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Evaluation of the Antimicrobial Susceptibilities of Coagulase - Negative Staphylococci by E-Test

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ABSTRACT. Coagulase-negative staphylococci (CoNS) have recently emerged as important nosocomial pathogens. In this study, the susceptibility of 53 isolates of CoNS (42 *S. epidermidis*, 6 *S. haemolyticus*, 3 *S. caprae*, 1 *S. lugdunensis* and 1 *S. chromogenes)* obtained over a 1 year period at a Brazilian Hospital were tested for vancomycin, teicoplanin, cephalothin, penicillin, oxacillin, and chloramphenicol. The minimal inhibitory concentration (MICs) were determined by the E-test using saturated swabs or the flooding method. No isolates showed resistance to vancomycin, 3 (5.7%) were resistant to teicoplanin, 5 (9.4%) to cephalotin, most of them, (47, 88.6%) were resistant to penicillin, 19 (35.8%) to oxacillin and 27 (50.9%) to chloramphenicol. The study shows that the increased resistance of CoNS can cause serious infections of difficult treatment. The E-test proved to be a convenient and reliable method for MIC determination of CoNS isolates and the qualitative and quantitative results obtained by flooding were much more reproducible than the results obtained using swabs.
Key words: Antimicrobial Susceptibilities, Staphylococci

RESUMO. Os estafilococos coagulase-negativa (ECN) surgiram recentemente como patógenos nosocomiais importantes. Neste estudo, a susceptibilidade de 53 amostras de ECN (42 *S. epidermidis*, 6 *S. haemolyticus*, 3 *S. caprae*, 1 *S. lugdunensis* e 1 *S. chromogenes*) obtidos no período de 1 ano em um Hospital Brasileiro foram testados para vancomicina, teicoplanina, cefalotina, penicilina, oxacilina e cloranfenicol. As concentrações inibitórias mínimas (CIM) foram determinadas pelo E-test usando o método da inundação e swabs saturados. Nenhuma amostra mostrou resistência à vancomicina, 3 (5,7%) foram resistentes à teicoplanina, 5 (9,4%) à cefalotina, a maioria delas 47 (88,6%) foram resistentes à penicilina, 19 (35,8%) à oxacilina e 27 (50,9%) ao cloranfenicol. O estudo mostra que a alta resistência dos ECN podem causar sérias infecções de difícil tratamento. O E-test se mostrou um método conveniente e confiável para determinação da CIM de amostras de ECN e os resultados qualitativos e quantitativos obtidos pelo método de inundação foram mais precisos que os resultados obtidos usando swabs.
Palabras Clave: Susceptibilidade Antimicrobiana, Estafilococos

INTRODUCCION

Coagulase-negative staphylococci (CoNS) have recently emerged as important nosocomial pathogens.[2,3] Serious infection caused by these organisms can be difficult to treat because of their resistance to many antibiotics and because sometimes they are considered to be contaminants and the patients are not immediately treated.

The E-test method is based on the diffusion of a continuous concentration gradient of an antimicrobial agent from a plastic strip into an agar medium. This *in vitro* technique was created to overcome several of the disadvantages of the disk diffusion and dilution techniques and also to retain the principle of the agar dilution method by producing an accurate and reproducible quantitative reference minimal inhibitory concentration (MIC) result. Overall, the E-test results have compared favourably with those of the reference methods, even though there have been a few instances of disagreement between methods, principally due to differences in inoculum, media and supplements.[4,14]

The E-test has permitted precise detection of non-epidemic and epidemic common source isolates among identical antibiogram patterns produced by routine susceptibility tests, such as Bauer-Kirby, Micro-scan, and Vitek, that only produce broad category interpretations or have few dilutions. The latter methods have also produced false-susceptible or false resistant results.[6,17]

Fifty-three isolates of coagulase-negative staphylococci

species from hospitalized patients were evaluated *in vitro* for antibiotic susceptibility using E-test strips, a novel and convenient method for accurate susceptibility testing.

The objective of the present study was to correlate the clinical and microbiologic data to actualize and give directions to the antibiotic therapy and to compare the results of antimicrobial susceptibility obtained by two different methods (swab and inoculation) applied simultaneously and analysed by three different examiners.

