Brazilian Journal of Microbiology 45, 1, 235-237 (2014)
ISSN 1678-4405

Short Communication

Comparison of cefoxitin disk diffusion test and *mecA* gene PCR results for methicillin resistance detection in *Staphylococcus intermedius* group isolates from canine origin in Brazil

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Submitted: December 20, 2012; Approved: September 9, 2013.

Abstract

The study evaluated cefoxitin disk diffusion tests breakpoints and their correlation to *mecA* gene PCR results for detecting Methicillin-resistant *Staphylococcus intermedius* Group (MRSP) isolates from dogs in Brazil. Agreement using proposed breakpoint (resistant ≤ 30 mm) was encouraging. The current study reinforces that an epidemiological breakpoint can be established to predict presence of MRSP.

Key words: methicillin-resistant *Staphylococcus intermedius* group, cefoxitin, disk diffusion test.

Staphylococcus intermedius Group has been recognized as an opportunistic pathogen in many kinds of animals, especially in dogs and cats (van Duijkeren *et al.*, 2011a). Since its first description it has been shown to be the species of the *Staphylococcus intermedius* group (SIG) that predominantly colonizes dogs, also representing a leading cause of canine topic infections (Penna *et al.*, 2010; Perreten *et al.*, 2010). Some recent reports indicated that *S. intermedius* Group could occasionally cause infections and colonize human (Paul *et al.*, 2011; Stegmann *et al.*, 2010; van Duijkeren *et al.*, 2011b), suggesting that *S. intermedius* Group is a zoonotic pathogen and public health issue.

More recently methicillin resistant *S. intermedius* Group (MRSP) has been reported as the predominant coagulase-positive methicillin resistant staphylococci in dogs, what poses a therapeutic challenge due to the limited treatment options (Bryan *et al.*, 2012). Methicillin resistance of *Staphylococcus intermedius* Group has been detected by disk diffusion test (DDT) employing oxacillin disk (Papich, 2010). However, *mecA* gene detection by polymerase chain reaction (PCR) is the most accurate methods for prediction of methicillin resistance in staphylococci (CLSI, 2012).

Cefoxitin disk diffusion susceptibility testing is being widely used for *Staphylococcus aureus* and coagulase-negative staphylococci (CNS) isolated from human beings, with better results than oxacillin disk (CLSI, 2012). Unlike similar testing with oxacillin, cefoxitin disk diffusion testing does not require additional supplementation of media or altered incubation conditions (Skov *et al.*, 2003). Zones of growth inhibition may also be more clearly demarcated and easier to interpret (Bemis *et al.*, 2012). Despite this, the using of cefoxitin disk has not been validated for screening methicillin resistance in coagulase-positive staphylococci isolates other than *S. aureus* from animal origin. In that direction, attempts to interpret results from isolates of animal origin using the standards determined for *S. aureus* were not successful, and a breakpoint of 30 mm in cefoxitin DDT for *S. intermedius* Group of canine origin have been proposed (Bemis *et al.*, 2012). Nevertheless, the authors state that additional testing with *S. intermedius* Group isolates from different geographic regions is needed. Therefore, the purpose of the present study was to evaluate cefoxitin disk diffusion tests breakpoints and their correlation to *mecA* gene PCR results for methicillin resistance detection in *S. intermedius* Group isolates from canine origin in Brazil.

A total of 83 isolates of *S. intermedius* Group from unmedicated dogs with external otitis (OE, 52 isolates) and healthy dogs (HD, 31 isolates) were studied. A sterile cotton swab was used to collect samples of ear exudates. From the healthy animals swab was taken from one anterior nostril. The cotton swabs were inoculated in Brain Heart Infusion broth (Difco, Franklin Lakes, NJ, USA) and incubated

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at 37 °C. Only one sample from each dog was studied, even in the cases of dogs with otitis externa if both ears presented with clinical signs.

Dogs with otitis extrena included had to present with clinical signs in at least one ear. Signs of otitis externa included local pain, pruritus, erythema, ear discharge and desquamation. Ears were screened with cytological for evidence of cocci and subsequent pure cultures of staphylococci-like bacteria as seen on Gram stain of cultured colonies were included. The included healthy dogs with no history of any infection related symptoms at the time of the evaluation and no history for at least one month prior to sampling.

All isolates were were classified according to reference methods as previously described (Penna *et al.*, 2010). Isolates in pure culture were identified on the basis of colony morphology, Gram staining, pigment production, haemolysis on 5% bovine blood agar and biochemical reactions, including catalase activity test, resistance to Bacitracin 0.04 U, acid production in Hugh-Leifson's OF base medium, tube coagulase test, acetoin production, urease, novobiocin resistance, deoxyribonuclease test, ornithine and arginine utilization and aerobic fermentation of sucrose, D-mannose, D-cellobiose, D-xylose, L-arabinose, raffinose, D-trehalose, maltose and D-mannitol.

All the *S. intermedius* Group isolates were tested for susceptibility to cefoxitin (30 μg) by the agar disc diffusion method on Mueller Hinton Agar (Difco) (CLSI, 2012). A PCR targeting the *mecA* gene was employed to confirm the resistance to methicillin (Zhang *et al.*, 2005).

