Role of Nonfermenting Gram-Negative Bacilli Responsible for Respiratory Tract Infections seen in the Laboratory of the University Hospital of Befelatananana Antananarivo Madagascar

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The majority of nonfermenting gram-negative bacilli are responsible for respiratory tract infections. The aims of this study are to identify the nonfermenting gram-negative bacilli species in respiratory tract infections, to describe their antibiotic resistance and to describe the factors associated with these bacteria. It is a prospective study of 54 respiratory samples for a period of six months from October 2019 to March 2020 in the laboratory of the University Hospital of Befelatanana Antananarivo. Among the 54 respiratory samples, the microbiological results showed 12 (22.2%) cases of nonfermenting gram-negative bacilli. These bacilli were represented by 7 (58.3%) cases of Acinetobacter baumannii, 4 (33.3%) cases of Pseudomonas aeruginosa and 1 (8.3%) case of Stenotrophomonas maltophilia. Concerning the associated factors, subjects under 40 years (24%) (p=0.77; NS), men (30.8%) (p=0.03) and subjects hospitalized in intensive care units (40.7%) (p=0.003) were the most affected by the nonfermenting gram-negative bacilli. Regarding antibiotic resistance, it varies from 71.4% to 100% for Acinetobacter baumannii and 0% to 100% for Pseudomonas aeruginosa. The Stenotrophomonas maltophilia isolate had a broad spectrum ß-lactamase but was sensitive to the other antibiotics. Nonfermenting gram-negative bacilli are responsible for severe nosocomial pneumonia and the majorities are multi-resistant bacteria.

Keywords: Acinetobacter baumannii, Pseudomonas aeruginosa, Stenotrophomonas maltophilia, antibiotic resistance, carbapenemase.

INTRODUCTION

Nonfermenting gram-negative bacilli (NFGNB) are a taxonomically diverse group of aerobic, non-sporing, bacilli that either do not utilize glucose as a source of energy or utilize it oxidatively (Malini et al., 2009). NFGNB are defined as strictly aerobic and non-spore forming group of bacteria that do not ferment carbohydrates but generate energy required for their metabolic activities by oxidative pathway (Chiu et al., 2015). Intrinsic resistance of NFGNB to the commonly used antiseptics and their ability to...
survive under a wide range of environmental conditions have aided them to occupy different settings. They have been isolated from soil, aquatic environment, and hospital environments such as anesthesia equipment, sinks, intravenous fluids, and even distilled water. Their survival in different hospital settings facilitated them to transfer from patient to patient through fomites or the hands of medical staff causing hospital acquired infections (Rit et al., 2013). NFGNB are taxonomically diverse group of bacteria that have been considered as commensals or contaminants for many years. However, numerous recent studies revealed that NFGNB are important cause of different types of nosocomial infections, including ventilator-associated pneumonia, septicemia, urinary tract infection, and surgical site infection (Bitew, 2019). NFGNB are innately resistant to many antibiotics and are known to produce extended spectrum β-lactamases and metallo β-lactamases. Currently, they exhibit resistance not only to β-lactam and the other groups of antibiotics, but also to carbapenems (Gniadek et al., 2016). It is important to correctly identify all clinically significant NFGNB considering the intrinsic multidrug resistance exhibited by these bacteria. To better understand the role of NFGNB in respiratory tract infections, the aims of this study are to identify the NFGNB species in respiratory tract infections, to describe their antibiotic resistance and to describe the factors associated with these bacteria.

MATERIALS AND METHODS

It is a prospective study of 54 respiratory samples for a period of six months from October 2019 to March 2020 in the laboratory of the University Hospital of Befelatanana Antananarivo Madagascar. This study includes all respiratory samples of patients who have applied for microbiological examination. These respiratory samples are either sputum, bronchial aspiration fluids or bronchoalveolar fluids. This study excludes any non-compliant respiratory samples such as salivary specimens and respiratory samples contained in a transport medium other than the laboratory vial (sterile red cap vial). In the laboratory, the respiratory samples are cultured in petri dishes, containing ordinary agar called Uriselect® and in sheep blood agar prepared from a mixture of Columbia agar® and sheep blood. Afterwards, the petri dishes are incubated in the oven at 37 °C for 48 hours. Blood agars are incubated in an atmosphere rich in CO2 to facilitate the culture of certain bacteria. Then, a microscope examination is performed after gram staining to quantify epithelial cells and leukocytes and to identify bacteria. After 48 hours, the positivity criteria are the presence of bacterial colonies ≥ 10⁷ CFUs (colony-forming units) per mL, the number of epithelial cells ≤ 25 and the number of leukocytes ≥ 25. Then, the bacterial colonies are identified by the various bacterial identification tests available in the laboratory. Finally, the antibiogram corresponding to the identified bacterial species is carried out. Resistance to antibiotics was determined by the Mueller / Hinton agar diffusion method, according to the recommendations of the "comité de l'antibiogramme de la sociétéfrançaise de Microbiologie" (2019). The antibiotics tested are represented by penicillins (ticarcillin), 3rd generation cephalosporins (3CG), penemes (imipenem), aminoglycosides (gentamycin, tobramycin, amikacin), quinolones (ciprofloxacin), and sulfonamides (cotrimoxazole). The dependent variable is constituted by the positivity of the microbiological culture showing NFGNB and the results of antibiogram. The independent variables consist of the type of respiratory sample, the gender, the age, the clinical information, the departments, the result of microbiological culture of the pulmonary secretion samples and the results of antibiogram. This study was authorized by the Director of Establishment of the University Hospital of Befelatanana and the Department Head of the laboratory before its implementation. This study respected the notion of anonymity and confidentiality. The data entry and processing were performed on the software Epi-info 3.5.2. The comparison of percentages used the Chi square tests. The statistical significance threshold used was p = 0.05.

