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# Activity of vancomycin, ciprofloxacin, daptomycin and linezolid against coagulase-negative staphylococci bacteremia

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## ABSTRACT

**Objective.** Multiresistant coagulase-negative staphylococci (CNS) infections are mainly increased in hospitalized patients. We have studied the activity of vancomycin, ciprofloxacin, daptomycin and linezolid in methicillin-resistant CNS strains, isolated from true blood cultures.

**Methods.** We collected 87 strains of different CNS species from positive blood cultures. Staphylococci were identified by MicroScan Walkaway (Dade Behring, Siemens) and with the Api ID 32 Staph (BioMerieux, France). The susceptibility to oxacillin, vancomycin and ciprofloxacin was performed by automatic microdilution plate as cited above. The susceptibility to daptomycin and linezolid was performed by Etest (AB BioMerieux, Solna, Sweden). Interpretative criteria were done following the CLSI guidelines.

**Results.** Eighty-seven CNS strains were studied: 55 (63%) were *S. epidermidis,* 15 (17%) *S. haemolyticus*, 10 (12%) *S. hominis,* and 7 (8%) other species. Fifty-three (61%) strains showed loss of susceptibility to vancomycin, MIC = 2 mg/L. Ciprofloxacin resistance, MIC > 2 mg/L, was observed in 56 (64%) strains. Daptomycin resistance was not observed, with a susceptibility range between 0.032-1 mg/L and modal value of 0.25 mg/L. Ten strains (11.5%) resistant to linezolid were observed. Nine patients were in ICU, where the average length of stay was 38 days (range 16-58 days) and one belonged to Hepato-Pancreatic Surgery, where he stayed for 64 days.

**Conclusions.** Low susceptibility to vancomycin is frecuent in the CNS strains studied in our hospital. Daptomycin shows a high efficacy against CNS, and it could be useful for the treatment of primary bacteremia or catheter associated bacteremia. The massive and continuous use of linezolid has led to the appearance of resistance.

**Keywords: Coagulase-Negative-Staphylococci, blood culture, antimicrobial resistance.**

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## Actividad de vancomicina, ciprofloxacino, daptomicina y linezolid frente *Staphylococcus* coagulasa negativo aislados en hemocultivos

## RESUMEN

**Objetivo.** Las infecciones por *Staphylococcus* coagulasa negativos (CNS) resistentes a meticilina aumentado considerablemente en los pacientes hospitalizados. Hemos estudiado la actividad de vancomicina, ciprofloxacino, daptomicina y linezolid en cepas de CNS resistente a meticilina aisladas en hemocultivos clínicamente significativos.

**Material y Métodos.** Se estudiaron 87 cepas de distintas especies de CNS de hemocultivos positivos. Los estafilococos fueron identificados mediante el sistema automático MicroScan Walkaway (Dade Behring, Siemens) y con Api ID 32 Staph (BioMerieux, Francia). La sensibilidad a oxacilina, vancomicina y ciprofloxacino fue realizada por dicho sistema MicroScan. La susceptibilidad frente a daptomicina y linezolid fue realizada mediante Etest (AB BioMerieux, Solna, Suecia). Para los criterios de interpretación se siguieron las indicaciones del CLSI.

**Resultados.** Se estudiaron 87 cepas, 55 (63%) fueron *S. epidermidis*, 15 (17%) fueron *S. haemolyticus*, 10 (12%) fueron *S. hominis*, y 7 (8%) pertenecieron a otras especies. 53 (61%) cepas presentaron una MIC para vancomicina de 2 mg/L. La resistencia a ciprofloxacino, MIC > 2 mg/L fue observada en 56 (64%) cepas. No se encontraron resistencia a daptomicina, con un rango de sensibilidad entre 0.032-1 mg/L y un valor modal de 0,25 mg/L. Se aislaron 10 (11,5%) cepas resistentes a linezolid. Nueve pacientes estuvieron ingresados en la Unidad de Cuidados Intensivos, donde la estancia media fue de 38 días (rango 16-58 días), y uno perteneció al Servicio de Cirugía Hepato-Pancreática, con una estancia de 64 días.

**Conclusiones.** Es frecuente aislar cepas de CNS con pérdida de sensibilidad para vancomicina en nuestro hospital, mientras que daptomicina presenta una alta sensibilidad frente a este tipo de microorganismos. El uso masivo y continuado de linezolid ha llevado a la aparición de resistencias.

