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Antimicrobial sensitivity and adherence study in strains of coagulase-negative *Staphylococcus* spp.

Isabel L. Barberis,* María C. Pájaro,*
Sergio D. Godino,* Liliana Pascual,*,**
María D. Daniele*

ABSTRACT. Antimicrobial sensitivity was evaluated from 120 strains of coagulase-negative *Staphylococcus* (CoNS), isolated from urinary infections. The isolated species were identified by conventional methods and sensitivity to vancomycin, tetracyclin, norfloxacin, cephalothin erythromycin, clindamycin, oxacillin, penicillin, cyprofloxacin, ampicillin and ampicillin/sulbactam was tested by the agar dilution technique and the diffusion in disc technique. The distribution of the species was: *Staphylococcus epidermidis* 26 (21.6%), *S. haemolyticus* 48 (40.0%), *S. saprophyticus* 40 (33.4%) and *S. simulans* 6 (5%). CoNS strains extracted from urinary infections showed a high percentage of vancomycin and tetracyclin sensitivity, and a low sensitivity to β-lactamic antibiotics, except ampicillin/sulbactam and cephalotin. Isolation percentage resistance to oxacillin was less than 48%. Strains resistant to oxacillin were considered as resistant to both penicillin and ampicillin. From 42% to 80% of *S. haemolyticus, S. saprophyticus, Staphylococcus epidermidis* and *S. simulans* were positive to the adherence test. Results from this work showed that slime producing CoNS strains, isolated in Río Cuarto Hospital, had different percentages of resistance to the studied antimicrobial agents.

Key words: Coagulase-Negative *Staphylococcus*, adherence, glycocalyx, antibiotics.

RESUMEN. La susceptibilidad antimicrobiana de 120 aislados de estafilococos coagulasa negativos (CoNS) fue evaluada. Los aislados fueron identificados a nivel de especie por métodos convencionales y la susceptibilidad a vancomicina, tetraciclina, norfloxacina, cefalotina, eritromicina, clindamicina, oxacilina, penicilina, ciprofloxacin, ampicilina y ampicilina/sulbactam fue probada por las técnicas de dilución en agar y de difusión en disco. La distribución de especies fue la siguiente: *Staphylococcus epidermidis* 26 (21.6%), *S. haemolyticus* 48 (40%), *S. saprophyticus* 40 (33.4%), y *S. simulans* 6 (5%). Las cepas CoNS provenientes de infecciones urinarias presentaron un alto porcentaje de sensibilidad a vancomicina y tetraciclina y una baja sensibilidad a antibióticos β-lactámicos, excepto ampicilina/sulbactam y cefalotina. El porcentaje de aislados resistentes a oxacilina fue menor al 48%. Esos aislados con reducida susceptibilidad a oxacilina fueron resistentes a penicilina y ampicilina, pero se mantuvieron sensibles a vancomicina. Del 42 al 81% de las cepas de aislados clínicos de *S. epidermidis, S. haemolyticus, S. saprophyticus,* y *S. simulans* fueron positivos en la prueba de adherencia. Nuestros resultados demostraron que la resistencia a un conjunto de once agentes antimicrobianos ha emergido entre algunos aislados en nuestra Institución.

Palabras clave: *Staphylococcus* coagulasa negativo, adherencia, antibióticos, glicocalix.

INTRODUCTION

Coagulase-Negative staphylococci emerged as important pathogens by the late 70's, when knowledge of their Biology and sensitivity to antimicrobial agents increased, thus assuming their value as real pathogens and not polluting agents. They are frequently opportunistic pathogens, but none of them should be underestimated, until their clinical relevance is resolved.[8]

Of all CoNS species, *S. epidermidis* seems to have the greatest pathogenic potential, and is present in a larger diversity of clinical processes. It is the most common cause for prosthesis-valve endocarditis, but it is also responsible in a small percentage of native-valve endocarditis. *S. epidermidis* is the primary pathogen in infections associated to cerebro-spinal fluid in shunt receiving patients, as much as it is in peritonitis in ambulatory patients subjected to dialysis, and it is also commonly isolated from infections of the urinary tract.[9,11,18]

Among CoNS, *S. haemolyticus* is the second most frequently isolated species from clinical infections. *S. saprophyticus* has been clearly identified as a species which commonly produces urinary tract infections (UTI), especially in sexually active young women.[9] Other CoNS species such as *S. hominis, S. warneri, S. simulans, S. cohnii, S. saccharolyticus, S. capitis* and *S. xylosus* present a low incidence in a variety of human infections.[15]

Some strains excrete a glycocalyx cover that helps them resist humoral and cell-mediated immunological mechanisms, as well as antibiotics. This adherent cover, or "slime", allows bacteria to adhere to surfaces, particularly those of patients with catheters and prosthesis or immuno-compromised patients.[20] Adherence is evidenced by slime production, and this could be quite important for colonization, being this, a virulence factor.[20] Christensen et al[3] described a test for adherence, or slime production, that can measure virulence, and therefore, clinical importance.

