Antibiotic resistances and plasmids in *Staphylococcus aureus* from Italian hospitals

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Summary. A total of 473 *Staphylococcus aureus* isolates from six Italian hospitals was examined for susceptibility to several antimicrobial agents and for plasmid content. Methicillin-resistant *S. aureus* (MRSA) were characterised by a plasmid of mol. wt (10^6) 18–22 or 25 that carried the determinants for penicillinase production, resistance to cadmium ions and resistance to tetracycline. MRSA isolates usually harboured other smaller plasmids of mol. wt (10^6) 2.8, 2.6 and 1.65 that encoded resistance to tetracycline, chloramphenicol and erythromycin, respectively, and cryptic plasmids of mol. wt (10^6) c. 2 and 1 were found frequently. Methicillin-sensitive *S. aureus* (MSSA) that produced penicillinase often carried plasmids of mol. wt (10^6) 11 or 13. No particular difference was found in plasmid patterns of strains from the various sources. Analysis of plasmids by *EcoRI* digestion showed that plasmids of similar mol. wt and phenotypic characteristics may have different restriction patterns, but often share one or more fragments in common.

Introduction

Staphylococcus aureus strains frequently cause nosocomial outbreaks of infection (Lyon *et al.*, 1983; Lacey *et al.*, 1984). The severity of infections depends on the ability of these micro-organisms to adapt to the presence of antimicrobial drugs and to accumulate antibiotic-resistance characters (Lacey, 1975). The incidence of antibiotic resistance in *S. aureus* increased progressively in the 1960s and 1970s (Lacey *et al.*, 1984). It has been demonstrated that the introduction of each new antimicrobial agent has been followed by the appearance and rapid spread of resistant strains of *S. aureus* (Shanson, 1981). Vancomycin and teicoplanin seem to be the only antibiotics against which resistance is unknown (Kirby, 1984).

S. aureus strains resistant to methicillin (MRSA) and to a series of other antibiotics have re-emerged since the mid-1970s and caused serious clinical problems in hospitals in several countries that include USA (Wenzel, 1982), Australia (Lyon *et al.*, 1983), Greece (Giamarellou *et al.*, 1981) and Ireland (Dowd *et al.*, 1983). In Italy, MRSA strains responsible for nosocomial infections are isolated frequently (Andreoni *et al.*, 1985; Schito, 1985).

Since 1983, *S. aureus* isolates from the Spedali Civili of Brescia have been examined for suscep-

tibility to antimicrobial agents and for the presence of plasmids. Recently, *S. aureus* isolates from hospitals in five other Italian towns have been investigated to determine whether their plasmids were similar and to survey the spread of the resistance determinants in Italy.

Materials and methods

Bacterial strains

The strains were collected and identified as *S. aureus* at the Laboratory of Microbiology of the Spedali Civili of Brescia on the basis of Gram's stain, colony morphology, production of coagulase and fermentation of mannitol (Baird-Parker *et al.*, 1976). The isolates were stored in glycerol 15% v/v at -70°C .

A total of 418 clinical isolates was obtained from different patients in various units—intensive care (27%), surgical (31%), burns and plastic surgery (9%), medical (17%) and infectious diseases (16%)—of the Spedali Civili of Brescia during the period Sep. 1983–Apr. 1985. Strains were isolated from: blood (30.4%); wound swabs, pus and secretions (34.8%); cerebrospinal fluid (1.2%); pleural fluid (3.7%); peritoneal fluid (3.1%); bronchial secretions (8.7%); bile (3.1%); other materials (15%).

Further *S. aureus* strains were kindly provided by: Professor C. Chezzi, Institute of Microbiology, Ospedale Maggiore, Parma (17 strains); Professor A. Guarino, Hospital of Avellino (9 strains); Professor G. Satta, Institute of Microbiology of Cagliari (10 strains); Professor A. La Manna, Institute of Microbiology of Firenze

(10 strains); Professor P. Martinetto, Institute of Microbiology of Torino (9 strains).

S. aureus strains carrying the plasmids pII 147, pI 258 and R_w 8, used as mol.-wt standards, were kindly provided by Dr K. Wachsmuth, Centers for Disease Control, Atlanta, GA, USA.

