Epidemiol. Infect. (1994), **113**, 463–470

Staphylococcus aureus strains of type 95. Spread of a single clone

V. T. ROSDAHL[1]*, W. WITTE[2], M. MUSSER[3] AND J. O. JARLØV[1]

[1] *Staphylococcus Laboratory, Statens Seruminstitut, Artillerivej 5, DK-2300 Copenhagen S, Denmark*

[2] *Robert-Koch-Institut, Bereich Wernigerode, Wernigerode, Germany*

[3] *Department of Pathology, Baylor College of Medicine, Houston, Texas, USA*

(*Accepted 4 July 1994*)

SUMMARY

Staphylococcus aureus strains of type 95 in Denmark have increased to a frequency of 20% of the total *S. aureus* population. A clonal origin and possible subdivision of these strains have been discussed. In the present investigation 35 epidemiologically unrelated *S. aureus* strains of type 95 as well as reference strains of other types have been analysed by other typing techniques including lectin-typing, multilocus enzyme electrophoresis and pulsed-field gel electrophoresis of genomic restriction fragments. No subdivision could be achieved based on any of these methods and a clonal origin seems therefore possible.

INTRODUCTION

During the last 30 years the predominant *Staphylococcus aureus* population isolated from hospitalized patients has changed several times both in phage types and antibiotic resistance [1–3]. New *S. aureus* clones have developed from old ones by prophage-introduction into ancestor strains as already described for the old strains of the 52, 52A, 80, 81 complex [4] and the subsequent strains of the 83A complex [5]. Rapid spread of new clones might occur if the properties of these new clones like antibiotic resistance patterns are favoured by the actual selection pressure like antibiotic usage.

In recent years *S. aureus* strains of phage type 95, resistant to penicillin only, have increased in frequency in Denmark from 3·8% in 1977 to 19·3% in 1993. This increase has occurred more slowly than the one observed for the introduction of multiply resistant strains of the 83A complex in the period 1965–75 [6]. It has been suggested that this difference in the rate of spread might be due to two different mechanisms. With the old multiply resistant strains of the 83A complex, different prophages were introduced on different occasions into Group III strains causing a shift to the 83A complex and the high usage of antibiotics, especially tetracycline, was an effective selection pressure combined with frequent cross-infection. With the recent strains of type 95, however, only penicillin usage might act as the selective pressure and would select not only strains of type 95 but also strains of many different phage types; in addition improved hospital hygiene had

* Correspondence and requests for reprints to: Vibeke Thamdrup Rosdahl, Staphylococcus Laboratory, Statens Seruministut, Artillerivej 5, DK-2300 Copenhagen S, Denmark.

reduced cross-infection and preliminary investigations seemed to indicate that strains of type 95 belong to only one or two bacterial clones [6, 7], which spread slowly.

The present investigations were undertaken in order to evaluate the hypothesis that strains of type 95 belonged to only one clone. In order to do so further typing with a number of newer typing methods was performed including multilocus enzyme electrophoresis profiles, analysis of chromosomal DNA by pulse field gel electrophoresis and lectin-typing. The present investigations were also undertaken in order to detect whether any of these newer typing methods could be used for subdivision of isolates of type 95. Such a subdivision might be valuable since the increasing number of type 95 strains has made epidemiological studies on cross-infection with these strains difficult; the detection of several isolates of this type might not indicate a common origin of the isolates.

MATERIALS AND METHODS

Bacterial strains

During the years 1977–90 *S. aureus* strains of type 95 comprised 1567 of the 10494 strains isolated from Danish patients hospitalized with bacteraemia [3, 8]. Resistance to penicillin, methicillin, streptomycin, gentamicin, tetracycline and erythromycin was recorded on all strains and since 1985 arsenate resistance was investigated on all strains. Further typing with newer methods was performed on 35 bacteraemia strains of type 95. The strains were selected at random and originated from 18 different hospitals all over Denmark. Twenty of the strains (nos 1–10 and 26–35) collected during 1977–81 had previously been analysed for arsenate-resistance and beta-lactamase production [7], and comprised 10 strains with high beta-lactamase production and As-resistance (strain nos 1, 5, 7, 9, 10 and 31–35) and 10 susceptible strains with medium production. In addition, 15 As-susceptible type 95 bacteraemia-strains from 1985–90 were included (strain nos 11–25). A total of 21 *S. aureus* propagating strains from the basic phage-typing set was investigated as reference strains including strain 29, 52, 52A, 80, 3A, 71, 55, 6, 42E, 47, 53, 54, 75, 77, 83A, 84, 85, 81, 94, 96 and 95 [1]. In the pulsed-field gel electrophoresis four animal reference strains H1, B5, 737 and A1591 (see Fig. 2) were included. Table 1 indicates which strains have been investigated by which methods.