The ability of an organism to produce slime would be significantly associated with its capability to produce diverse ill-

* Departamento de Microbiología, Universidad Nacional de Río Cuarto, Córdoba Argentina.
** Laboratorio de Bacteriología, Hospital de Río Cuarto, Córdoba Argentina.

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nesses.[19] Slime production by CoNS could play an important role in the adherence of these microorganisms to the mucous epithelia. During the last years, an increase in the number of clinical samples with CoNS isolating has been observed.

The frequency of ampicillin resistance of *S. epidermidis* has also increased.[16,19] A high frequency of colonization by multiresitant CoNS strains in hospital workers has been evidenced in a recent study. Vancomycin resistant strains of methicillin resistant *staphylococcus* represent a potential therapeutic problem.[16,19]

The object of this work is to study the sensitivity patterns of CoNS species to 11 antimicrobial agents of clinical use, as well as the probable relationship between antibiotic resistance and slime production of CoNS species most frequently isolated in Río Cuarto, Argentina.

MATERIAL AND METHODS

Strains: This study included 120 CoNS species, isolated from urine at the Bacteriology Laboratory of the Hospital Central de Río Cuarto, Argentina.

All strains were Gram-positive coccus, catalase positive. The guideline that was followed, in order to consider the studied bacterial strains as causes of urinary tract infections, was the presence of more than 10^5 UFC/ml of the bacteria, and a pathologic urinary sediment. Strains were isolated from samples of non-hospitalized female patients, within 19 and 40 years old.

Identification: Coagulase reaction was performed for all strains, in slide and tube, using rabbit blood. Coagulase negative strains were identified upon the basis of a variety of conventional phenotypic characteristics, following the method of Kloos.[10]

Sensitivity study by the disc diffusion method: Diffusion in Muller Hinton agar plate (AMH) technique was performed, using antibiotics according to the NCCLS rules.[14] The sensitivity spectrum of CoNS was tested, to 11 antibiotics commonly used in urinary infections. A suspension was prepared, with colonies from an 18 h, 37°C culture, re-suspended in sterile tubes containing Soya Tripticase Medium (STM) until a turbidity equivalence to that of the 0.5 Mc Farland scale tube (nephelometer) was achieved. This methodology was implemented for each bacterial species in study. The bacterial suspension was absorbed with a sterile hyssop, eliminating the liquid excess by pressing the hyssop against the walls of the tube. Afterwards, the suspension was plated in several directions until the plate's surface was homogeneously covered, and the testing antibiotic disks were applied. The plates were incubated at 35°C during 24 h aerobically, determining sensitivity or resistance for each antibiotic by measuring the diameter of the bacterial growth inhibition haloes. Selected antibiotics for this study, were: vancomycin, tetracyclin, norfloxacin, cephalotin, erythromycin, clindamycin, oxacillin, penicillin, cyprofloxacin, ampicillin, and ampicillin/sulbactam.

The criteria for the interpretation of inhibition haloes, was the recommended by the NCCLS.[14] Values were measured by triplicate, and *S. epidermidis* ATCC 12228 was implemented as sensitivity control.

Determination of the minimal inhibitory concentration (MIC), plate dilution method: Colonies isolated in STA plate from 18-24 h incubations were re-suspended in sterile medium, and turbidity was adjusted to that of the 0.5 standard of the McFarland scale. AMH plates with 2% NaCl were prepared, using serial dilutions at double antibiotic, and punctual inoculation in the plate was realized with a 1 mm ase (.001 ml), for a later 24 h incubation at 35°C.[4] Also, a control plate without antibiotic was inoculated to testify the viability of the cultures.

MIC was defined according to the international rules suggested by NCCLS, as the lowest antibiotic concentration that inhibited visible growth (except two colonies) after 24 h incubation at 35°C.[14]

The cut point for sensitivity to each of the tested antimicrobial agents corresponded to the MIC. Values were measured by triplicate and *S. epidermidis* ATCC 12228 was implemented as a sensitivity control.

Adherence or slime: Tests of adherence to glass were performed for all strains, as described by Christensen et al.[3] Slime production was observed as a film adhered to the bottom of the tube. A sterile polyester or cotton hyssop was used to transfer bacteria from a Soya Tripticase Agar (STA) plate incubated 24 h in a 5 ml STM tube. The hyssop was agitated in the medium until an inoculum with a turbidity approximated to that of the tube NR 1 of the McFarland scale was achieved. The tubes were incubated aerobically at 35°C for 18-20 h. Afterwards, content was discarded from the tubes, these were washed with distilled water and a safranin solution was added for 30 min at room temperature. Safranin was discarded and the tubes were gently washed with phosphate saline buffer, then inverted to eliminate remaining buffer and then dried. Dried tubes were observed with a fluorescent lamp to make evident the presence of a film adhered to the inner walls of the tubes. Results were registered as follows: negative (absence of film), weakly positive, moderately positive and strongly positive. As a positive control, *S. epidermidis* ATCC 35984 was used.