Chemicals and antibiotics

Chemicals were obtained from Sigma Chemicals Co., P.O. Box 14508, St Louis, MO., 63178 USA. Antibiotics and other inhibitors were obtained from Sigma except for amikacin, which was from Bristol Italiana (Sermone, Latina, Italy).

Antibiotic susceptibility tests were performed by the Kirby Bauer method (Bauer *et al.*, 1966), with Mueller-Hinton agar plates (BBL Microbiology Systems, Cockeysville, MD, USA) and Oxoid disks (Oxoid, Basingstoke, Hampshire) containing the following antibiotics: benzylpenicillin 10 units, tetracycline 30 µg, erythromycin 15 µg, amikacin 30 µg, gentamicin 10 µg, chloramphenicol 30 µg, trimethoprim 1.25 µg, sulphamethoxazole 25 µg, ampicillin 10 µg, methicillin 5 µg, cefotaxime 30 µg, cephalixin 30 µg, rifampicin 30 µg; vancomycin 30 µg, streptomycin 10 µg.

Susceptibility to cadmium ions was tested by disk diffusion on Mueller-Hinton agar plates with paper disks impregnated with 20 µl of a 0.01M solution of cadmium sulphate.

The disk susceptibility tests were performed with an inoculum of $c. 10^4$ cfu from an overnight culture.

Minimum inhibitory concentrations (MICs) were determined by the Sceptor System (BBL Microbiology Systems, Becton Dickinson) for the following antibiotics: methicillin (0.25–8 mg/L), penicillin (0.12–16 mg/L), ampicillin (0.25–16 mg/L), cephalothin (0.5–32 mg/L), amikacin (0.5–32 mg/L), gentamicin (0.12–8 mg/L), clindamycin (0.12–8 mg/L), erythromycin (0.12–8 mg/L), tetracycline (0.5–32 mg/L), chloramphenicol (0.5–32 mg/L), vancomycin (0.25–16 mg/L), trimethoprim/sulphamethoxazole (0.5/9.5–16/304 mg/L). The Sceptor System was also used to detect penicillinase production.

For multiresistant strains, MICs were determined also by the broth dilution technique (Mueller-Hinton broth, BBL) at the following drug concentrations (mg/L): amikacin, kanamycin, tobramycin, gentamicin, streptomycin, tetracycline, chloramphenicol, benzylpenicillin, ampicillin (16–256); and methicillin, cephalothin, erythromycin (2–64).

For the sensitivity testing an inoculum of $c. 10^4$ cfu/ml was used.

Isolation of plasmid DNA

Initially, plasmid DNA was isolated by a modification of the Birnboim procedure (Birnboim and Doly, 1979). The cells, grown overnight in Luria Bertani broth (Bacto

Tryptone 1%, Bacto Yeast extract 0.5%, NaCl 1%, pH 7.5), were harvested by centrifugation of 0.5 ml of broth culture at 10 000g for 1 min (Eppendorf centrifuge 5412) and resuspended in 100 µl of the following lysis solution: lysostaphin (Sigma) 20 mg/L in 50 mM glucose, 25 mM Tris HCl, 10 mM EDTA pH 8. Tubes were incubated at 37°C for 45 min. Lysis was achieved by adding 200 µl of sodium dodecyl sulphate 1% in 0.2N NaOH, incubating at 0°C for 5 min and then adding 150 µl of 3M sodium acetate (pH 4.8). The lysates were incubated at 0°C for at least 60 min and then centrifuged at 10 000g for 7 min. Cold ethanol (1 ml) was added to the supernate and tubes were kept at –70°C for 30 min. After centrifugation at 10 000g for 8 min, the precipitate that contained the plasmid DNA was resuspended in 100 µl of 0.1M sodium acetate, 0.05M Tris HCl (pH 8). A 200 µl volume of ethanol was added to the DNA preparation and the tubes were held at –70°C for 30 min. The final pellet obtained by centrifugation at 10 000g for 8 min, was resuspended in 10 mM Tris HCl, 1 mM EDTA (pH 8).

Recently, we have extracted the plasmid DNA by the more rapid method of Dunkle and Sippell (1984).

Samples were electrophoresed in agarose gel 0.75% in TPE (0.08M phosphate Tris, 0.002M EDTA) in a horizontal gel electrophoresis system (BRL, mod. H4) for 14 h at 20 V at room temperature.

