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Journal of Microbiological Methods 71 (2007) 186–190
Journal of Microbiological Methods
www.elsevier.com/locate/jmicmeth

Quantitative disk diffusion as a convenient method for determining minimum inhibitory concentrations of oxacillin for staphylococci strains

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Received 3 July 2007; accepted 20 August 2007
Available online 5 September 2007

Abstract

In this study the susceptibility of 58 coagulase-negative staphylococci (CoNS) strains and 58 *Staphylococcus aureus* strains to oxacillin was evaluated by a novel method called quantitative disk diffusion (DD) method. The results obtained were compared to phenotypic methods as agar dilution (AD) for oxacillin, disk diffusion (DD) for cefoxitin, and related to the presence of the *mecA* gene detected by PCR. Minimum inhibitory concentrations (MIC) determined by the quantitative DD method were equivalent to MICs determined in the AD method for *S. aureus* (Student's *t* test, $p=0.99$) and CoNS (Student's *t* test, $p=0.97$). Incongruent results between PCR *mecA* gene determinations and the quantitative DD method were obtained in 8 strains (5 *S. aureus* and 3 CoNS) where the *mecA* gene expression was blocked. However, oxacillin resistance was detected by the proposed method even in staphylococci strains showing low-level or heterogeneous resistance to the antibiotic while other phenotypic methods failed. The single quantitative DD method is not expensive, it can be performed in any laboratory and permits accurate identification of oxacillin resistant staphylococci.

Keywords: Staphylococci; Oxacillin; *mecA* gene; Antimicrobial resistance; Cefoxitin

1. Introduction

Detection of oxacillin resistance in staphylococci strains is problematic despite the guidelines published by the Clinical Laboratory and Standards Institute (CLSI, 2007). The polymerase chain reaction (PCR), described as a gold standard method detects the *mecA* gene (Berger-Bächi and Rohrer, 2002) which codifies a penicillin binding protein (PBP2a) with low-level affinity to β-lactams (Chambers, 1997). However, since many laboratories are not equipped for molecular methodology, in recent years many studies were directed to the development of other standardized susceptibility testing methods (Sakoulas et al., 2001; Ferreira et al., 2003; Caieirão et al., 2004; Ghoshal et al., 2004; Velasco et al., 2005). These tests are attractive due to simplicity, reproducibility, and lack of requirements for specialized equipment.

Recently published results from our laboratory showed that association of the cefoxitin disk diffusion (DD) and agar dilution (AD) methods for determining oxacillin minimum inhibitory concentrations (MICs) was the best approach to detect antibiotic resistance in staphylococci (Palazzo and Darini, 2006). However, MIC determinations by AD are very laborious and the Etest, that could be a substitute, is very expensive. Thus, we are now proposing a single quantitative DD method for accurately determining oxacillin resistance in staphylococci.

2. Materials and methods

2.1. Strain collection

Staphylococci strains analyzed in this study are part of a laboratory collection stored at the Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Brazil, which includes 58 *S. aureus* and 58 coagulase-negative

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doi:10.1016/j.mimet.2007.08.014

Table 1
Estimated MIC values based on the quantitative DD method

Strains	Inhibition zone diameters (mm) in disks containing oxacillin in mg/mL												
	256	128	64	32	16	8	4	2	1	0.5	0.25	0.125	MIC value (mg/mL)[a]
S. epidermidis	15	14	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	≥ 256
S. epidermidis	22	20	18	17	15	13	10	NZ	NZ	NZ	NZ	NZ	32
S. haemolyticus	35	32	30	26	24	22	17	15	13	11	9	NZ	4
S. aureus	36	35	31	29	27	25	22	18	16	13	10	NZ	0.25
S. aureus	10	8	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	≥ 256
S. aureus	38	36	35	34	32	28	27	23	21	18	13	11	0.125
S. aureus ATCC 25923[b]	40	38	36	35	33	31	30	26	24	16	13	12	
S. aureus ATCC 29213[c]	40	38	36	35	34	32	28	25	22	19	15	NZ	0.125