RESULTS

Identification. Of the 120 CoNS isolated species, 48 were identified as *S. haemolyticus*, 26 as *S. saprophyticus*, 40 as *S. epidermidis* and 6 as *S. simulans*.

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Sensitivity patterns. Sensitivity patterns of CoNS species to 11 antibiotics most commonly used clinically are shown in Table 1.

This work has demonstrated a clear difference in the sensitivities of the diverse CoNS species. Frequencies of sensitivity to erythromycin was of a 90% for *S. simulans* and 40% for *S. epidermidis*; to norfloxacin, sensitivity from *S. saprophyticus* and *S. haemolyticus* was of 80%, compared with a 30% from *S. simulans*.

CoNS strains isolated from urinary tract showed a high sensitivity percentage to vancomycin and tetracyclin, and a low sensitivity to β-lactamic antibiotics, except ampicillin/sulbactam and cephalotin.

All *S. saprophyticus* strains were sensitive to vancomycin, and presented a high sensitivity to cephalotin, ampicillin/sulbactam, tetracyclin, norfloxacin and cyprofloxacin. This species showed a moderated sensitivity to erythromycin, clindamycin and oxacillin. Only 10% of the strains showed sensitivity to penicillin, while all strains were resistant to ampicillin.

S. haemolyticus presented a high sensitivity to most of the studied antibiotics. Only 8% of the strains were sensitive to penicillin, and 2% to ampicillin.

S. epidermidis sensitivities to vancomycin, tetracyclin, cephalotin, cyprofloxacin and ampicillin/sulbactam ranged from 80% to 95%. All strains were resistant to ampicillin, and only 8% were sensitive to penicillin.

All *S. simulans* strains were sensitive to vancomycin and cyprofloxacin, besides showing a high sensitivity to most antibiotics (80%-90%). 70% of the strains were resistant to norfloxacin, 90% to penicillin and all to ampicillin.

S. saprophyticus, *S. simulans*, *S. epidermidis* and *S. haemolyticus* showed a moderate sensitivity to oxacillin (< 60%). From 80% to 95% of CoNS strains were sensitive to tetracyclin, cephalotin and ampicillin/sulbactam (Table 1).

Strains that were resistant to oxacillin showed a diameter of the halo of bacterial growth inhibition ≤ 17 mm and a MIC of 16 μg/ml, but remained sensitive to vancomycin (MIC ≤ 4 μg/ml). Resistance to vancomycin (MIC ≥ 32 μg/ml) was detected in 2.5% of *S. epidermidis* and in 5% of *S. haemolyticus*, while an intermediate resistance to vancomycin (MIC 8-16 μg/ml) was found in 2.5% of *S. epidermidis*. Strains that were resistant to oxacillin were considered as resistant to penicillin and ampicillin. The cut points to the different antimicrobial agents tested, with respect to the diffusion and dilution tests of sensitivity in agar, are shown in Table 2. Also, a correlation between both antibiogramic techniques employed was observed.

Adherence or slime. In this study, frequency of slime production was determined for 120 CoNS strains, classifying them according to the intensity of production as: negative, weakly positive, moderately positive and strongly positive.

From the total number of strains tested for adherence the number of positive ones were: 20 *S. haemolyticus*, 19 *S. saprophyticus*, 32 *S. epidermidis* and 4 *S. simulans*. Percentages of positives ranged from 42% to 80%, moderately positive being the most common classification (Table 3).

All moderately and strongly positive slime producing CoNS strains presented the highest resistance percentage to

Table 1. Percentage of sensitivity of CoNS species to 11 antimicrobial agents.

Sensitivity Percentage* Antibiotics	CoNS Species *S. haemolyticus* n = 48	*S. saprophyticus* n = 26	*S. epidermidis* n = 40	*S. simulans* n = 6
Vancomycine	95	100	95	100
Tetracyclin	93	85	80	80
Norfloxacin	80	80	46	30
Cephalotin	92	95	85	85
Erythromycin	60	76	40	90
Clindamycin	60	72	70	75
Oxacillin	52	57	56	58
Penicillin	8	10	8	10
Cyprofloxacin	80	80	85	100
Ampicillin	2	0	0	0
A/Sulbactam	94	90	84	85

*Percentages are the mean value of triplicate experiments by the disk diffusion method.

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the tested antibiotics, which was not observed from weakly positive strains.

The *S. epidermidis* strain that was resistant to vancomycin, with an MIC of 32 μg/ml, was a weak slime producer.