MATERIAL AND METHODS

In this study, we examined 53 CoNS isolated from patients with a possible infection caused by these pathogens, from blood cultures,[43] intravascular catheters[9] and cerebrospinal fluid[1] collected from 46 patients at a Brazilian Hospital from May 1995 to May 1996.

All isolates were identified as *Staphylococcus* by Gram stain, catalase production and resistance to bacitracin (0.4 U) and as coagulase-negative staphylococci by the standard tube test for free coagulase and by the slide test for bound.[8]

Isolates were stored temporarily in glycerol (15%) at -20°C and subcultured onto 5% sheep blood agar prior to testing.

API 20 Staph and ID 32 Staph (bioMérieux-France) were used to identify the species. When the identification percentage was lower than 95% by API 20 Staph, single and complementary tests such as PYR (Difco, Detroit, MI), polymixin and novobiocin resistance (Difco, Detroit, MI), and the oxidase test were used, according to the results presented by Kloos & Bannerman[7]. And when doubts persisted after these procedures, the ID 32 Staph (bioMérieux-France) was used.

For the E-test, 150 mm-diameter Müeller-Hinton agar plates (Difco, Detroit, MI) were inoculated in two different ways. The first inoculation was done using swabs saturated with suspensions prepared with overnight cultures equivalent to a 0.5 Mac Farland standard. For the second inoculation, 150 mm Müeller-Hinton plates were flooded with each isolate cultured in brain heart infusion (BHI, Difco, Detroit, MI) inoculated approximately 2 h before and the growth was observed up to the turbity of 0.5 Mac Farland standard. Excess fluid was removed and plates were dried.

The antibiotics tested were vancomycin, teicoplanin, cephalothin, penicillin G, oxacillin and chloramphenicol. The six antimicrobial agent-coated test strips were placed in separated quadrants on each plate in accordance to the manufacturer instructions. The ranges of antimicrobial concentrations in E-test strips (AB Biodisk, Solna, Sweden) were 0.016 to 256 μg/ml. Susceptibility or resistance to the antibiotics tested was analysed according to the guidelines of the National Committee for Clinical Laboratory Standard.[15]

The reference strain *S. aureus* ATCC 25923 was included in the study as a quality control indicator.

The results were read after 18 to 24 h of incubation at 37°C by three different readers and the results of the 3 readings and of the different inoculation methods were compared.

The records of 46 patients were reviewed retrospectively to determine the clinical significance of the bacteria isolated and correlate it with the susceptibility test.

The staphylococci were considered multiresistant when they were resistant to methicillin or oxacillin and to 3 or more antimicrobial agents.[1]

RESULTS

The 53 coagulase-negative staphylococcus species identified by API-Staph using the complementary biochemical tests described above, were 42 *S. epidermidis*, 6 *S. haemolyticus*, 3 *S. caprae*, 1 *S. lugdunensis* and 1 *S. chromogenes*. Of the 42 *S. epidermidis* isolates, 28 were identified only using the API-Staph and the percentage of identification was higher than 95%. Thirteen *S. epidermidis*, whose percentage of identification by API 20 Staph was lower than 95% were identified by the polymixin resistance test, and 1 *S. epidermidis* isolate, whose percentage was in doubt between *S. epidermidis* and *S. sciuri* was identified by the oxidase test. Of the 6 *S. haemolyticus* isolates obtained, 3 were identified only by API-Staph and the PYR test was necessary for the other 3 to reach a final conclusion. The 3 *S. caprae* isolates obtained were identified only by the API 20 Staph with very good percentages of identification. *S. chromogenes* was identified only by the API 20 Staph. The ID 32 Staph was necessary to identify 1 *S. lugdunensis*.

The MIC determination obtained for each isolate by the E-test method were read by 3 different readers and the results were compared. Agreement was 98% for the antibiotics tested by the flooding method and 85% for the swab method.

The MIC results obtained by the two methods were converted to qualitative categories (susceptible, intermediate and resistant) using NCCLS guidelines and were compared.

