Hospital strain colonization by *Staphylococcus epidermidis*

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The skin and mucous membranes of healthy subjects are colonized by strains of *Staphylococcus epidermidis* showing a high diversity of genomic DNA polymorphisms. Prolonged hospitalization and the use of invasive procedures promote changes in the microbiota with subsequent colonization by hospital strains. We report here a patient with prolonged hospitalization due to chronic pancreatitis who was treated with multiple antibiotics, invasive procedures and abdominal surgery. We studied the dynamics of skin colonization by *S. epidermidis* leading to the development of catheter-related infections and compared the genotypic profile of clinical and microbiota strains by pulsed field gel electrophoresis. During hospitalization, the normal *S. epidermidis* skin microbiota exhibiting a polymorphic genomic DNA profile was replaced with a hospital-acquired biofilm-producer *S. epidermidis* strain that subsequently caused repetitive catheter-related infections.

Key words: *Staphylococcus epidermidis*; Microbiota; Biofilm; Central venous catheter

Staphylococcus epidermidis colonizes the skin and mucous membranes and represents the main bacterium in the normal human microbiota (1). Over the last 20 years, *S. epidermidis* has been increasingly reported to be the causative agent of hospital infections, mainly associated with prosthetic devices such as central venous catheters (CVC) (2).

The pathogenesis of CVC colonization by *S. epidermidis* sequentially includes adhesion, persistence and later dissemination of *S. epidermidis* to the bloodstream (3). Biofilm formation is the main virulent factor resulting from the biosynthesis of polysaccharide intercellular adhesion, promoting cellular aggregation, biofilm accumulation (4-6) and offering a protective shield against the immune system and against antibiotic therapy (7).

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This report illustrates the importance of prolonged hospitalization, invasive procedures and exposure to multiple antibiotics in the change of the normal *S. epidermidis* skin/mucous microbiota to a highly adaptable hospital strain responsible for the development of catheter-related infections.

A 45-year-old male was hospitalized on October 21, 2001 at the University Hospital of UNICAMP, Brazil, due to chronic pancreatitis and pancreatic pseudocyst. On the 4th day of hospitalization (D4), parenteral nutrition was started through a peripheral vein and continued for 4 days. On D8, a CVC was inserted into the right subclavial vein. On D16, the patient presented fever and nocturnal sweating and the CVC was removed.

The catheter tip was cultured and two blood samples were collected simultaneously for culture. The microbiological study of the catheter tip, according to Maki's semiquantitative technique (8), and the blood cultures yielded positive results for *S. epidermidis* susceptible to multiple antibiotics, except penicillin-G (Table 1; D16). At that time, the microbiota of the patient's palms, axillae, site of CVC insertion, and nasal mucous membrane were rigorously examined for the presence of *S. epidermidis*. The *S. epidermidis* strains isolated from the skin/mucous microbiota and from the catheter tip were genotyped using pulsed field gel electrophoresis (9) and tested for biofilm production by the Congo red agar technique (10).

The automated Vitek® 2 system (BioMérieux Vitek, Inc., USA) was used to identify all strains and to test their

antibiotic susceptibility. The genotyping of the microbiota strains revealed *S. epidermidis* with polyclonal profiles susceptible to multiple antibiotics (Figure 1, lanes 2 to 6). The comparison of the DNA genomic profiles of *S. epidermidis* isolated from the CVC tip (Figure 1, lane 2) with microbiota strains (Figure 1, lanes 3 to 6) confirmed that the genomic profiles were not related.

On D25, parenteral nutrition was initiated via a CVC inserted into the left jugular vein. On D33, the patient presented fever and inflammatory signs at the site of catheter implantation and ceftazidime was started on D35. The CVC was removed on D37 and inserted into the right jugular vein, and parenteral nutrition was continued. The catheter tip was cultured and blood cultures were performed. The microbiological results revealed positive blood

Table 1. Minimum inhibitory concentration (MIC) to antimicrobial drugs of *Staphylococcus epidermidis* strains isolated from central vascular catheter tips during the patient's hospitalization.

