Simultaneous PCR detection of ica cluster and methicillin and mupirocin resistance genes in catheter-isolated Staphylococcus

Summary. Recent data show that more than 50% of catheter-associated bloodstream infections are caused by staphylococci. Staphylococcal infections produced by intercellular-adhesion cluster (ica) carriers can be even more problematic due to the presence of methicillin and mupirocin resistance genes. In the present study, a multiplex PCR protocol that allows the simultaneous identification of staphylococci and detection of both the ica and methicillin and/or mupirocin resistance genes was developed. Furthermore, the method allows differential detection of the ica locus from Staphylococcus aureus and Staphylococcus epidermidis. [Int Microbiol 2004; 7(1):63–66]

Key words: Staphylococcus aureus · Staphylococcus epidermidis · intercellular adhesion gene cluster · antibiotic resistance · multiplex PCR

Introduction

A major achievement within the medical field in the twentieth century was the invention and development of prosthetic surgical implants. However, such implants frequently become infected by bacteria [7,18,23]. This is a serious complication, especially when the infection is caused by multiresistant bacteria, which are difficult to eradicate from the prosthetic material. Additionally, the efficiency of some bacteria in their ability to colonize indwelling medical devices, such as catheters [6,7], poses particular problems. These bacteria develop a highly consolidated structure: the biofilm [6,7,25]. In the biofilm, microbial populations reside within a matrix that facilitates cell-to-cell and/or cell-surface adherence, resulting in an inherent structural resistance towards antimicrobial agents, such as antibiotics, disinfectants, and germicides [7,8,19,24]. In the case of staphylococci, formation of the biofilm requires polysaccharidic intercellular adhesin (PIA), which is synthesized by enzymes encoded by the intercellular adhesion cluster (ica) [5,11,14].

Recent data show that 50% of pathogens isolated from
hospital-acquired bloodstream infections, normally associated with the use of central-venous catheters, are staphylococci [3,4,9]. Furthermore, many Staphylococcus aureus and Staphylococcus epidermidis strains carry the ica cluster. Staphylococcal infections produced by ica carriers can, in turn, be even more problematic due to the presence of methicillin and mupirocin resistance genes [10,15,20].

Nosocomial infections due to methicillin-resistant staphylococci have increased subsequent to the widespread use of β-lactam antibiotics. The transmission of resistant strains among hospital departments, and the ability of bacteria to transmit resistance genes are well-known [18,22]. With respect to mupirocin, several recent studies indicate the utility of topical mupirocin application in order to avoid the colonization of indwelling medical devices. While mupirocin is not an antibiotic for treatment of infections once they occur, it is used prophylactically to prevent S. aureus strains from reaching the inner part of the device. However, this application has led to the selection of highly mupirocin-resistant staphylococci [2,4,16]. For example, in our hospital, the incidence of mupirocin resistance has increased sharply from 7.7% in 1998 to 19% in 2000 [22].

Since most S. aureus are ica carriers, the colonization of devices by isolates harboring both resistance genes and adhesion genes has become a serious problem. Rapid detection of the ica locus in hospital staphylococcal isolates, together with simultaneous detection of antibiotic-resistance genes, may allow the development of methods to prevent or reduce the ability of bacteria to invade indwelling medical devices. It should be noted that the presence of the ica locus does not guarantee its expression; thus, it does not directly reflect biofilm formation. However, anticipating and detecting the possibility of biofilm colonization of catheters before it actually occurs would be of great help in preventing infection. For this reason, we developed a method to simultaneously detect ica and methicillin- and mupirocin-resistance markers. The method described here is a rapid and easy means to verify that catheters are not being colonized by mupirocin-resistant, methicillin-resistant staphylococci harboring the ica cluster. The presence within the hospital of these staphylococci would not only make the preventive use of mupirocin useless, it could also lead to an increased risk of infections.

antibiotic-resistance patterns were determined according to the standard laboratory criteria of the Microbiology Service of our hospital. S. aureus 229 and S. epidermidis 1855-25 and 9295-79 were methicillin and mupirocin resistant, whereas S. epidermidis 9295-79 was mupirocin and methicillin sensitive.

