and bacteriophage 29 used in the transduction experiments have been described[2]. Phage 407-1 was obtained from Dr. LOUIS BLOUSE (Brooks Air Force Base, TX). Three plasmids from *Bacillus thuringiensis* var. *thuringiensis*, HD-2a, HD-2b and HD-2c, and one from *S. aureus*, pRW-8, were gifts from Ms. BARBARA BROWN (University of Georgia). Plasmid pBR-322 was a gift from Dr. JEFFREY ROBBINS (University of Missouri).

Biotype identification and production of urease, gelatinase, caseinase, lysozyme, phosphatase, DNase, hemolysin and lipolytic activity by the donor, cured, and transduced strains were determined[1]. Phage typing and the typing phages used have been described[3]. Antibiotic sensitivity to 15 antibiotics was determined by the disk diffusion method[4]. The macro broth dilution method[5] was used to determine the minimal inhibitory concentration (MIC) of parent, cured, and transduced cultures to six antibiotics. Determination of heavy metal resistance was according to NOVICK and ROTH[6].

The transduction procedure was the same as reported by OLSON, *et al.*[2] except that the medium used for selection of resistant transductants contained 3 μg of tobramycin/ml. Loss of resistance to antibiotics upon exposure to chemical agents was the same as reported by OLSON, *et al.*[2] except that the sodium dodecyl sulfate (SDS)-exposed cells were replicated onto medium containing either 3 μg of tobramycin or 90 μg of kanamycin/ml of medium. The preparation of crude lysates for electrophoresis was the same as reported by us recently[7] except for the procedure used for deproteinization of the lysates and precipitation of DNA[8]. For agarose gel electrophoresis, the electrophoresis buffer consisted of

0.089 M Tris, 0.089 M boric acid, and 2.5 mM EDTA (pH 8.5). Prior to use, ethidium bromide (EtBr) was added to a final concentration of 0.5 μg/ml of electrophoresis buffer. Agarose (type II) was dissolved to a final concentration of 0.4% in electrophoresis buffer containing EtBr and added to a vertical slab gel (15 cm×15 cm×3 cm). RNase-treated DNA[7] from cleared lysates was mixed with 10 μl of a solution of 0.025% bromophenol blue and 50% glycerol and 10 μl of 10×electrophoresis buffer and then heated at 60°C for 3 minutes. Samples (10~15 μl) were then applied to the wells of a vertical agarose slab gel and subjected to electrophoresis for 2 hours at 85 volts. After electrophoresis, DNA bands were visualized on a short wave ultraviolet light box and photographed with a Polaroid 10.2 cm×12.7 cm press-type camera with type 52 Polapan-Polaroid film.

Results and Discussion

The donor and recipient strains, along with their biotypes, phage types, and antibiotic susceptibilities are listed in Table 1. Donor strain 870 was resistant to penicillin (Pc), ampicillin (Am), trimethoprim-sulfamethoxazole (Ts), streptomycin (S), kanamycin (Km), tobramycin (Tm), and neomycin (Nm). Recipient 158 was sensitive to all antibiotics tested. Recipient 870c was an isolate from a curing experiment and was sensitive to Tm, Km, and Nm; it was derived from donor strain 870. Antibiotic susceptibilities indicated the joint transduction of resistance to Tm, Km, and Nm at frequencies ranging from 1.8×10^{-7} to 9.5×10^{-7} transductants/plaque-forming units (PFU). Results of MIC's showed that in addition to transfer of resistance to Tm, Km, and Nm, resistance to gentamicin A (Gm-A) and butirosin (Bu) was also transferred. Intermediate resistance to amikacin (An) was also observed. The transduction of Nm resistance has been reported previously but these investigators[9] did not report the concomitant transduction of Tm or Km resistance. In a later study, however, SANTANAM and KAYSER[10] isolated the enzyme that mediated Nm resistance, and found it also inactivated Tm and Km. This enzyme was a nucleotidyl transferase which catalyzed the nucleotidylation of the 4′-hydroxyl group of these antibiotics. In addition, MIC data indicated that bacteria producing this enzyme also were resistant to Gm-A and Bu, with intermediate resistance to An. SANTANAM and KAYSER concluded that in addition to transduction

Table 1. Biotype, phage type, and antibiotic susceptibilities of donor, recipient, cured and transduced strains of *S. epidermidis*.

