Interpretive criteria to differentiate low- and high-level mupirocin resistance in *Staphylococcus aureus*

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Meticillin-resistant *Staphylococcus aureus* isolates were classified into three mupirocin susceptibility groups by the disc diffusion method using 5 and 200 µg mupirocin discs. The zone diameter observed for a 5 µg disc distinguished Mup^S from the resistant strains (either Mup^{RL} or Mup^{RH}). On the other hand, a 200 µg disc distinguished the high-resistance Mup^{RH} strains from the other two (Mup^S or Mup^{RL}). Thus, the concomitant use of 5 and 200 µg mupirocin discs allowed the clear distinction among the three mupirocin susceptibility groups, Mup^S, Mup^{RL} or Mup^{RH}.

INTRODUCTION

Mupirocin is an effective antibiotic for the elimination of methicillin-resistant *Staphylococcus aureus* (MRSA) that colonize the nasopharynx. It has been used to control the spread of these micro-organisms among patients during outbreaks (Eltringham, 1997). Mupirocin-resistant strains are grouped into two distinct categories: low level (Mup^{RL}), with MICs of 8–256 µg ml⁻¹, and high level (Mup^{RH}), with MICs ≥ 512 µg ml⁻¹ (Eltringham, 1997). Susceptible strains are defined as those with a MIC ≤ 4 µg, showing zone diameters of ≥ 14 mm around 5 µg mupirocin discs (Finlay *et al.*, 1997; Fuchs *et al.*, 1990). Strains presenting diameters < 14 mm are considered to be mupirocin resistant (either Mup^{RL} or Mup^{RH}). High-level mupirocin resistance has been associated with failure to clear the organism from patients. However, it has been suggested that Mup^{RL} nasal isolates can still be controlled with mupirocin therapy, as the ointment used contains a much higher mupirocin concentration (20 000 µg ml⁻¹) than the Mup^{RL} MICs (Hudson, 1994). The dilution method is considered the 'gold standard' for the determination of mupirocin-resistance levels. We have recently shown that the mupirocin Etest (AB Biodisk) with tetrazolium reduction is more accurate, thus bringing the Etest closer to the gold standard agar dilution method for determining the mupirocin MICs for *S. aureus* strains (Mondino *et al.*, 2003). However, both agar dilution and Etest are too expensive and laborious for routine application. Clinical laboratories are able to differentiate Mup^S strains using the 5 µg disc, but the resistant strains can only be distinguished empirically, as Mup^{RH} isolates show heavy growth around the 5 µg

mupirocin disc, whereas Mup^{RL} isolates produced hazy zones of inhibition (Deshpande *et al.*, 2002).

At present, there are no criteria for the disc diffusion method to differentiate between low- and high-level mupirocin resistance. Therefore, we propose the concomitant use of 5 and 200 µg mupirocin discs to distinguish clearly the three susceptibility groups of mupirocin-resistant *S. aureus* strains.

METHODS

Strains and culture conditions. We selected 45 MRSA isolates previously classified by the agar dilution method (Santos *et al.*, 1999), as susceptible (13 Mup^S) or low-level (16 Mup^{RL}) and high-level (16 Mup^{RH}) mupirocin-resistant strains. The strains were isolated from patients attending the Teaching Hospital of the Federal University of Rio de Janeiro, Brazil. PFGE showed that the isolates belonged to eight different clones or subclones (Santos *et al.*, 1999). In addition, susceptible ATCC 25923 and Mup^{RH} HU1A (Bastos *et al.*, 1999) control strains were analysed. PCR analysis revealed that only the Mup^{RH} strains possessed the *mupA* gene (Nunes *et al.*, 1999). In the second part of the study, we validated the proposed classification criteria by analysing 79 nosocomial *S. aureus* strains.

Disc diffusion susceptibility tests. Disc diffusion tests were carried out according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2006). Plates containing Mueller–Hinton broth (Difco) were swabbed in three directions with 0.5 McFarland inocula and 6 mm discs containing 5 or 200 µg mupirocin (Oxoid) were applied. Following incubation at 35 °C for 18 h, the diameters of the inhibition zones were determined in mm. The results of mupirocin susceptibility testing related to the 5 µg disc were interpreted as described by Fuchs *et al.* (1990). All strains were retested using Mueller–Hinton broth purchased from bioMérieux.

Abbreviation: MRSA, methicillin-resistant *Staphylococcus aureus*.

Detection of the *mupA* gene by PCR. All the strains were analysed by PCR for the presence of the *mupA* gene, also known as *ileS-2*, responsible for high-level mupirocin resistance (Nunes *et al.*, 1999).

Scatterplot. The results were compared by scatterplot analysis. The criteria established by Fuchs *et al.* (1990) were employed for the 5 µg mupirocin disc.

