FEMS Microbiology Letters 95 (1992) 181–186

Published by Elsevier

FEMSLE 04995

Pulsed-field gel electrophoresis of genomic restriction fragments as a tool for the epidemiological analysis of *Staphylococcus aureus* and coagulase-negative staphylococci

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Received 20 May 1992
Accepted 26 May 1992

Key words: *Staphylococcus aureus*; Coagulase-negative staphylococci; Epidemiological analysis; Pulsed-field gel electrophoresis

1. SUMMARY

Thirteen *Staphylococcus aureus* and *S. epidermidis* strains obtained from nose and hand of two employees and one patient of a medical ward as well as two *S. hemolyticus* strains were analysed according to their restriction fragment length patterns (RFLP) by pulsed-field gel electrophoresis (PFGE) using the restriction enzymes *Sma*I and *Sst* II. Species identification of the isolates was performed by a system which includes 20 biochemical reactions. Furthermore, the antibiotic resistance patterns of the strains were determined. While several isolates exhibited identical antibiotic susceptibilities and biochemical profiles, differences in the RFLP were obtained. In three cases, *S. epidermidis* strains colonizing the skin showed an identical restriction profile as isolates from the mucous membranes of the same person. We concluded that the analysis of staphylococcal strains by PFGE is an important epidemiological tool with high discrimination power.

2. INTRODUCTION

Staphylococcus aureus belongs to the most common cause of nosocomial infections. In the last years, also coagulase-negative staphylococci (CNS) emerged as a significant pathogen in medical device-related infections and in immunocompromised patients [2,5,6,8–10,14,17,18]. For epidemiological studies, the discrimination power of commonly used typing systems involving biotyping, antibiogram, lysotyping and plasmid analysis is often unsatisfactory. Pulsed-field gel electrophoresis (PFGE) as a relatively new technique for analysing large DNA fragments [15,16] has recently proved to be useful for the genetic analysis and molecular epidemiology of Gram-negative [1,3,4,12] and Gram-positive [5,10,14] bacteria. A

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rapid approach to genome analysis of both *S. aureus* strains and CNS including *S. hemolyticus* by PFGE for epidemiological purposes is presented here.

3. MATERIALS AND METHODS

3.1. Staphylococcal strains

Strains of *S. aureus* and *S. epidermidis* were isolated by swabbing the anterior nares and the non-dominant hand of two employees (designated as A and B) and one patient (designated as C) of a medical ward at the Medizinische Poliklinik Würzburg. Swabbing of the nose of A was repeated 1 week later (Table 1). *S. hemolyticus* BP 135/91 was isolated from a blood culture at a medical ward of the Klinik für Innere Medizin der Humboldt-Universität Berlin; *S. hemolyticus* CCM 1798 represents a reference strain. Swabs were initially streaked out on whole blood agar (5% defibrinated human erythrocytes) and incubated overnight at 37°C. Staphylococci were identified by colonial appearance and Gram's stain. Single colonies were picked and subcultured on blood agar for 2 days at 37°C.

3.2. Typing of staphylococcal strains

S. aureus was identified by positive coagulase activity (rabbit plasma, BioMerieux, Nürtingen, FRG), DNase-test (Bacto DNase test agar, Difco, Detroit, MI) and detection of clumping factor by slide agglutination of fibrinogen-sensitized sheep erythrocytes (Staphyslide test, BioMerieux, Nürtingen, FRG). Species determination of coagulase-negative stahylococci was performed by using a numeric profile based upon 20 biochemical reactions according to the manufacturer's instructions (API-Staph, BioMerieux, Nürtingen, FRG). Antibiotic susceptibility was determined by standard disk diffusion method on Mueller-Hinton agar plates.

