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Diversity of plasmids in *Staphylococcus saprophyticus* isolated from urinary tract infections in women

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SUMMARY

A group of 150 *Staphylococcus saprophyticus* strains isolated from urinary tract infections in women were included in this study. Antimicrobial susceptibility tests showed that these isolates were sensitive to most antimicrobial agents. All strains were sensitive to penicillin, cephalothin, gentamicin, kanamycin, trimethoprim and nitrofurantoin. Resistance to tetracycline was present in 10·6% of the strains, to chloramphenicol in 4%, to erythromycin in 1·3% and to streptomycin in 1·3%. All strains were resistant to cadmium chloride as well as to novobiocin and nalidixic acid. Plasmid analysis showed that 82% of the strains harboured plasmids, some of them with complex plasmid profiles. Most plasmids were considered to be cryptic, although antibiotic resistance plasmids were identified in 18 isolates. Tetracycline resistance was encoded by a plasmid of *c*. 2·8 MDa, chloramphenicol resistance by a plasmid of *c*. 2·9 MDa and erythromycin resistance by a plasmid of *c*. 1·6 MDa. Streptomycin resistance could not be linked to the presence of any specific plasmid. Plasmid profiling seemed to be a good method for differentiating among *S. saprophyticus* strains.

INTRODUCTION

The role of *Staphylococcus saprophyticus* as a urinary pathogen in women was first described in Torres Pereira (1962) more than 25 years ago. This finding was soon confirmed in Great Britain (Mitchell, 1964; Alder, Brown & Mitchell, 1966; Mitchell & Baird-Parker, 1967), and in recent reports from the USA (Latham, Running & Stamm, 1983), Sweden (Hovelius & Mardh, 1984) and the UK (Pead, Maskell & Morris, 1985). *S. saprophyticus* has been considered, after *Escherichia coli*, the most frequent agent of urinary tract infection in young women.

S. saprophyticus isolates are reported to be generally sensitive to most antibiotics (Torres Pereira, 1962; Hovelius & Mardh, 1984; Pead, Maskell & Morris, 1985), in contrast to many isolates of other human pathogenic *Staphylococcus* species, especially *S. aureus* and *S. epidermidis*, which can be multiply antibiotic resistant. Plasmid analysis has become a useful tool in the study of several aspects of the genetics and epidemiology of these microorganisms but in contrast with *S. aureus* and *S. epidermidis*, little is known about plasmids

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in *S. saprophyticus*. In this study we report the results of plasmid analysis of 150 clinical isolates of *S. saprophyticus* and the relationship of some of the plasmids with antibiotic resistances.

MATERIALS AND METHODS

Bacterial strains and plasmids

A group of 150 *S. saprophyticus* strains were included in this study. All were isolated in Lisbon from outpatient women with urinary tract infection. The isolates were identified as *S. saprophyticus* by a specific method based on novobiocin resistance and mannose inhibition previously described (Melo Cristino *et al.* 1987).

S. aureus strain 8325 carrying the plasmids pI524, pT181, pE194 and pC194, used as molecular weight standards, was kindly provided at the Division of Hospital Infection, Central Public Health Laboratory, London, by Dr J. Naidoo.

Antimicrobial susceptibility tests

Susceptibility tests were performed by a disk diffusion method as described previously (Melo Cristino *et al.* 1986). The following antimicrobial agents were tested: penicillin 10 IU, cephalothin 30 μg, tetracycline 30 μg, erythromycin 15 μg, clindamycin 2 μg, chloramphenicol 30 μg, gentamicin 10 μg, streptomycin 10 μg, kanamycin 30 μg, trimethoprim 2·5 μg, nitrofurantoin 300 μg, nalidixic acid 30 μg and novobiocin 5 μg. The susceptibility to cadmium was determined on DST agar plates incorporating 5 μg/ml cadmium chloride.

Beta-lactamase production

The tests for beta-lactamase production were performed with the chromogenic cephalosporin nitrocefin (Oxoid), using an inoculum obtained on DST agar from around a methicillin-containing disk.

Serology

Slide agglutination was performed according to the technique of Torres Pereira (1962). Agglutinating sera are prepared with the original strain isolated at that time.