	CIP	CLI	ERY	GEN	OXA	PEN	RIF	TET	SXT	VAN
Reference values										
Susceptible	≤1	≤0.5	≤0.5	≤4	≤0.25	≤0.12	≤1	≤4	≤2/38	≤4
Intermediate	2	1-2	1-4	8	–	–	2	8	-	8-16
Resistant	≥4	≥4	≥8	≥16	≥0.5	≥0.25	≥4	≥16	≥8/152	≥32
Day of hospitalization										
D16	0.5	0.5	0.5	2	2	16	1	1	10	2
D37	4	0.5	0.5	2	8	16	1	1	160	2
D54	4	0.5	0.5	16	8	16	1	1	160	2
D66	4	0.5	0.5	16	8	16	1	1	160	2
D97	4	0.5	0.5	16	8	16	1	1	160	2

Data are reported as MIC values in µg/mL. CIP = ciprofloxacin; CLI = clindamycin; ERY = erythromycin; GEN = gentamicin; OXA = oxacillin; PEN = penicillin-G; RIF = rifampin; TET = tetracycline; SXT = trimethoprim/sulfamethoxazole; VAN = vancomycin. Reference values according to the NCCLS M100-S12 (M7) (20).

Figure 1. Genomic DNA profiles of *Staphylococcus epidermidis* strains obtained using *Sma*I restriction endonuclease enzyme followed by pulsed field gel electrophoresis. *Lanes 1* and *15*, lambda ladder marker; D16: lane 2, isolate from catheter tip; *lanes 3 to 6*, microbiota; D37: lane 7, isolate from catheter tip; *lanes 8* to *10*, representative microbiota strains of predominant (16/18) profile A; D54: lane 11, catheter tip; *lanes 12* and *13*, representative microbiota strains of predominant (8/9) profile A; D66: lane 14, isolate from catheter tip. D16, D37, D54, and D66 indicate the days when the specimens were obtained.
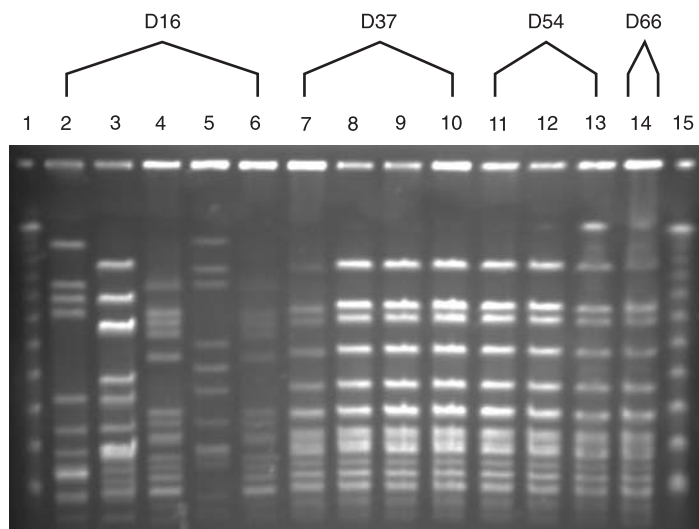
cultures for *Enterobacter cloacae*, and *S. epidermidis* was isolated from the CVC tip. At that time, a new *S. epidermidis* microbiota investigation was carried out, when 18 different *S. epidermidis* strains were collected. Genotyping revealed *S. epidermidis* strains with an oligoclonal profile, 16 of which were identical to the strain from the catheter tip (profile A) while two other strains had profile O1. Profile A produced biofilm and was resistant to multiple antibiotics, including oxacillin (Figure 1, lanes 8 to 10). The strain isolated from the catheter tip was genotypically and phenotypically identical to the microbiota strains (Figure 1, lane 6).

The patient had persistent fever until D54 when another blood culture was collected. The CVC was removed, the catheter tip was cultured and imipenem was administered for two consecutive days. However, it should be pointed out that ciprofloxacin had already been given for four consecutive days since D51. Blood cultures were positive for *E. cloacae* and the catheter tip was positive for a biofilm-producing and resistant strain of *S. epidermidis*.

A new CVC was inserted into the left jugular vein, and cefepime was added to the therapy and administered until D67. Another thorough search was performed in the patient's microbiota, and *S. epidermidis* was isolated in nine cultures from different sites, including the insertion area of the CVC. The predominant microbiota strain (8/9) and catheter tip *S. epidermidis* strains had identical genomic DNA profile A. On D66, the CVC was removed for technical reasons and the tip was cultured and resulted positive for *S. epidermidis* genotypically identical to the strains from previous episodes (D37 and D54; Figure 1, lane 13). On D71, the patient received ceftazidime and underwent surgical enucleation of the pancreas head and a pancreato-jejunal shunt. After surgery, the patient received levofloxacin. On D77, the patient had septic shock and the peritoneal fluid and surgical incision cultures were positive for *Pseudomonas aeruginosa* and *E. cloacae*. Vancomycin and imipenem were introduced. On D79, resuturing of the enteric-enteric anastomosis and laparoscopic trans-anastomotic jejunostomy were performed. The administration of vancomycin and imipenem was continued for 21 days. On D97, a new episode of *S. epidermidis* CVC infection was observed; however, for technical reasons, genotyping analysis was not performed. No new episodes of CVC infection were observed up to D145, when the patient was discharged.