3.3. DNA preparation

DNA isolation was essentially performed according to the method of Patel et al. [13], Smith et al. [15,16] and Goering et al. [7] with some modifications. *S. aureus* strains were grown overnight at 37°C in 10 ml of LB medium (10.0 g/l caseinhydrolysate, 5.0 g/l yeast extract, 5.0 g/l sodium chloride). For CNS strains, LB medium was supplemented with 1% glycine. In case of *S. hemolyticus* strains ampicillin was added at sublethal dosage. Bacteria were harvested, washed twice in SE buffer (75 mM NaCl, 25 mM EDTA, pH 7.4) and resuspended in 1 ml SE buffer. In a small prewarmed tube, 0.5 ml of the cell suspension was quickly mixed with 0.5 ml of 2% LGT-agarose (Biometra), 10 μl lysostaphin (for CNS 30 μl from a 10 mg/ml stock solution lysostaphin, Sigma) and 2 μl RNase (10 mg/ml stock solution, Merck). After insert casting, agarose blocks were placed into a glass tube with 5 ml EC buffer (6 mM Tris · HCl, pH 7.6, 1 M NaCl, 100 mM EDTA, 0.5% Brij 58, 0.2% deoxycholate acid, 0.5% N-lauryl-sarcosyl) containing 10 mg/ml lysozyme (Merck) and incubated for 1 h at 37°C with gentle shaking. The solution was changed to 5 ml EC buffer supplemented with 1 mg/ml proteinase K (Merck) and inserts were incubated again for 2 h at 55°C in a waterbath with gentle shaking. After decanting the buffer, the agarose blocks were washed three times with 10 ml of TE buffer (10 mM Tris · HCl, 1 mM EDTA, pH 7.5) and stored at 4°C in TE until restriction endonuclease cleavage.

3.4. Restriction enzyme digestion and PFGE

For restriction enzyme cleavage, *Sma*I (Pharmacia, Freiburg, FRG) and *Sst*II (BRL, Neu Isenburg, FRG) were used. Agarose blocks containing the whole genomic DNA were equilibrated at 50°C for 2 h in 1 ml of restriction enzyme buffer. Cleavage was performed in 100 μl of fresh buffer with 20 units of the enzyme according to the manufacturers instructions. Electrophoresis was performed with the CHEF-DRII-System (Bio-Rad, Richmond, CA) in 1% agarose gels at 4°C in 0.5 × TBE buffer. Constant voltage of 200 V was applied with an increasing pulse time of 5–35 s over a period of 26 h. *S. aureus* NCTC 8325-4 digested with *Sma*I, lambda concatamers (Bio-Rad) and yeast chromosomes (*S. cerevisiae*, Bio-Rad) were used as DNA size markers. Gels were stained with ethidium bromide and photographed under UV light.

4. RESULTS

4.1. Biotyping and antibiotic susceptibilities

Isolation of staphylococci was performed as described above. Biotyping of the strains yielded four *S. aureus* strains, nine *S. epidermidis* strains and two strains of *S. hemolyticus* (Table 1). Antibiotic susceptibilities are also shown in Table 1.

4.2. Pulsed-field patterns of staphylococcal strains

Cleavage of isolated DNA with either *Sma*I or *Sst* II resulted in clear restricted enzyme patterns with about 10 recognizable fragments ranging in size from 45 to 550 kb (Fig. 1). Isolates with identical *Sma*I fragment patterns also showed identical *Sst* II profiles, whereas strains with differences in their *Sma*I restriction patterns also displayed various *Sst* II fragment profiles. Strains with a distinct biotype were also different in genome analysis. However, strains exhibiting identical biotypes also showed totally different restriction enzyme profiles in some cases (Table 1: strains 1, 2 vs. 7, 8; strains 3, 4 vs. 5 vs. 6 vs. 9, 10; strain). *S. epidermidis* isolates 3, 4, 5, 6 and *S. aureus* isolates 1, 2, 7, 8, respectively, showed identical biotypes and antibiotic susceptibilities. In microbiological routine practice these isolates would have been regarded in each case as the same strains. PFGE, however, revealed in case of the four *S. epidermidis* isolates not less than three and in case of *S. aureus* strains two different genotypes (Fig. 1). In addition, the two *S. hemolyticus* strains exhibited differences in their cleavage patterns. These data suggest that the

