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Multiply- and methicillin-resistant *Staphylococcus aureus* strains isolated in the German Democratic Republic in 1985 and 1986

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SUMMARY

Multiply- and methicillin-resistant *Staphylococcus aureus* (MRSA) strains have been isolated from five small outbreaks of nosocomial infection in five different hospitals. The MRSA were typed by phage patterns, biochemical traits, resistance phenotypes and plasmid patterns. Three different groups of strains can be distinguished. The MRSA from three outbreaks in one country share identical characters.

Phage typing by the use of the International Basic Set for Phage Typing staphylococci as well as experimental phages does not completely discriminate between the strains. Attribution of several resistance determinants to plasmids in two of the described strain groups proved valuable for strain differentiation.

These multiply-resistant strains are sensitive to vancomycin and to rifampicin.

INTRODUCTION

The occurrence of multiply- and methicillin-resistant *Staphylococcus aureus* (MRSA) in connexion with nosocomial infections has been reported from several European countries (El, Solh, Moreau & Ehrlich, 1986*a*; Kayser, 1980; Naidoo *et al.* 1983; Witte *et al.* 1986) and also from the United States of America (Haley *et al.* 1982) and from Australia (Pavillard *et al.* 1982), where epidemic spread was observed.

Although these strains share similar phenotypes of resistance to antimicrobial agents they can differ in their phage patterns (Marples, Richardson & DeSaxe, 1986). Their resistance determinants are also obviously different (El Sohl *et al.* 1986*b*, El Sohl, Moreau & Ehrlich, 1986*a*; Gillespie & Skurray, 1986; Witte *et al.* 1986). This is especially reflected by the chromosomal or plasmid-location of their gentamicin-resistance determinants as well as by the nature of the β-lactamase-plasmids (El Sohl, Moreau & Ehrlich, 1986*a*; Gillespie & Skurray, 1986; Naidoo *et al.* 1983; Witte & Dünnhaupt, 1984). In the German Democratic Republic until 1984 MRSA were seen at large teaching paediatric hospitals, where they mainly caused sporadic infections (Witte, Nguyen van Dip & Dünnhaupt, 1986). However, within the last 2 years MRSA have been isolated in connexion with outbreaks in hospitals located in geographically separated cities.

In this paper the occurrence of MRSA in these hospitals and the characteristics of the strains are described. In different outbreaks clearly different but also rather uniform strains are observed.

MATERIAL AND METHODS

Staphylococcal strains. The wild-type strains were sent to the Institut für Experimentelle Epidemiologie for typing. For all further investigations they were cultivated on sheep blood agar.

The following strains were used as recipients for transduction-experiments: strain 8325-4 (Novick, 1967) and the rec⁻-derivative RN 981 (Wyman, Goering & Novick, 1974).

Nutrient media (produced by Staatliches Institut für Immunpräparate und Nährmedien, Berlin-Weissensee, GDR). Nutrient agar I with the addition of calcium chloride (10^{-3} M) for phage typing. Nutrient medium L4 (Reissbrodt, Witte & Rische, 1983) was used for resistance determinations.

Phage typing. Performed according to the methods of Blair & Williams (1961) by use of the International Basic Set for Phage Typing Staphylococci and the experimental phages 88, 89, 90, 91, 92 and A994. The biochemical traits, such as coagulase for human and bovine plasma, crystal-violet type, haemolysin type and fibrinolysin formation which are used for discrimination of host-specific varities of the species *Staphlococcus aureus* have been determined as described by Meyer, Ziomek & Rische (1973).

Resistance determinations. MICs were determined by the broth-microdilution test as described by Thrupp (1981).

Transduction of plasmids and chromosomal resistance determinants. Experimental phage A994 (derived from phage 85, Witte, Nguyen van Dip & Dünnhaupt, 1986) was used; the experimental procedure is described by Witte (1976). The transductants were selected on nutrient medium L4 containing cadmium sulphate (200 μg/ml), chloramphenicol (10 μg/ml, erythromycin (2 μg/ml), gentamicin (1 μg/ml) and oxytetracycline (10 μg/ml).

Demonstration of plasmid DNA. Two methods were used for the extraction of plasmid DNA: the 'alkaline-SDS-method' according to Kado & Liu (1981) and the 'boiling method' according to Goering & Ruff (1983).