The *mecA* gene was detected in 14 (17.3%) isolates, both from dogs with OE (17.3%) and HD (16.1%). Zone diameters obtained in DDT with cefoxitin disk were analyzed to the 83 samples and compared to the established breakpoints to CNS and *S. aureus* (Table 1). The cefoxitin growth inhibition zone diameters were distributed in a bimodal fashion (Figure 1). Also, to each zone diameter sensitivity and specificity were calculated using a ROC curve.

Table 1 - Table 1. Comparison of the cefoxitin disk diffusion test considering different breakpoints with the *mec*A detection by PCR as a predictor of methicillin resistance in SIG.

	Number of the isolates			
	PCR	Cefoxitin disk diffusion		
		S. aureus breakpoint[a]	CNS breakpointb	Bemis and coworkers (2012) breakpointc
Resistant	14	1	5	14
Susceptible	69	82	78	69
Total	83	83	83	83

[a]Resistant ≤ 21 mm (CLSI, 2012); [b]Resistant ≤ 24 mm (CLSI, 2012); [c]Resistant ≤ 30 mm (Bemis *et al.*, 2012).

Sensitivity and specificity of the zone diameter breakpoint criteria set were 100% when a breakpoint of 30 mm was adopted for predicting methicillin resistance. With that breakpoint, results of DDT using cefoxitin disk agreed 100% with *mecA* gene detection (kappa statistic; κ = 1.000).

Studies conducted in the USA, the cefoxitin DDT using interpretive criteria recommended for human isolates of CNS (breakpoint of 24 mm) (CLSI, 2012) generated unacceptably high levels of major errors (resistant isolates called susceptible) and low agreement with *mecA* gene detection by PCR (Bemis *et al.*, 2009; Schissler *et al.*, 2009) Similar results were observed in the present study. If those criteria were applied, the same major errors would occur, and nine of the 14 *S. intermedius* Group isolates would be erroneously categorized as susceptible; hence the sensitivity of DDT would only reach 36%, with a low concordance to molecular test (k = 0.479), although specificity would still be 100%. When standards for *S. aureus* of human ori-

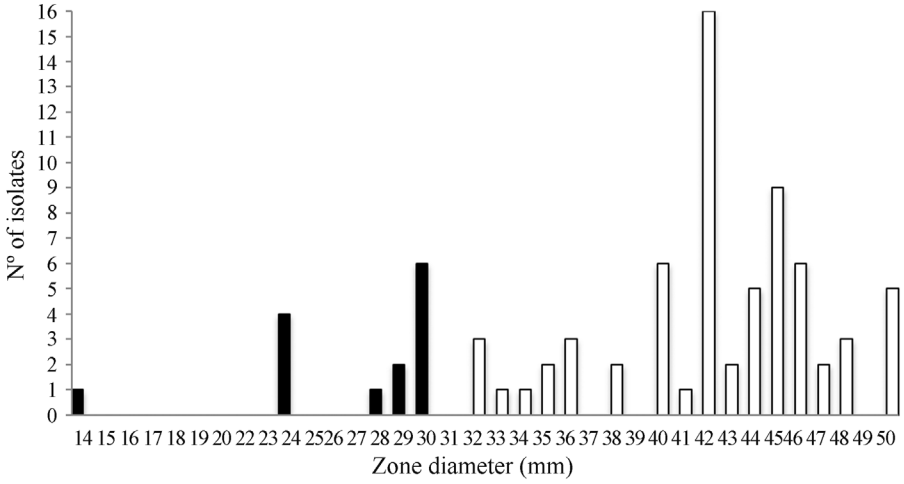

Figure 1 - Cefoxitin disk diffusion growth inhibition zone diameters obtained from *S. intermedius* Group isolates from dogs. Black bars indicate *mecA* gene polymerase chain reaction (PCR) positive; white bars indicate *mecA* gene PCR negative.

gin are employed (breakpoint of 21 mm) (CLSI, 2012), concordance was even lower (k = 0.113), and sensitivity of cefoxitin DDT was 7%, since only one isolate was categorized as resistant.

The major errors that have been reported and also observed in the present study clearly demonstrate that suggested standards must be revised for the adequate phenotypical detection of *S. intermedius* Group of canine origin. Conversely, the overall strong agreement between cefoxitin DDT using the proposed breakpoint (resistant ≤ 30 mm) and *mecA* gene detection by PCR is encouraging.

Establishment of *S. intermedius* Group specific cefoxitin DDT interpretive criteria is an achievable goal. PCR may not be available for all laboratories around the globe, and also presents greater costs. Therefore, reliable standardization of DDT, including the breakpoints, are essential for interlaboratory comparisons and additional understanding on methicillin resistance of *S. intermedius* Group isolates from canine origin in different geographic regions.

This research was supported by the National Council for Scientific and Technological Development (Capes) and Faperj (E-26/110.095/2009). The authors wish to thank Dr. Marcia Giambiagi de Marval, Dr Beatriz Meurer Moreira, Dr Miliane Moreira.

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