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Changing patterns among the subgroups of strains of *Staphylococcus aureus* of phage group II in Danish hospitals from 1961–91

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SUMMARY

During the period 1961–91 a total of 567635 strains of *Staphylococcus aureus* from hospitalized patients in Denmark have been characterized according to their antibiotic resistance, site of isolation and phage type. Strains of phage group II (typed by the phages 3A, 3C, 55 and 71) have been analysed further. The occurrence of group II strains was relatively constant (approximately 16%) from 1961 until 1983. Since then the frequency of group II strains increased; in 1991 they accounted for 22·7% of all *S. aureus* strains isolated. Strains of group II can, on the basis of their phage types, be divided in four subgroups: 3A, 71, 71+ and the 'rest of group II'. Furthermore, within these groups strains may differ from one another in respect to their sensitivity to phages.

The increased isolation of group II strains during recent years was because of an increase in strains of subgroups 71+ and the 'rest of group II strains'. In 1991 these two subgroups accounted for 89·7% of all group II strains. Furthermore, an increasing number of group II strains, 71·4% in 1991, was typable only at RTD × 100. The increase in the number of group II strains was even throughout Denmark. All four subgroups of group II have, during the observation period, become more frequently resistant to penicillin and/or tetracycline. Strains typed at 100 × RTD of subgroup 71+ and the 'rest of group II' are more frequently antibiotic resistant than the rest of the group II strains.

Strains of the increasing subgroups occurred most often in abscesses.

INTRODUCTION

During the last 30 years the epidemiology of *Staphylococcus aureus* in Denmark has been followed closely. This has been possible because of the centralized phage-typing in Denmark. Approximately 30000 *S. aureus* isolates from 20000 hospitalized patients have been investigated every year since 1960 [1–3]. *S. aureus* strains of phage group II occurred with about the same frequency, approximately 16%, until the year 1983. Since then group II strains have increased in frequency. Since 1988 they have become the most frequently isolated phage group of *S. aureus* [4]. Strains of group II have been divided into four subgroups according to their phage type. They have been further divided into strains reacting with phages

at routine test dilution (RTD) or only at the higher concentrations of 100 × and 1000 × RTD. They can also be characterized by antibiotic resistance and site of isolation. The present investigation was undertaken in order to analyse and illustrate the epidemiology of this increase by following the occurrence of the different subgroups among strains correlated with antibiotic resistance pattern, and anatomical site of isolation.

METHODS

The present investigation was based on the central register of all phage-typed *S. aureus* strains isolated from patients in Danish hospitals during the period 1961–91.

S. aureus strains

A mean of 18312 strains (range 12003–22327) were phage-typed annually, giving a total number of 567635 strains. Each year only one isolate was included per patient. This means in practice that we are dealing with one isolate per patient except for a few possible overlaps during the turn of the year. Specific information about site of isolation and subgroup was available from the period 1967–91, representing 485734 strains. From 1970–91 information about the strength of phage reaction was also available. From 1978 isolates that were non typable at RTD and RTD × 100 were typed at RTD × 1000.

Phage typing

The method of Blair and Williams was used [5] with the 23 phages of the current international set. The phages could be used at concentrations of RTD, 100 × RTD and 1000 × RTD. Only isolates not typable at RTD were further typed at 100 × RTD, and if non-typable at 100 × RTD then typing at 1000 × RTD was performed. Typing with 1000 × RTD has thus been continued since 1978. Subdivision into phage groups was accomplished according to Parker [6].

There are four phages belonging to group II: 3A, 3C, 55 and 71. Based on phage types, strains of phage group II were divided into four subgroups as shown in Table 1.

Antibiotic susceptibility testing

The susceptibility of *S. aureus* to antibiotics was tested locally by an agar-diffusion technique using either Neosensitabs (Rosco diagnostica, Taastrup, Denmark) [7] or disk diffusion with [8, 9] or without [10] prediffusion. During the period 1964–91 results were available for 93·5% of the strains. These tests included penicillin and in most cases methicillin, streptomycin, gentamicin, erythromycin and tetracycline.