The two different methods, based on different principles for the denaturation and removal of the chromosomal DNA, were used in order to be more sure of the detection of plasmids with higher molecular masses in wild-type strains. Both methods gave the same results. For the investigation of transductants for plasmid DNA the 'boiling method' was used.

For the procedure of the 'alkaline-SDS-method' *S. aureus* was grown overnight at 37 °C on nutrient agar. The cells were suspended in 0·9 % NaCl, sedimented and resuspended in STE buffer (8 % saccharose, 50 mM EDTA, 50 mM Tris.HCl, pH 8·0); after addition of lysostaphin (100 μg/ml) the cells were incubated for 15 min at 37 °C in Eppendorf tubes. All further steps correspond to the procedure described by Kado & Liu (1981). The following plasmids served as molecular-weight standards: pCRG 1600, 35 MDa (Goering & Ruff, 1983); pII147, 21 MDa,

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pC221, 3·1 MDa (Ruby & Novick, 1975); pE2222, 1·8 MDa (Iordanescu & Surdeanu, 1980). The DNA isolated was subjected to vertical agarose-gel-electrophoresis according to Maniatis, Fritsch & Sambrook (1982).

RESULTS

Outbreaks in hospitals caused by MRSA

Outbreak A

From the end of 1985 until the present, MRSA were isolated from infections in various teaching hospitals of a university medical school 'O', where the following clinics are involved: skin (17 wound infections), oncology (1 wound infection), surgery (2 wound infections), and internal medicine (1 septicaemia). From each of these cases one MRSA isolate has been subjected to typing, including plasmid profiles. Transduction experiments and plasmid analysis for one of each kind of transductant were performed on five isolates from skin and on all isolates from the other clinics. The isolates from outbreak A exhibit a unique pattern of characteristics (strain-group A in Table 1).

From two wound infections in dermatology of the same medical school, two MRSA isolates were obtained with a pattern of characteristics according to strain-group B in Table 1.

Outbreak B

In municipal hospital 'B' five cases of wound infections with MRSA occurred in surgery. The five MRSA isolates from these patients were typed and further investigated for the location of their resistance-determinants. The pattern of their characteristics is shown as strain-group B in Table 1.

Outbreak C_1

From November 1985 until February 1986 MRSA have caused eight cases of infection in the surgical intensive-care unit of country hospital 'X'. (Five cases of septicemia with one of them fatal and three wound infections). The eight isolates corresponding to each of these cases were typed and analysed for their resistance determinants. They exhibit the unique pattern of characteristics of strain-group C in Table 1.

Outbreak C_2

From December 1985 until May 1986, 21 cases of post-operative wound infection with MRSA have been recorded in orthopaedic clinic 'D' in county 'X'; all of the corresponding 21 isolates were typed and examined for plasmid profiles, 10 of them were subjected to the further study on the location of their resistance determinants. They show the characteristics of strain-group C in Table 1.

For the purpose of specialized treatment and diagnostics patients are exchanged between hospitals 'X' and 'D'; however, this connexion could not be demonstrated for those patients suffering from infections with MRSA.

Table 1. *Characteristics of MRSA from epidemics in five hospitals*

Grouping of the strains	Phage-patterns: Intern. basic Set	Phage-patterns: Experimental phages	Host-specific-variety	Resistance phenotype	Resistance plasmids	Obviously chromosomal determinants
(A) Epidemic A	54, 77 + RTD	A994 RTD, 90, 91, 92, 100 RTD	n.a. CV-A	Pn,* Ox†, Me, Sm, Gm, Cm, Tc, Em, Lm; Cd, Hg	pP 18 MDa (bla, mer, cad, ebr), pC 3 MDa, pE 1·8 MDa	tmn, gen
(B) Epidemic B	NT, 100 RTD	A994 RTD, 89, 100 RTD	hominis	P*, Ox†, Me, Sm, Gm, Cm, Tc, Mn, Em, Lm, Cd, Hg, Eb	Not detectable	Demonstrated: tmn, gen, erm
(C) Epidemics C_1, C_2, C_3	85, + RTD	A994 RTD, 89, 91 100 RTD	hominis	Pn* Ox†, Me, Sm, Gm, Cm, Tc, Em, Lm, Cd, Hg	pC 3 MDa, pT 2, 7 MDa, pE 1, 8 MDa	demonstrated: gen

Abbreviations. bla, β-Lactamase; cad, cadmium resistance; ebr, ethidium bromide resistance; erm, erythromycin resistance; gen, gentamicin resistance; tmn, oxytetracycline-minocycline resistance; pP, penicillinase-plasmid; pC, chloramphenicol-resistance plasmid; pE, erythromycin-resistance plasmid; pT, oxytetracycline-resistance plasmid. For abbreviations of the antibiotic resistance phenotypes see Table 2.