Plasmid isolation and agarose gel electrophoresis

Plasmid DNA was isolated by a technique adapted from modifications of the methods of Wilson, Totten & Baldwin (1978) and of Kado & Liu (1981). The cells after overnight growth on DST Agar were scraped off from about a 0·5 cm^2 area of the agar surface with a wooden applicator, and suspended in 40 μl of a NaCl/EDTA solution (2·5 M-NaCl, 0·05 M-EDTA, pH 7·5) in Eppendorf micro-centrifuge tubes. Lysates were prepared by the addition of 10 μl of lysostaphin at a final concentration of 100 μg/ml, and 10 μl of lysozyme at a final concentration of 4 mg/ml, and incubation at 37 °C for 60 min in the waterbath. Lysis was achieved by the addition of 150 μl of the lysing solution (3 % SDS in 0·05 M-Tris HCl, pH 12·6) and mixed with a wooden applicator. The lysate after incubation at 56 °C for 45 min was added with 200 μl of a phenol/chloroform (1:1, w/v)

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solution. The solution was emulsified by vortexing briefly, and centrifuged at 10000 g for 10 min. After centrifugation, 120 μl of the upper aqueous phase were transferred to a new tube and 30 μl of a 0·025% bromophenol blue in 50% glycerol solution was added.

The samples were electrophoresed in 0·8% agarose gels in Tris-borate buffer (0·089 M-Tris, 0·0025 M-EDTA, 0·089 M boric acid) at 110 V for 2·5 h. Gels were stained in ethidium bromide (5 μg/ml) and the DNA bands visualized on a TM 40 UV-transilluminator. Photography was carried out with a Polaroid MP-4 Camera on 665 film. Plasmid sizes were calculated according to the method of Meyers *et al.* (1976).

Curing experiments

Plasmid elimination experiments were performed in Nutrient Broth no. 2 (Oxoid). Strains were grown overnight at 42 °C (Asheshov, 1966), or were grown in broth containing 3 μg/ml ethidium bromide at 37 °C and subcultured in these conditions for 10 days, as described by Noble & Rahman (1986). The cultures were diluted and spread on DST agar plates. Isolated colonies were plated on a new DST agar plate and on DST agar plates containing different antimicrobial agents in the following concentrations (μg/ml): tetracycline 10, erythromycin 10, chloramphenicol 20, streptomycin 25, and cadmium chloride 5. Cured colonies purified from the plain DST agar plate were examined for resistance markers and submitted to plasmid analysis.

RESULTS

Susceptibility to antimicrobial agents

All 150 *S. saprophyticus* strains were sensitive to penicillin, cephalothin, gentamicin, kanamycin, trimethoprim and nitrofurantoin. Sixteen (10·6%) strains were resistant to tetracycline, 6 (4%) to chloramphenicol, 2 (1·3%) to erythromycin (inducible phenotype), and 2 (1·3%) to streptomycin. Five strains were resistant to two antibiotics (Table 1). All strains were resistant to cadmium chloride and, as expected, all were resistant to novobiocin and nalidixic acid.

Beta-lactamase production

The chromogenic cephalosporin assay did not reveal beta-lactamase production in any of the 150 isolates

Serology

Antigen 51 was present in 134 (89·3%) strains.

Plasmid analysis

All isolates were screened for the presence of plasmid DNA. Plasmid content of the 150 *S. saprophyticus* strains is indicated on Table 1. Most of the strains (82%) harboured plasmids, some of them showing complex plasmid profiles, with up to six DNA bands (one case). Twenty-seven strains (18%) had no plasmids.

Resistance determinants which were lost with a plasmid after a curing procedure were considered to be mediated by the plasmid. As shown on Table 1,

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Table 1. *Plasmid content of 150* Staphylococcus saprophyticus *strains isolated in Lisbon*

	No. of plasmids per strain					
	None	One	Two	Three	Four	More than four
No. of strains	27	41	49	19	12	2
(%)	(18·0)	(27·3)	(32·0)	(13·3)	(8·0)	(1·3)
No. of strains with resistance plasmids		1*	2*	8†	7‡	0

Resistances to: * tetracycline; † tetracyline (4 strains), chloramphenicol (1 strain), tetracyline and chloramphenicol (2 strains), erythromycin and chloramphenicol (1 strain); ‡ tetracycline (4 strains), chloramphenicol (1 strain), tetracycline and chloramphenicol (1 strain), tetracycline and erythromycin (1 strain).