**Palabras clave: Staphylococcus coagulasa negativo, hemocultivo, resistencia antimicrobiana.**

## INTRODUCTION

Coagulase-negative staphylococci (CNS) are among the major bacteria producing bacteremia in seriously ill patients, especially in connection with catheters, bone and joint prostheses or pacemakers[1,2]. It has been observed that the elevation in the MIC of vancomycin to 2 mg/L results in loss of *in vivo* antibiotic activity[3], making it necessary to seek new alternatives to glycopeptides for the treatment of Gram-positive cocci infections. The aim of the study was to value the activity of vancomycin, ciprofloxacin, daptomycin, and linezolid in methicillin-resistant CNS strains isolated from true blood cultures.

## MATERIALS AND METHODS

From April to December 2009 we collected 87 strains, one per patient, of different species of methicillin-resistant CNS from positive blood cultures of patients admitted to various hospital services. The following requirements were necessary to consider a CNS responsible of a true bacteremia: a) to produce a primary bacteremia, or catheter-associated bacteremia; b)to be isolated in pure culture in at least four of six vials of blood cultures; c) no isolation of another organisms from sterile samples of the patient; d) no more clinically significant organisms isolated from nonsterile samples, as urine, sputum or tracheobronchial aspirate; e) provide signs or symptoms of infection process; f) patients received specific antistaphylococcal treatment, and treatment was succesfull.

Positive bottles were inoculated onto blood, polyvitex and Sabouraud agar plates. They were incubated at 35°C with 5% $CO_2$ overnight. The samples without growth were reincubated 24 hours additionally before discarding as negative. To rule out anaerobic bacteria, a SCS agar plate was incubated at 35ºC in anaerobiosis during 72 hours.

Staphylococci were identified to genus level by colony morphology, Gram stain, positive catalase and coagulase-negative tests. Identification at the species level was performed by MipancroScan Walkaway Automatic System, using the Combo 24 panels (Dade Behring, Siemens). Api ID 32 Staph (BioMerieux, France) was used in those cases where identification was not reliable.

The susceptibility to oxacillin, vancomycin and ciprofloxacin was performed by automatic microdilution plate as cited above. The oxacillin resistance was confirmed using the pbp2´ latex agglutination kit (Oxoid, England), and the susceptibility or resistance of clindamycin was done using erythromycin disk (15µg) and clindamycin disk (2µg) on Mueller-Hinton agar plate. The susceptibility to daptomycin and linezolid was performed by Etest (AB BioMerieux, Solna, Sweden) on Mueller-Hinton agar plate (BioMerieux, France). Interpretative criteria were done following the CLSI guidelines[4]. Linezolid-resistant strains were confirmed at the Spanish reference laboratory (Centro Nacional de Microbiología, Madrid, Spain) using agar microdilution following the CLSI directions[5].

## RESULTS

Of 87 CNS strains studied, 55 (63%) were *S. epidermidis,* 15 (17%) *S. haemolyticus*, 10 (12%) *S. hominis,* and 7 (8%) other species. For services, 41 (47%) patients belonged to the Unit of Infectious Pathology, 33 (38%) to the ICU, 7 (8%) to Nephrology and 6 (7%) remaining to other services. 34 (39%) strains showed a vancomycin MIC □)1 mg/L, while in 53 (61%) the MIC was 2 mg/L. By species, *S. epidermidis* showed 40 (73%) strains with low susceptibility to vancomycin, *S. haemolyticus* showed 9 (60%) and *S. hominis* showed 2 (20%). Concerning ciprofloxacin, the MIC > 2 mg/L, was observed in 56 (64%) strains: 35 *S. epidermidis,* 12 *S. haemolyticus*, 5 *S. hominis* and 4 other species. Diferents CNS species and their MIC are showed in table 1. Table 2 shows the MIC ranges and the modal value by species for daptomycin and linezolid. We isolated 10 (11.5%) strains resistant to linezolid, 5 *S. epidermidis*, 4 *S. haemolyticus* and 1 CNS. Nine patients were in ICU, where the average length of stay was 38 days (range 16-58 days) and one belonged to Hepato-Pan creatic Surgery, where he stayed for 65 days.