NZ: no inhibition zone.
[a]MIC values were calculated based on breakpoints to DD method of oxacillin (CLSI, 2007) for *S. aureus*/*S. lugdunensis* as: susceptible ≥ 13 mm, intermediate 11–12 mm, resistant ≤ 10 mm. For CoNS as: susceptible ≥ 18 mm and resistant ≤ 17 mm.
[b]The inhibition zone diameter for *S. aureus* ATCC 25923 using 1 μg disk of oxacillin is 18 to 24 mm.
[c]The MIC for *S. aureus* ATCC 29213 is 0.12 to 0.5 μg/mL.

staphylococci (CoNS). The bacterial strains were recovered from blood, intravascular catheters, surgical wounds, and cerebrospinal and peritoneal fluids. Species distribution among the 58 CoNS studied was *S. epidermidis* (39 strains; 67.2%), followed by *S. haemolyticus* (10 strains; 17.2%), *S. hominis* (4 strains, 6.9%), *S. warneri* (2 strains, 3.4%) and one isolate each of *S. gallinarum*, *S. chromogenes* and *S. lugdunensis*.

2.2. Antimicrobial susceptibility testing

The MIC values were determined by the quantitative DD method with disks containing oxacillin concentrations (0.125 to 256 μg/mL) provided by Cecon (São Paulo, Brazil). The disks were applied on the surface of plates, containing Mueller–Hinton agar supplemented or not with 2% NaCl, previously streaked with the bacterial suspension adjusted to 0.5 Mac Farland (10^8 CFU/mL) standard by Densimat (bioMèrieux, Roma, Italy). Plate readings were taken after 24 and 48 h of incubation at 35 °C. The determination of oxacillin MIC values based on the quantitative DD method proposed in this study is illustrated in Table 1 and Fig. 1, which show data for 3 *S. aureus* and 3 CoNS strains as well as for the control strains. The registered MIC values correspond to oxacillin disk concentrations when the inhibitory zone diameter matches the breakpoint established by CLSI. *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213 were used as control in all antimicrobial susceptibility tests.

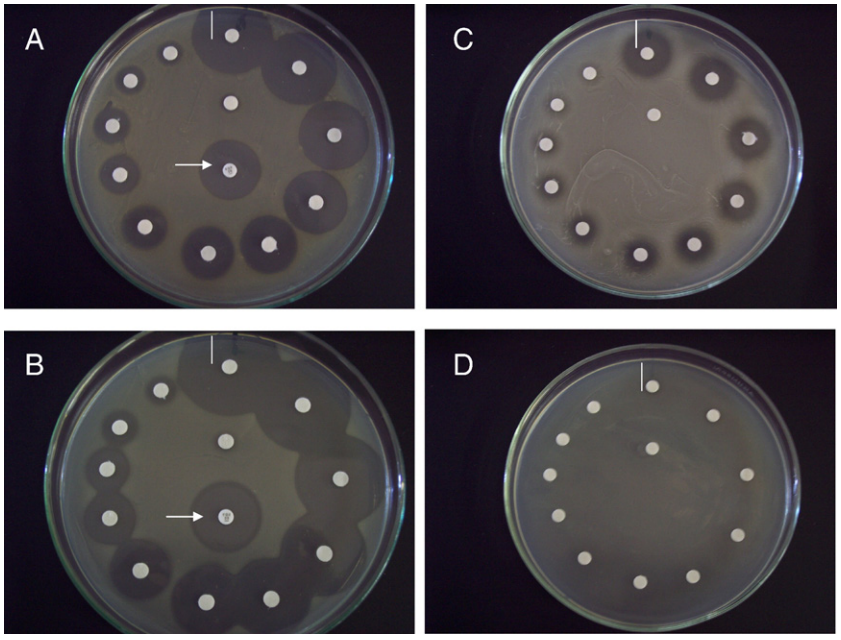

Fig. 1. Representation of MIC determination by quantitative DD method. The disks containing oxacillin with concentrations 0.125 to 256 μg/mL were plotted in Mueller–Hinton agar plates in clockwise starting at the marked point. A and B: *S. aureus* strains susceptible to oxacillin by quantitative DD method. The arrows indicate the cefoxitin disks that were plotted in those plates. C and D: CoNS strains oxacillin resistant by quantitative DD method.