Research
Volume 6:2,2020

Journal of Antimicrobial Agents

ISSN: 2472-1212 Open Access

Antibiotic Resistance of Coagulase-Negative Staphylococci Isolated in the Laboratory

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Abstract

Introduction Coagulase-negative staphylococci (CoNS) such as *Staphylococcus epidermidis, Staphylococcus haemolyticus* and *Staphylococcus saprophyticus* live as commensals on the human skin and mucous membranes. They can be potentially pathogenic when isolated from immunocompromised patients such as: HIV positive, cancer chemotherapy, hemodialysis, diabetics, etc.

Methodology This was a prospective study aimed at collecting data on biological sample received at Fann's CHNU bacteriology laboratory in Dakar from April 1, 2018 to March 31, 2019. The classical bacteriology techniques were used and the antibiotic susceptibility performed according to the Recommendations of the Antibiogram Committee of the French Society of Microbiology (CA-SFM 2016).

Results We isolated 86 strains of CoNS, distributed as follows: 45.3% (n=39) of *S. epidermidis* and 54.7% (n=47) of *S. saprophyticus*. Among the 39 strains of *S. epidermidis* isolated, we obtained 3 strains (3.5%) resistant to all beta-lactams (methicillin-resistant) and of the 47 strains of *S. saprophyticus*, 4 strains (4.7%) are methicillin-resistant. We isolated 39 strains of *S. epidermidis*, among which 3 strains (3.5%) are resistant to all aminoglycosides (KTG phenotype) and of the 47 strains of *S. saprophyticus*, 4 strains (4.7%) are KTG phenotype. All CoNSs strains were susceptibility to vancomycin.

Discussion Hospitalized patients represented 65.7%, and blood cultures 51.1%, which can be explained by the state of immunosuppression from some patients and the lack of asepsis during care. Strains of CoNS resistant to oxacillin are called méti-R strain, thus resistant to all betalactamines. Aminoglycosides were inactive in the 8.2% (n=7) of CoNS. Vancomycin was active on all CoNS; confirming that diminished susceptibility to glycopeptides is exceptional.

Conclusion CoNS are frequently isolated in the laboratory. The site of infection, immune status, purity of cultures and the antibiotic resistance informs about the clinical pathogenic role of CoNS.

Keywords: Antibiotics• Coagulase-Negative Staphylococci • Pathogenicity • Resistance

Introduction

Coagulase-Negative Staphylococci (CoNS) are bacteria that live as commensals on the skin and mucous membranes of humans or animals. Some species such as *Staphylococcus epidermidis, Staphylococcus haemolyticus* and *Staphylococcus saprophyticus* are often isolated alone or in associated with other germs in biological samples [1,2].

They can be potentially pathogenic when isolated from immunocompromised patients such as: HIV positive, cancer chemotherapy, hemodialysis, diabetics, etc.

Of all the bacteria responsible for an opportunistic infection, they are among the first pathogens responsible for bacteremia in neutropenic patients often carrying venous catheters in hospitalized patients [3].

Their involvement in the infectious process must be appreciated by microbiological and epidemiological criteria.

With the advent of antibiotic resistance, CoNSs can develop antibiotic resistance by acquisition of plasmids and/or transposons [4-6].

The antibiotic resistance of a CoNS strain can be a determining factor in favor of its pathogenicity.

This is why; we undertook the work whose objective was to evaluate the part occupied by the CNS and especially their implication in the bacterial infections at CHNU of Fann and to determine their profile of resistance to antibiotics.

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Received: January 12, 2020; **Accepted**: April 23, 2020; **Published**: April 27, 2020

Methods

The study was carried out at the bacteriology laboratory of Fann CHNU in Dakar from April 1, 2018 to March 31, 2019. We frequently isolate these CoNSs but are not always considered as potentially pathogenic responsible for the infection. Bacteria have been isolated from pathological products, including 86 strains of CoNSs.

The socio-demographic information (age, sex) of the patients and the immune status are mentioned in a register. The collection of pathological products was carried out in the laboratory or at the patient's bedside for hospitalized patients with rigorous aseptic measures to avoid any contamination.