The susceptibility profile obtained on the basis of the MICs (E-test method) for the 53 coagulase-negative staphylococci isolates to the six antibiotics tested is shown in Fig. 1. For vancomycin no isolates showed resistance and 5 (9.4%) showed intermediate susceptibility. Of these 5 CoNS, 4 were *S. epidermidis* isolated from blood culture and 1 was *S. haemolyticus* isolated from cerebrospinal fluid. The highest MIC found for vancomycin was 12 μg/ml for 1 *S. epidermidis* isolate from blood culture, that was susceptible to oxacillin, cephalotin and teicoplanin. Three isolates (5.7%) were resistant to teicoplanin, and 9 (16.9%) were intermediate. Two teicoplanin-resistant isolates were *S. haemolyticus*, one from a catheter (MIC 32 μg/ml) and

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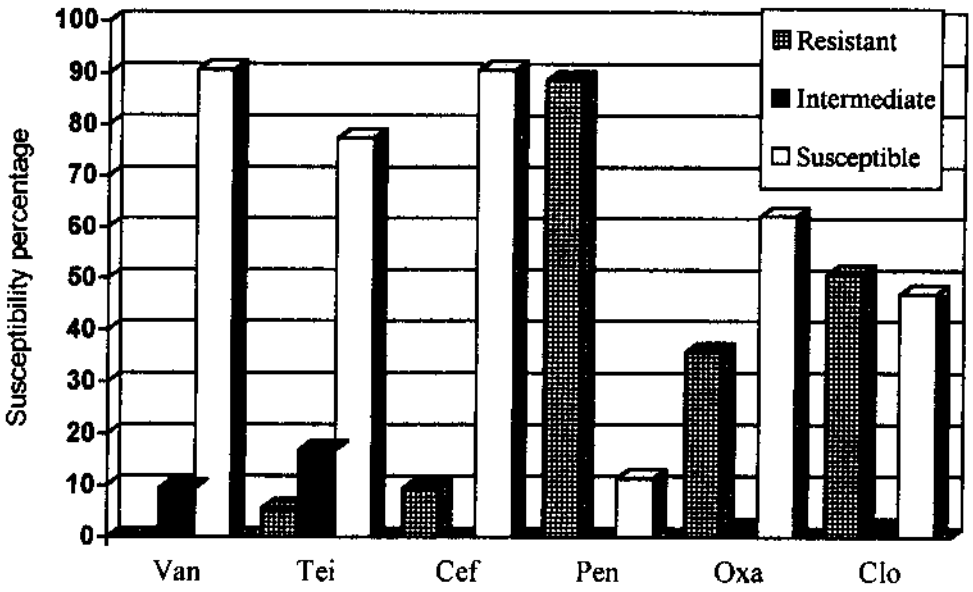

Fig. 1- Profile of the susceptibility of the CoNS species isolated to the six antibiotics in the E-test.

the other from a blood culture (MIC 64 μg/ml). The other teicoplanin-resistant (MIC 32 μg/ml) and 8 intermediate isolates were *S. epidermidis*, except for 1 intermediate isolate (*S. caprae)*. Of these 10 isolates, 8 were from blood cultures and 2 from catheters. The MIC results for these glycopeptides are illustrated in Fig. 2. The equivalent MIC breakpoints were 8.0 μg/ml (susceptibility) and 32.0 μg/ml (resistance) for teicoplanin and 4.0 μg/ml and 32.0 μg/ml for vancomycin.

Five isolates (9.4%) were resistant to cephalothin and no intermediate results were obtained. Forty-seven isolates (88.6%) were resistant to penicillin and 6 (11.3%) were susceptible. Nineteen (35.8%) were resistant and 1 (1.9%) was intermediate to oxacillin and 27 (50.9%) isolates were resistant to chloramphenicol.

The MICs for the 42 *S. epidermidis* identified were evaluated for each antibiotic. For vancomycin the MIC ranges were narrow, most of them varying from 2.0 to 4.0 μg/ml (90.6% of the isolates). No isolates were identified as vancomycin resistant. For teicoplanin most of the MIC ranged also from 4.0 to 12.0 μg/ml, but 3 isolates (5.7%) were resistant. The MICs for penicillin and oxacillin were more variable, for penicillin the MICs ranged from 0.016 to 256 μg/ml, showing a wide difference in isolate behaviour in relation to this antibiotic. For oxacillin the MICs ranged from 0.094 to 256 μg/ml and we detected a significant proportion of resistant isolates.