† n.a. CV-A = not allotted to one of the known host-specific varieties, but with crystal violet-type A.

* Parallel resistance to penicillins. † Parallel resistance to cephalosporins

Outbreak C_3

In September 1986 in the orthopaedic clinic of the university medical school 'X' eight infections with MRSA (two in hip-joint prosthesis and in external fixation) were recorded. The isolates of each of these infections were investigated by typing, plasmid profiles and transduction analysis, their characteristics also correspond to strain-group C in Table 1. The identical patterns of characteristics of the isolates from epidemics C_1, C_2 and C_3 suggest a clonal inter-hospital spread of a particular MRSA strain.

Characteristics of the MRSA

The isolates were subjected to a complex typing by phage typing, biochemical characterization (host-specific varieties), resistance phenotype and attribution of the resistance determinants to definite plasmids or to the chromosome (Rische & Witte, 1985). Table 1 shows the results of this complex typing: the resistance phenotype of the relevant strains is registered in Table 2.

Although multiply-resistant to β-lactams and to the older standard antibiotics, all of the MRSA investigated are still sensitive to vancomycin, rifampicin, ciprofloxacin, and with the exception of the three strains from outbreak A, also to fusidic acid.

Strain-group A (from outbreak A)

These isolates exhibit a phage pattern with strong reactions with group-III phages and with the experimental phage A994, and weaker reactions with the experimental phages 90, 91 and 92. Besides multiple resistance to antibiotics they exhibit resistance to trimethoprim sulphonamide, to cadmium, to mercury, and to ethidium bromide and quaternary ammonium compounds. The minimal inhibitory concentrations for ethidium bromide are 128–256 μg/ml, for the quaternary ammonium compound benzalkonium chloride 8–16 μg/ml (control strain *S. aureus* 8325–4 with 8 and 2 μg/ml respectively). When wild isolates of group A are investigated for their plasmid profiles, plasmids with molecular masses of 18, 3 and 1·8 MDa are found. In order to get more information on the genetic location of the resistance determinants, transductants obtained after different kinds of selection for resistance were further analysed for their resistance phenotypes and the attribution of them to plasmids. The transductants obtained after selection for resistance to cadmium harbour a plasmid which obviously determines resistance to cadmium, to mercury, to ethidium bromide and quaternary ammonium compounds and the β-lactamase. It has a molecular mass of 18 MDa. Transductants selected for resistance to chloramphenicol possess a plasmid of 3 MDa. In the transductants selected for erythromycin resistance and exhibiting constitutive resistance to lincomycin, a plasmid with a molecular mass of 1·8 MDa is found. The determinants for the resistance to oxytetracycline-minocycline and for the resistance to gentamicin could be transduced only to strain 8325–4 after UV irradiation of the transducing lysate and not to the rec$^-$ recipient RN981. This indicates a chromosomal location of both determinants. The determinant for trimethoprim resistance could be transduced as well to strain 8325–4 as to the rec$^-$ recipient. It cannot be attributed to one of the plasmids present in MRSA of this group and possibly represents a chromosomally located transposon.

Table 2. *Resistance to antimicrobial agents in MRSA from epidemics in hospitals*

Grouping of the strains	Minimal inhibitory concentrations (μg/ml)																				
	Pn	Ox	Me	Ct	Cx	Gm	Am	Sm	Tc	Mn	Em	Lm	Cm	Fs	Vm	Rf	Cp	Tp/Sa	Cd	Eb	Hg
(A) Epidemic A	0,25	32	32	32–64	32–64	16–32	0·5–1.0	128	128	0·025	32	2	64	0·5–1·0 16·0*	0·25–0·5	0·063	0·25–0·5	128	128	128–256	16
(B) Epidemic B	0,25	64	64	64	64	16	0·5–1·0	128	128	32	32	2	64	0·5	0·25	0·063	0·5	0·25	128	8	16
(C) Epidemics C_1, C_2, C_3	0,25	64	64	64	32–64	16–32	1·0	128	128	0·025	32	2	64	0·5	0·25	0·063	0·25 0.5	0·25	128	8	16

Abbreviations. Pn, penicillin; Ox, oxacillin; Me, methicillin; Ct, cefotiam; Cx, cefotaxime; Gm, gentamicin; Am, amikacin; Sm, streptomycin; Tc, oxytetracycline; Mn, minocyclin; Em, erythromycin; Lm, lincomycin; Cm, chloramphenicol; Fs, fusidic acid; Vm, vancomycin; Rf, rifampicin; Cp, ciprofloxacin; Tp/Sa, trimethoprim/sulphonamide; Cd, cadmium-sulphate; Eb, ethidiumbromide; Hg, mercury chloride.