Depending on the pathological product (urine, pus, blood, vaginal aspiration), the appropriate culture media were inoculated and the bacterial identification was carried out by conventional bacteriological methods. An identified strain CoNS was considered pathogenic and an antibiogram was performed if:

- The germ is isolated on two consecutive blood cultures.
- The germ is isolated alone (pure culture) from pus or vaginal aspiration
- The germ is isolated from an urine sample with leucocyturia
- The germ is isolated from a sample taken from an immunocompromised patient.

The isolation of the strains is done on a selective Chapman medium. After 18 to 24 hours of incubation, the bacterial colonies were identified first by the morphology (Gram positive cocci), then by the hydrogen peroxide enzymatic test (positive) and finally by the pathogenicity tests (search for free coagulase and DNAse, sensitivity to Novobiocin 5 µg and hydrolysis of urea).

Antibiotic resistance (antibiogram) was performed according to the recommendations of the antibiogram committee of the French microbiology society (CA-SFM 2016). The method of broadcasting discs on Müeller-Hinton was used.

Resistance to antibiotics were carried out using the diffusion disk method on Müeller-Hinton agar. The following disks (Bio-Rad, France) were used: penicillin G (PENG-1 IU 6 µg (10 IU)), cefoxitin (FOX-30 µg), chloramphenicol (CHL-30 µg), tetracycline (TET-30) µg), kanamycin (KMN-30 µg), tobramycin (TOB-10 µg), gentamicin (GMN-30 µg), erythromycin (ERY-15 µg), lincomycin (LCN-15 µg), pristinamycin (PTN-15 µg), pefloxacin (PEF-5 µg), norfloxacin (NOR-10 µg), ciprofloxacin (CIP-5 µg), fusidic acid (FAD-10 µg), vancomycin (VAN-30 µg). *Escherichia coli* ATCC 25922 and *S. aureus* ATCC 29213 were used as reference strains for antibiotic discs control.

The data was saved with the Microsoft Excel 2013 software. Statistical calculations and diagrampers were performed with the same software. Of a small sample (86), statistical analysis was not relevant.

Results

Distribution of strains CoNSs according to the species

Of 3856 biological samples received, 1924 germs have been isolated, 86 of which are CoNSs isolated from blood cultures, urine, pus and vaginal aspirations.

They are distributed as follows: 45.3% (n=39) represented by *S. epidermidis* and 54.7% (n=47) by *S. Saprophyticus*(Figure 1).

Distribution of strains CoNSs according to the type of sample

In the various pathological products where we isolated the CoNSs, 44 strains were isolated from the blood culture (51.1%), 17 strains in the urine (19.8%), 14 strains in the pus samples (16,30%) and 11 strains in vaginal aspirations (2.8%) (Figure 2).

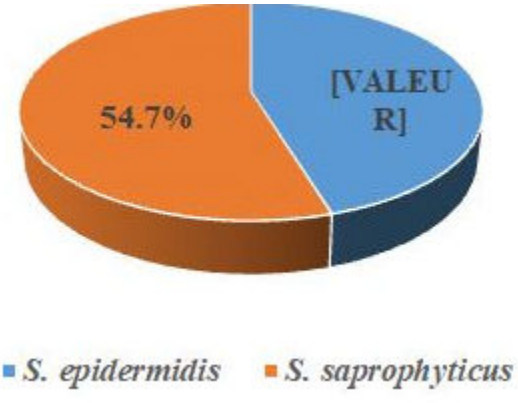

Figure 1: Distribution of strains CoNSs according to the species.

