Journal of Global Antimicrobial Resistance 4 (2016) 16–20

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Correlation of mupirocin resistance with biofilm production in methicillin-resistant *Staphylococcus aureus* from surgical site infections in a tertiary centre, Egypt

Ghada I. Barakat *, Yasmin M. Nabil

Medical Microbiology and Immunology Department, Faculty of Medicine, Mansoura University, Gomhoria Street, Mansoura 35516, Egypt

ARTICLE INFO

Article history:
Received 21 April 2015
Received in revised form 20 November 2015
Accepted 27 November 2015
Available online 8 January 2016

Keywords:
Mupirocin
Biofilm
MRSA

ABSTRACT

The aim of this study was to detect mupirocin-resistant isolates from pus/wound swabs taken postoperatively in a tertiary centre in Egypt and to determine their ability to form biofilm in order to establish its correlation with mupirocin resistance. This was a prospective study including 513 pus/wound swabs from patients suffering from postoperative surgical site infections over the period July 2013–January 2015. Samples were cultured and isolates were identified by coagulase activity, DNase test, mannitol fermentation by mannitol salt agar followed by API Staph 32. Oxacillin agar screen test, agar dilution test for mupirocin, and *mupA* gene detection by PCR were performed for all methicillin-resistant *Staphylococcus aureus* (MRSA) isolates. Biofilm detection was carried out by the microtitre plate and Congo red agar methods. Of the 161 *S. aureus* isolates identified, 73 (45.3%) were MRSA, among which 82.2% were mupirocin-susceptible and 17.8% were mupirocin-resistant. Among the resistant isolates, 38.5% showed low-level resistance and 61.5% were high-level mupirocin-resistant. The *mupA* gene was detected in 75.0% of high-level mupirocin-resistant strains and in none of the low-level mupirocin-resistant strains. Among the mupirocin-susceptible isolates, 95.0% were biofilm-producers and 5.0% did not produce biofilm. All mupirocin-resistant isolates produced biofilm. Moreover, 15.3% of high-level mupirocin-resistant strains were negative for the *mupA* gene but showed evidence of biofilm formation. In conclusion, biofilm formation may be suggested to play a role in mupirocin resistance besides the presence of a genetic element encoding abnormal isoleucyl-tRNA synthetase, however further studies are needed to confirm these findings.

© 2016 International Society for Chemotherapy of Infection and Cancer. Published by Elsevier Ltd. All rights reserved.

1. Introduction

One of the most serious problems worldwide is the treatment of infections caused by antibiotic-resistant bacteria, especially methicillin-resistant *Staphylococcus aureus* (MRSA) [1]. MRSA strains can spread readily in hospitals from colonised or infected persons [2]. Mupirocin is an important topical antibiotic for skin and soft-tissue infections as well as for the eradication of MRSA carriage. It acts through binding specifically to the bacterial isoleucyl-tRNA synthetase enzyme and inhibits protein synthesis [3]. With the increased use of mupirocin, low- and high-level resistance has been reported during treatment with nasal mupirocin [4].

Three categories of mupirocin susceptibility have been described for *S. aureus*. These categories are mupirocin susceptibility with minimum inhibitory concentrations (MICs) of 4 μg/mL, low-level mupirocin resistance with MICs of 8–256 μg/mL and high-level mupirocin resistance with MICs of 512 μg/mL. Isolates with MICs of 128 μg/mL or 256 μg/mL are uncommon. Most isolates that demonstrate high-level mupirocin resistance have the acquired plasmid-mediated *mupA* gene that encodes a novel isoleucyl-tRNA synthetase [5]. Isolates with low-level mupirocin resistance usually have mutations in the native isoleucyl-tRNA synthetase gene *ileS*. Isolates that are *mupA*-positive by PCR but are mupirocin-susceptible have also been reported. This has been attributed to a frameshift mutation in the *mupA* gene that inactivates the gene product [6]. Isolates with this frameshift mutation revert to the wild-type sequence and show restoration of high-level mupirocin resistance at a high frequency ($>10^6$). Few isolates have been identified that demonstrate high-level

* Corresponding author. Tel.: +20 10 0058 2546.
E-mail address: Barakat.ghada@yahoo.com (G.I. Barakat).

http://dx.doi.org/10.1016/j.jgar.2015.11.010
2213-7165/© 2016 International Society for Chemotherapy of Infection and Cancer. Published by Elsevier Ltd. All rights reserved.

mupirocin resistance but are *mupA*-negative by PCR despite the use of multiple primer sets (A. Simor, personal communication). These isolates may carry a novel mechanism of mupirocin resistance. The *mupA* gene is typically located on mobile genetic elements, which facilitates the dissemination of this resistance mechanism. The *mupA* gene is plasmid-mediated and some of these plasmids are conjugative [7]. Insertion sequences have been identified flanking the *mupA* gene in plasmids, which might facilitate movement of the *mupA* gene between plasmids by recombination [8].

Biofilm formation is another mechanism of antibiotic resistance and is one of the virulence factors of MRSA. Biofilm formation causes treatment failure as well as chronic and recurrent staphylococcal infections especially in burn patients [9]. Biofilm acts as a barrier to antimicrobial agents and the host immune system, which assists sustained bacterial colonisation. Biofilms are organised communities of bacterial cells that are accumulated in a polymeric matrix produced by bacteria. Biofilm can be attached to living or inanimate surfaces [10]. It has been demonstrated that attachment of staphylococci to surfaces such as host tissues is the first stage of infection [11]. Molecular studies have shown that during the late stages of attachment, organisms attach to each other to form biofilms. This is done through polysaccharide intercellular adhesin (PIA), which is synthesised by products of the *icaABCD* operon [12]. The aim of this study was to determine the prevalence of the *mupA* gene in MRSA isolated from surgical site infections as well as the prevalence of biofilm formation among them to detect the resistance patterns among these isolates in Mansoura University Hospitals (Mansoura, Egypt).