Antibiotic and arsenate-resistance

This was performed as previously described by the disk-diffusion techniques [8] and MIC determination on solid media [9].

Phage typing

This was performed according to the methods of Blair & Williams [10] using the international set of typing phages. All strains of phage type 95 were recorded separately.

Multilocus enzyme electrophoresis

This was carried out as described by Musser and colleagues [11] including 20 enzymes. Each isolate was characterized by its combination of alleles of the 20

Table 1. Staphylococcus aureus *investigated by the different methods included*

Strains	From years	Investigated (+) or not investigated (0) by the methods: Isoenzyme	Pulsed-field	Lectin	Beta-lactamase and As-resistance
1–10	1977–81	+	+	+	+
11–25	1985–90	0	+	+	0
26–35	1977–81	+	0	+	+
The 21 propagating strain		0	+	+	0
The four animal strains		0	+	0	0

enzyme loci and distinctive combinations of electromorphs, corresponding to unique multilocus genotypes, were designated as electrophoretic types [ETs].

Pulsed-field gel electrophoresis of genomic Sma*I restriction fragments*

The technique was performed both with a standard technique [12] as well as with a technique which allows the separation of the low molecular weight fragments (1·2% agarose, pulse-ramps: 1–4 s for 6 h followed by 5–50 s for 15 h followed by 60–90 s for 12 h).

Lectin-typing

This was performed by binding the unknown bacteria to the wells of a microtitration plate, followed by addition of the biotinylated lectin. After repeated washing the degree of lectin binding to the bacteria was measured with a peroxidase reaction.

The method has previously been described for *S. epidermidis* [13] and for methicillin-resistant *S. aureus* [14]. The following lectins were applied: Wheat-germ agglutinin, WGA, Soy-bean lectin, SBA, *Lycopersicon esculentum* agglutinin, LEA and Concanavalin A, ConA.

RESULTS

Antibiotic and arsenate resistance

Among the 1567 phage type 95 strains isolated from bacteraemia cases between 1977 and 1990 antibiotic resistance occurred with the following frequencies: penicillin, 90·2%; methicillin, 0·006%; streptomycin, 0·3%; gentamicin, 0·3%; erythromycin, 2·1%; tetracycline, 1·1%. Arsenate resistance occurred in 47 (4·5%) of the 1041 type 95 bacteraemia strains isolated during the years 1985–90, varying annually between 3·9 and 5·2%.

Multilocus enzyme electrophoresis

A total of 20 phage type 95 bacteraemia strains from the period 1977–81 was analysed (strain nos 1–10 and 26–35). They originated from 15 different hospitals and were not epidemiologically related. Ten of the strains had previously [7] been shown to be As-resistant and to produce large amounts of beta-lactamase, whereas

