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Identification of methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from burn patients by multiplex PCR

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ARTICLE INFO

Article history:
Accepted 25 August 2014

Keywords:
Methicillin resistance
Staphylococcus aureus
Multiplex PCR
Burn patients

ABSTRACT

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase-negative staphylococci (MRCoNS) as important human pathogens are causes of nosocomial infections worldwide. Burn patients are at a higher risk of local and systemic infections with these microorganisms.

Objective: A screening method for MRSA by using a multiplex polymerase chain reaction (PCR) targeting the 16S ribosomal RNA (rRNA), *mec*A, and *nuc* genes was developed. The aim of the present study was to investigate the potential of this PCR assay for the detection of MRSA strains in samples from burn patients.

Methods: During an 11-month period, 230 isolates (53.11%) of *Staphylococcus* spp. were collected from burn patients. The isolates were identified as *S. aureus* by using standard culture and biochemical tests. DNA was extracted from bacterial colonies and multiplex PCR was used to detect MRSA and MRCoNS strains.

Results: Of the staphylococci isolates, 149 (64.9%) were identified as *S. aureus* and 81 (35.21%) were described as CoNS. Among the latter, 51 (62.97%) were reported to be MRCoNS. From the total *S. aureus* isolates, 132 (88.6%) were detected as MRSA and 17 (11.4%) were methicillin-susceptible *S. aureus* (MSSA). The presence of the *mec*A gene in all isolates was confirmed by using multiplex PCR as a gold standard method.

Conclusion: This study presented a high MRSA rate in the region under investigation. The 16S rRNA–*mec*A–*nuc* multiplex PCR is a good tool for the rapid characterization of MRSA strains. This paper emphasizes the need for preventive measures and choosing effective antimicrobials against MRSA and MRCoNS infections in the burn units.

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http://dx.doi.org/10.1016/j.burns.2014.08.018
0305-4179/

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first reported in the 1960s, soon after the introduction of the penicillinase-resistant β-lactam antibiotic, methicillin [1,2]. Today, MRSA is a common cause of severe nosocomial infections worldwide, and is spread by hand and airborne particles, and via environmental routes [3,4]. The burn wound is particularly susceptible to bacterial colonization and infection, due to the physical disruption of the normal skin barrier and the accompanying reduction in cell-mediated immunity [5]. Therefore, patients with extensive burns are especially susceptible to infection with MRSA [1,5]. Colonization with MRSA increases the risk of bacteremia, septicemia, and serious clinical complications including the loss of skin grafts in burn patients [3,4].

In recent years, the percentage of patients with proven microbiological MRSA infection in industrial countries has increased dramatically [5,6]. The fact that staphylococci can survive intracellularly in polymorphonuclear leukocytes (PMNs) is a further problem. Additionally, the bactericidal function of PMNs is decreased in patients with burns, allowing the bacteria to survive longer [3,5]. As methicillin resistance is mediated by the penicillin-binding protein (PBP2a) encoded by the *mecA* gene, which is often heterogeneously expressed in staphylococci, detection of the *mecA* gene by polymerase chain reaction (PCR) is ''gold standard'' for the detection of methicillin resistance [7]. In the case of severe infections, it is clinically useful to provide rapid identification and antimicrobial susceptibility test results. Several multiplex PCR techniques have been described for the characterization of staphylococci in recent years [7,8]. Sequence analysis of the 16S ribosomal RNA (rRNA) gene has been widely used to identify bacterial species. Bacterial 16S rRNA genes generally contain nine ''hypervariable regions'' that demonstrate considerable sequence diversity among different bacterial species and can be used for species identification [9,10].

This study describes a PCR assay that targets three genes: *mecA*, a determinant of methicillin resistance; *nuc*, which encodes the *S. aureus*-specific region of the thermonuclease gene; and a genus-specific 16S rRNA sequence used as an internal amplification control for staphylococcal DNA. The aim of the present study was to investigate the potential of this PCR assay for the detection of MRSA strains in samples from burn patients.