Restriction enzyme analysis

Plasmid DNA was cut with *EcoRI* according to the manufacturer's directions (BRL lyphozyme; Bethesda Research Laboratories, Gaithersburg, MD, 20760, USA). Phage lambda DNA (BRL) was used as a control and the fragments separated by electrophoresis in agarose gel 0.75% in TPE for 14 h at 20 V at room temperature.

Curing experiments

Plasmids were eliminated by growth in Luria-Bertani broth for 18–24 h at 44°C (Asheshov, 1966) or by growth at 37°C in presence of ethidium bromide or acridine orange at various concentrations (Bouanchaud *et al.*, 1968).

Results

Resistance to antimicrobial agents of strains isolated from Brescia Hospital

More than 60% of 418 isolates tested between Sep. 1983 and Apr. 1985 were found to be multiresistant (table I). The large number of such isolates was probably due to the widespread

Table I. Patterns of antibiotic resistance in isolates of *S. aureus* from Brescia

Resistance pattern	Percentage of <i>Staph. aureus</i> isolates collected during (number of isolates)		
	Sept.–Dec. 1983 (87)	Jan.–Dec. 1984 (202)	Jan.–Apr. 1985 (129)
None	11.5	13.3	19.3
Penicillin	6.9	17.1	14.7
Penicillin–tetracycline or Penicillin–erythromycin	4.6	5.0	1.7
Multiple (MRSA)	77.0	64.6	64.3

dissemination of a few strains responsible for nosocomial epidemics during the period of study. During the same period an increasing frequency of isolation of *S. aureus* susceptible to all the tested drugs or resistant to penicillin only, can be seen (table I).

The multiresistant isolates had similar resistance patterns; they were resistant to: methicillin (MIC > 64 mg/L), penicillin (> 256 mg/L), gentamicin (> 256 mg/L), tetracycline (> 256 mg/L), erythromycin (> 64 mg/L), rifampicin, streptomycin and sulphamethoxazole (MICs not determined). Susceptibility to amikacin, netilmicin, chloramphenicol and trimethoprim were variable but all the isolates tested were susceptible to vancomycin (MIC 1 mg/L). About 70% of MRSA strains were resistant to cadmium ions.

On the basis of the resistance patterns and plasmid profiles (see below) of the MRSA isolates, it was possible to observe that nosocomial infections were caused mainly by two distinct strains. These strains were isolated, with great frequency from patients undergoing antibiotic therapy, in the intensive care and burns and plastic surgery units. The incidence of multiresistant strains was lower in other units.

Plasmid profiles of strains isolated from Brescia Hospital

Plasmids and resistance phenotype have been correlated by curing experiments. The isolated plasmids have been labelled for convenience. Among the isolates examined, those susceptible to all antibiotics did not contain plasmids, except three strains in which a cryptic plasmid of mol. wt (10^6) c. 2 (pBS4384) was found (fig. 1, lane A).

Isolates resistant to penicillin and susceptible to all the other antibiotics were frequent. Such isolates

Fig. 1. Plasmid patterns of *S. aureus* isolated in Brescia. Lanes A, B and C show the profiles of MSSA strains, lanes D and E show the profiles of MRSA strains. A: plasmid pBS4384; B: pBS1884; C: pBS3583; D: pBS283, pBS3784, pBS2585, pBS4985; E: pBS983, pBS2585, pBS1285. Plasmid mol. wts (10^6) are shown on the right. CHR = chromosomal DNA.

harboured only one plasmid of mol. wt (10^6) 13 (pBS1884) or 11 (pBS3583) (fig. 1, lanes B and C). Plasmid pBS1884 confers resistance to penicillin and to cadmium ions; pBS3583 encodes only penicillinase production. In some penicillin-resistant isolates no plasmid DNA was found.

S. aureus strains resistant to penicillin and to tetracycline or erythromycin have been isolated occasionally. These strains carried a plasmid of mol. wt 11×10^6 , identical to pBS3583, and a smaller plasmid of mol. wt 2.8×10^6 in tetracycline-resistant isolates and 1.65×10^6 in the erythromycin-resistant isolates.

