# Use of the D Test Method to Detect Inducible Clindamycin Resistance in Coagulase Negative Staphylococci (CoNS)

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**According to the National Committee for Clinical Laboratory Standards (NCCLS, 2004), a method to evaluate the inducible clindamycin resistance in accordance with an approach of the disks of erythromycin and clindamycin – the D test – has been reported. We analyzed the performance of this method in 200 coagulase negative staphylococci (CoNS) strains obtained from blood cultures of hospitalized patients at a general hospital in Southern Brazil. Twenty-seven clinical isolates with suitable profile (erythromycin-resistant and clindamycin-susceptible) were evaluated for the D test realization. Thus, only 5 CoNS were D test positive. The D test method showed to be simple and an important technique in the detection of inducible clindamycin resistance.**
**Key-Words: Clindamycin, resistance, D test.**

The determination of antimicrobial susceptibility of a clinical isolate is often crucial for the optimal antimicrobial therapy of infected patients. This is particularly important considering the increase of resistance and the emergence of multidrug-resistant microorganisms [1-3]. Several authors have screened clinical isolates of erythromycin-resistant *Staphylococcus aureus* and coagulase negative staphylococci (CoNS) for genes encoding resistance to macrolides, lincosamides and streptogramins type B ($MLS_B$) [4-10].

Resistance to macrolides (e.g. erythromycin) can occur by two different mechanisms: efflux due to macrolide streptogramin resistance (*msrA* gene) and ribosome alteration due to erythromycin ribosome methylase (*erm* gene) [11,12]. Macrolide resistance due to efflux encoded by *msrA* has been more prevalent in CoNS than in *S. aureus* [13].

Different mechanisms of acquired MLS resistance have been found in Gram-positive bacteria [11,12]. The first mechanism of resistance to macrolide described was due to posttranscriptional modifications of the 23S rRNA by the adenine-*N*-6-methyltransferase. Target modification alters a site in 23S rRNA common to the binding of $MLS_B$ antibiotics. Modification of the ribosomal target confers cross-resistance to $MLS_B$ antibiotics ($MLS_B$-resistant phenotype) and remains the most frequent mechanism of resistance. In general, genes encoding these methylases have been designated *erm* (erythromycin ribosome methylation). Expression of resistance to $MLS_B$ in staphylococci may be constitutive ($MLS_{Bc}$) or inducible ($MLS_{Bi}$). When expression is constitutive, the strains are resistant to all $MLS_B$ type antibiotics. When expression is inducible, the strains are resistant to 14- and 15-membered macrolides only [11,12].

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For $MLS_{Bi}$ strains, erythromycin will induce production of the methylase, which allows clindamycin resistance to be expressed. Inducible clindamycin resistance can be detected with a simple disk approximation test, commonly referred as the D test [14]. For this test, an erythromycin disk is placed 15 mM to 26 mM (edge to edge) from a clindamycin disk in a standard disk diffusion test. Following incubation, a flattening of the zone in the area between the disks where both drugs have diffused indicates that the organism has inducible clindamycin resistance [14-16].

The purpose of this study was to characterize the antimicrobial susceptibility patterns (erythromycin, clindamycin and oxacillin) and to evaluate, according to the D test [17], all coagulase negative staphylococci from a collection of 200 clinical isolates from blood cultures that had the necessary characteristics for this study: resistance to erythromycin and susceptibility to clindamycin. We analyzed 200 consecutives clinical isolates of CoNS obtained from patients admitted in a general hospital, in Porto Alegre city, in Southern Brazil, between January and June 2002. All the isolates were obtained from blood cultures.

The samples were identified (only for species-level identification) through MicroScan, panel Pos-Combo 13 (Dade Behring – Deerfield, Illinois, USA). For selection criteria, a method for determining susceptibility to clindamycin (disk diffusion) was performed rather separated on the plate and so it was not confused with the performance of the D test itself.

The susceptibility tests – disks for the following agents at the amounts specified: 15 μg erythromycin, 2 μg clindamycin and 1μg oxacillin (Difco Laboratories, Detroit, Mich.) – were performed by the agar disk diffusion method (Kirby-Bauer), according to the guidelines of the National Committee for Clinical Laboratory Standards.

D test Method – for this test, erythromycin disks were placed at 15 mM and 26 mM (edge to edge) from clindamycin disks, as recommended (NCCLS, 2004) on Muller-Hinton agar plate (Oxoid – Hampshire, England). Moreover, the disks also were placed at a distance of 10 mm. According to evaluation criteria of NCCLS 2004, the flattened (positive test) or not

(negative test) clindamycin zone between erythromycin and clindamycin disks was verified.

We performed the clindamycin induction test on CoNS that had the following profile: resistant or with intermediate resistance to erythromycin and susceptible to clindamycin, using routine antimicrobial susceptibility test. Twenty-seven CoNS of our collection (n=200) had this profile. *S. aureus* ATCC 25923 was used for quality control (QC) of clindamycin and erythromycin disks, according to the standard disk diffusion QC procedure. The susceptibility patterns for CoNS isolates are showed in the Table 1. One hundred thirty-three (66.5%) out of the isolates were oxacillin-resistant. The full and intermediate resistance were 63% and 3.5%, respectively, for erithromycin and 53.5% and 1% respectively, for clindamycin. The phenotypic pattern compatible for realization of the D test was obtained by 27 CoNS isolates (13.5% of the total). Thus, only 5 (18.5%) were positive for inducible clindamycin resistance. The test was more visible when the erythromycin disk was placed 15 mM or 10 mM from the clindamycin disk. Positive D test reactions were showed when intermediate resistance to erythromycin was included as well as full resistance. In total, 5 positive reactions were observed, 3 (14.3%) for full resistance and 2 (33.4%) for intermediate resistance to erythromycin. We observed distinct species in these 5 positive D test CoNS isolates: 2 *Staphylococcus epidermidis*, 2 *Staphylococcus haemolyticus* and 1 *Staphylococcus simulans*. Both *S. epidermidis* (2) and *S. haemolyticus* (2) were carriers of the *mecA* gene – oxacillin-resistant, whereas *S. simulans* was not carrier (data not showed). The distances between disks more suitable to detection of the induction of resistance were 15 mM (standard) and 10 mM (no standard). The Figure 1 showed the induction of the resistance at a distance of 10 mm.

