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ORIGINAL PAPER

Clonal Analysis of *Staphylococcus aureus* Strains Isolated in Obstetric-Gynaecological Hospital

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Abstract

Epidemiological studies were carried out on 135 isolates of *Staphylococcus aureus* strains originating from medical staff, patients, and hospital environment. Restriction fragment length polymorphism (RFLP) analysis revealed genetic diversity of *S. aureus* isolates. Some clones were transmitted among nurses, doctors and patients. Our studies also demonstrate contamination of the hospital environment with *S. aureus* strains and there is a possibility that the patients acquire staphylococci from the environment. Moreover, we found that many medical staff workers were colonized with *S. aureus* and the transmission of these strains to patients is possible.

Key words: *Staphylococcus aureus*, epidemiological studies, RFLP analysis

Introduction

Staphylococcus aureus has been recognised as an important pathogen causing many infections such as septicemia, pneumonia, wound infections, septic arthritis, osteomyelitis and postsurgical toxic shock syndrome (Kloos and Bannerman, 1999). On the other hand, many healthy people are persistently or intermittently colonized with *S. aureus* at their anterior nares. Approximately 20% of individuals almost always carry one type of strain, 60% harbor *S. aureus* intermittently. About 20% of people almost never carry *S. aureus*. Colonization of human noses by *S. aureus* appears to play a role in the epidemiology and pathogenesis of infection (Kluytmans *et al.*, 1997). *S. aureus* is one of leading agents of nosocomial infections therefore for public health epidemiologists and clinicians involved in patient management of prime importance is understanding the dynamics of the spread and transmission the of bacteria within a hospital, this being crucial for their control and eradication. Molecular typing approaches have been used to a great advantage in identifying and monitoring the local and international spread of *S. aureus* strains (Štěpán *et al.*, 2004).

The aim of the present study was to evaluate the clonal composition of *Staphylococcus aureus* strains isolated from specimens taken from patients, medical staff and hospital environment at the Obstetric-Gynaecological Clinic Hospital in Poznań, Poland. Moreover, we wanted to elucidate the spread of clones in different departments and the possible transmission routes of these clones.

Experimental

Material and Methods

Bacterial strains. *S. aureus* strains were isolated from clinical specimens obtained from neonates and adult patients treated in different medical units in a hospital in Poznań. Several members of the healthcare staff were screened for *S. aureus* nasal, throat and hand carriage. Also specimens from medical equipment and hospital environment were taken. All strains were identified as *S. aureus* by analysis of cell morphology, Gram stain, and catalase production, using the latex coagulase test and the ID 32 Staph Kit (bioMérieux, France). Methicillin susceptibility test