Occurrence of group II strains at different hospitals in Denmark

A more detailed survey of the spread of group II strains was conducted in the period 1976–91 by analysis of their occurrence in 15 larger hospitals. They accounted for 51·4% of all strains received for phage-typing during the years 1976–91.

Table 1. *The four subgroups of* S. aureus *belonging to the group II complex*

Designation in text	Phage-type combination
3A	3A only
71	71 only
71+	71+ any other phage reaction
Rest of group II	Any complex reaction excluding 71

Isolation sites in relation to the four subgroups of group II

Information about isolation sites was based on information from clinical departments.

Statistical analysis

The Chi-square test was used to evaluate the association between *S. aureus* group II and the anatomical site of isolation.

RESULTS

Occurrence of group II strains

Among the 567635 *S. aureus* strains phage-typed during the years 1961–91 the frequency of strains of group II was relatively constant until the year of 1983; since then it has increased (Fig. 1). In 1991 strains of group II accounted for 22·7% of all *S. aureus* strains isolated. Strains of phage group II are thus presently the most frequently occurring phage group. This increase in frequency was because of an increase in isolation of two of the subgroups: 71+ and 'rest of group II'. Frequency of strains lysed by 3A and 71 alone, remained constant. Today *S. aureus* strains of 71+ and 'rest of group II' account for 89·7% of all group II strains isolated.

Changes in lysis with different phage concentrations

Since 1980 a gradual increase in the proportion of *S. aureus* strains reacting with phages at RTD × 100 and not at RTD has occurred for all four subgroups. This was most pronounced for the 71+ and the rest of group II strains (Figs. 2, 3). The occurrence of *S. aureus* strains reacting at RTD × 1000 was nearly constant for strains of all four subgroups (data not shown).

Antimicrobial susceptibility

Multiresistant *S. aureus* were a problem in Denmark until 1975 (Fig. 4). The frequency of resistance among strains of the four group II strains to methicillin, streptomycin and gentamicin was unchanged (about 0·2%) during the observation period (data not shown). Each year only about 0·1–0·5% of these strains demonstrated resistance to methicillin and no difference was found among strains of the four subgroups (data not shown). Few strains were resistant to erythromycin: but a slight increase in frequency has been observed since 1986; in this respect strains of group II did not differ from strains of other phage types (Fig. 4). Penicillin resistance among all *S. aureus* strains has increased from 58·0 to 87·8% during the observation period (Fig. 5). *S. aureus* strains of the subgroups