For chloramphenicol the MICs ranged from 1.5 to 256 μg/ml. The MIC values for cephalothin ranged from 0.19 to 1.0 μg/ml (76.2%) indicating that most of the isolates are still susceptible to this antibiotic.

DISCUSSION

The E-test has been found to be especially useful for susceptibility testing of many fungi and bacteria.[4,11,19,20]

In this study, the E-test was found to be convenient and reliable for MIC determinations of CoNS isolates to many antibiotics, and for detecting high-level resistance to teicoplanin.

The qualitative results of the E-test obtained by flooding were much more reproducible than the results obtained using the swabs. Consequently, the quantitative E-test results showed less variability when we used the flooding inoculation method instead of the swab inoculation method. Fluctuations in the readings by the three examiners were common when the swab inoculation method was used. The swab marks made when inoculating the plates may explain the reading variability. However, the MIC break point was readily identified when using flooding method.

When reading antibiograms using disks and when the resistance level is close to the break point of susceptibility or is in the intermediate zone, it is difficult to know for sure if the organisms are susceptible or not. In contrast, when reading the inhibitory zone produced by E-test strips this did not occur, especially when we used the flooding method, since the MIC breakpoint is well defined.

Once familiarity with the E-test flooding was gained, its ease and convenience of use made it more practical and preferable to the swab method for determining the MIC of antimicrobial agents. Some mucoid strains of the CoNS on occasion exhibited an indistinct intersection between the inhibitory zone and the E-test strips, making precise deter-

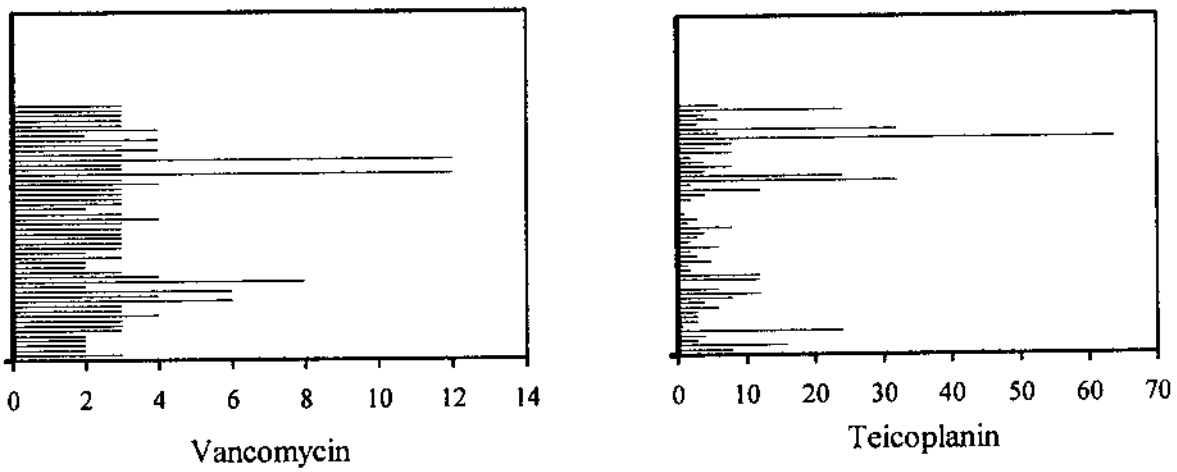

Fig. 2- MIC variations for 53 CoNS isolates in mg/ml.

mination of the MIC problematic.

On the whole, the advantages offered by the E-test flooding method are great.

An important feature of the CoNS studied is their multiple resistance to antimicrobial agents, indicating a growing necessity of studying the MICs for these isolates.[3]

Most of the coagulase-negative staphylococci proved to be resistant to penicillin, explaining the increasing importance of these organisms as nosocomial pathogens. No isolates were resistant to vancomycin and 5.7% were resistant to teicoplanin, indicating that there are strains resistant to these glycopeptides which can become important problems in the future.