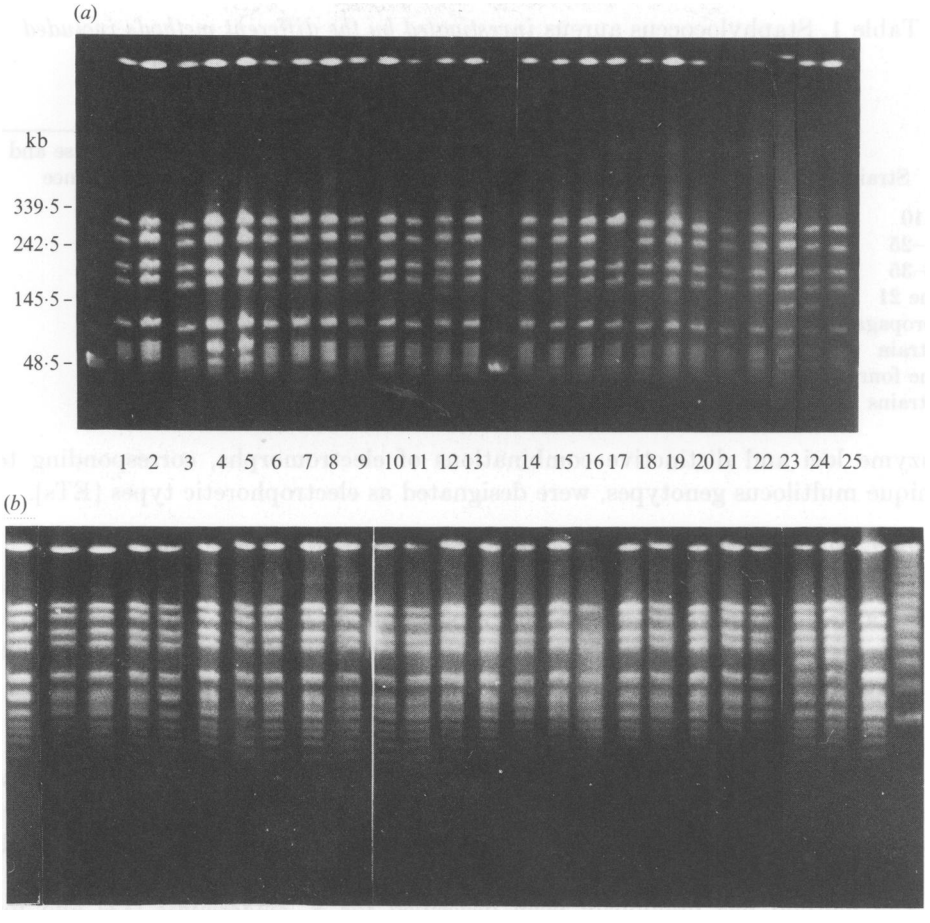

Fig. 1. (*a*) Genomic DNA fragment patterns of 25 *S. aureus* strains (numbered 1–25) of phage type 95, *Sma*I-digestion and 'standard-procedure' (12) of pulsed-field gel electrophoresis. (*b*) Genomic DNA fragment patterns of 25 *S. aureus* strains of phage type 95, *Sma*I-digestion and 'low molecular mass procedure' of pulsed-field gel electrophoresis. Lane 1–25 corresponding to strain 1–25 as in (*a*).

the remaining 10 were As-susceptible and had a medium beta-lactamase production (see Material and methods). Eighteen of the 20 strains had the same multilocus genotype: 125,5,5,5,7,5,4,5,5,5,5,3,5,0,8,7,5,6,5,5; the remaining two strains (nos 3 and 8) had the same genotype: 125,5,5,5,7,5,7,5,5,5,5,3,5,0,8,7,5,6,5,5; however, this differed from the 18 other strains by only one enzyme. The two discrepant strains were both As-susceptible. These results might indicate that there are no clonal differences between As-susceptible and As-resistant strains of phage type 95.

Pulsed-field gel electrophoresis of genomic restriction fragments

Investigations with this technique were performed on ten of the bacteraemia strains analysed by multilocus enzyme electrophoresis (nos 1–10), with 5 As-resistant and 5 As-susceptible strains and including the two strains, which differed in multilocus genotype.

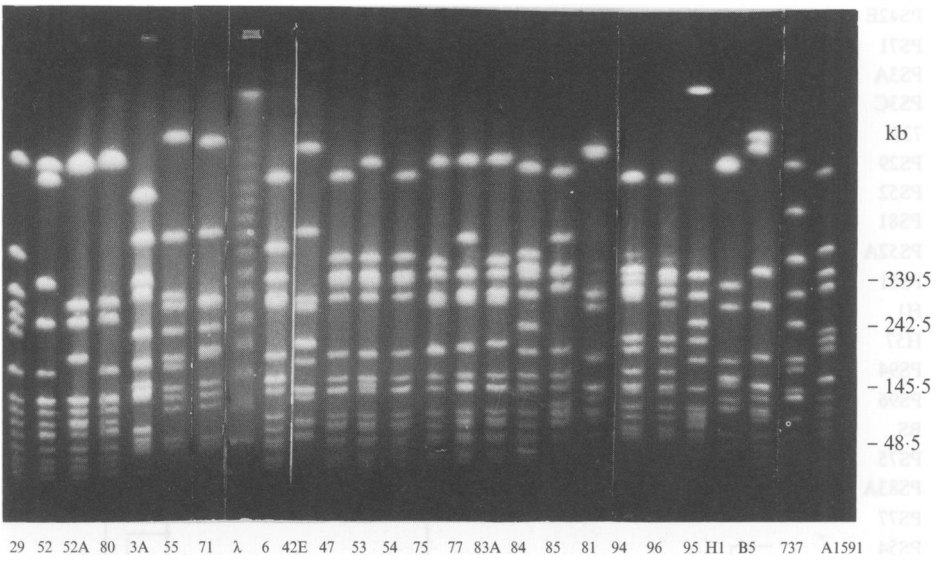

Fig. 2. Genomic DNA fragment patterns of the propagating strains in the International Basic set for phage typing (strain 29–95) and the four reference strains for animal specific varieties of *S. aureus* (H1 *S. aureus* var. hominis, B5 *S. aureus* var. bovis, 737 *S. aureus* var. ovis, 1591 *S. aureus* var. gallinae).