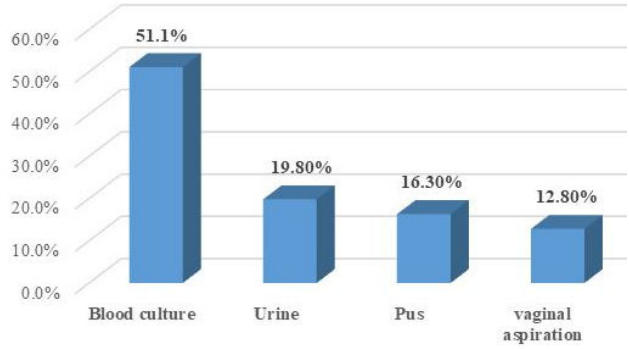

Figure 2: Distribution of strains CoNSs according to the type of sample

Bêta-lactam resistance profile of CoNSs

Among the 39 strains of *S. epidermidis* isolated, we obtained 3 strains (3.5%) resistant to all beta-lactams (methicillin-resistant) and of the 47 strains of *S. saprophyticus*, 4 strains (4.7%) are methicillin-resistant (Figure 3).

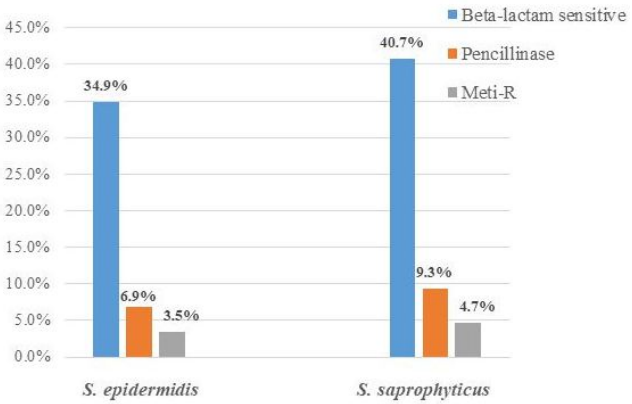

Figure 3:CoNSs Beta-lactam resistance profile

- Bêta-lactam sensitive : sensitive to all beta-lactams
- Penicillinase: resistant to penicillin A, G and M
- Meti-R: resistant to all beta-lactams

Aminoglycoside resistance profile of CoNSs

We isolated 39 strains of *S. epidermidis*, among which 3 strains (3.5%) are resistant to all aminoglycosides (KTG phenotype) and of the 47 strains of *S. saprophyticus*, 4 strains (4.7%) are KTG phenotype (Figure 4).

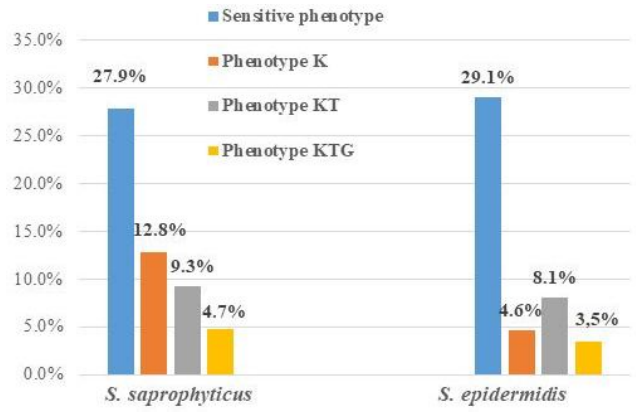

Figure 4: Aminoglycoside resistance profile of CoNSs

Sensitive phenotype: sensitive to all aminoglycosides

K phenotype: resistant to kanamycin and amikacin

KT phenotype: resistant to kanamycin, amikacin and tobramycin

KTG phenotype: resistant to all aminoglycosides

Resistance profile of CoNSs to macrolides and related

Among the CoNSs strains, 20.9% of *S. saprophyticus* and 23.2% of *S. epidermidis* were sensitive to macrolides and related. The CoNS strains were resistant to macrolides and related to 4.7% for *S. saprophyticus* and 1.2% for *S. epidermidis* (Table 1).

