Original article

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. Usually considered non-pathogenic, they can still be responsible for infections under several circumstances. 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. Methicillin-resistant CoNSs accounted for 8.2%. Aminoglycosides were inactive in 8.2% of CoNSs. All 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.

1. Introduction

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 association with other germs in biological samples [1,2]. They have long been considered non-pathogenic, but recent research shows that they can be potentially pathogenic [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 multidrug resistance of a CoNS strain may be a guiding element in favor of its pathogenicity. For this reason, we undertook this work, which aimed to evaluate the role played by CoNSs at CHNU Fann bacterial infections and to determine their antibiotic resistance profile.

Methodology

The study was carried out in the bacteriology laboratory of CHNU Fann in Dakar from April 1, 2018 to March 31, 2019. Bacteria were isolated from biological sample, including 86 strains of CoNS. This is a prospective study to collect data on biological samples received in the laboratory.

The socio-demographic information (age, sex), the origin (hospitalized or not), and the immune status of the patient are mentioned on the register. Some samples were taken in the laboratory with strict asepsis while others were made at the...
patient's bed (patient hospitalized) especially the blood cultures. Depending on the pathological product, conventional bacteriology techniques were used for the identification of staphylococci. An identified strain of CoNS was considered pathogenic and an antibiogram performed only if:
- the germ is isolated on two consecutive blood cultures.
- the germ is isolated from polymicrobial sample alone in pure culture
- 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 selective medium of Chapman. After 18 to 24 hours of incubation, the bacterial colonies will be identified first by morphology (Gram-positive cocci), then by the hydrogen peroxide enzymatic test (positive) and finally by the pathogenicity tests (research free coagulase and DNAse, susceptibility to Novobiocin 5 μg and hydrolysis of urea).

Resistance to antibiotics (antibiogram) was examined according to the guidelines of the antibiogram committee of the French microbiology society (CA-SFM 2016).

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 diagrammers were performed with the same software. Of a small sample (86), statistical analysis was not relevant.

Results

1. Distribution of strains according to the species

Of the 3856 biological samples received, 1924 germs were isolated, of which 86 were CoNSs, 45.3% (n = 39) represented by S. epidermidis and 54.7% (n = 47) represented by S. saprophyticus (Figure a).

Table 1: Profile of resistance to macrolides and related antibiotics

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>S. saprophyticus</th>
<th>S. epidermidis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitive phenotype</td>
<td>20.9%</td>
<td>23.2%</td>
</tr>
<tr>
<td>Inducible MLSb</td>
<td>23.3%</td>
<td>13.9%</td>
</tr>
<tr>
<td>Phenotype L.</td>
<td>5.8%</td>
<td>7.0%</td>
</tr>
<tr>
<td>MLSb Phenotype associated with SA</td>
<td>4.7%</td>
<td>1.2%</td>
</tr>
<tr>
<td>Total</td>
<td>54.7%</td>
<td>45.3%</td>
</tr>
</tbody>
</table>

Figure a: Distribution of strains according to the species

2. Distribution of strains according to the type of sample

CoNSs strains were mostly isolated from blood cultures, followed by urine specimens (Figure b).

3. Beta-lactam resistance profile

The methicillin-resistant CoNS strains were 8.2% and the penicillinase-producing strains 16.2% (Figure c).
4. **Aminoglycoside resistance profile**

The majority of CoNS strains were susceptible to aminoglycosides with 57% and 8.2% of our isolate was resistant to aminoglycosides (Figure d).

**Discussion**

Little information is available on the antibiotic resistance of coagulase-negative staphylococci. This study provides data on antibiotic resistance of CoNS strains isolated from biological samples.

CoNSs are important opportunistic pathogens, we note that the majority of strains were isolated from hospitalized patients with 65.7%, and in blood culture with 51.1% (n = 44), which can be explained by the immune status of hospitalized patients and lack of asepsis in hospitalization services during care. In the study by Choi S. et al., 24 patients had blood cultures positive for S. saprophyticus, among them 07 patients were considered to have clinically significant bacteremia [7].

The antibiogram showed resistance to oxacillin with 8.2% (n = 7); these meta-R strains (resistant to methicillin) are phenotypically resistant to all betalactamines used in treatment. These are penicillins, aminopenicillins, isoxazolylpenicillins (oxacillin, cloxacillin, cephalosporins, penicillins with inhibitors, cephalosporins with inhibitors and carbapenems). This was confirmed by Bhargava and Zhang [8] in their study where all methicillin-resistant strains were phenotypically resistant to penicillin and oxacillin. In the clinic, the treatment of methicillin-resistant Staphylococcal infections is a serious problem [9]. The resistance mechanism in methicillin resistance CoNS is related to the presence of mecA gene. The mecA gene encodes a new penicillin-binding protein 2a, responsible for phenotypic resistance.

Aminoglycosides were resistant in 8.2% (n = 7) of CoNS. They remain a class of antibiotics playing an important role in the treatment of hospital infections. Quinolones, macrolides and related antibiotics were inactive in 5.9% (n = 5) CoNS. Recently, a high prevalence (45.8%) of macrolide-lincosamide-streptogramin resistance (MLS) has been reported [10]. In breeding, resistance to macrolides and lincosamides has been observed in several CoNS, including S. epidermidis isolated from cows with mastitis [11].

In our study, all CoNS strains were sensitive to vancomycin; which shows that the diminished sensitivity to glycopeptides remains exceptional [12]. Vancomycin must be protected to maintain its effectiveness in the treatment of staphylococcal infections.

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

**Conclusion**

CoNS are isolated on biological samples in the laboratory, but their involvement in human pathology is based on clinical and epidemiological criteria. The site of infection, immune status, purity of culture and resistance phenotype provide information on the clinical pathogenic role of CoNS.

**Référence :**