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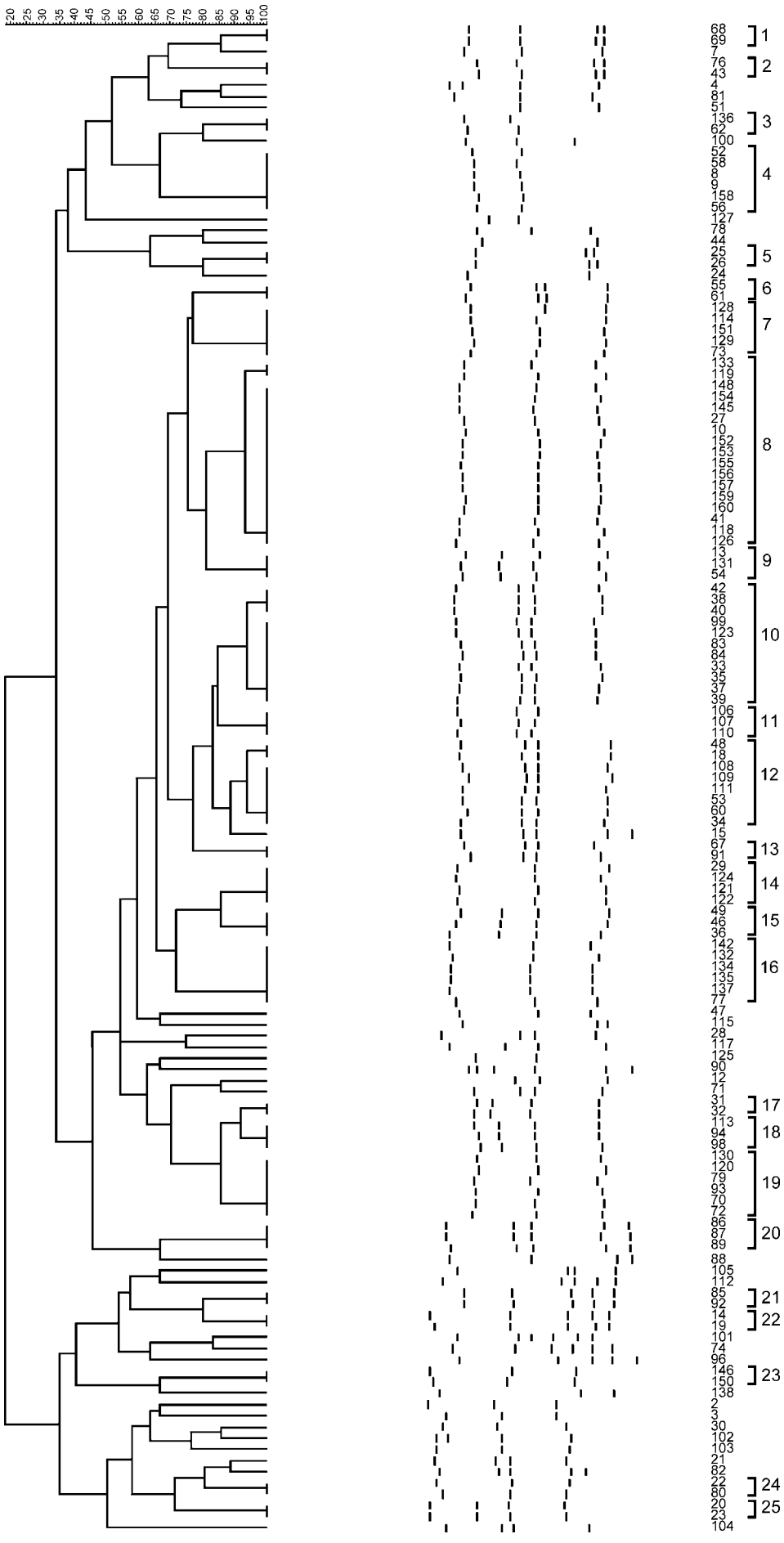

Fig. 1. Dendrogram showing genetic relatedness of 135 strains of *S. aureus* determined by analysis of RFLP fingerprint patterns using Dice similarity coefficient and UPGMA cluster method.

was determined by disc diffusion method and the results were interpreted in accordance to the criteria of the Clinical Laboratory Standards. The presence of the *mecA* gene was determined by PCR as described previously (Geha *et al.*, 1994).

RFLP analysis. Chromosomal DNA from the *S. aureus* strains were extracted according to methods described by Pitcher *et al.* (1989). A PCR was applied to simultaneously amplified part of the hypervariable region (HVR), a part of *spa* gene and a part of the *coa* gene based on primers established by Wichelhaus *et al.*, 2001. The PCR product was incubated overnight with 10 units of *Hae*II restriction enzyme (MBI Fermentas) at 37°C. The resulting fragments were separated in 1.5% agarose gel. The DNA in gels were documented with V.99 Bio-Print system (Vilber Lourmat, Torcy, France). A computer analysis was carried out using GelCompar II (version 3.0; Applied Maths, Kortrijk, Belgium) software. Similarity between fingerprints was calculated with the Dice coefficient. Cluster analysis was performed using the unweighted pair-group method with average linkages (UPGMA).