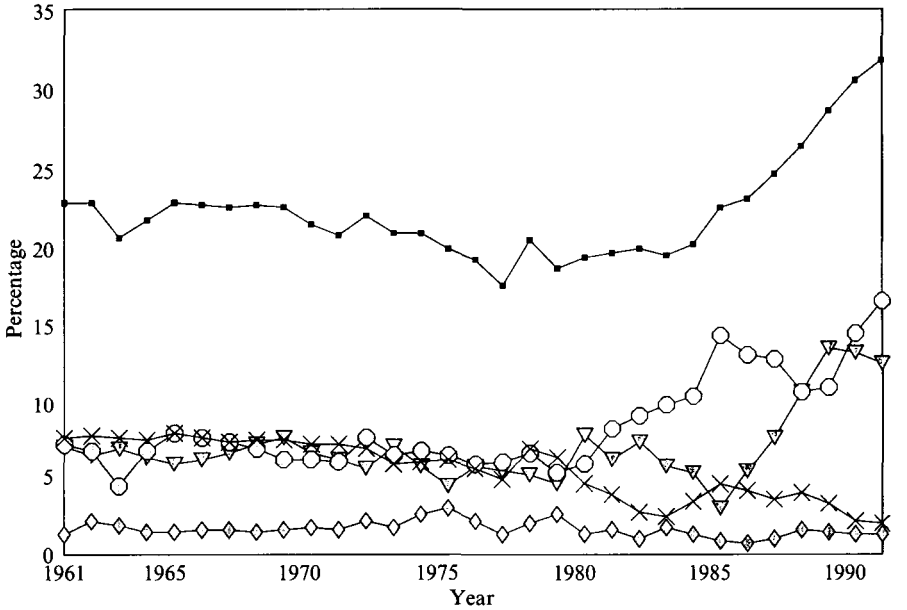

Fig. 1. Frequency of subgroups of the group II of *S. aureus* during the years 1961–91. Squares represent all group II strains; crosses, 3A; diamonds, 71; circles, 71+; and inverted triangles, rest of group II.

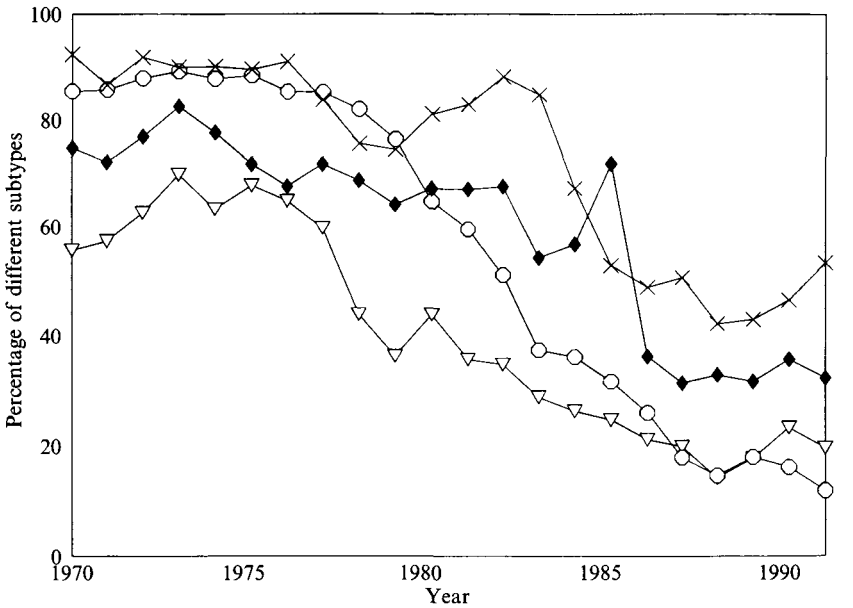

Fig. 2. Frequency of subgroups of the group II of *S. aureus* in relation to RTD during the years 1970–91. Crosses represent 3A; diamonds, 71; circles 71+; and inverted triangles, rest of group II.

71, 71+ and 'rest of group II' were more frequently sensitive to penicillin compared with all phage types at the beginning of the observation period; but since 1982 strains of 71+ and rest of group II have become more frequently resistant to penicillin that strains of other phage types while strains of the 71

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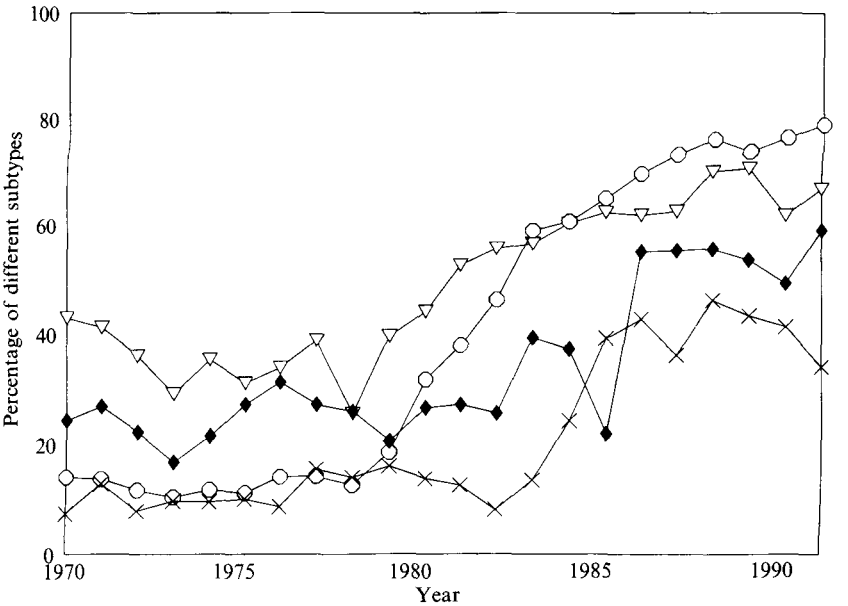