Strain	Biotype	Phage type	Reaction to:*														
			Pc	Am	Ox	Cf	E	Te	C	CC	ST	S	Km	Tm	An	Nm	Gm
870	1	29/113/ 113A/188	R**	R	R	S	S	S	S	S	R	R	R	R	S	R	S
870c***	1	29/113/ 113A/188	R	R	R	S	S	S	S	S	R	R	S	S	S	S	S
870ct ($Tm^rKm^rNm^r$)****	1	29/113/ 113A/188	R	R	R	S	S	S	S	S	R	R	R	R	S	R	S
158	3	29/108/ 113	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
158t ($Tm^rKm^rNm^r$)*****	3	29/108/ 113	S	S	S	S	S	S	S	S	S	S	R	R	S	R	S

* Pc, penicillin; Am, ampicillin; Ox, oxacillin; Cf, cephalothin; E, erythromycin; Te, tetracycline; C, chloramphenicol; CC, clindamycin; ST, sulfamethoxazole-trimethoprim; S, streptomycin; Km, kanamycin; Tm, tobramycin; An, amikacin; Nm, neomycin; and Gm, gentamicin

** R, resistant; S, sensitive

*** Cured isolate of donor strain 870

**** $Tm^rKm^rNm^r$ transductant of 870c

***** $Tm^rKm^rNm^r$ transductant of 158

of Nm resistance which they reported earlier[9], resistance to Tm, Km, Gm-A and Bu had also been transduced. Results presented in this investigation agree with the conclusions of SANTANAM and KAYSER[10]. However, to determine whether genetic markers other than those for resistance to antibiotics were transduced jointly, the parent and transduced recipients were characterized more fully. No change was noted between parent and transduced recipients for urease, gelatinase, caseinase, lysozyme, phosphatase, DNase, hemolysin, and lipolytic activity. Heavy metal resistance also remained unchanged for parent and transduced recipients.

Resistance to Tm and Km was lost upon growth in 0.003% SDS from donor strain 870 at a rate of 10% of 520 colonies screened. Twenty percent of the cells from cured colonies were tested and were identical in both phage type and antibiotic susceptibility. Cured cells also lost resistance to Nm, as determined by the disk diffusion test and resistance to Gm-A and Bu, as determined by the MIC. When cured isolates were tested for changes in biochemical activities and loss of heavy metal resistance, none were different from donor strain 870. The high (10%) rate of curing noted in this study exceeded that of ROSENDORF and KAYSER[9], although SDS was not used in their curing experiments.

The plasmid DNA of *S. epidermidis* donor strain 870, its cured derivative, 870c, and transduced recipients 870ct ($Tm^rKm^rNm^r$) and 158t ($Tm^rKm^rNm^r$) were examined by agarose gel electrophoresis (Fig. 1). Lane 1 contains the DNA of 870 with eight prominent bands, and a heavier broad band at the top which is chromosomal DNA which is not removed in the extraction procedure. Lane 2 contains the DNA of 870c, cured of resistance to Tm, Km and Nm. Its DNA contains only seven prominent bands plus the chromosomal band at the top. The fourth band from the bottom in lane 1 is absent in lane 2. Lane 3 contains the DNA of a transductant of 870c, resistant to Tm, Km and Nm. Eight prominent bands are present, including the fourth band from the bottom, and a chromosomal band. Lane 4 contains the DNA of the recipient, 158, before transduction. Three prominent bands plus a chromosomal band at the top are present. Lane 5 contains the DNA of a transductant of 158, resistant to Tm,