* Three strains resistant among 21 from this epidemic.

Strain-group B (from outbreak B)

These isolates are non-typable at 100 RTD by the phages of the International Basic Set for Phage Typing Staphylococci; they exhibit reactions with the experimental phages 89 and A994.

According to their biochemical properties they belong to the host-specific variety hominis of the species *Staphylococcus aureus*. Although these isolates exhibit a multiply-resistance phenotype, no plasmid DNA can be detected, neither by use of the method of Kado & Liu (1981) or by the 'boiling method' (Goering & Ruff, 1983). The determinants for the resistances to oxytetracycline-minocycline and for gentamicin resistance behave like chromosomal genes when transduced to strain 8325–4; also no plasmid DNA can be detected in these transductants. The same is valid for transductants selected for resistance to erythromycin. However, in this case transduction to the rec$^-$ recipient was possible, which suggests a chromosomal transposon determining erythromycin-lincomycin resistance.

Strain-group C (from outbreaks C_1, C_2 and C_3)

When phase typed these isolates react with phage 85 and with the experimental phages 89, 91 and A994. By this phage pattern and by their resistance-phenotype they are similar to the strains from epidemic B. However, these isolates harbour three different plasmids (3·0, 2·7, 1·8 MDa).

The analysis of corresponding transductants reveals that several of the resistance determinants can be attributed to plasmids: resistance to chloramphenicol to a plasmid with a molecular mass of 3 MDa, resistance to oxytetracycline to a plasmid of 2·7 MDa, and the constitutive resistance to erythromycin-lincomycin to a 1·8 MDa plasmid. The determinant for gentamicin resistance could be transduced to strain 8325–4 like a chromosomal gene as described for the strains of group A. Attempts to transduce the determinants for the resistances to penicillins, to cadmium and to mercury have been unsuccessful. Since these determinants cannot be attributed to one of the plasmid detectable in the wild strains, they might be located on the chromosome.

DISCUSSION

Initially, MRSA have been observed in connexion with sporadic infections in larger medical centres in the German Democratic Republic (Witte, Nguyen van Dip & Dünhaupt 1986), but within the last 2 years small nosocomial outbreaks with MRSA also occurred in this country. The same situation was earlier observed in the USA (Haley *et al* 1982; Locksley *et al.* 1982), in Australia (Pavillard *et al.* 1982; Vickery, Beard-Pegler & Rountree, 1983) and also in the United Kingdom (Marples, Richardson & De Saxe, 1986). The reasons for the recently reported spread of MRSA are not clear. Before 1983 no resistance to gentamicin was detected in MRSA from this country; however, a possible selective pressure cannot account for the reported outbreaks since gentamicin is used very rarely in the hospitals where outbreaks C_2 and C_3 occurred. Also resistance to quaternary ammonium compounds is unlikely to contribute to a selection of MRSA in hospitals as suggested by Townsend *et al.* (1983): only the strains from outbreak

A exhibit this kind of resistance. Another possibility which could favour the selection of MRSA in hospitals is the therapeutic use of the third-generation cephalosporins.

The MRSA described in this study show reactions with group-III phages and/or with experimental phages already known to give reactions with MRSA. In this respect they are similar to the MRSA from other countries.