Results and Discussion

All strains included in this study were methicillin/oxacillin sensitive phenotypically and did not harbour the *mecA* gene. Our results demonstrated that many clones of *S. aureus* were coexisting in one hospital. This conclusion is based upon the results generated by clonal analysis of 135 strains of *S. aureus* (Fig. 1). We revealed 25 clusters at the 90% similarity level (Table I). Strains within these clusters were considered to be genetically related. We identified three major clones (cluster 8, 10, and 12), which included 28% strains of *S. aureus*. As shown in the dendrogram, there are five smaller clusters consisting from six to four strains. In addition, we found a considerable number of minor clusters, each harboring three or two strains. Minor clusters reached 36 strains (29%). Moreover, we identified 32 single strains with unique genotypes. The fact, that 25 clusters were identified next to 32 unique genotypes indicating a large genetic diversity among isolates of *S. aureus* obtained from the hospital. Similar result was obtained previously by Van Dijk *et al.*, 2002, who described genetic diversity among *S. aureus* isolates from a Dutch Teaching Hospital.

Although *S. aureus* has been described as the normal flora of nasal carriage, several epidemiological studies indicated that nasal carriage have increased risk for staphylococcal infections especially in specific group of patients (Archer and Climo 2001; Kooistra-Smid *et al.*, 2004; Melless *et al.*, 2004). *Staphylococcus* sp. infections are most commonly acquired from

Table I
Results of *S. aureus* clinical isolate typing by using RFLP analysis

Cluster	Strain No	Source of isolation
1	MPU S 68, 69	nasal swab of medical staff
2	MPU S 43	clothes of medical staff
	MPU S 76	throat swab of medical staff
3	MPU S 62, 136	throat swab of medical staff
4	MPU S 8, 9	abscess of neonates
	MPU S 52, 58, 56	nasal swab of medical staff
	MPU S 158	hand basin
5	MPU S 25	throat swab of medical staff
	MPU S 26	hands of medical staff
6	MPU S 55, 61	nasal swab of medical staff
7	MPU S 73, 128	throat swab of medical staff
	MPU S 114, 151	wound of patient
	MPU S 129	patient's bed
8	MPU S 10, 126, 133, 152, 155	throat swab of medical staff
	MPU S 27, 41	clothes of medical staff
	MPU S 118, 156, 119, 145, 148	nasal swab of medical staff
	MPU S 153, 160	patient's bed
	MPU S 154	hand basin
	MPU S 157	vagina of patient
	MPU S 159	throat swab of neonate
9	MPU S 13, 54	throat swab of medical staff
	MPU S 131	skin of neonate
10	MPU S 33, 39, 42, 83, 99	clothes of medical staff
	MPU S 35, 84	nasal swab of medical staff
	MPU S 37, 38	hands of medical staff
	MPU S 40	skin of neonate
	MPU S 123	scale in delivery room
11	MPU S 106	hand basin
	MPU S 107	abscess of patient
	MPU S 110	clothes of medical staff
12	MPU S 18, 48, 108	nasal swab of medical staff
	MPU S 34, 53	throat swab of medical staff
	MPU S 60	abscess of neonate
	MPU S 109	vagina of patient
	MPU S 111	hands of medical staff
13	MPU S 67, 91	patient's bed
14	MPU S 29	throat swab of medical staff
	MPU S 121	hand basin
	MPU S 122, 124	patient's bed
15	MPU S 36	clothes of medical staff
	MPU S 46, 49	skin of neonate
16	MPU S 77, 142	hand basin
	MPU S 132, 135	patient's bed
	MPU S 134	catheter of neonate
	MPU S 137	nasal swab of medical staff
17	MPU S 31	nasal swab of medical staff
	MPU S 32	hands of medical staff
18	MPU S 94	hand basin
	MPU S 98	patient's bed
	MPU S 113	vagina of patient
19	MPU S 70, 72, 130	nasal swabs of medical staff
	MPU S 79	hands of medical staff
	MPU S 93	patient's bed
	MPU S 120	scale