Each MRSA isolate contained between 1–4 plasmids, but most showed a uniform plasmid profile of four plasmids of mol. wt (10^6) c. 25 (pBS283), 2.8 (pBS3784), 2.6 (pBS2585) and 1.65 (pBS4985) (fig. 1, lane D). Curing experiments indicated that plasmid pBS283 encodes penicillinase production, resistance to cadmium ions and resistance to tetracycline; pBS3784 confers resistance to tetracycline; pBS2585 carries the determinant for chloramphenicol resistance and pBS4985 is responsible for erythromycin resistance. Isolates that lost the plasmid pBS3784 retained tetracycline resistance and only the simultaneous elimination of both pBS283 and pBS3784 caused loss of the

resistant phenotype. In these isolates, therefore, the determinant for tetracycline resistance appeared to be present on two different plasmids.

Other resistances in these isolates (streptomycin, gentamicin, amikacin, methicillin, cefotaxime, cephalixin, sulphamethoxazole and rifampicin) were encoded by chromosomal genes.

Other MRSA strains isolated frequently carried three plasmids of mol. wt (10^6) c. 20 (pBS983), 2.6 (this plasmid confers chloramphenicol resistance and it was considered to be identical to pBS2585), and 1 (pBS1285) (fig. 1, lane E). Plasmid pBS983 encoded resistance to penicillin and tetracycline; pBS1285 remained cryptic. In these isolates, the resistances to gentamicin, methicillin, erythromycin, trimethoprim, sulphamethoxazole, rifampicin and streptomycin were chromosomally encoded.

Recently, multiresistant strains totally devoid of plasmids have been isolated, suggesting that all the resistance determinants in these strains are integrated into the chromosome.

Characteristics of the plasmids isolated in Brescia are listed in table II.

Antibiotic resistances and plasmid profiles of isolates from five other Italian towns

The isolates received from the other Italian hospitals had the resistance characteristics and plasmids shown in table III.

MRSA strains showed similar plasmid patterns to those found in MRSA strains from Brescia. A plasmid of mol. wt (10^6) c. 25 or 18–22 was always present, conferring various resistance phenotypes (table III). Smaller plasmids of mol. wt (10^6) 2.8, 2.6 and 1.65, encoding resistance to tetracycline, chloramphenicol and erythromycin respectively, could also be found.

Methicillin-susceptible isolates that were resistant to penicillin harboured plasmids of mol. wt (10^6) 11, 13 or 18. The plasmids of mol. wt (10^6) 11 and 13 were similar to pBS3583 and pBS1884; the 18×10^6 -mol. wt plasmid was found only in one isolate from Cagliari.

Restriction enzyme analysis

The relationship between isolated plasmids was verified by *EcoRI* digestion, and the fragments of the plasmids isolated in Brescia (pBS283, pBS983, pBS1884, pBS3583) are shown in fig. 2 (lanes B, C, D, E).

Endonuclease digestion of pBS283, pBS983 and pBS1884 generated six, five and three fragments respectively. Digestion of pBS3583 generated only

Table II. Characteristics of plasmids in isolates from Brescia

Plasmid	Mol. wt (10^6)	Resistance phenotype
pBS283	c. 25	penicillin–tetracycline–cadmium
pBS983	c. 20	penicillin–tetracycline
pBS1884	13	penicillin–cadmium
pBS3583	11	penicillin
pBS3784	2.8	tetracycline
pBS2585	2.6	chloramphenicol
pBS4384	2	cryptic
pBS4985	1.65	erythromycin
pBS1285	1	cryptic

one fragment that migrated more slowly than the unrestricted plasmid.

Treatment of pBS3784, pBS2585, pBS4384, pBS4985 and pBS1285 with *EcoRI* generated two bands that probably corresponded to the open circular and covalently-closed circular forms (data not shown).

The plasmids pBS283 and pBS983 shared three *EcoRI* fragments of about 7.3, 4.5 and 3.2 Kb. These fragments were also obtained from plasmid pII 147 (fig. 2, lane F); pBS283 also shared with pII 147 a fourth fragment of about 6.8 Kb.

Restriction analysis of some plasmids isolated in the other towns showed fragment patterns identical to the ones isolated in Brescia. In particular, plasmids of mol. wt 25×10^6 , harboured by isolates from all the towns, except Avellino, shared all the *EcoRI* fragments with pBS283. One isolate from Firenze (FI 6) carried a 25×10^6 -mol. wt plasmid with a different restriction pattern; it showed the absence of the 6.8-Kb fragment and the presence of additional smaller fragments (fig. 2, lane G). This plasmid, however, conferred the same resistance characteristics as pBS283.