Fig. 1. Agarose gel electrophoresis of plasmid DNA from *S. epidermidis* strains 870, 158 and derivatives.
(1) 870 ($Tm^rKm^rNm^r$); (2) 870c (cured of Tm-KmNm resistances); (3) 870ct ($Tm^rKm^rNm^r$ transductant); (4) 158 ($Tm^sKm^sNm^s$); (5) 158t (Tm^r-Km^rNm^r transductant); (6) HD-2 (7.2, 6.2, and 5.2×10^6 daltons); (7) pBR-322 (2.6×10^6 daltons); and pRW-8 (1.9×10^6 daltons).

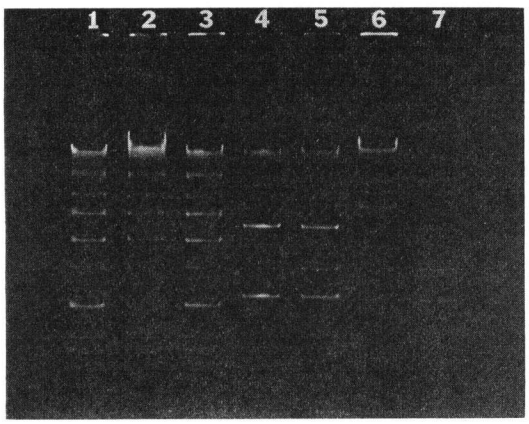
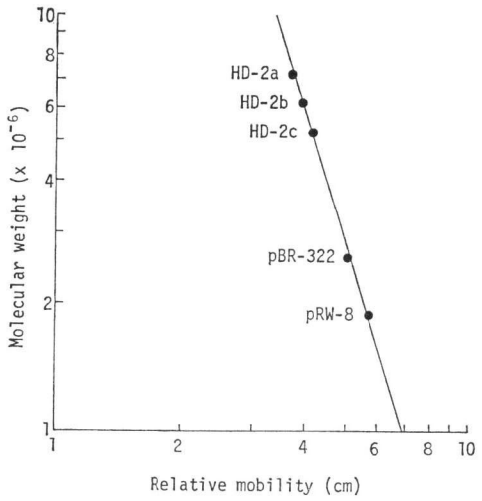


Fig. 2. Molecular weight *versus* relative mobility of plasmid DNA of known molecular weights.
HD-2a (7.2×10^6 daltons), HD-2b (6.2×10^6 daltons), HD-2c (5.2×10^6 daltons), pBR-322 (2.6×10^6 daltons), and pRW-8 (1.9×10^6 daltons).



Km and Nm, and with the appearance of the TmKmNm resistance plasmid band also seen in lanes 1 and 3. Lane 6 contains three plasmid species of HD with molecular weights of 7.2, 6.2 and 5.2×10^6 daltons, designated HD-2a, HD-2b and HD-2c respectively, plus a chromosomal band at the top. Lane 7 contains the two plasmid species, pBR-322 (MW$=2.6 \times 10^6$ daltons) and pRW-8 (MW$=1.9 \times 10^6$ daltons). The pRW-8 species is found nearest the bottom of the lane, with the pBR-322 species directly above it. In addition, an open circle form of a plasmid is seen above the pBR-322 band.

Purified DNA from the well-characterized plasmids, HD-2a, HD-2b, HD-2c, pBR-322, and pRW-8 were used as internal molecular weight standards for determination of the molecular weight of the TmKmNm resistance plasmid. The relative mobility of these plasmid markers was inversely related to the logarithm of their molecular weights (Fig. 2). The molecular weight of the TmKmNm resistance plasmid was determined by interpolation and calculated to be 2.85×10^6 daltons. On the other hand, using electron microscopy ROSENDORF and KAYSER[9] determined the molecular weight of this plasmid to be 2.66×10^6 daltons. We do not find our results to be significantly different than those of these investigators due to errors inherent in the calculation of molecular weight by either of these methods.

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