Studies conducted on the dynamics of epidermal colonization by *S. epidermidis* prior to CVC-related infection have suggested that changes from a polyclonal, multiple antibiotic susceptible microbiota to an oligoclonal profile precede bloodstream infections caused by *S. epidermidis*

in hospitalized patients (11). In the present patient, the replacement of the normal skin/mucous microbiota with a hospital-acquired resistant strain of *S. epidermidis* was well documented by molecular typing.

The use of broad-spectrum antibiotics is the most important factor causing selective pressure on the normal microbiota. The present patient was on prolonged and multiple antibiotic therapy throughout the hospitalization period. Some antibiotics, such as ciprofloxacin, are excreted by the sudoriparous glands and may alter the skin microbiota, including *S. epidermidis* strains (12).

S. epidermidis strains isolated on the 16th day of hospitalization were susceptible to the tested antibiotic (Table 1), did not produce biofilm and had polymorphic genotype profiles. At that time, the patient had not received an antibiotic. A different profile was observed in the second episode: most of the microbiota strains collected on day 37 were genotypically identical and coincident with CVC strains, producing biofilm and exhibiting a profile of multi-resistance to antibiotics, although the antibiotic therapy was not specifically directed at *S. epidermidis*, but at *E. cloacae*, which was simultaneously isolated from the blood culture.

Although in that episode the catheter tip had more than 15 colonies, the clinical team considered that *S. epidermidis* worked as a colonizing microorganism rather than as an infectious agent. This shows some differences in the management of infection by the clinical team and in the criteria used by the Nosocomial Infection Committee for surveillance. This finding reflects the difficulty in defining the presence of *S. epidermidis* as colonization or infection, mainly when a second potential pathogen is present.

Although a patient's skin microbiota is frequently regarded as the main source of infection, the investigation of outbreaks associated with *S. epidermidis* has detected the presence of endemic strains within medical centers (13). During a period of 7 months we analyzed the genotypic profile of *S. epidermidis* of skin microbiota and clinical blood samples and/or CVC infection of several patients and the predominant genotypic profile, denominated A, was observed in 31.2% (77/247) of the strains analyzed (Blum-Menezes D, Bratfich OJ, Moretti ML, unpublished data). The present patient was part of this surveillance study.

The selective pressure of antibiotics on *S. epidermidis* from normal microbiota might have caused in our patient the colonization of skin/mucous membrane with a predominant hospital-acquired *S. epidermidis* strain, demonstrating a more resistant antibiotic profile. This biofilm-producing strain was well adapted to our hospital conditions, and was found to colonize and cause bloodstream

infections in other patients in our hospital, as cited above.

Biofilm-producing bacteria are highly resistant to antibiotics due to the biofilm environment, which represents a mechanical barrier against antibiotics. The expression of resistance mechanisms in biofilm-producing bacteria can be due to the "special lifestyle" present inside the biofilm, which is highly resistant to host defenses and to antibiotics (14). It is interesting to emphasize that, although the susceptibility to *in vitro* glycopeptides and to other antibiotic classes was demonstrated, the same clone colonized the patient for long periods of time. The discrepancy between *in vivo* biofilm-producing strains and therapeutic failure in the presence of an apparently favorable *in vitro* susceptibility, analyzed in planktonic conditions, has been discussed (15).

The presence of prosthetic devices, such as a CVC in our patient, represented an important risk factor for the development of CVC-related bloodstream infections (16). The multiple insertions of CVCs in our patient broke the integrity of the skin, therefore promoting the invasion of the endemic multiresistant biofilm-producing *S. epidermidis*, which colonized the skin/mucous membrane. Our patient also presented a systemic infection caused by *E. cloacae* at the same time. Recently, some reports have demon-

strated an association between *S. epidermidis* bloodstream infection and other microorganisms (17,18). The production of biofilm increases the resistance to antibiotics (19) and promotes a symbiotic relation among different microorganisms (18). In the present case, the constant presence of a biofilm-producing *S. epidermidis* may have created a favorable environment for the colonization and infection by other typical nosocomial microorganisms such as *E. cloacae* and *P. aeruginosa.* Therefore, we emphasize the importance of biofilm-producing strains as a reservoir for other typical pathogens, considering the recurrence of episodes of colonization by *S. epidermidis* and *E. cloacae.*

The present report clearly demonstrated the hospital acquisition of an endemic resistant strain of *S. epidermidis* and the subsequent steps of skin colonization directly related to repetitive CVC infections by the same strain.

Acknowledgments

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