RESULTS

Criteria to differentiate resistant from susceptible mupirocin strains are well established using the disc diffusion method with the 5 µg disc. However, this breakpoint does not differentiate between low-level (Mup^{RL}) and high-level (Mup^{RH}) resistant *S. aureus* strains. Therefore, we decided to verify the use of the 5 µg disc concomitant with the 200 µg mupirocin disc on 45 well-characterized MRSA strains to attempt to distinguish Mup^{RL} from Mup^{RH} mupirocin-resistant strains. The inhibition zones around the 5 and 200 µg mupirocin discs allowed us to classify the strains into the three mupirocin susceptibility groups (Mup^S , Mup^{RL} and Mup^{RH}). No significant differences in the size of the inhibition zones were observed when culture medium from a different company was used.

The disc diffusion results using the 5 and 200 µg mupirocin discs were plotted on a scatterplot (Fig. 1). As previously shown, the scatterplot revealed that the 5 µg disc distinguished two groups, susceptible and resistant strains, when a zone size breakpoint of ≥ 14 mm was used. However, the 5 µg disc was unable to differentiate the two mupirocin-resistant subgroups, which are classified according to their MICs (Mup^{RL} 8–256 µg ml⁻¹, Mup^{RH} ≥ 512 µg ml⁻¹). The scatterplot for the 200 µg disc showed that, using a zone diameter breakpoint of ≥ 14 mm, the strains could be differentiated as belonging to either Mup^{RH} or Mup^{RL}/Mup^S (Fig. 1). The latter included the low-level resistant Mup^{RL} and the susceptible Mup^S strains, which could not be distinguished clearly using the 200 µg disc. Therefore, we propose concomitant use of the 5 and 200 µg mupirocin discs to differentiate the three categories of mupirocin susceptibility presented by *S. aureus* strains (Table 1). Our results suggest that the absence of a zone for the 5 µg disc indicates resistance (Mup^{RL} or Mup^{RH}) to mupirocin; the absence of a zone with the 200 µg disc indicates high-level mupirocin resistance. The absence of a zone with the 5 µg disc and an inhibition zone (≥ 14 mm) around the 200 µg disc are characteristic for Mup^{RL} , as shown in Table 1.

For validation of these criteria, 79 nosocomial *S. aureus* strains were tested using our method, resulting in 72 Mup^S , 3 Mup^{RL} and 4 Mup^{RH} strains. PCR detected the *mupA* gene only in the four Mup^{RH} strains.

DISCUSSION

The need for antimicrobial susceptibility testing of *S. aureus* isolates is becoming more urgent. *S. aureus* is the most frequently isolated pathogen from nosocomial

Fig. 1. Scatterplots of mupirocin MICs comparing the inhibitory zone diameters for 5 (a) and 200 µg (b) discs, obtained for 45 MRSA isolates. The vertical broken lines represent the zone diameter breakpoints. For the 5 µg disc, a breakpoint of ≥ 14 mm has been suggested by Fuchs *et al.* (1990). A proposed breakpoint zone (≥ 14 mm) was also used for the 200 µg disc.

infections and, due to an increased number of infections caused by MRSA strains, chemotherapy has become difficult (Lowy, 2003). Mupirocin ointment has been used in nasal decolonization to control MRSA outbreaks, but mupirocin resistance has been observed. The ease of use of the disc diffusion susceptibility test makes it adequate for routine use. This methodology employing a 5 µg mupirocin disc has been established to differentiate resistant from susceptible strains (Finlay *et al.*, 1997; Fuchs *et al.*, 1990). However, the 5 µg mupirocin disc does not differentiate resistant Mup^{RL} and Mup^{RH} strains. In addition, Palepou *et al.* (1998) stated that the use of either a 5 or a

Table 1. Criteria proposed to determine the categories of mupirocin susceptibility by the disc diffusion method

Inhibition zone diameter (mm)		Interpretive criteria
5 µg disc	200 µg disc	
≥ 14	≥ 14	Mup^S
—	≥ 14	Mup^{RL}
—	—	Mup^{RH}

—, Absence of inhibition zone.

200 µg disc alone did not reliably distinguish Mup^{RL} from Mup^{RH} strains. Therefore, the purpose of this study was to evaluate the concomitant use of 5 and 200 µg mupirocin discs to distinguish the three mupirocin susceptibility categories of *S. aureus* isolates.

We analysed a total of 124 *S. aureus* isolates and found 20 Mup^{RH}, 19 Mup^{RL} and 85 Mup^S. We established the criteria using 45 *S. aureus* strains (16 Mup^{RH}, 16 Mup^{RL} and 13 Mup^S) that had been well characterized by the agar dilution method and PFGE (Santos *et al.*, 1999), PCR (Nunes *et al.*, 1999) and Etest (Mondino *et al.*, 2003). We validated the criteria by analysing another 79 *S. aureus* nosocomial strains. Additional studies should be performed on coagulase-negative *Staphylococcus* strains in order to establish the criteria required to determine mupirocin susceptibility in this group of micro-organisms.

Clearance of *S. aureus* nasal colonization can reduce the subsequent risk of development of infection by MRSA, in addition to reducing the spread of these micro-organisms. Therefore, the criteria established in this study could help to identify *S. aureus* strains with low-level mupirocin resistance in a fast and feasible way. It could be used as an important epidemiological tool in hospitals using large amounts of mupirocin, to guide the possible need for more rigid infection-control measures.

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