**Table 1** **Vancomycin susceptibility of different CNS.**

| CNS | Vancomycin MIC ≤1 mg/L n/N (%) | Vancomycin MIC=2 mg/L n/N (%) |
|---|---|---|
| *S. epidermidis* | 15/55 (27) | 40/55 (73) |
| *S. haemolyticus* | 6/15 (40) | 9/15 (60) |
| *S. hominis* | 8/10 (80) | 2/10 (20) |
| Other species* | 5/7 (71) | 2/7 (29) |
| **Total percentage** | **39%** | **61%** |

N = total number of strains

* 1 ***S. intermedius***, 2 ***Staphylococcus spp***, 1 ***S. chleiferi***, and 3 ***S. simulans***.

**Table 2** **Activity of daptomycin, linezolid, and ciprofloxacin against CNS strains.**

| | Daptomycin | | | Linezolid | | | Ciprofloxacin*** | | |
|---|---|---|---|---|---|---|---|---|---|
| CNS (n) | MIC range (mg/L) | Mode (mg/L) | Susceptibility (%) | MIC range (mg/L) | Mode (mg/L) | Susceptibility (%) | Values (mg/L) | Mode (mg/L) | Susceptibility (%) |
| *S. epidermidis* (55) | 0.064-1 | 0.25 | 100 | 0.125-256 | 0.25 | 91 | ≤1/>2 | >2 | 35 |
| *S. haemolyticus* (15) | 0.047-0.25 | 0.094 | 100 | 0.38-256 | 0.75 | 73 | ≤1/>2 | >2 | 20 |
| *S. hominis* (10) | 0.047-0.19 | 0.125 | 100 | 0.38-1 | 0.75 | 100 | ≤1/>2 | ≤1/>2 | 50 |
| Other species* (7) | 0.032-0.38 | -** | 100 | 0.5-256 | 1 | 86 | ≤1/>2 | >2 | 43 |
| **Total percentage** | - | - | 100% | - | - | 82% | - | - | 31% |

* 1 ***S. intermedius***, 2 ***Staphylococcus*** spp, 1 ***S. chleiferi***, and 3 ***S. simulans***.
**No value is repeated.
***Ciprofloxacin MIC was tested by MicroScan

## DISCUSSION

In developed countries, the increased survival of patients with serious illnesses, together with the implantation of differents foreign bodies, as pacemakers, protheses, and catheters, have favored the emergence of infections caused by opportunists microorganisms, as CNS.

Vancomycin has been the main choice for the treatment of meticillin-resistent CNS infections. However the continuous use of this antibiotic has increased MICs from 0.5 mg/L to 2 mg/L in a large number of strains. Although they are still microbiologically considered as susceptible, this rise in MICs values has developed *in vivo* clinical consequences: decreasing efficacy of antibiotic leading to therapeutic failure, monitoring needs in arthritis, osteomyelitis and pneumonia due to the poor tissue penetration[6], major side effects (bone marrow suppression or ototoxicity), increase hospital costs, more time for bacterium clarification from the blood and therefore, therapeutic failure of bacteremia with increased of mortality (OR 6.39).

Our work was conducted with methicillin resistant strains. This situation is increasingly common in hospitals for CNS true bacteremia, since 1987 when the meticillin resistance was 2% until 2008 when this resistance reached 90%[7,8]. Currently, meticillin resistance in CNS in our hospital reaches 86% of the strains. Furthermore, 61% of these strains had a loss of susceptibility to vancomycin. Although we did not find any MIC ≥4 mg/L strain, which would mean *in vitro* antimicrobial resistance, we have concerned with the most isolates show an elevation of the MIC, involves a loss of clinical efficacy *in vivo,* with the risk of increasing the morbidity and mortality of the patients. This situation requires the use of other antibiotics families instead of vancomycin.

In the other hand, 64% of strains were resistant to ciprofloxacin. This percentage is consistent with those published by authors who study the susceptibility to macrolides, lincosamides, aminoglycosides, fluoroquinolones, and cotrimoxazole against CNS[9-11]. In 39 (45%) strains we observed co-occurrence of resistance to ciprofloxacin and loss of sensitivity to vancomycin (p = 0.025). Thus, 29 of 55 cases in *S. epidermidis,* 7 of 15 in *S. haemolyticus,* and 2 of 10 in *S. hominis* were observed. Therefore, beside the relationship between fluoroquinolone and methicillin situations described by Hamilton *et al,*[12] there is a clear relationship between ciprofloxacin resistance and loss of susceptibility to vancomycin in *S. epidermidis*. This occurs due to the presence of CNS gene *mecA*, placed in the staphylococcal chromosomal *cassette* (SCC *mec)* type I, II or III, that gives them the ability to develop resistance against differents antimicrobial families, among which are beta-lactams and fluoroquinolones[2].