Plasmid cure of chloramphenicol resistance was not achieved in 5 strains (see discussion).

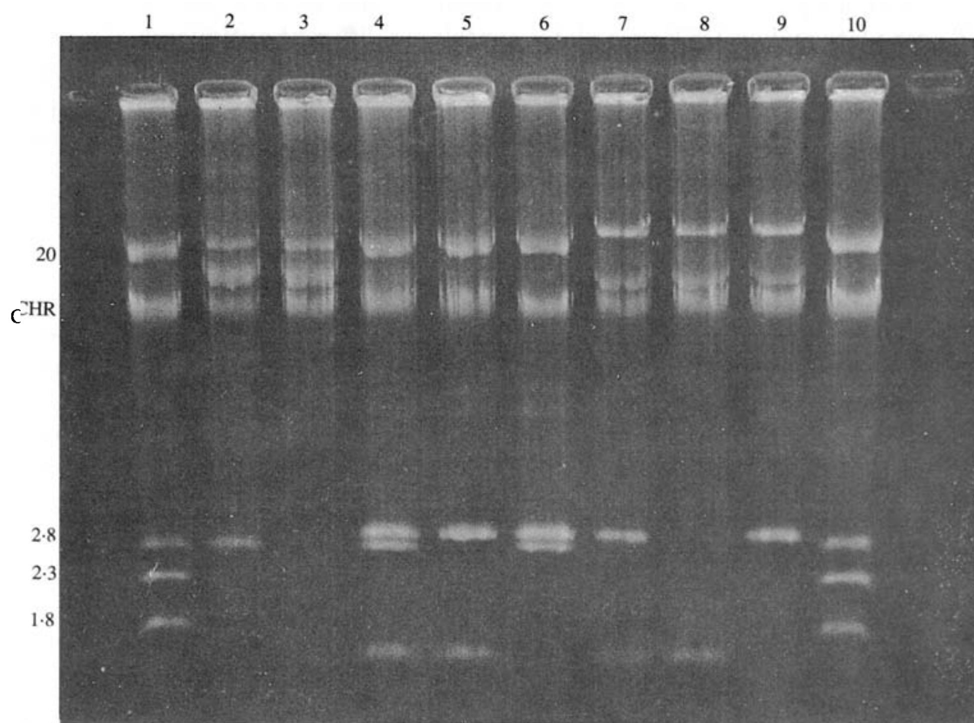

Fig. 1. Agarose gel electrophoresis of DNA from *Staphylococcus saprophyticus* selected strains containing antibiotic resistance plasmids. Lanes 1 and 10, standard *Staphylococcus aureus* plasmids pI524, pT181, pE194 and pC194 with sizes (in MDa) shown on the left; lane 2, tetracycline resistant strain R182; lane 3, strain R182 cured from tetracycline resistance; lane 4, tetracycline and erythromycin resistant strain ICP2327; lane 5, strain ICP2327 cured from tetracycline resistance; lane 6, strain ICP2327 cured from erythromycin resistance; lane 7, chloramphenicol and erythromycin-resistant strain ICP2225; lane 8, strain ICP2225 cured from chloramphenicol resistance; lane 9, strain ICP2225 cured from erythromycin resistance. The chromosomal band is labelled CHR.

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resistance determinants were much more frequent in strains with 3 and 4 plasmids (15 strains out of 31 = 48·3%) than in strains with no plasmids or with fewer than 3 (3 strains out of 117 = 2·5%).

Tetracycline resistance was found in 16 strains, 15 of which harboured a plasmid of *c*. 2·8 MDa. Cure of this tetracycline resistance plasmid was achieved in seven of the strains. The other resistant strain contained only one plasmid migrating slower than the chromosomal DNA band. Attempts were made to cure this strain from tetracycline resistance, but this was not achieved.

Inducible erythromycin resistance was found to be mediated by a plasmid of *c*. 1·6 MDa in the two resistant strains.

Six strains were chloramphenicol resistant, but this resistance marker could be cured in only one strain. A plasmid of *c*. 2·9 MDa encoded the resistance. All other resistant strains possessed a plasmid that migrated in the same position, but this resistance marker could not be cured from them.

Streptomycin and cadmium resistances could not be linked to any specific plasmid.

Fig. 1 shows an agarose gel electrophoresis of DNA from resistant *S. saprophyticus* strains before and after the curing of some resistance markers.