RESULTS

Microbiological results of the respiratory samples

Among the 54 respiratory samples, the microbiological results showed 12 (22.2%) cases of NFGNB (Figure 1). The negative results represent the 23 (42.6%) respiratory samples in which no bacteria were found.

![Figure 1: Microbiological results of the respiratory samples](image)

These bacilli were represented by 7 (58.3%) cases of A. baumannii, 4 (33.3%) cases of P. aeruginosa and 1 (8.3%) case of S. maltophilia (Figure 2).

![Figure 2: Nonfermenting gram-negative bacilli (NFGNB) species](image)
Associated factors with NFGNB in respiratory tract infections

Concerning the associated factors, the average age is 46.8 years and the sex ratio is 2.6. Subjects under 40 years (24%) (p=0.77; NS), men (30.8%) (p=0.03) and subjects hospitalized in intensive care units (40.7%) (p=0.003) were the most affected by NFGNB (Table 1).

Table 1: Associated factors with NFGNB in respiratory tract infections

<table>
<thead>
<tr>
<th>Gender</th>
<th>Female</th>
<th>Male</th>
<th>n</th>
<th>%</th>
<th>n</th>
<th>%</th>
<th>Total</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>&lt;40</td>
<td>&gt;40</td>
<td>24.0</td>
<td>19</td>
<td>76.0</td>
<td>25</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>Departments</td>
<td>Intensive care units</td>
<td>40.7</td>
<td>27</td>
<td>59.3</td>
<td>27</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other departments</td>
<td>3.7</td>
<td>26</td>
<td>96.3</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Antibiotic resistance of NFGNB strains

Regarding antibiotic resistance, they vary from 71.4% to 100% for A. baumannii (Figure 3) and 0% to 100% for P. aeruginosa (Figure 4).

Antibiotic resistance of S. maltophilia

Table 2: Antibiotic resistance of S. maltophilia

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Isolate of S. maltophilia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotrimoxazole</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>Resistant</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>Sensitive</td>
</tr>
<tr>
<td>3CG</td>
<td>Resistant</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Imipenem</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Amikacin</td>
<td>Sensitive</td>
</tr>
</tbody>
</table>

DISCUSSION

The current study has shown the isolation of NFGNB in 22.2% of respiratory samples. This result is higher compared to another study carried out in Coastal Karnataka which found 5% of NFGBN (Chawla et al., 2013). Similarly, Malini et al. (2009) from Kolar in India have documented the isolation of 6.8% (25 of 365) of NFGNB in respiratory samples. The importance of isolation of non-fermenters has increased in last decade, after more and more reports are correlating them with the either infection outbreaks in hospitals, or healthcare-associated infections (Chawla et al., 2013). Earlier the identification of non-fermenters, based on biochemical tests, was cumbersome and many non-fermenters were misidentified. But, now with the availability of commercial systems, the identification has become easier.

P. aeruginosa and A. baumannii are the most common NFGNB isolated in our study. Other studies have also found the same results (Malini et al., 2009; Hurley, 2016; Kaier, 2019).