Fig. 3. Frequency of subgroups of the group II of *S. aureus* in relation to RTD × 100 during the years 1970–91. Crosses represent 3A; diamonds, 71; circles, 71+; and inverted triangles, rest of group II.

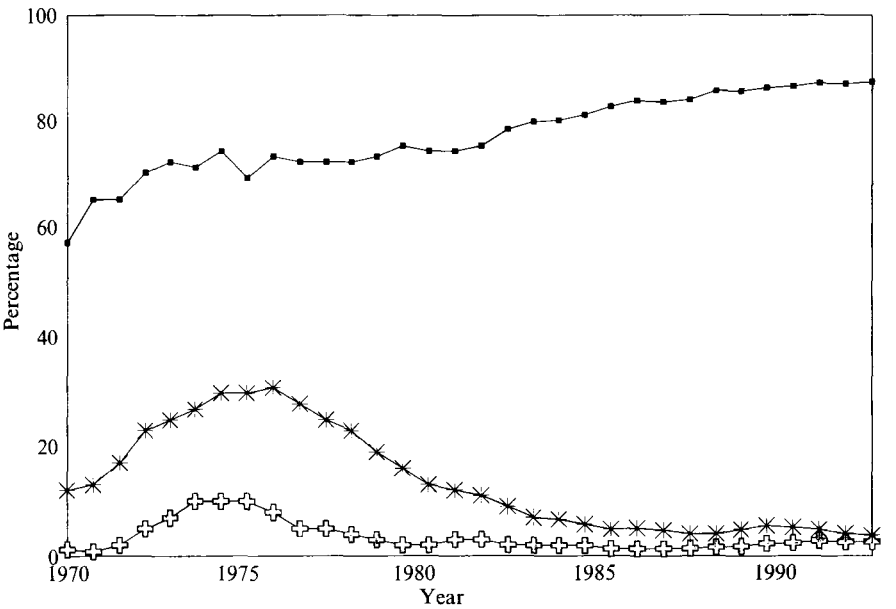

Fig. 4. Antimicrobial resistance among Danish *S. aureus* strains of all phage-type patterns to three different antibiotics during the years 1960–91. Squares represent resistance to penicillin; crosses, tetracycline; and diamonds, erythromycin.

typable subgroup remained more often sensitive. Strains of the 3A typable subgroup were close to the average for all strains (Fig. 5). The rapid increase in resistance to penicillin of strains of the four subgroups (Fig. 5) occurred simultaneously with the shift to non-typability at RTD (Figs 2, 3). In the period

