when an antisera to formalin-killed whole cell was included in the intermediate gel a very distinct specific antigen appeared. This was the only precipitate that stained with polysaccharide stain. Precipitates expressed in the presence of formalin-killed whole cell antisera are supposed to be located superficially, so this specific antigen can be presumed to be a specific capsular polysaccharide. When different staining techniques were applied to the gels, all precipitates stained with Coomassie blue, except the mentioned polysaccharide and a diffuse precipitate which was present in every test near the antigen well. Upon SDS-PAGE of sonicated antigens the pattern obtained with the five different serotypes indicated a high degree of homogeneity, the number of protein bands being greater than in the crossed immunoelectrophoresis plates.

Although further studies including larger numbers of strains are needed to substantiate these findings, the results presented here suggest that the protein antigens of pneumococci are not a suitable epidemiological marker.

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Heat Treatment to Increase Phage Typability of *Staphylococcus aureus*

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Heat treatment was used to reduce the number of *Staphylococcus aureus* strains that were not typable with the basic set of phages. All strains were phage typed according to the standard method after growth in broth at 37 °C or 48 °C. Forty-eight of 72 nontypable strains could be phage typed after heat treatment of the bacterial cultures. The page lysability increased with the higher incubation temperature of the broth, but the mean variability in the phage pattern of a strain was not significantly affected. The phage typing results of strains sampled over a period of several months were in accordance with the epidemiology, suggesting that phage typing after incubation at 48 °C is a stable and useful epidemiological tool.

The phage-typing pattern of *Staphylococcus aureus* is an important epidemiological marker. Phage typing has been successfully performed using an international set of phages for over 25 years (1, 2). In our hospital the nontypable strains among the more than 3,500 strains typed per year account for more than 20 %. This poses a problem when hospital-acquired infections are suspected. Several authors have reported alternative ways of analysing outbreaks with nontypable *Staphylococcus aureus* strains which involve reverse typing (3), toxin production, plasmid patterns and new phages (4, 5, 6). The aim of this investigation was to reduce the number of nontypable strains by treating them with heat and to investigate the reproducibility of such treatment.

Materials and Methods. Seventy-two strains of *Staphylococcus aureus,* nontypable by our standard routine testing, and 11 typable strains, all isolates from inpatients, were used. The staphylococci isolates were preserved in agar stab cultures and subcultured to a blood agar plate before phage typing. The strains were typed using Routine Test Dilution (RTD) and 100 X RTD of 25 phages in the current international basic set. The isolates were incubated in broth culture for 2 h at 37 °C. Strains that were nontypable with the standard method were incubated in broth culture for 1 1/2 h at 48 °C and retested at RTD and 100 X RTD. The bacterial cultures were incubated

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stationary in a water bath (optical density = 40-50 depending on the strain). There was no difference in OD in a strain after incubation at 37 °C or 48 °C. Thirty strains, which were nontypable at 37 °C, were retyped 6–8 times after incubation at 48 °C. Eleven typable control strains were retyped 4–6 times at 37 °C. A strain was considered typable if it gave at least 50 lysis plaques per inoculum (++ reaction) with at least one phage. Strains with a variation in the phage pattern of no more than two phages were considered to belong to the same parental strain.

Results and Discussion. Forty-eight of 72 strains of *Staphylococcus aureus* not typable at 37 °C with RTD or 100 X RTD could be typed after incubation at 48 °C. Eighteen of these strains were typable at 37 °C when retested. The variation in the phage pattern in the strains retyped 6–8 times at 48 °C was not larger than those retyped 4–6 times at 37 °C. Table 1 shows the variability in the phage pattern of two of the strains, one tested at 37 °C and one after incubation at 48 °C. The strains were sampled on one occasion and were not epidemiologically related.

The phage-typing results of nontypable strains isolated from the burns of two patients during 1 1/2 months in the Burns Department are shown in Table 2. The strains of each patient belonged to different basic patterns, thus a cross-infection in the ward could be ruled out.

Table 1: Variations in the phage pattern of two *Staphylococcus aureus* strains after incubation at different temperatures.

Phage type (100 Routine Test Dilution)	
Strain a, typable at 37 °C	Strain b, typable at 48 °C
1. 47/54/84/81	1. 52/80/53/75
2. 47	2. 47/75
3.[a] 47/53/54	3. 47
4.[b] 47/53/54/75/84/81	4. 47
	5. 47
	6. 52/80/47/53
	7. 80/47
	8. 47

[a] 53 at RTD
[b] 47/53 at RTD

The appearance of nontypable strains in an epidemiological situation is a problem. Nontypable strains are common in our hospital, and it cannot be assumed that they are derived from the same parental strain. Since heat treatment considerably increased phage susceptibility, we included it in our routine in order to identify several epidemics. This method has also been applied successfully by other authors (5). The reproducibility was acceptable when the same strain was retested. There was no increase in inhibition reactions after 48 °C incubation compared to 37 °C incubation.