Table 1: Resistance profile of SCNs to macrolides and related

Phenotypes	*S. saprophyticus*	*S. epidermidis*
Sensitive phenotype	20,9%	23,2%
Inducible MLSB	23,3%	13,9%
Phenotype L	5,8%	7,0%
MLSB Phenotype associated with SA	4,7%	1,2%
Total	54,7%	45,3%

Sensitive phenotype: strain sensitive to all MLS

Inducible MLSB: strain resistant to erythromycin and sensitive to other macrolides, lincosamides and streptogramins

Phenotype L: strain resistant only to lincomycin

MLSB phenotype associated with SA: strain resistant to all antibiotics of the MLS group

Discussion

We know little about the involvement of the CoNS in bacterial infections in our hospitals in Dakar. They are frequently isolated in the laboratory from pathological products but are almost not considered to be responsible for bacterial infections.

CoNS are important opportunistic pathogens, methicillin-resistant coagulase negative staphylococci have become one of the main pathogens responsible for bacteremia in neutropenic patients often carrying central venous catheters [3].

The strains were mainly found in hospitalized patients carrying venous catheters with 65.7% (n=56) against 34.3 (n=30) for non-hospitalized patients. The strains were isolated mainly in blood culture with 44 strains including 25 strains of *S. epidermidis* and 19 strains of *S. saprophyticus*, ie 51.1%. This observation can be explained by the state of immunosuppression of HIV positive patients, diabetics or under cancer chemotherapy. These results are comparable to those of Chapin KC et al. who have found a predominance of staphylococci in blood culture [7]. In the study by Choi S. et al., 24 patients had blood cultures positive for *S. saprophyticus*, of which 07 patients were considered to have clinically significant bacteremia [8].

The antibiogram showed a resistance to oxacillin of 8.2% (n=7) in the two CoNSs species; these meti-R strains (resistant to methicillin) are phenotypically resistant to all the betalactamines used in the treatment. These are penicillins, aminopenicillins, isoxazolylpenicillins (oxacillin, cloxacillin, cephalosporins, penicillins with inhibitors, cephalosporins with inhibitors and carbapenems). This was confirmed by Bhargava and Zhang in their study where all strains resistant to methicillin were phenotypically resistant to penicillin and oxacillin [9]. From a therapeutic point of view, methicillin-resistant staph infections are a serious problem [10]. The mechanism of resistance to methicillin resistance CoNSs is linked to the presence of the mecA gene. The mecA gene codes for a new penicillin 2a binding protein, responsible for phenotypic resistance [11,12].

Among the antibiotics inactive on the CoNSs, we have the aminoglycosides 3.5% (n=3) of *S. epidermidis* and 4.7% (n=4) of *S. saprophyticus* which are resistant to all aminoglycosides. It is a class of antibiotics that continues to be effective in the treatment of MRSA infections. Quinolones, macrolides and related antibiotics (n=5) were inactive in 4.7% for *S. saprophyticus* and 1.2% for *S. epidermidis*. Very recently, a high prevalence (45.8%) of resistance to macrolides, lincosamides and streptogramins (MLS) has been reported [13,14].

The methicillin resistance of CoNS, strains is crossed between all the antibiotics of the bêta-lactam family. These strains are mainly found in hospitals, where they are associated with numerous resistances to other families of antibiotics thus imposing the use of glycopeptides. In our study, all strains of CoNS were sensitive to vancomycin; which confirms that the reduced sensitivity to glycopeptides is exceptional [15].

To avoid the development of resistance to vancomycin CoNS, it is important to rationalize its prescription. Use vancomycin only if treatment with beta-lactams, aminoglycosides, macrolides and related does not work, to prevent the development of resistance.

The limitations of this study were the lack of molecular identification of strains and resistance genes. Phenotypic identification of CoNSs species is often difficult with conventional methods.

Conclusion

With the advent of antibiotic resistance and the frequent isolation in the CoNS laboratory of pathological products, we find that they can be responsible for bacterial infections and can develop resistance to antibiotics, as the results of our work show.

The site of the infection, the immune status, the pure culture and the resistance phenotype guide the pathogenic role or not in the clinic. Compliance with aseptic rules during care and at the time of sample collection is very important to limit contamination by the CoNSs. Thus, they can be incriminated in infections even in an immunocompetent person.