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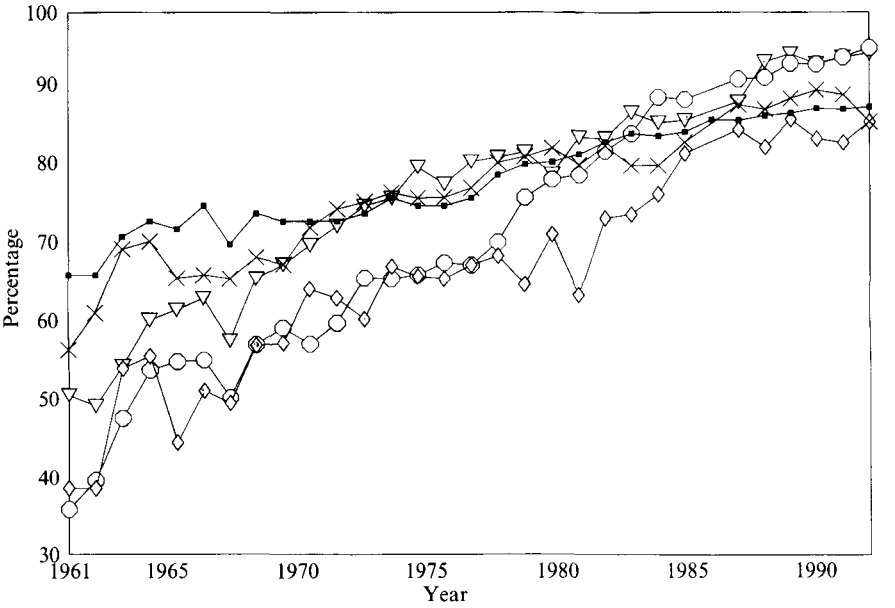

Fig. 5. Antimicrobial resistance to penicillin of the group II of *S. aureus* during the years 1961–91. Squares represent all strains: crosses represent 3A; diamonds, 71; circles, 71+; and inverted triangles, rest of group II.

Table 2. *Occurrence of resistance to penicillin among strains of the four subgroups of group II of* Staphylococcus aureus *since 1970 divided in strains lysed at RTD and RTD × 100*

Material (Total)	Percent of resistant strains				Net percent increase*
	1970–4	1975–9	1980–4	1985–91	
Whole of Denmark: all phage-types	74·3	78·3	83·6	86·9	14·4
All group II strains	70·3	76·0	83·9	92·0	27·7
3A at RTD (8339)	75·0	79·4	80·5	82·9	6·1
3A at RTD × 100 (2183)	70·6	82·5	84·3	94·4	28·6
71 at RTD (1915)	60·6	63·5	66·7	74·9	10·9
71 at RTD × 100 (1225)	76·6	75·4	84·7	91·3	17·7
71+ at RTD (8762)	61·9	69·6	75·6	81·9	34·9
71+ at RTD × 100 (11604)	72·1	84·0	94·5	95·4	30·5
Rest of group II at RTD	76·5	79·0	84·0	90·1	21·1
Rest of group II at RTD × 100	71·6	81·1	86·7	94·4	26·5

* Net percent increase: the rate of 1991 minus the rate of 1970.

1985–91 *S. aureus* strains of phage group II lysed at RTD were more often sensitive to penicillin while the strains lysed at RTD × 100 were more often resistant (Table 2). The increase in frequency of penicillin resistance was more pronounced for all group II *S. aureus* strains compared with strains of all phage types except strains of subgroup 3A and 71 lysed at RTD (Table 2).

Until 1977 strains of the four group II subgroups were usually (about 95%) sensitive to tetracycline (Fig. 6), independently of the different subgroups and

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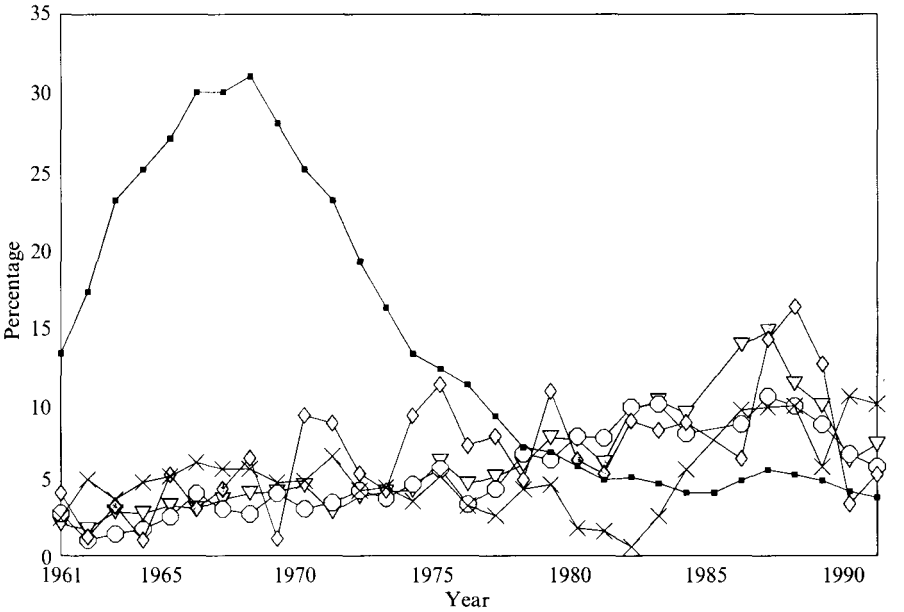