These findings suggested that the phage pattern after incubation at 48 °C is as stable an epidemiological marker as after incubation at 37 °C. Alterations in the lipid content of the cell wall and/or protein A could account for the difference in phage susceptibility after different incubation temperatures. The lipid content has been shown to increase after growth at lower temperatures (28 °C) (7) and protein A is involved in the phage adsorption (8). The optimal incubation temperature giving the highest production of protein A in our strains has not yet been determined. Although it has been shown that lysogeny may also affect the phage typing pattern (9), it is not known whether this is responsible for variations in the typing pattern in the investigated strains.

In summary, heat treatment of nontypable strains isolated in epidemiological situations is a valuable method for increasing phage susceptibility.

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Table 2: Five nontypable (at 37 °C, RTD or 100 RTD) strains sampled from different body sites (wounds 1–3) in two patients during 1 1/2 months in the Burns Department, which were phage-typed simultaneously with the same test dilutions after 48 °C incubation.

Patient A		Patient B	
Sample site	Phage type (100 RTD)	Sample site	Phage type (100 RTD)
wound 1	29/52/79/6	nose	80/53/84/85
wound 2	29/ 52/79/6	perineum	80/53/84/85
wound 2	72/79/6	wound 2	85
wound 3	52	wound 1	85
wound 3	52/84	wound 3	80/53/85

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Possible Virulence Marker for *Streptococcus agalactiae* (Lancefield Group B)

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Mouse passage of a stock strain of each of the serotypes Ia, Ib, Ia/c, II and III of *Streptococcus agalactiae* was followed by increased virulence. The change was associated with an increased content of sialic acid and also with the appearance of an antigen common to all passaged strains. This antigen was subsequently detected in all but one of 12 strains isolated from infected babies or diabetic adults, but in none of 12 organisms recovered from carriers.

Group B streptococci (GBS) are subdivided into major serotypes (Ia, Ib, II and III) on the basis of the presence of antigens that contain terminal acid-labile sialic acid. Type specific protein antigens can also be demonstrated, one of which – designated C – is often present in strains of serotype Ia; these strains are referred to as representatives of serotype Ia/c (1). The type polysaccharides can be demonstrated in electron micrographs using ferritin-conjugated antisera as complete capsules, the size of which differs from strain to strain (2, 3). Virulence for mice is not uniformly present in GBS. The widely used reference strain of serotype Ia (090R) is virulent while the comparable strain of serotype III (D136C) is not (4). Among the same serotype, strains show differences in virulence (5), in adherence to epithelial cells (6) and in resistance to phagocytosis (7). In previous studies our group has reported an increase in sialic acid content and virulence, and a decrease in bioluminescence and phagocytosis after several mouse passages (8). We report here a concomitant change in type antigens which could be detected in immunodiffusion tests and suggest it could be related to virulence.

Materials and Methods. Lancefield's GBS reference strains of types Ia (090), Ib (H36B), Ia/c (A909), II (18RS21) and III (D136C) were kindly supplied by G. Colman, Public Health Laboratory Service, London, UK. From these reference strains virulent strains – designated Ia_t, Ib_t, Ia/c_t, II_t and III_t respectively – were obtained by serial passages in mice as previously reported (8). After 15 passages the sialic acid content and LD50 were determined. A total of twelve strains from healthy carriers (pregnant woman and healthy neonates) composed of types Ia (three strains), Ia/c (one strain), IIR (one strain), III (two strains), IIIR (one strain), and R (one strain) were tested for type antigens in immunodiffusion. Antigens were also extracted from 12 strains isolated from blood cultures or skin lesions in diabetic patients. They comprised serotypes Ia (four strains), Ia/c (four strains), and III/R (four strains). Representatives of type Ib or type II were not found among our isolates from clinical cases. Antisera against types Ia, Ib, Ia/c, II and III as well as antisera against the variants derived from the reference strains by mouse passage were prepared by intravenous inoculation of formalin killed cells (9). Antigens for immunodiffusion test were prepared by hydrochloric extraction (0.5 ml N/5 HCl, 1 h at 50 °C) of the cells from 50 ml cultures. Sialic acid content was determined by extraction of the acid from lyophilized bacterial cells using the method described by Shigeoka et al. (10). Reference strains were grown in Todd Hewitt broth for 18 h and serial dilutions of these cultures in the same medium were inoculated intraperitoneally into 12 g weight Swiss albino mice (8).

Results and Discussion. After 15 mouse passages stock strains showed an increase in virulence, this increase being greatest for the strain of serotype III (Table 1). This change was paralleled by an increase in the sialic acid content in all serotypes which was also maximal in serotype III (0.09–0.27 μmol/mg protein) (Figure 1).

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