The DNA extraction method has been described previously [20,21]. After overnight culture on brain-heart infusion agar plates, one or two colonies (one from a S. aureus isolate and one from a S. epidermidis isolate) were suspended in 20 ml of sterile distilled water, and the suspension was then heated at 100°C for 20 min. From this suspension, a 5-ml aliquot was directly used as template for PCR amplification.

### Multiplex PCR amplification

The oligonucleotide primers have all been previously described [1,10,13,17,20]. icaAB: 5'-TTATCAATGCG-CCGAGTTGTGC-3' and icaAB-R: 5'-TTATCAATGCGAGTTGTGC-3' from partial icaAB genes of S. epidermidis, icaA-S: 5'-AAAATTGTGGCG- GTTACAGG-3' and icaA-E: 5'-TCTGGGCTGACACATGTGG-3' from partial icaAB genes of S. aureus, FemB1: 5'-TTACAGAGTTAACTGTT-TACC-3' and FemB2: 5'-ATACAAATTCAGCAGCCTC-3' from femB, MupA: 5'-TATATATTGCGAGTTATTG-3' and MupB: 5'- AATGAAACTCAGTGAAAAGTGTTG-3' from ileS-2 and MecA1: 5'- GTGAAATGATCGAAGCTCGAATA-3' and MecA2: 5'-CAGATCC- TACATTGTTCCGCTTAA-3' from mecA. Multiplex PCR assays were all carried out using the bacterial suspension obtained after the rapid DNA extraction method described above. A 5-ml aliquot of this suspension was added to 45 µl of PCR mixture consisting of 1× reaction buffer [16 mM (NH4)2SO4, 67 mM Tris-HCl (pH 8.8), 0.2 mM of each of the four dNTPs (Promega, Madison, WI, USA), 3.5 mM MgCl2, 3.6 µM of each femB primer, 0.2 µM of each ileS-2 primer, 0.1 µM of each mecA primer, and 0.8 µM of each icaAB primer and 1.25 U of Taq DNA polymerase (Bioline, UK). For each sample, one reaction was done with the femB pair of primers to identify S. aureus strains, with the mecA and ileS-2 pairs of primers to detect both resistance markers, and with the two icaAB pairs of primers to detect the ica cluster from S. aureus, S. epidermidis, or simultaneously from both. In order to reduce the formation of nonspecific extension products, a “hot-start” PCR protocol was used. All multiple PCR assays were carried out with a negative control containing all of the reagents without DNA template. DNA was amplified in a GeneAmp PCR system 9700 thermocycler (PE, Applied Biosystems, CA, USA) with the following thermal cycling profile: an initial denaturation step at 94°C for 5 min was followed by 10 cycles of amplification (denaturation at 94°C for 30 s, annealing at 66°C for 45 s, and extension at 72°C for 60 s); 5 cycles of amplification (denaturation at 94°C for 45 s, annealing at 64°C for 45 s, and extension at 72°C for 60 s); and 25 cycles of amplification (denaturation at 94°C for 45 s, annealing at 50°C for 45 s, and extension at 72°C for 60 s), ending with a final extension step at 72°C for 10 min. After PCR amplification, 5 µl were removed and subjected to electrophoresis to estimate the size of the amplification products by comparison with a 100-bp molecular size standard ladder (Roche Diagnostics, Mannheim, Germany). The gel was stained with ethidium bromide and the amplicons were visualized using a UV light.

The reaction conditions for the multiplex PCR assay were optimized to ensure that all of the target gene sequences were satisfactorily amplified. The primers used in this study differ in annealing temperatures, which increased the possibility of occurrence of unwanted bands originating from nonspeciﬁc ampliﬁcation. Therefore, both a “hot-start” and three rounds of amplification with different annealing temperatures were carried out. In addition, there is evidence indicating that multiplex PCR with targets that differ widely in size often favors amplification of the shorter target over the longer one, resulting in different amounts of ampliﬁed products [21]. For this reason, in order to optimize the conditions for the multiplex PCR analysis, different primer concentrations, template DNA preparations, and MgCl2 concentrations were assayed. As described above, the ﬁnal quantities generated optimal results were 3.5 mM MgCl2, 180 pmol of each femB primer, 10 pmol of each ileS-2 primer, 5 pmol of each mecA primer, 40 pmol of each icaAB primer and 5 µl of the DNA solution obtained with our DNA extraction method.