In addition 15 phage type 95 As-susceptible bacteraemia strains from the years 1985–90 (nos 11–25) were included in order to look at more recent strains plus the 21 propagating *S. aureus* strains from the basic set for phage typing as well as four reference strains for animal specific varieties of *S. aureus* [15] were analysed to compare with patterns from strains of other phage types.

Figure 1 *a* and *b* illustrates the gel electrophoresis patterns of the type 95 strains after treatment with *Sma*I restriction endonuclease. A standard procedure (Fig. 1 *a*) and a procedure which allows separation of the low molecular weight fragments (Fig. 1 *b*) were performed. All 25 type 95 strains gave a very uniform picture; the only small discrepancies were in strain 17, an As-susceptible strain from 1990, which lacked a fragment of about 260 kb and strain 7, an As-resistant strain from 1979 of the dominant multilocus genotype, which lacked one of the smaller fragments.

Figure 2 shows the investigations of the propagating strains of different phage types and in Fig. 3 a dendrogram illustrates the relation of strains of different phage types according to the performed gel electrophoresis patterns.

Lectin-typing

Lectin-typing was performed on 56 isolates: these comprised the 20 bacteraemia isolates also investigated by multilocus enzyme electrophoresis, the 15 newer isolates included in the pulsed-field gel electrophoresis and the propagating strains from the basic phage-typing set. WGA, SBA, LEA and ConA were the four lectins included, but variation in the lectin binding for all strains investigated was only found in LEA and WGA. In Fig. 4 is shown the binding of the 56 isolates to respectively LEA and WGA; it is seen that the majority of the type 95 isolates had very similar values. There were no differences between As-resistant and As-