A greater variety of restriction patterns emerged in the $(18–22) \times 10^6$ -mol. wt plasmids (fig. 2, lanes H, I, J, K, L, M).

Plasmids with an *EcoRI* digestion profile identical to pBS983 were found in isolates from all the towns, except Avellino. Isolates from Avellino (AV 3–5–7) harboured a 20×10^6 -mol. wt plasmid with the same restriction pattern as that present in isolates from Parma (PR 12–13–15) (fig. 2, lane K) and Cagliari (CA 2) (fig. 2, lane H). These plasmids shared two *EcoRI* fragments with pBS283.

Restriction endonuclease digestion of the 22×10^6 -mol. wt plasmid carried by the isolate PR 3 produced a series of fragments, three of which were common to pBS283 (fig. 2, lane I). The restriction patterns of the 22×10^6 -mol. wt plasmid isolated

Table III. Characteristics of isolates from five other Italian towns

Isolates (number per centre)	Resistance profile of isolates										Plasmid mol. wt (10 ⁶) (associated phenotype)
	Met	Pen	Ak	Gm	Cm	Tc	Em	Sxt	Rf	Va	
Parma (17)											
PR 1	R	R	S	R	S	R	R	R	R	S	20 (Pen-Tc); 1 (cry)
PR 3	R	R	S	R	S	S	R	S	R	S	22 (Pen-Gm); 1 (cry)
PR 6-7-8 14-18	R	R	R	R	S	R	R	S	R	S	22 (Pen-Tc-Gm); 1.65 (Em)
PR 4	R	R	R	R	S	R	R	S	R	S	25 (Pen-Tc-Cd); 1.65 (Em)
PR 5	R	R	S	R	R	R	R	R	R	S	20 (Pen-Tc); 2.6 (Cm)
PR 12-13 15	R	R	R	R	S	S	R	S	S	S	20 (Pen-Gm)
PR 10	S	S	S	S	S	S	S	S	S	S	2 (cry)
PR 11	S	S	S	S	R	S	R	S	S	S	2.6 (Cm); 1.65 (Em)
PR 9-17	S	R	S	S	S	S	S	S	S	S	11 (Pen)
PR 16	S	R	S	S	S	S	S	S	S	S	no plasmid
Cagliari (10)											
CA 2	R	R	S	R	S	S	R	S	S	S	20 (Pen-Gm)
CA 6	R	R	S	R	S	R	R	R	R	S	20 (Pen-Tc); 1 (cry)
CA 9	R	R	S	R	S	R	R	S	S	S	25 (Pen-Tc-Cd)
CA 1	S	R	S	S	S	S	S	S	S	S	11 (Pen)
CA 3-10	S	R	S	S	S	S	S	S	S	S	18 (Pen)
CA 4	S	R	S	S	S	S	S	S	S	S	no plasmid
CA 7-8	S	R	S	S	S	R	S	S	S	S	13 (Pen-Cd); 2.8 (Tc)
CA 5	S	S	S	S	S	S	S	S	S	S	2 (cry)
Avellino (9)											
AV 3-5-7	R	R	S	R	S	R	R	S	S	S	20 (Pen-Gm); 2.8 (Tc)
AV 1	S	R	S	S	S	S	S	S	S	S	13 (Pen-Cd)
AV 6-12	S	R	S	S	S	S	S	S	S	S	no plasmid
AV 10	S	S	S	R	S	S	S	S	S	S	no plasmid
AV 2-13	S	S	S	S	S	S	S	S	S	S	2 (cry)
Firenze (10)											
FI 1	R	R	S	R	R	R	R	S	S	S	25 (Pen-Tc-Cd); 2.6 (Cm)
FI 2-8	R	R	S	R	S	R	R	S	R	S	25 (Pen-Tc-Cd); 2.8 (Tc)
FI 4-9	R	R	S	R	S	R	R	S	R	S	25 (Pen-Tc-Cd); 2.8 (Tc); 1.65 (Em)
FI 3	R	R	R	R	S	R	R	R	R	S	20 (Pen-Tc); 1.65 (Em)
FI 5-10	R	R	R	R	S	R	R	S	R	S	25 (Pen-Tc-Cd); 1.65 (Em); 2 (cry); 2.8 (Tc)
FI 6	R	R	R	R	R	R	R	R	R	S	25 (Pen-Tc-Cd); 1.65 (Em); 2.6 (Cm); 1 (cry)
FI 7	R	R	R	R	S	R	R	R	R	S	20 (Pen-Tc)
Torino (9)											
TO 1	R	R	S	R	S	R	R	S	R	S	25 (Pen-Tc-Cd); 2.8 (Tc); 2 (cry)
TO 5-6-7	R	R	S	R	S	R	R	S	R	S	25 (Pen-Tc-Cd)
TO 2	R	R	S	R	S	R	R	R	R	S	20 (Pen-Tc); 1 (cry)
TO 8	R	R	S	R	S	S	R	S	S	S	no plasmid
TO 9	R	R	S	R	S	S	R	S	S	S	22 (Pen-Gm)
TO 12	R	R	S	S	R	R	R	S	R	S	25 (Pen-Tc-Cd); 1.65 (Em); 2.4 (Cm); 2.8 (Tc)
TO 4	S	R	S	S	S	S	S	S	S	S	no plasmid