Fig. 6. Antimicrobial resistance to tetracycline of the group II of *S. aureus* during the years 1961–91. Squares represent all strains; crosses represent 3A; diamonds, 71; circles, 71+; and inverted triangles, rest of group II.

Table 3. *Occurrence of resistance to tetracycline among strains of the four subgroups of group II of* Staphylococcus aureus *since 1970 divided in strains lysed at RTD and RTD × 100*

Material (Total number of strains 1970–91)	Percent of resistant strains				Net percent increase*
	1970–4	1975–9	1980–4	1985–91	
Whole of Denmark: all phage-types (35140)	18·8	9·1	4·9	4·7	−21·2
All group II strains (3613)	4·4	5·4	7·4	9·4	2·0
3A at RTD (347)	4·7	3·1	1·3	3·2	−1·8
3A at RTD × 100 (269)	4·4	3·1	6·8	15·8	15·6
71 at RTD (202)	7·7	8·5	4·0	6·8	−3·4
71 at RTD × 100 (117)	5·5	8·1	10·7	12·2	−0·2
71+ at RTD (577)	3·9	5·4	5·5	6·6	4·7
71+ at RTD × 100 (835)	3·5	4·6	11·7	8·2	1·4
Rest of group II at RTD (394)	4·0	5·4	5·8	9·8	3·4
Rest of group II at RTD × 100 (696)	3·8	6·6	10·1	9·9	2·6

* Net percent increase: the rate of 1991 minus the rate of 1970.

typability at RTD (Table 3). The increase in tetracycline resistance in the sixties was caused by multiple-resistant strains of phage groups other than group II [1]. In the period 1985–91 strains of the four group II subgroups became more resistant to tetracycline as compared with all *S. aureus* isolates except subgroup of 3A lysed at RTD (Fig. 5 and Table 3). The group II strains lysed at RTD were more often sensitive to tetracycline than strains non-typable at RTD. These

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Table 4. *Occurrence of* Staphylococcus aureus *of group II in 15 different hospitals since 1976. The absolute numbers and percentage of all strains are stated for the period from 1976–91. For the year 1991 the occurrence of 71+ and rest of group II is stated*