In our study, while all isolates were found to remain susceptible to vancomycin, others were found to have become less susceptible to teicoplanin, in agreement with other investigators.[5, 13] This is particularly true for CoNS isolated from patients in Europe, where teicoplanin is approved for general use. In one study from France the isolates did not show reduced susceptibility to vancomycin but teicoplanin resistance was correlated with either vancomycin or teicoplanin use.[13] It is also important to notice the low rate of resistance to oxacilin, less than 40%, which is lower than the one observed at another Brazilian University Hospital[16]. The susceptibility of the isolates to cephalothin was high and superior to their susceptibility to oxacillin and teicoplanin. These data can help the physicians to prescribe antimicrobial drugs for nosocomial infections.

Among the 42 strains of *S. epidermidis* isolated in the present study, 36% (15) were resistant to one or more antibiotic families (penicillins, cephalosporins, imipenem, aminoglycosides, tetracycline, sulfonamides, chloramphenicol, clindamycin., macrolides and glycopeptides) and all were resistant to oxacillin. In a study conducted at the same hospital in 1994, approximately 26% of the *S. epidermidis* strains were resistant to oxacillin,[12] showing that the susceptibility of these bacteria to this antibiotic was considerably reduced over this 3-year period. A retrospective 9-year study carried out in Greece also reported an increase in the profile of CoNS resistance, especially to oxacillin, with resistance increasing from 11-21% in 1986 to 51-75% in 1994.[10]

Of these 15 multiresistant *S. epidermidis* strains, 6 were isolated from patients submitted to bone marrow transplantation. The clinical impression was that staphylococci colonised the intravenous catheter and caused bacteremia, emphasising the importance of these microorganisms as emerging pathogens in immunocompromised patients. The multiresistance of these strains probably results from selection by the prolonged broad-spectrum antibiotic treatment used during the period immediately after transplant.

Five other multiresistant *S. epidermidis* strains were also isolated from patients who were immunosuppressed for different reasons, and who were probably infected by this micro-organism or developed bacteremia.

Cercenado et al.[4] reported a percentage of CoNS isolates that were teicoplanin-resistant, including two isolates of *S. haemolyticus*. Concerning the 6 *S. haemolyticus* strains isolated in the present study, 3 of them showed resistance to 3 or more antibiotic families. These three strains were isolated from patients with severe heart disease, cancer of the uterus and AIDS, respectively. All of them had been implanted with a central venous catheter which may have been the focal point of origin of bacteremia and of blood infection with multiresistant *S. haemolyticus*.

Two multiresistant *S. caprae* strains were isolated from the same patient periodically submitted to total parenteral nutrition. The other *S. caprae* strain was considered to be probably responsible for a pulmonary infection in a patient

with adenocarcinoma and liver metastases, although it was sensitive to all drugs tested in the present study.

The *S. lugdunensis* strain isolated from a new-born without an apparent focal point of infection showed a considerably broad susceptibility profile and probably represented contamination of the blood culture. However, it should be pointed out that bacterial endocarditis due to this CoNS species involves a high mortality rate, with 8 of the 12 patients reported in the literature dying of this complication.[9]

The only *S. chromogenes* strain isolated, which was resistant only to chloramphenicol and penicillin G, possibly caused bacteremia in a patient submitted to total parenteral nutrition.

The increasing recognition of the pathogenic potential of various species of staphylococci and the emergence of drug resistance amongst them denote the need to adopt better laboratory procedures to identify and understand the diversity of staphylococci isolated from clinical material.

The E-test allowed the simultaneous testing of 6 antimicrobial agents per 150 mm agar plate. The same Muller-Hinton agar plates used for disk diffusion testing can be used for the E-test, a fact that makes the procedure easier. The E-test strips can be stored at -20° C for at least 1 year and can be used to test single isolates as needed.

Perhaps the only disadvantage of the E-test method use in Brazil is its relatively higher cost (approximately US $7.00 per strip). Unfortunately, routine use of the E-test procedure for hospitalised patients is impracticable, except for some specific clinical cases where the E-test strips can be used to better monitor the therapeutic conduct.

However, we do recommend the E-test for testing CoNS isolates in situations in which the clinical data of the patient confirm the CoNS as the main pathogen, when quantitative susceptibility data are clinically necessary or when qualitative tests methods can cause doubts in terms of therapeutic conduct because the reading ranges are close to the susceptibility limits.

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