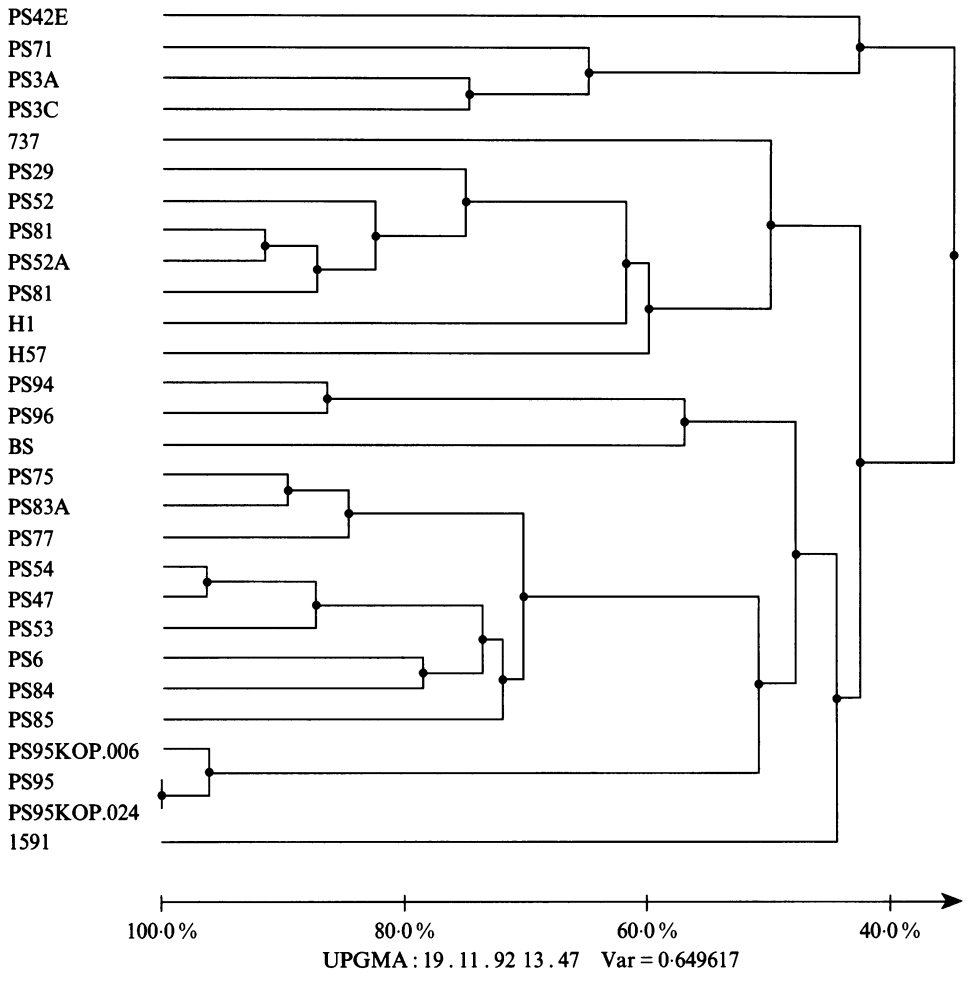

Fig. 3. Dendrogram of similarity derived from genomic DNA fragment patterns of the propagating strains in the International Basic set for phage typing two Danish type 95 strains (6+24) and the four reference strains mentioned in Fig. 2 for animal specific varieties of *S. aureus*.

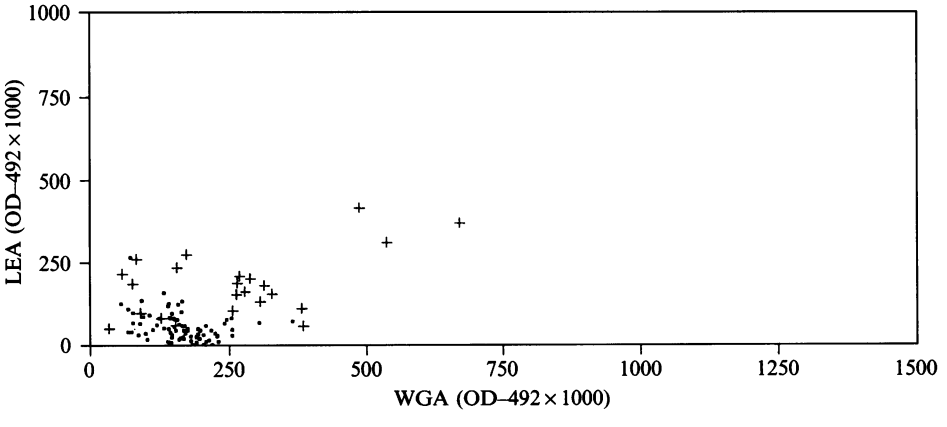

Fig. 4. Reaction with LEA- and WGA-lectins (expressed as OD at 492 × 1000) among the 35 type 95 strains and the propagating strains of other phage types.

susceptible strains. Neither did the four strains with slight differences in respectively multilocus enzyme-electrophoresis and pulsed-field electrophoresis show any differences from the other type 95 strains in lectin-typing.

DISCUSSION

Previous investigations on *S. aureus* strains of type 95 including only beta-lactamase production and heavy metal resistance pointed to the possibility that all the As-resistant strains belonged to one single clone, and that the remaining few strains with As-resistance and high beta-lactamase production might either be a totally separate, not so widespread, clone or might be a variant of the original clone with a change in the beta-lactamase plasmid including introduction of As-resistance [7]. In the present investigations three newer typing methods: multilocus enzyme electrophoresis, pulsed-field gel electrophoresis and lectin-typing have been selected to represent three fundamentally different typing principles: phenotypically expressed differences in the produced isoenzymes and surface-components as well as differences in the gene-sequences. All three methods clearly demonstrated that similar typing patterns were obtained for all type 95 strains including both As-resistant and As-susceptible strains. Minor differences were observed such as a one enzyme difference in two strains and one restriction fragment band in two strains. These variations occurred on different strains, however. These smaller differences might, like the As-resistance and high beta-lactamase production, be occasional variants within the basic type 95 clone. This assumption of occasional small variations might further be supported by the fact that these variants did not occur in any special hospital or in any special observation period.