### Materials and methods

#### Bacterial isolates, identiﬁcation, susceptibility testing, and DNA extraction method

S. epidermidis strains 1855-25, 9295-79, and 9951-79 were isolated from catheters obtained from the Oncology Service of our hospital (Nuestra Señora de Candelaria University Hospital, Santa Cruz de Tenerife, Spain). S. aureus strain 229 was isolated from the sputum of a patient with sepsis. All strains were biochemically identiﬁed, and
Results and Discussion

Prior to optimizing the multiple PCR, the PCR protocol was evaluated in order to ensure that it was adequate for the individual amplification of all five DNA fragments. Each individual amplification yielded a fragment of the expected size, i.e. 750-, 651-, 546-, 456-, and 310-bp, respectively. Figure 1 shows an agarose gel that illustrates results typically obtained with the optimized PCR assay. Amplification of icaAB, femB, ileS-2 and mecA targets produced distinct, easily recognizable bands corresponding to their respective molecular size (lanes 3–7). The icaAB fragments were clearly differentiated when amplified from S. aureus or from S. epidermidis (lanes 8–10); femB was always amplified in the case of S. aureus strains (lane 9) and never in the case of S. epidermidis (lane 8). Results obtained for the mecA and ileS-2 fragments were in accordance with the resistance pattern of the isolates: methicillin- and mupirocin-resistant isolates S. epidermidis 1855-25 and S. aureus 229 presented the mecA fragment and showed the ileS-2 marker (lanes 8–10). PCR from mixed S. aureus 229 and S. epidermidis 9951-79 colonies permitted the simultaneous detection of the five different targets (lane 10).

This protocol, including the rapid extraction of DNA from single colonies and electrophoretic analysis of the amplified products on an agarose gel, was completed in less than 6 h. Moreover, it can be used for S. aureus and S. epidermidis and for a mixture of both staphylococci.

Nowadays, since few antibiotics, including mupirocin, constitute the last resort against MRSA, and due to the increasing incidence of catheter-associated staphylococcal bloodstream infections, a fast, sensitive laboratory method to immediately detect multiple-resistant staphylococci harboring the ica cluster is urgently needed. Although most S. aureus carry the ica cluster, confirmation of its presence in a particular isolate is a necessary step in preventing colonization by potentially biofilm-forming strains. The method described herein is highly sensitive and specific, fast and feasible, and thus provides a new tool for controlling catheter-borne infections.

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References

Detección simultánea del cluster *ica* y de los genes de resistencia a meticilina y mupiricina en *Staphylococcus aureus* aislados de catéteres

**Resumen.** Estudios recientes han demostrado que más del 50% de las septicemias asociadas al uso de catéteres están causadas por estafilococos. Las infecciones estafilocócicas producidas por cepas portadoras del operón de adhesión intercelular (*ica*) pueden agravarse por la presencia de genes de resistencia a la meticilina y/o a la mupiricina. Hemos desarrollado un protocolo de PCR múltiple que permite, simultáneamente, identificar estafilococos y detectar la presencia de *ica* y de los genes de resistencia a la meticilina y/o a la mupiricina. Además, dicho método permite la detección diferencial del locus *ica* de *Staphylococcus aureus* y/o *S. epidermidis*. [Int Microbiol 2004; 7(1):63–66]

**Palabras clave:** *Staphylococcus aureus* · *Staphylococcus epidermidis* · cluster génico de adhesión intercelular · resistencia a antibióticos · PCR múltiple

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**Resumo.** Estudos recentes têm demonstrado que mais de 50% das septicemias associadas ao uso de catéteres são causadas por estafilococos. As infecções estafilocócicas produzidas por cepas portadoras de operón de adesão intercelular (*ica*) podem agravarse pela presença de genes de resistência a meticilina e/ou a mupiricina. Foi desenvolvido um protocolo de PCR múltiplo que permite, simultaneamente, identificar estafilococos e detectar a presença de *ica* e dos genes de resistência a meticilina e/ou a mupiricina. Este método permite a detecção diferencial do locus *ica* de *Staphylococcus aureus* e/ou de *S. epidermidis*. [Int Microbiol 2004; 7(1):63–66]

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