Met, methicillin; Pen, penicillin; Ak, amikacin; Gm, gentamicin; Cm, chloramphenicol; Tc, tetracycline; Em, erythromycin; Sxt, trimethoprim/sulphamethoxazole; Rf, rifampicin; Va, vancomycin; Cd, cadmium ions; cry, cryptic.

Fig. 2. *EcoRI* restriction digests of isolated plasmid DNA from *S. aureus*. Lanes A and O: phage lambda DNA; B: pBS283; C: pBS983; D: pBS1884; E: pBS3583; F: pII147; G: plasmids from FI6; H: plasmid from CA2; I: plasmids from PR3; J: plasmids from PR6; K: plasmid from PR13; L: plasmids from PR14; M: plasmid from TO9; N: plasmid from CA3.

from PR 6 and PR 14 strains also produced two bands common to pBS983 and pBS283 (fig. 2, lanes J and L).

Analysis of the 22×10^6 -mol. wt plasmid found in strain TO 9 generated three fragments, one of which was common to pBS283 (fig. 2, lane M), while plasmids of mol. wt (10^6) 11 and 13 showed patterns identical to pBS3583 and pBS1884.

The 18×10^6 -mol. wt plasmid carried by isolate CA 3, isolated in Cagliari, showed a digestion profile different from the previous ones (fig. 2, lane N).

Discussion

Methicillin-resistant and methicillin-sensitive isolates of *S. aureus* from six hospitals in different Italian towns were analysed to investigate the genetic nature and the spread of the resistance determinants. Many of the methicillin-susceptible (MSSA) isolates from the various sources produced penicillinase; generally this trait was associated with the presence of a plasmid of mol. wt (10^6) 11 or 13 but in a few strains was chromosomally encoded. Plasmids responsible for penicillinase production in MSSA were never found in MRSA.

The MRSA were characterised by a plasmid of mol. wt (10^6) 18–22 or 25. This plasmid carried more than one resistance determinant. In the isolates from Brescia, such a plasmid always encoded penicillinase production and resistance to tetracycline (plasmid pBS983); it sometimes also encoded resistance to cadmium ions (plasmid pBS283). The plasmids of similar mol. wt present in isolates from other towns often carried the same resistance determinants but plasmids possessing different arrays of resistances were also detected. The plasmids of mol. wt (10^6) 18–22 or 25, responsible for penicillinase production, were associated frequently with gentamicin or gentamicin and tetracycline resistance determinants.

Restriction analysis showed that plasmids with the same size and *EcoRI* restriction patterns as pBS283 and pBS983 were harboured by strains from all sources, except one (Avellino). The wide occurrence of such plasmids may indicate either spread from hospital to hospital of a strain bearing them, or a genetic transfer of these factors amongst different MRSA strains. Plasmids of similar mol. wt but conferring different resistance phenotypes showed different restriction profiles.