	No. of strains (% occurrence)					
	All group II strains		71+		Rest of group II	
Area/hospital	1991 occurrence	Net percent increase*	1991 occurrence	Net percent increase	1991 occurrence	Net percent increase
Whole of Denmark	3642 (22·7)	9·2	1871 (11·6)	7·5	1397 (8·7)	4·7
Northern Jutland/'N'	70 (20·5)	6·7	38 (11·1)	9·6	29 (8·5)	4·8
Northern Jutland/'S'	117 (19·0)	8·4	58 (9·4)	6·7	53 (8·6)	4·8
Aarhus/'M'	167 (23·5)	11·8	92 (12·9)	9·7	55 (7·7)	4·3
Aarhus/'C'	57 (21·4)	6·2	25 (9·4)	3·9	27 (10·1)	4·9
Vejle	68 (22·5)	7·6	38 (12·6)	9·5	21 (7·0)	2·8
Odense	184 (31·5)	18·1	88 (15·0)	10·9	71 (12·9)	9·0
South Zealand	81 (23·1)	13·7	40 (11·4)	8·7	32 (9·1)	4·9
North Zealand	140 (23·0)	5·1	56 (9·2)	2·2	70 (11·5)	7·0
Great Copenhagen/'F'	110 (21·4)	8·6	49 (9·5)	6·1	50 (9·7)	6·1
Greater Copenhagen/'H'	320 (24·2)	13·2	143 (10·8)	7·5	130 (9·8)	7·0
Greater Copenhagen/'B'	223 (23·4)	11·2	133 (14·0)	10·3	76 (8·0)	4·7
Great Copenhagen/'R'	386 (26·2)	11·0	215 (14·6)	11·8	138 (9·9)	4·8
Greater Copenhagen/'L'	34 (18·0)	3·2	20 (8·2)	4·5	12 (6·3)	1·4
Greater Copenhagen/'G'	50 (17·4)	0·7	23 (9·5)	5·6	22 (9·1)	7·5
Greater Copenhagen/'S'	51 (21·0)	11·0	25 (8·7)	3·7	21 (7·3)	2·2

* Net percent increase: the rate of 1991 minus the rate of 1976.

particular group II strains were more frequently tetracycline resistant than *S. aureus* of other phage groups.

Occurrence of group II strains at different hospitals in Denmark

The frequency of strains of all group II strains increased in all 15 hospitals from 1976 to 91. The net increased varied between hospitals (Table 4). For strains of subgroup 3A the net decrease varied between 6·8 and 0·9% (average for the whole of Denmark 2·5%). For strains of subgroup 71 the net decrease varied between 1·5 to a net increase to 0·9% (average for the whole of Denmark a net decrease to 0·6%) (data not shown). For strains of subgroup 71+ the net increased varied

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Table 5. *Association of the four subgroups of the group II complex of* Staphylococcus aureus *with anatomical site of isolation during the years 1967–91*

Source	All strains (485734) Number of isolates	All strains (485734) Percent of all strains	Phage-type 3A (17188) Percent of source strains	Phage-type 71 (5540) Percent of source strains	Phage-type 71+ (29330) Percent of source strains	Phage-type 'rest of group II' (23427) Percent of source strains
Any site	485734	100·0	3·5	1·1	6·0	4·8
Sites:						
Sputum	45444	9·4	4·7	0·9	5·1	4·5
Wounds	42668	8·8	2·9	1·1	5·1	4·2
Cicatrices	40569	8·4	3·2	0·8	4·7	4·4
Urine	36069	7·4	3·8	0·6	3·3	3·8
Trachea	30343	6·2	4·1	0·8	6·1	4·7
Abscesses	30048	6·2	1·9	1·2	10·7	6·2
Hands	21436	4·4	3·0	1·5	8·4	5·6
Throat	19253	4·0	5·9	1·7	7·2	5·5
Ear	18779	3·9	4·8	1·4	5·7	5·3
Nose	14197	2·9	4·8	1·7	5·7	5·3
Blood	13642	2·8	3·0	0·8	5·8	4·7

Isolation sites with occurrence lower than 1% are not included.

between 2·2 and 11·8% (average for the whole of Denmark 7·5%). For strains of the rest of group II the net increase varied between 1·4 and 9·0% (average for the whole of Denmark 4·7%) (Table 4).

The increasing trend for the 71+ and 'rest of group II' was apparent in all 15 hospitals. Odense showed the highest net increase (Table 4).