2. Materials and methods

A total of 433 samples were collected from burn patients, admitted to the Taleghani Burn Hospital, Ahvaz, Iran, during an 11-month period, from January to November 2013. Each sample was collected from the individual patients. The types of samples were blood, urine, deep wound, abscess, and endotracheal secretion. The samples were cultured on appropriate culture media including blood agar, mannitol salt agar, and MacConkey agar (Merck, Darmstadt, Germany), and the grown colonies were examined conventionally for the detection of *S. aureus* strains according to colony and microscopic morphology; catalase, coagulase, and DNase tests; and novobiocin sensitivity [11].

2.1. Antimicrobial susceptibility testing

2.1.1. Cefoxitin and oxacillin disk diffusion method

The Kirby–Bauer disk diffusion method as recommended by clinical and laboratory standards institute (CLSI) using cefoxitin (30 μg) and oxacillin (1 μg) disks (Mast Co., UK) was performed [12]. The standard bacterial suspension of staphylococci with turbidity equal to 0.5 McFarland was inoculated on Mueller–Hinton agar (MHA) (Merck, Darmstadt, Germany) with the subsequent application of an oxacillin disk. The plates were incubated at 35° C for 24 h and the inhibition zone around the disk was then measured. The results were evaluated according to the interpretive criteria of CLSI. The inhibition zones for the oxacillin (1 μg) disk with diameters $\leq$ 17 mm for coagulase-negative staphylococci (CoNS) and $\leq$10 mm for *S. aureus* were considered to be resistant. Moreover, all isolates were tested by the disk diffusion method for resistance to cefoxitin (30 μg), to distinguish MRSA from MSSA. According to the CLSI guideline, for *S. aureus*, inhibition-zone diameters of $\leq$19 mm and $\geq$20 mm were considered to be resistant and susceptible, respectively, and a zone diameter of $\leq$ 24 was considered to be resistant for CoNS.

2.1.2. Epsilometer test

The Epsilometer test (E-test) was performed for quantitative antimicrobial susceptibility testing, whereby a preformed antimicrobial gradient from a plastic-coated strip (Liofilchem, Italy) diffuses into an agar medium inoculated with the test organism [13]. A standard bacterial suspension equal to 0.5 McFarland inoculated on MHA plates, E-test strips were placed on the medium surface and incubated at 35° C for 24 h. The E-test minimal inhibitory concentration (MIC) results were read where the edge of the inhibition ellipse intersects the MIC scale on the strip. According to the manufacturer's instructions and CLSI, [12] the MIC break points for defining interpretative MRSA and methicillin-resistant CoNS (MRCoNS) were 4 and 0.5 μg/mL, respectively.

2.1.3. S. aureus antibiotic susceptibility pattern

The susceptibility patterns of *S. aureus* isolates to the following antibiotics were measured by using the protocol described in the previous Section (2.1.1.):amikacin (30 μg), azithromycin (15 μg), ciprofloxacin (5 μg), trimethoprim–sulfamethoxazole (25 μg), doxycycline (30 μg), gentamicin (10 μg), linezolid (30 μg), ofloxacin (5 μg), rifampicin (5 μg), teicoplanin (30 μg), tigecycline (15 μg), vancomycin (30 μg), tobramycin (10 μg), and quinupristin/dalfopristin (15 μg) (Mast Co., Bootle, UK). *S. aureus* ATCC 25923 was used as the reference strain for quality-control purpose.

2.1.4. Oxacillin–salt agar screening

This screening test was used for confirmation of the presence of MRSA. The test was performed according to CLSI guidelines [12]. For each isolate, 1 μl of standard 0.5 McFarland suspension was streaked on an MHA plate supplemented with 4% NaCl and 6 μg of oxacillin per mL. The plates were incubated in