Daptomycin is a lipopeptide that acts depolarizing the bacterial cytoplasmic membrane by binding to a bivalent calcium-dependent mechanism. The results are an inhibition of bacterial protein synthesis, RNA and DNA. In June 2006 it was approved by the FDA *(Food and Drugs Administration)* for use in bacteremia and endocarditis caused by *Staphylococcus*. In our study, all strains were susceptible to daptomycin with a low level MIC. All CNS showed very close values between minimum and maximum MIC ranges. The MIC mode was 0.25 mg/L in *S. epidermidis* and 0.125 mg/L in *S. hominis,* while it was still lower in *S. haemolyticus*. Therefore the loss of vancomycin susceptibility did not influence in daptomycin susceptibility. This happens because the antibiotic action mechanism is unique, and it is very difficult the appearance of cross reactions, even if it has not been a widely used antibiotic. Elsewhere, there are reported strains with reduced susceptibility or resistant to daptomycin[13]. This loss of susceptibility is determined by increased cell wall thickness after others antibiotics treatment, which impedes the passage of the antimicrobial to the plasma membrane, but once in place, efficiency is the same. These authors recommend to test their susceptibility and make partnerships with other groups of antimicrobials, including aminoglycosides, rifampycin, or fluoroquinolones[14].

In our institution we have not found any strain of these features. Moreover, thanks to fast bactericidal effect of daptomycin on the CNS, it is doubtly that this synergistic effect happens when other antibiotics families are coadministered[15-17], because the time action is very different for each antibiotics. However, we consider that it is useful to test their susceptibility in the microbiology laboratory, both to ensure the therapeutic success

alone, and as epidemiological and preventive purposes, to monitor the possible emergence of strains with high MIC.

Excluding the seven resistant strains, linezolid presented good MIC levels, below the cutoff sensitivity value (1-4 mg/L). In our study, the susceptibility range was higher than that offered by daptomycin, both overall and distributed by CNS species (table 2). This raising in the linezolid MIC does not make it less effective than daptomycin. In fact, in ICU patients, mortality rates are significantly lower when we do an aggressive antistaphylococcal treatment with linezolid instead of vancomycin. One reason is that linezolid has an inhibition in toxins and immunomodulators bacterial release, which favors the elimination of the infection. In the other hand, these patients frequently develop ventilator-associated pneumonia, that require a treatment with linezolid, instead daptomycin that is inhibited by the lung surfactant, making it ineffective.

Since 2000, when linezolid was approved for use in the U.S., sporadic resistance has been reported in CNS mainly in *S. haemolyticus*. Thus, Ross *et al.*[18] in 2005 found an isolate of *S. epidermidis* with MIC > 8 mg/L between 870 CNS studied, and one year later, Potoski *et al.*[19] isolated and identified the same clone of *S. epidermidis* with MIC > 256 mg/L by pulsed field electrophoresis in 25 strains studied. The development of resistance occurs by two independent mutations: G2447U and G22576U, relating to the previous employment and continuing linezolid[20].

In our study, we isolated and identified 10 (11.5%) strains resistant to linezolid. Five *S. epidermidis*, 4 *S. haemolyticus,* and 1 unidentified CNS from 9 ICU patients and one from Hepato-Pancreatic Surgery. All patients had prolonged ICU hospitalization and were previously treated with linezolid. All linezolid resistants strains were resistant to ciprofloxacin, in addition to methicillin, but only 5 of 10 presented elevation in the vancomycin MIC. John *et al*[2] analyzed 658 CNS strains and they did not find neither linezolid resistance nor relationship between *mecA* gene and oxacillin resistance. Today, there are not enough linezolid resistance CNS strains to do studies that link *mecA* gene with this antibiotic. We think that it is necessary to verify susceptibility to linezolid in the laboratory when it is used as treatment.

## CONCLUSIONS

Due to the high percentage of vancomycin MIC = 2 mg/L strains, this antibiotic should not be used empirically in the treatment of infections caused by CNS in our hospital.

Our results show a high efficacy of daptomycin for CNS, and this antibiotic could be useful for the treatment of primary bacteremia or catheter associated bacteremia in severe ill patients, while in patients with high risk to develop pneumonia linezolid should be used instead.

The massive and continuous use of linezolid has led to the appearance of resistance, so that antibiotics rotations would be desirable in patients treated for long periods of time.

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