Among *S. aureus* strains which type both with phage 95 and other phages, co-reaction with phages of group I is often seen, which could point to a close relationship between strains of type 95 and group I strains. One might speculate whether type 95 have developed by prophage introduction into strains of group I. The present investigations with other typing methods show some similarity between type 95 strains and the propagating strain 29 and 52 in lectin-types but not with the strains of 52A/79 and 80 also of group I. In pulsed-field gel electrophoresis no similarities between type 95 strains and strains of group I were observed. A nationwide continuous surveillance of the *S. aureus* population has only been performed in Denmark and it is therefore difficult to know if the spread observed in Denmark has also occurred in other countries. In Germany the frequency of *S. aureus* strains of type 95 was around 0·8% among strains of different origin until 1992 and rose to 7% in 1993. Strains of type 95 seems to have a high colonization capacity. A patient colonized or infected by a strain of type 95 very seldom changed to staphylococci of other phage-type patterns [16]. Resistance to methicillin has until now been very rare if at all existing [6] in strains of type 95. In Denmark methicillin-resistant *S. aureus* has since 1984 occurred with a frequency of only 0·2%, with approximately half of the resistant strains imported with patients coming from hospitals abroad [17]. Just recently a MRSA epidemic was recorded in six Berlin hospitals due to dissemination of a strain which exhibited the genome DNA fragment pattern of type 95 *S. aureus*. The role of type 95 strains might therefore change in the future.

REFERENCES

1. Parker MT. The significance of phage-typing patterns in *Staphylococcus aureus*. In: Easmon CSF, Adlam C, eds. Staphylococci and staphylococcal infections. Vol 1. London: Academic Press, 1983; 33–62.
2. Altemeier WA, Lewis SA. Cyclic variations in emerging phage types and antibiotic resistance of staphylococcus. Surgery 1978; **84**: 534–41.
3. Rosdahl VT, Espersen F, Frimodt-Møller N, Skinhøj P. Changing *Staphylococcus aureus* epidemiology; 30 years experience. Zentr Bakt 1993; Suppl 26: in press.
4. Asheshov EH, Winkler KC. *Staphylococcus aureus* strains in the '52, 52A, 80, 81 complex'. Nature 1966; **209**: 638–9.
5. Jevons MP, John M, Parker MT. Cultural characters of a newly recognized group of hospital staphylococci. J Clin Pathol 1966; **19**: 305–12.
6. Schønheyder H, Jensen KT, Pers C, Korsager B, Rosdahl VT. Spread of *Staphylococcus aureus* strains of phage-type 95 in Denmark 1968–1989. J Hosp Infect 1992; **20**: 25–34.
7. Rosdahl VT, Rosendal K. Unusual properties of *Staphylococcus aureus* strains belonging to the new epidemic phage type 95. J Med Microbiol 1985; **20**: 325–33.
8. Rosendal K, Jensen O, Faber V, Bentzon MW. Frequency, phage types and antibiotic resistance of *Staphylococcus aureus* isolated from blood cultures in Denmark 1975–1981. Scand J Infect Dis 1983; Suppl **41**: 19–26.
9. Rosdahl VT, Rosendal K. Correlation of penicillinase production with phage type and susceptibility to antibiotics and heavy metals in *Staphylococcus aureus*. J Med Microbiol; **16**: 391–9.
10. Blair JE, Williams REO. Phage typing of staphylococci. Bull WHO 1961; **24**: 771–84.
11. Musser JM, Schlievert PM, Chow AM, et al. A single clone of *Staphylococcus aureus* causes the majority of cases of toxic shock syndrome. Proc Natl Acad Sci USA 1990; **87**: 225–9.
12. Witte W, Grimm H. Occurrence of quinolone resistance in *Staphylococcus aureus* from nosocomial infections. Epidemiol Infect 1992; **109**: 413–21.
13. Jarløv JO, Hansen JES, Rosdahl VT, Espersen F. The typing of *Staphylococcus epidermidis* by a lectin-binding assay. J Med Microbiol 1992; **37**: 195–200.
14. Jarløv JO, Rosdahl VT, Yeo M, Marples RR. Lectin-typing of methicillin-resistant *Staphylococcus aureus* from Singapore, England & Wales, and Denmark. J Med Microbiol 1993; **39**: 305–9.
15. Witte W, Hummel R, Meyer W, Exner H, Wundrak R. On the ecology of *Staphylococcus aureus*: characterization of strains from chicken. Z Allg Mikrobiol 1977; **17**: 639–47.
16. Rosdahl VT, Laursen H, Bentzon MW, Kjældgaard P, Thomsen M. Colonizing ability among *Staphylococcus aureus* strains isolated in 1985 and 1986. Correlation to phage-type. J Hosp Infect 1988; **12**: 151–62.
17. Rosdahl VT, Knudsen AM. The decline of methicillin resistance among Danish *Staphylococcus aureus* strains. Infect Control Hosp Epidemiol 1991; **12**: 83–8.