Our results show that approximately 50% of slime producers *S. epidermidis*, *S. saprophyticus* and *S. haemolyticus* strains presented resistance to 2 or more antimicrobial agents, and that strongly positive slime producers were more resistant to some antimicrobial agents.

DISCUSSION

Throughout the last years, the frequency of *S. epidermidis* isolation has increased, as much as methicillin resistance has increased in such microorganism.[5] In this study, the frequencies of sensitivity to oxacillin observed from *S. epidermidis* and *S. haemolyticus* were 56% and 52%, respectively. These results are not comparable to those obtained by Del' Alamo in the study realized in São Paulo, Brazil (19.2% and 4.2%).[4] Results obtained in this study are comparable to those found by Laverdiere et al in 1998,[11] where percentages of resistance to norfloxacin, cyprofloxacin and cephalotin of 15%, 14% and 10% were found, respectively. Results for norfloxacin from *S. epidermidis* and *S. simulans* are not comparable to those of Laverdiere et al.[12]

The high frequency of sensitivity to cyprofloxacin (80% to 100%) found in this study might be related to the limited use of fluorochinolones in our environment.

Isolated CoNS presented a high sensitivity (≥ 80%) for tetracyclin, cephalotin, cyprofloxacin, ampicillin/sulbactam, and for vancomycin ≥ 95%. These results are comparable to those carried out in Paris by Vu-Thien.[21]

In this study, we observed that methicillin resistant strains were frequently resistant to several antimicrobial agents, while CoNS oxacillin resistant strains were generally resistant to clindamycin and erythromycin. In a work realized in Brazil, the authors report oxacillin resistance percentages comparable to those obtained in this study.[4]

Sensitivity to cyprofloxacin was more frequent among oxacillin sensitive strains than among oxacillin resistant CoNs. In this present work, it was observed that norfloxacin and/or cyprofloxacin resistant *S. epidermidis strains w*ere simultaneously oxacillin resistant.

In 4 CoNS strains we observed that 2 S. haemolyticus and 1 S. epidermidis presented an intermediate resistance to vancomycin (MIC 8-16 mg/ml). They were classified as sensitive (> 17mm) by the disk diffusion method, while 1 S. epidermidis strain was considered resistant to an MIC of 32 mg/ml. Our results showed that the disk diffusion method cannot detect isolates with a diminished sensitivity to antimicrobial agents. Strausbaugh obtained similar results in *S. epidermidis* for vancomycin in 1999.[17]

Slime production has been reported in strains of all *Staphylococcus* species associated with human infections.[3,15,20] In this study we observed that 42% to 80% were positive to adherence. 80% of *S. epidermidis*, 73% of *S. saprophyticus*, 67% of *S. simulans* and 42% of *S. haemolyticus* isolated were positive for slime production. A couple of *S. simulans* strains were found that were moderately positive, and another couple weakly positive for the adherence test. These results are lightly inferior to those found by Drozenova et al.[6]

Table 2. Antimicrobial sensitivity patterns by the methods of disk diffusion and agar dilution of CoNS species.

Antibiotics	CoNS	
	Inhibition haloes (mm)	Cut point μg/ml
Vancomycine	≥ 17	< 4
Tetracyclin	≥ 19	< 4
Norfloxacin	≥ 17	< 4
Cephalotin	≥ 18	< 8
Erythromycin	≥ 23	< 0.5
Clindamycin	≥ 21	< 1
Oxacillin	≥ 18	< 8
Penicillin	≥ 29	< 0.12
Cyprofloxacin	≥ 21	< 1
Ampicillin	≥ 29	< 0.25
A/Sulbactam	≥ 15	< 8/4

Table 3. Classification of isolated CoNS species by slime production.

CoNS	N° (%) of positive strains	Slime production (%) Strongly positive	Moderately positive	Weakly positive
S. haemolyticus	42% (20/48)	3%	27%	12%
S. saprophyticus	73% (19/26)	7%	59%	7%
S. epidermidis	80% (32/40)	30%	40%	10%
S. simulans	67% (4/6)	0%	50%	50%

In this study, *S. epidermidis* presented a significant association between slime production and resistance to various antimicrobial agents. These results are similar to those obtained by Nayak et al,[13] who observed that slime producing *S. epidermidis* isolated from ocular infections was multiresistant to antimicrobial agents.

Results in this work suggest that slime producing CoNS was responsible for a significant decrease in sensitivity to some antimicrobial agents.

Our observations confirm that CoNS isolated in our zone presented resistance to several antibiotics of frequent use in the therapy of urinary infections.

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Correspondence to:

Isabel L. Barberis
lbarberis@exa.unrc.edu.ar
Address: Ruta 36 Km 601
Dpto. de Microbiología e Inmunología,
UNRC. 5800, Río Cuarto,
Córdoba, Argentina.