Notwithstanding the identification of genetic information encoded by some plasmids detected in *S. saprophyticus* strains included in this study, the great majority of plasmids present in these isolates were considered to be cryptic because their function in cell was not identified.

DISCUSSION

Staphylococcus saprophyticus has been known to be a human pathogen for some years. However, its pathogenicity is completely different from the pathogenicity of *S. aureus* and *S. epidermidis*, the two other frequent human pathogenic species since it is virtually limited to the urinary tract of young and otherwise healthy women. About 90% of the isolates reported in this study revealed antigen 51 characteristic of *S. saprophyticus* strains isolated from urinary tract infections. As reported previously (Torres Pereira & Melo Cristino, 1985) this antigen might be a specific virulence factor for the urinary tract. Few other infections have been described (Hovelius, Thelin & Mardh, 1979) which we could not confirm (unpublished results). Furthermore, *S. saprophyticus* has not been associated with cases of hospital infection. Finally, this microorganism is generally susceptible to most antimicrobial agents, especially those prescribed for patients with urinary tract infections (Torres Pereira, 1962; Latham, Running & Stamm, 1983; Hovelius & Mardh, 1984; Pead, Maskell & Morris, 1985).

Resistances to some antibiotics were detected in a few strains, and some of them could be linked to plasmids. Sixteen strains were resistant to tetracycline. In at least seven of them the resistance was encoded by a plasmid of *c*. 2·8 MDa as curing of tetracycline resistance from these isolates eliminated the plasmid. One tetracycline resistant strain lacked a *c*. 2·8 MDa plasmid and possessed only one plasmid migrating slower than the chromosomal DNA band. This resistance could not be cured despite examining 3600 colonies, 1400 of them after ten subcultures in nutrient broth containing ethidium bromide. Thus, in this isolate, tetracycline

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resistance was either encoded by a different plasmid which could not be eliminated or by the chromosome.

Chloramphenicol resistance was detected in six strains. A plasmid of *c*. 2·9 MDa proved to code for this resistance in one strain. Although all chloramphenicol resistant strains possessed a plasmid that migrated in the same position, in five isolates, this resistance marker could not be cured in spite of about 12800 colonies of these strains were examined either after growth at 42 °C or after ten subcultures in nutrient broth containing ethidium bromide. In *S. aureus* and in *S. epidermidis* chloramphenicol resistance has always been described to be mediated by small multicopy plasmids (Tennent, May & Skurray, 1986), some of them with a molecular weight as described for plasmids in *S. saprophyticus* reported here. The high copy number in the cells could be a difficulty preventing plasmid elimination in our technical conditions.

Inducible erythromycin resistance was detected in only two strains. In both this resistance was mediated by a plasmid of *c*. 1·6 MDa.

Although *S. saprophyticus* strains revealed some plasmids with molecular weights identical to plasmids coding for tetracycline, chloramphenicol and erythromycin resistance identified in *S. aureus* and in *S. epidermidis* (Cooksey & Baldwin, 1985; Tennent, May & Skurray, 1986; Thakker-Varia *et al.* 1987), further studies are needed to show if these plasmids are similar. Plasmid transfer from *S. epidermidis* and other coagulase negative Staphylococci into *S. saprophyticus* has been demonstrated by Naidoo & Noble (1987). This observation supports the occurrence of interspecific genetic transfer among several species of *Staphylococcus*, and might explain the existance of resistance plasmids identified in the strains reported here.

Cadmium resistance in *S. aureus* is often encoded by plasmids that migrate slower than the chromosomal DNA band, and is generally associated with penicillin resistance (Lyon & Skurray, 1987). All *S. saprophyticus* isolates reported here were resistant to cadmium chloride but sensitive to penicillin. Attempts were made, however, to cure cadmium resistance from some isolates which harboured plasmids, but the experiments were always unsuccessful. Plasmid free isolates of *S. saprophyticus* were also resistant to cadmium, the whole pointing to the resistance being mediated by the chromosome.

Plasmid profiles of *S. saprophyticus* showed that as in many *S. epidermidis*, these strains generally harbour several cryptic plasmids. As *S. saprophyticus* so far have not been associated with hospital infection, the need for effective typing methods has not been so critical as with other frequent hospital pathogens. As reported here, plasmid profiling seems to be a good method for differentiating among these strains.

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