Resistance in Gram-positive bacteria not only increases morbidity and mortality, but also the costs of management of hospitalized patients. Studies have indicated a great increase in the ratio of staphylococci resistance to MLS group and failure in the treatment with clindamycin in infections with microorganisms with inducible resistance to MLS group [17]. Reporting *Staphylococcus* spp. as susceptible to clindamycin without checking for inducible clindamycin resistance may result in inappropriate clindamycin therapy. As caution, to add comment of resistance based on detection of inducible clindamycin resistance has been proposed [18]. On the other hand, negative results for inducible clindamycin resistance confirm susceptibility to clindamycin and add comment that this *Staphylococcus* spp. does not demonstrate inducible clindamycin resistance *in vitro* [18].

The D test is acceptable for all *Staphylococcus spp*. including oxacillin-susceptible or oxacillin-resistant *S. aureus* or CoNS [18]. Many of the recently recognized methicillin-resistant *Staphylococcus aureus* (MRSA) that cause community-associated infections have the *msr*A gene, and oral clindamycin may be a treatment option for these patients. In this case, these *S. aureus* strains are susceptible to clindamycin and do not present inducible resistance to this antimicrobial agent. Although clindamycin can be effective in some patients, it is not recommended to use it before conduct the D test [18].

An important fact in our study was that we incorporated clinical isolates of CoNS that presented a profile of intermediate resistance to clindamycin. In fact, 2 isolates that showed compatible profile with the realization of the D test (erythromycin-intermediate and clindamycin-susceptible), resulted in resistance to clindamycin and positive D test. This isolates were identified as *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*. Outbreaks caused by multiresistent and offensive *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* have been reported in various nosocomial settings, as well as in individual intensive care units (ICU) or other units within a hospital [19]. Save this results, our *Staphylococcus* spp. isolates have resistance levels lower than in other countries (data not published was showed in the 104th General Meeting of the American Society for Microbiology, New Orleans, LA, 2004). Until now, there are not studies that report this test in Brazilian clinical isolates. This disk approximation test proved to be a good method to detect staphylococci strains with inducible clindamycin resistance. As demonstrated in the effectuated analyses, the method revealed to be adequate and viable for the evaluation of this phenotype of resistance when it was used 15 mM (standard) and 10 mM (no standard) of distance between the disks. At a distance of 26 mM between the disks, macrolide resistance was not detected in 2 isolates (1 *S. epidermidis* and 1 *S. haemolyticus*). In summary, the D test method revealed to be practical in the established conditions, being able to be used in the qualitative determination (phenotyping) of the resistance in coagulase negative staphylococci, mainly when the lowest standardized distance (15 mm) between disks was used. Additional advantages include decreased managements costs of treatment in resistant infections (by diagnostic confirmation), more rapidity in the results and its easy adaptation in the laboratorial routine.

The D test can be used as an auxiliary and alternative method to inducible clindamycin resistance detection in the routine of clinical laboratories. However, the confirmation of the *erm* gene in staphylococci strains with positive D test would assist in the standardization of the test (suitable distance between disks, sensitivity and specificity of the test). Moreover, the present study verified only 5 positive tests and a greater number would be required for validation of the interpretation of the distance between the disks.

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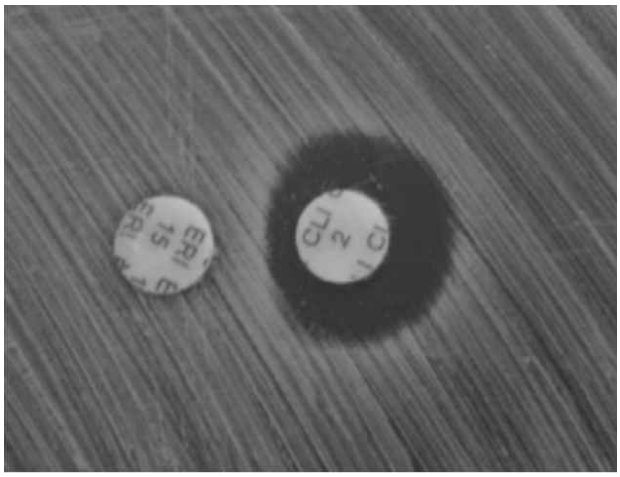
**Table 1.** Antimicrobial susceptibility patterns among coagulase negative staphylococci isolates

| Pattern | Antimicrobials | | |
|---|---|---|---|
| | Oxacillin (%) | Erythromycin (%) | Clindamycin (%) |
| Resistant | 133 (66.5) | 126 (63) | 107 (53.5) |
| Intermediate | - | 07 (3.5) | 02 (1) |
| Susceptible | 67 (33.5) | 67 (33.5) | 91 (45.5) |
| Total | 200 (100) | 200 (100) | 200 (100) |

**Figure 1**. D test positive result for *S. epidermidis* strain (distance of 10 mM between disks).



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