References

1. Mazzariol, Annarita, Giuliana Lo Cascio, Erika Kocsis and Laura Maccacaro et al. (2012) Outbreak of linezolid-resistant *Staphylococcus haemolyticus* in an Italian intensive care unit. Eur J Clin Microbiol Inf Dis 31: 523-527.
2. Piette, Anne and Gerda Verschraegen (2009) Role of coagulase-negative staphylococci in human disease. Vet Microbiol 34: 45-54.
3. Bouchami, Ons, WafaAchour and Assia Ben Hassen (2007) Prevalence and mechanisms of macrolide resistance among *Staphylococcus epidermidis* isolates from neutropenic patients in Tunisia. Clin Microbiol Infec 13: 103-106.
4. Lozano, Carmen, Carmen Aspiroz, Antonio Rezusta and Elena Gómez-Sanz et al. (2012) Identification of novel vga (A)-carrying plasmids and a Tn 5406-like transposon in meticillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* of human and animal origin. Int J Antimicrob Ag 40: 306-312.
5. Corrente, Marialaura, Maria D'Abramo, Francesca Latronico and Maria Fiorella Greco et al. (2009) Methicillin-resistant coagulase negative staphylococci isolated from horses. New Microbiol 32: 311.
6. Podkowik, Magdalena, Jarosław Bystroń and Jacek Bania (2012) Prevalence of antibiotic resistance genes in staphylococci isolated from ready-to-eat meat products. Pol J Vet Sci 15: 233-237.
7. Chapin, Kimberle and Michael Musgnug (2003) Evaluation of three rapid methods for the direct identification of *Staphylococcus aureus* from positive blood cultures. J Clin Microbiol 41: 4324-4327.
8. Choi, Sang-Ho, Jun Hee Woo, Jin-Yong Jeong and Nam Joong Kim et al. (2006) Clinical significance of *Staphylococcus saprophyticus* identified on blood culture in a tertiary care hospital. Diagn Microbiol Infect Dis 56: 337-339.
9. Bhargava, Kanika and Yifan Zhang (2012) Multidrug-resistant coagulase-negative staphylococci in food animals. J Appl Microbiol 113: 1027-1036.
10. Huber, Helen, Dominik Ziegler, Valentin Pflüger and Guido Vogel (2011) Prevalence and characteristics of methicillin-resistant coagulase-negative staphylococci from livestock, chicken carcasses, bulk tank milk, minced meat, and contact persons. BMC Vet Res 7: 6.
11. Chambers, Henry (1997) Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. Clin Microbiol Rev 10: 781-791.
12. Couto, Isabel, Ilda Santos Sanches, Raquel Sá-Leão and Hermínia de Lencastre (2000) Molecular characterization of *Staphylococcus sciuristrains* isolated from humans. J clin microbio 38: 1136-1143.
13. Le Bouter, Anne, Roland Leclercq and Vincent Cattoir (2011) Molecular basis of resistance to macrolides, lincosamides and streptogramins in *Staphylococcus saprophyticus* clinical isolates. Int J Antimicrob Agents 37: 118-123.
14. Niedja da Paz Pereira, Jussyêgles, Marcelle Aquino Rabelo, Jailton Lobo da Costa Lima and Armando Monteiro BezerraNeto et al. (2016) Phenotypic and molecular characterization of resistance to macrolides, lincosamides and type B streptogramin of clinical isolates of *Staphylococcus spp.* of a university hospital in Recife, Pernambuco, Brazil. Braz J Infect Dis 20: 276-281.
15. Weiss, Karl, Danielle Rouleau and Michél Laverdière (1996) Cystitis due to vancomycin intermediate *Staphylococcus saprophyticus*. J Antimicrob Chemother 37: 1039-1040.

How to cite this article: Habibou, Sarr, Diop Amadou, Dieye Baidy, Niang Aissatou Ahmet, Diallo Fatoumata, Diagne Rokhaya, Lo Seynabou, Dia Mouhamadou Lamine, Ka Roughyatou and Sow Ahmad Iyane. "Antibiotic Resistance of Coagulase-Negative Staphylococci Isolated in the Laboratory". J Antimicrob Agents 6 (2020)