Isolation sites in relation to the four subgroups of group II

The isolation frequencies of strains of the four subgroups from different anatomical sites in the period 1967–91 are shown in Table 5. The highest percentage of subgroup 3A strains was recorded for throat specimens and the lowest for abscesses; the observed differences were highly statistically significant ($P < 0{\cdot}00001$). The highest percentage of strains of subgroup 71 was for throat specimens and the lowest for urine specimens ($P < 0{\cdot}00001$). The highest rate for strains of subgroup 71+ was for abscesses, the lowest for urine ($P < 0{\cdot}00001$) and the highest percentage for strains of subgroup 'rest of group II' was for abscesses, the lowest for urine ($P < 0{\cdot}00001$). *S. aureus* strains from a few isolation sites showed some variation during the observation period. In the beginning of the observation period isolates from cicatrices and urine comprised less strains of subgroup 71+ and 'rest of group II' than isolates from other sites; but this difference has disappeared during recent years. During the first 20 years of the observation period strains of subgroup 71+ and 'rest of group II' were more frequent among abscess isolates than among isolates from other sites; but this difference has equalized during the last 5 years. Strains of type 3A were rarely isolated from abscesses especially during the first 15 years.

DISCUSSION

Nationwide centralized phage typing of *S. aureus* in Denmark over the last 30-odd years has enabled us to follow the dynamics of the staphylococcal population. Strains of group II have increased in frequency since 1984 [4] and are today the most frequent. This increase might be because of either a general rise in the number of all strains in this phage group, or a selected increase of one or a few clones.

The present investigation suggests that the latter explanation is true, as the increased frequency of group II strains is due to an increased number of strains of the two subgroups, 71+ and 'rest of group II' now comprising 89·7% of the group II. The most frequently isolated strains are those reacting with phages at 100 × RTD and not reacting at RTD, 66·8% of group II strains in 1991. Laboratories which perform typing at RTD only will thus be unable to detect the present rise in frequency of these strains, and a higher percentage of non-typable strains may be reported. A higher percentage of non-typable strains decreases discrimination of the typing methods. The increased number of strains of these two subgroups has occurred in hospitals all over Denmark, but with smaller local differences during the period.

The reason for this increase in strains of these two subgroups is a matter for speculation. Antibiotic resistance has previously been a major factor in the selection process. Strains of group II are today normally resistant to penicillin and are more likely to be tetracycline resistant than *S. aureus* strains of other phage

groups. Strains of the two increasing subgroups and especially those typed at 100 × RTD are today very frequently penicillin resistant, 95·4 and 94·4% respectively; strains of subgroup 3A typed at only RTD × 100 are also often penicillin resistant (94·4%). Selection of penicillin resistant strains may, therefore, not be the only mechanism for the increase of strains of the two subgroups. During the observation period the overall selection pressure by antibiotics has been constant in Denmark [11] but low even compared with the other Scandinavian countries [12, 13].

Penicillin resistance in *S. aureus* is nearly always mediated by beta-lactamase production. Group II strains have previously been shown to produce low levels of beta-lactamase and to have a chromosomally located gene [14, 15]. It might be of interest to compare the old and the more recent strains as well as strains of the different subgroups to see if they all have low chromosomally mediated beta-lactamase production, or if a high production, possibly mediated by acquired plasmids, has been introduced. The trend in tetracycline resistance apparently plays no role in the selection of the new dominating group II subgroups.

The increase in strains of subgroup 71+ and 'rest of group II' occurred at all isolation sites. The selection pressure with penicillin and tetracycline was the same at specific isolation sites during the period [16, 17]. The occurrence of the different subgroups at the different isolation sites seems to indicate that strains of the two new increasing subgroups are more able to produce abscesses than were the 'old' subgroups, whereas urinary tract infections are rarely caused by the 'new' subgroups. This might indicate that changes have occurred during the study period even within strains of the same subgroup, since it was observed that the two increasing subgroups rarely occurred in cicatrices early in the period, but have been isolated more frequently during recent years. The increased frequency of penicillin resistance in strains of all subgroups during the years may also indicate a gradual changes in the properties of subtypes of the group II strains. The increase of strains of certain subgroups of group II may be explained either by the selection of a few clones with special properties, or a gradual change in properties among some of the existing clones favouring their survival. The present investigations point to the last possibility, but further studies on the genetic properties, toxins and enzymes and colonization properties may elucidate this problem.

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