Characterisation of MRSA strains on the basis

of the phenotype conferred by their largest plasmids has been proposed (El Solh *et al.*, 1981; Townsend *et al.*, 1985) because this is more informative than is plasmid-pattern analysis. However, we have found plasmids with the same mol. wt that conferred identical resistance characteristics but that showed different restriction profiles. Thus, restriction analysis of the largest plasmids provides an important additional tool not only to confirm the identity between plasmids, but also to establish different arrangements of the resistance determinants. Different restriction patterns in plasmids conferring apparently identical phenotypes may indicate the presence of genetic factors coding for undetected characteristics, not necessarily related to antibiotic resistance. It may be opportune, therefore, to broaden the study of the phenotypes conferred by the largest plasmids by examining various physiological characteristics and amplifying the number of antimicrobial agents tested. Townsend *et al.* (1984b, 1985), for example, showed that plasmids of mol. wt (10^6) 15–22 can carry determinants for resistance to nucleic acid-binding compounds and to cationic surface active agents. We do not know if such determinants are also possessed by Italian isolates. Moreover, the results reported here are based only on curing experiments. To characterise plasmids completely, however, it would be necessary to transfer them to a recipient strain; experiments of this type are being performed in our laboratory at present.

Analysis of restriction patterns showed that plasmids with different profiles frequently shared one or more fragments. This may suggest that staphylococcal plasmids have originated from frequent rearrangements of a few resistance determinants. Various authors (Murphy and Novick, 1980; Novick *et al.*, 1981) have demonstrated that recombination can occur between plasmids and give rise to the genetic versatility of *S. aureus*. Frequent transposition of plasmid genes to or from the chromosome can also occur (Novick *et al.*, 1979; Phillips and Novick, 1979; Kahn and Novick, 1980). This may explain the recent isolation of multiresistant strains that are plasmid-free. In these strains all the resistance determinants must have been integrated in the chromosome. Such integration probably leads to a stabilisation of the resistance determinants (Lacey, 1984).

MRSA isolated in Brescia and the other five towns carried also smaller plasmids that encoded resistance to tetracycline or chloramphenicol. These plasmids appeared to be similar to those isolated in several countries (Lacey, 1975; Dowd *et al.*, 1983; Gillespie *et al.*, 1984; Lyon *et al.*, 1984).

In some strains, tetracycline resistance was associated with larger plasmids of mol. wt (10^6) 18–22 or 25 and was frequently present on two different plasmids simultaneously, possibly in a transitory state. All the isolates resistant to chloramphenicol harboured the 2.6×10^6 -mol. wt plasmid that seems to be, therefore, the only one responsible for this phenotype.

Erythromycin resistance was often encoded on a plasmid of mol. wt 1.65×10^6 . This observation is in accordance with those of several authors (Iordanescu, 1976; Horinouchi and Weisblum, 1980; Dyke and Noble, 1984; Weisblum, 1985) who have reported the resistance to erythromycin associated with small plasmids; other authors have demonstrated this trait as part of a penicillinase plasmid (Novick and Bouanchaud, 1971) or present on the chromosome (Kayser *et al.*, 1976). Thus, the erythromycin resistance determinant may be associated with different sites of bacterial DNA. Two transposons responsible for macrolide resistance have been described (Novick *et al.*, 1979; Phillips and Novick, 1979).

Small, cryptic, plasmids of mol. wt (10^6) 1 or 2 were found frequently. Other authors have described the isolation of small plasmids with no attributable function (Gillespie *et al.*, 1984; Lyon *et al.*, 1984). These plasmids are probably responsible for important accessory functions but could not be associated with resistance to any of the antimicrobial agents tested.

Plasmids coding for gentamicin resistance have been described (Wood *et al.*, 1977; Jaffe *et al.*, 1980; Townsend *et al.*, 1984a) but, in Italian isolates, gentamicin resistance was often chromosomally encoded. In some isolates the gentamicin-resistance determinant was plasmid encoded and since the genes coding for gentamicin resistance have been described on a transposon (Lyon *et al.*, 1984; Townsend *et al.*, 1984b), it is possible that in Italian strains they have undergone transposition on to the chromosome, as observed by Kayser (1976).

In summary, isolates of multiresistant *S. aureus* often exhibit similar plasmid patterns, but restriction enzyme analysis has revealed differences between the largest plasmids. Further studies are required to establish whether different plasmids have a different origin or derive from rearrangement of a few resistance determinants.

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