Among the isolates of A. baumannii, 100% are resistant to the 3 families of antibiotics (β-lactams, quinolones, sulfonamides,). Similarly, 71% have a carbapenemase and are resistant to all the antibiotics tested. Any of them did not produce extended spectrum β-lactamase highlighting other resistance mechanisms (impermeability of the wall, efflux pump, etc.). A similar study carried out in Arabia Saudi Arabia has identified strains of A. baumannii multi-resistant (94%) (Alyamani et al., 2015). The study carried out by Guckan et al. (2015) also highlighted the multi-resistant nature of the isolates of A. baumannii. These isolates were resistant to ciprofloxacin (97.3%), gentamicin (77.2%), trimethoprim-sulfamethoxazole (68.9%), imipenem (89.1%), meropenem (90.3%). However, colistin (5.5%) and netilmicin (19.5%) were effective on these strains. Another recent study in Iran showed resistance A. Baumannii with cefixime (99%),
ceftazidime (99%), ciprofloxacin (98%), meropenem (99%), trimethoprim-sulfamethoxazole (99%), imipenem (91.5%), ceftriaxone (99%), levofloxacin (96.5%), amikacin (70 %) and gentamycin (85%) (Goudarzi et al., 2013). In contrast, tigecycline (4%) and colistin (0%) are effective (Goudarzi et al., 2013). According to these 2 studies, colistin remains the molecule of choice for the treatment of infections caused by A. Baumannii (Guckkan et al., 2015; Goudarzi et al., 2013). The resistance rate varies widely between different hospitals and different types of hospital services. The mechanisms of resistance to antibiotics in multidrug-resistant bacteria are numerous and diverse. The resistance of A. baumannii strains to these families of antibiotics could be due to the misuse and inappropriate use of antibiotics. In addition, the resistance of the strains could be due to two factors favoring the emergence and dissemination of multidrug resistance including the selection pressure exerted by antibiotics and the spread of these bacteria by cross-transmission (Guessend et al., 2013). In view of the antimicrobial resistance of A. baumannii, detection in a patient affected by pneumonia must be the subject of an official declaration of the circulation of this strain at Coordination center for the fight against nosocomial infections (Vaux et al., 2013). Concerning the isolates of P. aeruginosa, they are less resistant than the strains of A. baumannii. However, this study found 25% of P. Aeruginosa isolates produce carbapenemase. Carbapenemase-producing bacteria are among the highly resistant emerging bacteria (Maamar et al., 2019). Infection with these microorganisms is responsible therapeutic difficulties or even dead ends. Of more, these germs represent a potential danger in as sources, reservoirs, and vehicles of carbapenemases as well as a risk of hospital epidemics which can quickly become uncontrollable (Maamar et al., 2019). Fortunately, amikacin is effective against P. aeruginosa strains. Indeed, the aminoglycosides, in particular amikacin, can be considered to be molecules of choice to integrate into the treatment regimen of infections with carbapenemase-producing bacteria (Maamar et al., 2019).

The current study has shown the isolation of one isolate of S. maltophilia. S. maltophilia is considered now as a common non-fermenter to cause infection in hospital settings (Velázquez-Acosta et al., 2018). These authors have reported 76 strains of Stenotrophomonas maltophilia responsible for nosocomial pneumonias. These nosocomial pneumonias are frequently associated with mechanical ventilation, tracheostomy, previous exposure to broad-spectrum antibiotics, the use of respiratory tract equipment such as nebulizers and therapy with aerosolized polymyxin (Chawla et al., 2019). In this study, the isolate of S. maltophilia had broad spectrum β-lactamase but was sensitive to the other antibiotics especially ciprofloxacin and cefotaxim (trimethoprim-sulfamethoxazole). Similarly, Malini et al. (2009) have documented 100% sensitivity to ciprofloxacin and trimethoprim-sulfamethoxazole. Despite trimethoprim-sulfamethoxazole often being the mainstay of treatment, resistance is widely encountered, and alternative regimens, including combination therapy, are often used (Abbott and Peleg, 2015).

Concerning the associated factors, men and subjects hospitalized in intensive care units are the most affected by NFGNB infections with significant differences. Indeed, men are the most affected because they are more numerous in the workplace than women and they are more exposed to many infections. Similarly, other studies have also highlighted the frequency of NFGNB in Intensive Care Units and their genetic potential to transmit diverse antibiotic resistance regardless of their ability to ferment glucose poses a major threat in hospitals. Invasive procedures such as mechanical ventilation, tracheostomy or the use of catheters favor nosocomial pneumonia in Intensive Care Units (Merzougui et al., 2018).

In brief, NFGNB are responsible for severe respiratory tract infections and the majorities are multi-resistant bacteria. These bacteria are common in Intensive Care Units. Thus, in view of the antimicrobial resistance of NFGNB, detection in a patient must be the subject of an official declaration of the circulation of this strain at Coordination center for the fight against nosocomial infections.

CONCLUSION

Any NFGNB isolated from respiratory tract infection should not be ignored as mere contaminant, but correlated clinically for its pathogenic potential and identified using standard methods so as to institute appropriate and timely antibiotic coverage.

REFERENCES


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