Comparison of the genetic arrangements of the resistance-determinants of these strains with those of strains of other origin show similarities but also clear differences. The chromosomal location of the gentamicin-resistance determinant is also reported from MRSA isolated in France (El Sohl, Moreau & Ehrlich, 1986) but these strains are sensitive to chloramphenicol and they carry a comparably small β-lactamase-plasmid of 14·5 MDa and two apparently cryptic plasmids. This kind of location of the gentamicin-resistance determinant is also known from MRSA from other European countries (Dowd, Cafferkey & Dougan, 1983; Kayser, Homberger & Devaud, 1981; Naidoo *et al*, 1983) but this is not specific for MRSA (Witte & Dünnhaupt, 1984). In contrast to this in MRSA from Australia (Gillespie & Skurray, 1986) and from the USA (Goering & Ruff, 1983) gentamicin resistance is determined by multi-resistance plasmids of two classes: large conjugative resistance plasmids in the USA and heavy-metal ion-resistance plasmids in Australia, probably also in the USA. The 'Australian' plasmids obviously acquired the gentamicin-resistance determinant on a transposon (Lyon, May & Skurray, 1984; Townsend *et al* 1984). Only strains from outbreak B harbour a β-lactamase plasmid which carries also determinants for resistance to heavy metal ions and to ethidium bromide–quaternary ammonium ions; plasmids of the same molecular mass and determining the same resistance phenotypes are known from Australian strains (Emslie, Townsend & Grubb, 1985). The small plasmids found in the MRSA of this study determine resistance to chloramphenicol, to oxytetracycline and to macrolides. Their molecular masses correspond to those known from multiply-resistant *S. aureus* (Locksley *et al.* 1982, Lyon *et al.* 1984). The presented data confirm the value of plasmid analysis for the differentiation of multiply-resistant *S. aureus* strains.

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REFERENCES

BLAIR, J. E. & WILLIAMS, R. E. O. (1961). Phage-typing of staphylococci. *Bulletin of the World Health Organization* **24**, 771–784.

DOWD, G., CAFFERKEY, M. & DOUGAN, G. (1983). Gentamicin and methicillin resistant *Staphylococcus aureus* in Dublin hospitals: molecular studies. *Journal of Medical Microbiology* **16**, 129–138.

EL SOLH, N., ALIGNET, J., BISMUTH, R., BURET, B. & FOUACE, I.-M. (1986*b*). Conjugative transfer of staphylococcal antibiotic resistance markers in the absence of detectable plasmid DNA. *Antimicrobial Agents and Chemotherapy* **30**, 161–169.

EL SOHL, N., MOREAU, N. & EHRLICH, S. (1986*a*). Molecular cloning and analysis of *Staphylococcus aureus* chromosomal aminoglycoside resistance genes. *Plasmid* **15**, 104–118.

EMSLIE, K. R., TOWNSEND, D. E. & GRUBB, W. B. (1985). A resistance determinant to nucleid acid-binding compounds in methicillin-resistant *Staphylococcus aureus*. *Journal of Medical Microbiology* **20**, 139–145.

GILLESPIE, M. T. & SKURRAY, R. A. (1986). Plasmids in multiresistant *Staphlococcus aureus*. *Microbiological Sciences* **3**, 53–58.

GOERING, R. V. & RUFF, E. A. (1983). Comparative analysis of conjugative plasmids mediating gentamicin resistance in *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* **24**, 450–452.

HALEY, R., HIGHTOWER, A., KHABBAZ, R. F., THORNSBERY, C., MARTONE, W. J., ALLEN, J. R. & HUGHES, J. M. (1982). The emergence of methicillin-resistant *Staphylococcus aureus* infections in United States hospitals. *Annals of International Medicine* **97**, 297–308.

IORDANUSCU, S. & SURREANU, M. (1980). New incompatibility groups for *Staphlococcus aureus* plasmids. *Plasmid* **4**, 256–260.

KADO, C. I. & LIU, S. T. (1981). Rapid procedure for detection and isolation of large and small plasmids. *Journal of Bacteriology* **145**, 1365–1373.

KAYSER, F. H. (1980). Die Resistenz methicillinresistenter Staphylokokken gegenüber neuen Cephalosporin-Antibiotika. *Infection* **8**, 165–170.

KAYSER, F. H., HOMBERGER, F. & DEVAUD, M. (1981). Aminocyclitol-modifying enzymes specified by choromsomal genes in *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* **19**, 766–772.

LOCKSLEY, R. M., COHEN, M. L., QUINN, T. C., TOMPKINS, L. S., COYLE, M. B., KIHARA, J. M. & COUNTS, G. W. (1982). Multiply resistant *Staphylococcus aureus*: introduction, transmission and evolution of nosocomial infection. *Annals of International Medicine* **97**, 317–324.

LYON, B. R., IURIO, J. L., MAY, J. W. & SKURRAY, R. A. (1984). Molecular epidemiology of multiresistant *Staphylococcus aureus* in Australian hospitals. *Journal of Medical Microbiology* **17**, 79–89.