Table 1

Staphylococcal strains analysed

Strain no.	Species	Source	Biotype Api-No.	Restriction Pattern *Sma*I	*Sst* II	Antibiotic susceptibilities AMP	OX	CTM	CN	TE	SXT	CIP
1	*S. aureus*	A nose	6736153	I	a	+	+	+	+	+	+	+
2	*S. aureus*	A ' nose	6736153	I	a	+	+	+	+	+	+	+
3	*S. epidermidis*	A nose	6706113	II	b	+	+	+	+	+	+	+
4	*S. epidermidis*	A nose	6706113	II	b	+	+	+	+	+	+	+
5	*S. epidermidis*	A hand	6706113	III	c	+	+	+	+	+	+	+
6	*S. epidermidis*	A hand	6706113	IV	d	+	+	+	+	+	+	+
7	*S. aureus*	B hand	6736153	V	e	+	+	+	+	+	+	+
8	*S. aureus*	B nose	6736153	V	e	+	+	+	+	+	+	+
9	*S. epidermidis*	B hand	6706113	VI	f	+	+	+	−	−	−	−
10	*S. epidermidis*	B nose	6706113	VI	f	+	+	+	−	−	−	−
11	*S. epidermidis*	C hand	6706153	VII	g	−	−	−	−	−	−	−
12	*S. epidermidis*	C nose	6706153	VII	g	−	−	−	−	−	−	−
13	*S. epidermidis*	C nose	6706153	VII	g	−	−	−	−	−	−	−
14	*S. hemolyticus* CCM1798	reference strain	6636151	VIII	h	−	+	+	+	+	+	+
15	*S. hemolyticus* BP135/91	blood culture *	6636151	IX	i	−	−	−	−	+	−	−
M	*S. aureus* NCTC 8325-4	reference strain	X size marker									

A,B, employees; C, patient of a medical ward. AMP, ampicillin; OX, oxacillin; CTM, cefotiam; CN, gentamicin; TE, tetracycline; SXT, cotrimoxazole; CIP, ciprofloxacin.

* Blood culture isolate from a catheter sepsis obtained on a medical ward of the Klinik für Innere Medizin der Humboldt-Universität zu Berlin.