2. Materials and methods

2.1. Bacterial isolation

A total of 513 pus/wound swabs obtained from patients admitted to Mansoura University Hospitals showing evidence of postoperative surgical site infections were included in this study. The samples were collected over a period of 18 months from July 2013 to January 2015. There were no outbreaks. Further patient evaluation consisted of clinical examination, reason for operative interference, and current signs and symptoms. Samples were obtained from patients with no known history of mupirocin prophylaxis or use even in patients with mupirocin-resistant strains. Mupirocin is not commonly used according to the antibiotic protocols in Mansoura University Hospitals.

Samples were sent immediately to the Medical Diagnostic and Infection Control Unit. Samples were inoculated onto Columbia blood agar and were incubated for 24 h at 37 °C.

Staphylococci were identified by colony morphology, haemolysis patterns, Gram staining, catalase activity, coagulase activity, DNase test, mannitol fermentation on mannitol salt agar followed by API Staph 32 (bioMérieux, Marcy-l'Étoile, France). Tests were carried out according to the manufacturers' instructions and the results were interpreted using the appropriate reference indices recommended by the manufacturer.

2.2. Detection of MRSA strains

Detection of MRSA strains was done by disk diffusion method according to the current Clinical and Laboratory Standards Institute (CLSI) guidelines with a 5 μg oxacillin disk (Oxoid Ltd., Basingstoke, UK) on Mueller–Hinton agar with incubation at 30 °C for 18–24 h. Isolates growing within 14 mm around the oxacillin disk were regarded as oxacillin-resistant and the results were confirmed by Etest performed according to the manufacturer's recommendations (AB bioMérieux, Marcy-l'Étoile, France) and interpreted based on the CLSI breakpoints.

Confirmation of MRSA was done by oxacillin agar screen test. Mueller–Hinton agar containing 2% NaCl and 6 μg/mL oxacillin was prepared. *S. aureus* suspensions were prepared equivalent to a 0.5 McFarland standard and were cultured on oxacillin salt agar medium incubated for 24 h at 30 °C. Growth inhibition zones were interpreted using CLSI guidelines. If any growth was detected, the isolate was considered MRSA [13].

2.3. Detection of mupirocin-resistant strains

Detection of mupirocin-resistant strains by agar dilution tests followed the guidelines provided by the CLSI. Serial two-fold dilutions of mupirocin (Oxoid Ltd.) were prepared in Muller–Hinton agar (Difco Laboratories, Detroit, MI). Strains were subcultured on tryptic soy agar (Difco Laboratories) and were then suspended in tryptic soy broth (Difco Laboratories) and adjusted to the turbidity of a 0.5 McFarland standard. The suspension was then diluted 1:10 and was inoculated on each plate coated with antibiotic-containing medium using a Steers replicator. The inoculated plates were incubated at 35 °C for 24 h. The lowest concentration of antibiotic that inhibited visible growth of an organism was considered the MIC, ignoring the growth of one isolate. Mupirocin resistance showed two phenotypes: low-level resistance (MIC usually between 8 μg/mL and 256 μg/mL); and high-level resistance (MIC $\geq$ 512 μg/mL) [14].

2.4. Detection of plasmid-mediated mupA gene by PCR

Mupirocin-resistant strains were selected and plasmid DNA was extracted using a QIAGEN Plasmid Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol where pure plasmid DNA is eluted in high-salt buffer and the DNA is concentrated and desalted by isopropanol precipitation and collected by centrifugation.

The primer pairs used for detection of the *mupA* gene were a forward primer with the sequence GGG CCT TAA TTT CGG ATA GTG CTC and a reverse primer with the sequence TAA TCT GGC TGC GGA AGT GAA ATC [15], which yielded a fragment of 456 bp using a thermal cycler (Perkin-Elmer, Norwalk, CT). The conditions used for amplification were: 95 °C for 15 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 68 °C for 90 s and extension at 72 °C for 90 s; and a final extension at 72 °C for 10 min [15]. The amplified products were run on a 2% agarose gel for 20 min at 200 V and were visualised with ethidium bromide.

2.5. Measuring biofilm production by MRSA strains

2.5.1. Microtitre plate method

Biofilm producing ability was measured by determination of adhesion to polystyrene microtitre plates according to the method described by Christensen et al. [15]. 96-well flat-bottomed microtitre plates (Greiner Bio-One, Frickenhausen, Germany) were filled with 100 μL of trypticase soy broth (TSB) with a bacterial suspension prepared from blood agar and adjusted to the opacity of a 0.5 McFarland standard. Negative control wells contained TSB only. The plates were incubated at 37 °C for 18 h followed by several washings with phosphate-buffered saline (pH 7.3). All tests were performed in triplicate. Sodium acetate (2%) was added as fixative and was then decanted and the wells were stained with crystal violet (0.1% w/v). The plates were then washed under running tap water, air-dried and read at 570 nm using a spectrophotometer (Tecan Austria GmbH, Salzburg, Austria). According to the original method, isolates with an optical density at 570 nm (OD_{570}) of $\leq$0.120 were considered as weak biofilm-producers/non-biofilm-producers,

Download English Version:

https://daneshyari.com/en/article/3405619

Download Persian Version:

https://daneshyari.com/article/3405619

Daneshyari.com