LYON, B. R., MAY, J. W. & SKURRAY, R. A. (1984). In 4001: a gentamicin and kanamycin resistance transposon in *Staphylococcus aureus*. *Molecular and General Genetics* **193**, 554–556.

MANIATIS, T., FRITSCH, E. F. & SAMBROOK, J. (1982). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

MARPLES, R. R., RICHARDSON, J. & DE SAXE, M. (1986). Bacteriological characters of strains of *Staphylococcus aureus* submitted to a reference laboratory related to methicillin resistance. *Journal of Hygiene* **96**, 217–223.

MEYER, W., ZIOMEK, D. & RISCHE, H. (1973). *Staphylococcus aureus*. In *Lysotypie und andere spezielle epidemiologische Laboratoriumsmethoden*. (ed. H. Rische), Jena: Gustav Fischer.

NAIDOO, J., NOBLE, W. C., WEISSMANN, A. & DYKE, K. G. H. (1983). Gentamicin-resistant staphylococci: genetics of an outbreak in a dermatology department. *Journal of Hygiene* **91**, 7–16.

NOVICK, R. P. (1967). Properties of cryptic high frequency transducing phage in *Staphylococcus aureus*. *Virology* **33**, 155–166.

PAVILLARD, R., HARVEY, K., DOUGLAS, D., HEWSTONE, A., ANDREW, J., COLLOPY, B., ASCHE, V., CARSON, P., DAVIDSON, A., GILBERT, G., SPICER, J. & TOSOLINI, F. (1982). Epidemic of hospital acquired infection due to methicillin-resistant *Staphylococcus aureus* in major Victorian hospitals. *Medical Journal of Australia* **1**, 451–454.

REISSBRODT, R., WITTE, W. & RISCHE, H. (1983). A new semidefined nutrient medium for bacterial susceptibility testing. *Journal of Hygiene, Microbiology, Epidemiology and Immunology (Prague)* **27**, 465–479.

RISCHE, H. & WITTE, W. (1985). The clone concept in the epidemiology of staphylococal infection. *Zentralblatt für Bakteriologie*, supplement **14**, 531–540.

RUBY, C. & NOVICK, R. P. (1975). Plasmid interactions in *Staphylococcus aureus*: nonadditivity of compatible plasmid DNA pools. *Proceedings of the National Academy of Sciences, U.S.A.* **72**, 5031–5035.

THRUPP, L. D. (1981). Susceptibility testing of antibiotics in liquid media. In *Antibiotics in Laboratory Medicine* (ed. V. Lorian). Baltimore and London: Williams & Wilkins.

TOWNSEND, D. E., GREED, L., ASHDOWN, N. & GRUBB, W. B. (1983). Plasmid mediated resistance to quarternary ammonium compounds in methicillin resistant *Staphylococcus aureus*. *Medical Journal of Australia* **2**, 310.

TOWNSEND, D. E., ASHDOWN, N., GREED, L. C. & GRUBB, W. B. (1984). Transposition of gentamicin resistance to staphylococcal plasmids encoding resistance to cationic agents. *Journal of Antimicrobial Chemotherapy* **14**, 115–124.

VICKERY, A., BEARD-PEGLER, M. A. & ROUNTREE, P. M. (1983). Strain differentiation in methicillin-resistant *Staphylococcus aureus*. *Pathology* **15**, 235–240.

WITTE, W. (1976). Restriktion und Modifikation bei *Staphylococcus aureus*. Eigenschaften von Resistenzplasmiden und Prophagen. *Zeitschrift für Allgemeine Mikrobiologie* **16**, 465–474.

WITTE, W. & DÜNNHAUPT, K. (1984). Occurrence of a nonplasmid-located determinant for gentamicin-resistance in strains of *Staphylococcus aureus*. *Journal of Hygiene* **93**, 1–8.

WITTE, W., GREEN, L., MISRA, T. & SILVER, S. (1986). Resistance to mercury and to cadmium in chromosomally resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* **29**, 663–669.

WITTE, W., NGUYEN VAN DIP & DÜNNHAUPT, K. (1986). Methicillin-resistant *Staphylococcus aureus* (MRSA) in the German Democratic Republic, incidence and strain characteristics. *Journal of Hygiene, Microbiology, Epidemiology and Immunology (Prague)* **30**, 185–193.

WYMAN, L., GOERING, R. V. & NOVICK, R. P. (1974). Genetics of chromosomal and plasmid recombination in *Staphylococcus aureus*. *Genetics* **76**, 681–702.