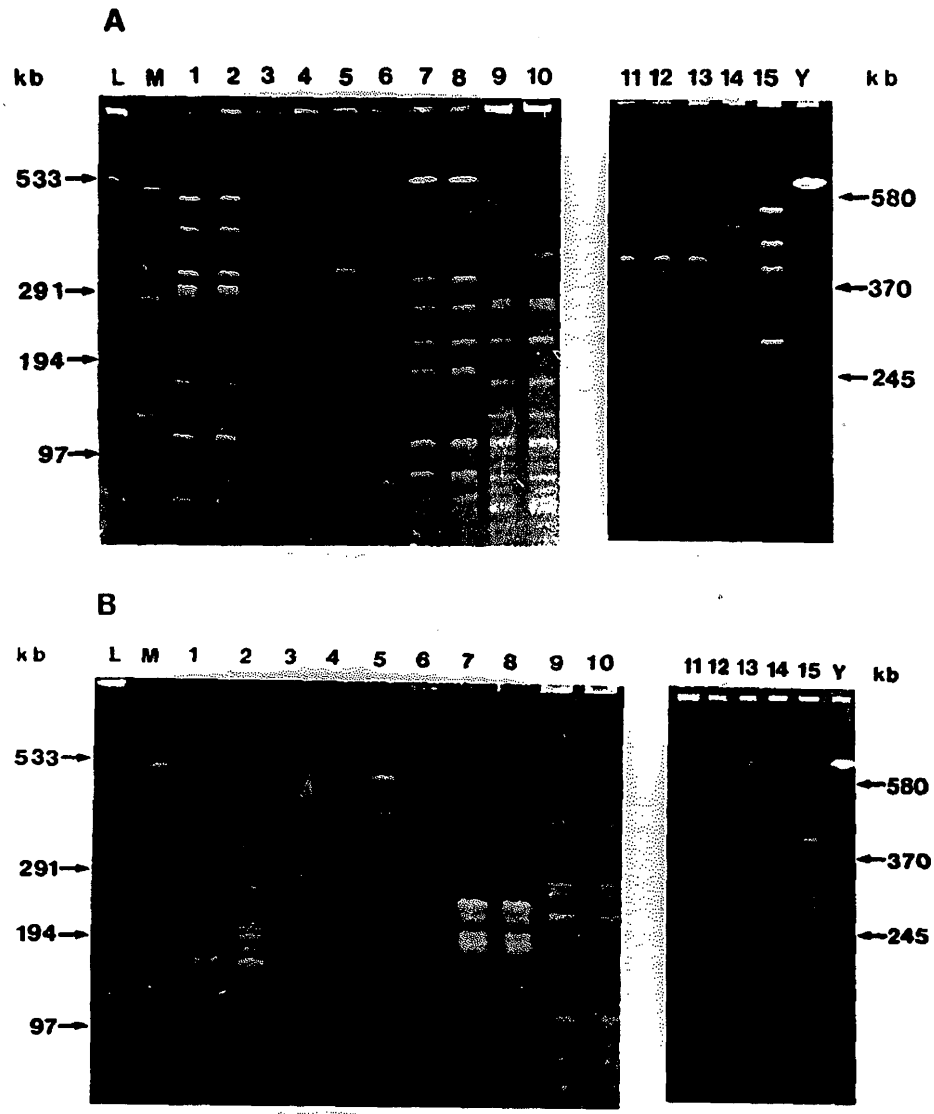

Fig. 1. Pulsed field gel electrophoresis of *Sma*I- (A) and *Sst* II- (B) cleaved genomic DNA of staphylococcal strains. Lanes 1–15, strains as listed in Table 1. L, lambda concatemers; M, *S. aureus* NCTC 8325-4; and Y, yeast chromosomes were used as size markers. DNA sizes are indicated.

discrimination power of DNA analysis by PFGE is clearly superior in comparison to other typing methods.

5. DISCUSSION

Staphylococci are widespread in nature. They are natural colonizers of the skin and mucous membranes of animals and man and produce a large variety of enzymes, toxins and extracellular substances [11]. For a long time, the coagulase-positive species *S. aureus* has been considered as the main pathogen for man causing skin infections and toxin-related diseases [11]. But in recent years, also coagulase-negative staphylococci (CNS) such as *S. epidermidis* and *S. hemolyticus* emerge in increasing numbers as etiological agents of nosocomial infections in oncological patients and on neonatal and intensive care units as well

as in medical device-related infections [2,5,6,8–10,14,17,18]. The accurate identification and discrimination of staphylococci is the precondition to follow routes of infections and to prevent further dissemination of possible pathogenic germs.

In the study presented here, 15 strains of *S. aureus* and CNS were isolated from different persons. The method of pulsed-field gel electrophoresis following cleavage of genomic DNA by rare restriction enzymes was used to identify individual strains among a group of isolates. In comparison to other typing methods such as biotyping or drug resistance patterns, PFGE proved to be more sensitive. In case of four *S. aureus* and four *S. epidermidis* isolates, identical antibiotic susceptibilities and biochemical profiles were obtained. These isolates would have been regarded as identical strains. Genome analysis by PFGE, however, yielded in case of *S. epidermidis* three and in case of *S. aureus* two different genotypes. Strains isolated from distinct persons always differed in their restriction enzyme profiles. In three cases, however, the *Sma*I and *Sst*II restriction fragment length pattern of isolates from nose swabs (Fig. 1, lanes 8, 10, 12, 13) were identical to the patterns obtained from skin isolates from the same person (lanes 7, 9, 11, respectively). This confirms the fact that staphylococci colonizing skin and mucous membranes are often of the same origin. In one person (A), swabbing of the nose was repeated 1 week later (A$^+$). The two strains of *S. aureus* isolated at these different opportunities showed an identical restriction enzyme pattern for both enzymes used (Fig. 1, lanes 1, 2), suggesting the persistence of *S. aureus* as a nasal colonizer and its genetic stability. The two *S. hemolyticus* strains analysed in this study exhibited cleavage patterns completely different to each other.

Our results, which are in agreement with recent data of different authors [5,7,10,14], showed that pulsed-field gel electrophoresis is a useful approach for epidemiological analysis of nosocomial infections and opens the way to finer molecular investigations of diseases cause by staphylococci.

ACKNOWLEDGEMENT

We thank Martina Schmittroth for excellent technical assistance. This work was supported by a DFG grant of the 'Graduiertenkolleg Infektiologie'.

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