Table I continued

Cluster	Strain No	Source of isolation
20	MPU S 86, 87 MPU S 89	medical equipment nasal swab of medical staff
21	MPU S 85, 92	nasal swabs of medical staff
22	MPU S 14 MPU S 19	skin of neonate hands of medical staff
23	MPU S 146 MPU S 150	throat swab of medical staff wound of neonate
24	MPU S 22, 80	nasal swab of medical staff
25	MPU S 20 MPU S 23	nasal swab of medical staff conjunctive of neonate

patients' own flora, however patients may become infected from other healthy carriers. It is worthy to note that *S. aureus* carriers can contaminate their clothes and their surroundings through air, dust *etc.* It is well known that decolonization may reduce the risk of *S. aureus* infections in carries and prevent transmission to other patients. Recently, Gilpin *et al.* (2010) indicated that the standard decolonization protocol did not result in long-term clearance of MRSA carriage for most patients. In this study we found that many nurses and doctors harbored and/or were colonized with *S. aureus* strains. We also found strains of *S. aureus* on nurses' hands and clothes.

We found six clusters (4, 7, 8, 10, 11, 16) comprising strains isolated from patients, nurses and hospital environment. It is difficult to determine exactly where and to whom transmission of *S. aureus* occurred. It is probably, that the cross-transmission of *S. aureus* occurred via hands, which may be contaminated by contact with colonized or infected body sites of medical staff or colonised or infected patient or with devises. Nurses' and doctors' hands could be contaminated by strains existing in the hospital environment. For example, cluster 4 included strains of *S. aureus* isolated from two neonates, three nurses and one strain from environment. It is important to note that these neonates suffered from skin infection. We also identified clusters (9, 12, 22, 23) that included only strains isolated from patients and medical staff. For example cluster 22 included one strain isolated from skin of neonate and one isolate originated from nurse's hands working in delivery room. Therefore, we think that the medical staff could be considered an important vector in the chain of *S. aureus* transmission. Previously, it has been reported that strains isolated from nurses' hands could be regarded as the source of staphylococcal scaled skin syndrome (SSSS) of neonates in a maternity unit in Paris (Helali *et al.*, 2005). Similary, Bertin *et al.*, 2006, indicated that strains isolated from a healthcare worker suffering from otitis externa and carrries *Staphylococcus aureus* could be responsible for the outbreak of bloodstream infections in a neonatal intensive care unit. Hand hygiene has been recognized as the key to prevent transmission of *S. aureus* strains and to reduce the nosocomial infections. Sroka *et al.* (2010) indicated that the increasing consumption of hand antiseptics was associated with a significant reduction of *S. aureus* rate.

We isolated strains from medical equipment, patients' beds or hand wash basins. Previously, several authors also demonstrated that hospital equipment and environment could be the reservoirs of *S. aureus* (Ohara *et al.*, 1998; Embil *et al.*, 2001; Hardly *et al.*, 2006; Sexton *et al.*, 2006). In addition, it is noteworthy that staphylococci can persist in clinical areas for a long period of time (Sexton *et al.*, 2006). Recently, Aldeyab *et al.* (2009) indicated that environmental decontamination using detergents and hypochlorite was effective in eliminating MRSA strains from hospital environment. We identified many clusters, which grouped strains isolated from patients and hospital environment. This suggests that patients acquired *S. aureus* from the hospital environment. However, we can not exclude the possibility that patients contaminate their surrounding such as hospital beds, hand wash basins *etc.* We found cluster 10, which included strains isolated from neonate, from scales used to weigh neonates after birth in the delivery room. In this cluster we also found strains, which were derived from cultures originated from healthcare workers. It might be speculate that strains obtained from scales were *via* contaminated nurse's hands transformed on childish skin. It is well known that shared equipment in common places is an additional source of dissemination of *S. aureus.*

Our results illustrate the great genetic diversity among *S. aureus* strains in a hospital. This study also reveals contamination of hospital environment with *S. aureus* strains and the need for more effective cleaning of the hospital environment in order to eliminate reservoirs of these strains. We believed that cross-transmission events can be reduced by strict hand hygiene and other prevention procedures.

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