Characterization of *Pseudomonas aeruginosa* and *Acinetobacter* spp. in respiratory secretions of hospitalized patients in intensive care

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Abstract

*Pseudomonas aeruginosa* and *Acinetobacter* spp. are the microorganisms most frequently associated with serious healthcare-associated infections and death. The objective of the research consisted of characterizing the isolates of *P. aeruginosa* and *A.* spp. in respiratory secretions of patients hospitalized in the Clinical Surgical Hospital of Santa Clara, Villa Clara, Cuba between January 2014 and December 2018. A descriptive, longitudinal and retrospective study was carried out with the isolates of *P. aeruginosa* and *A.* spp. from the Clinical Surgical Hospital “Arnaldo Milián Castro” in the city of Santa Clara, Villa Clara province, Cuba between the months of January 2014 to December 2018. For the realization of this work, a discretionary and intentional sampling by criterion was selected. The population consisted of 2 207 isolates, and the sample consisted of 249 *P. aeruginosa* and 705 *A.* spp. The data were obtained from the record books of the respiratory samples section of the Microbiology Laboratory and were arranged in a documentary observation guide. *A.* spp., was the most frequently isolated microorganism in the intensive care units, with a uniform behavior during the entire series studied, while *P. aeruginosa* predominated in the first and fourth trimesters. *A.* spp., showed almost absolute levels of resistance throughout the study, while *P. aeruginosa* showed low percentages of resistance with a propensity to increase resistance. It is concluded that both microorganisms were the most frequently isolated in respiratory secretions in ICUs, surpassing in recent years’ other microorganisms, such as Gram-positive cocci and enterobacteria in general isolates and in HAIs, with marked prevalence rates for both genera, as well as an evident resistance, characterized by differences in relation to percentages, but with a propensity to increase it.

Keywords: *Acinetobacter* spp; Intensive care; *Pseudomonas aeruginosa*; Resistance; Respiratory infections; Villa Clara

1. Introduction

Non-fermenting Gram-negative bacilli constitute a complex group of strict aerobic, non-sporulating microorganisms characterized by utilization of carbohydrates through respiratory metabolism [1, 2]. Because of their minimal nutritional requirements, tolerance to a wide variety of physical conditions and resistance to a large number of antimicrobials and disinfectants, they are considered to be universally distributed [1-3], occupying a large number of aquatic and terrestrial habitats, with high resistance to harsh environmental factors, allowing them to spread rapidly...
and develop resistance to all conventional antimicrobials [1-5]. They are very frequently isolated in the hospital environment, mainly in humid environments, such as respirators, bronchoscopes, dialysis equipment and even disinfectant solutions [1, 6, 7].

Currently, they have gained notorious importance due to their presence in hospital infections; among these the following stand out: *Pseudomonas aeruginosa* and *Acinetobacter* spp.; of the latter, *A. baumannii* is the species most frequently associated with severe healthcare-associated infections (HAI) and death [8-12]. A considerable percentage of patients admitted to intensive care units (ICUs) have an infection as a cause and an even higher percentage develop infections during their stay in the ICU. Gram-negative non fermenting bacilli (GNB) are among the most frequent agents causing HAIs in ICUs; in fact, they are among the so-called "problem bacteria"[2, 6, 13].

The management of patients admitted to the ICU usually involves the use of mechanical ventilation (MRA), bladder catheter (BV), central venous line (CV), among other procedures, which together with invasive monitoring and/or diagnostic methods increase the risk of infections by various microorganisms, among which BNF stand out. These bacteria usually have a diversity of antibiotic resistance mechanisms, both intrinsic and acquired, and consequently in ICUs there is a wide empirical use of broad-spectrum antimicrobials [3, 4, 6, 12].

*P. aeruginosa* is an opportunistic pathogen responsible for a wide range of infections, mainly HAIs [4, 5, 7, 10]. The vast majority of infections caused by this agent are related to hospital action, constituting a serious clinical problem. In addition, it could be mentioned that in almost all clinical cases of *P. aeruginosa* infection there is a compromise of the host defenses [1-7]. The respiratory tract is one of the most frequent sites of *P. aeruginosa* infection; this microorganism is a common cause of ventilator-associated ARI pneumonia (RAP). Identification of the responsible bacterium is based on culture of endotracheal tube aspirates and bronchoalveolar lavage fluid in the appropriate clinical setting [5-7]. In mechanically ventilated patients, pneumonia caused by *P. aeruginosa* is one of the most frequent and generally one of the most severe. Some studies have determined a mortality rate of 50-70% among affected patients. This is attributed both to the profile of the patients, critical and with underlying diseases, and to the virulence of the bacterium, indicating colonization rates of up to 54%. In cystic fibrosis (CF), *P. aeruginosa* infects up to 90% of adult patients, increasing mortality and pulmonary deterioration. This bacterium can survive and persist for some decades in the respiratory tract of CF patients, in whom a high frequency of hypermutable *P. aeruginosa* has been evidenced, suggesting a link between this phenotype and the evolution of antibiotic resistance [4-7]. Multidrug-resistant *P. aeruginosa* infection has been frequently described in patients with CF, in isolated outbreaks in ICUs or in patients with neoplasms [7, 13, 14].

*Acinetobacter* spp. was first described in 1911 as Micrococcus calco-aceticus, since then it has received different names and only since the 1950s has it received the generic name of *Acinetobacter*. In humans it colonizes the skin, wounds, respiratory and gastrointestinal tract [8-12]. It is a Gram-negative coccobacillus that, during the last three decades, has emerged from an organism of questionable pathogenicity to an important infectious agent in all hospitals worldwide [8, 9, 13, 16]. A spp. is a causative agent of infectious morbidity and mortality affecting mostly patients in ICUs. The most alarming aspect of this microorganism is its ability to accumulate diverse mechanisms of antimicrobial resistance and the emergence of strains resistant to almost all commercially available antimicrobials [1, 3, 8, 9]. Since the past decade, their prevalence has increased significantly and, with it, the mechanisms of resistance. According to the latest national surveillance study on nosocomial infection conducted by the working group on diseases and infections of the Spanish Society of Intensive Care, Critical Care and Coronary Units (SEMICYUC), the resistance mechanisms that *A. baumannii* has managed to develop occupy the first places as causes of HAIs affecting patients admitted to ICUs [8-13]. In recent years, in the United States (USA). A spp. has gone from being a pathogen found in ICUs to affecting patients admitted to other hospital units. Due to colonization of the oropharynx and tracheostomy tubes in ventilator patients, the upper respiratory tract is the most common site of A spp. infection [12, 13, 15]. The two most characteristic respiratory syndromes associated with A spp. infection are community-acquired pneumonia (CAP) and healthcare-associated pneumonia (HAP) [11-13]. In Saudi Arabia, A spp., is the most common pathogen associated with late-onset or recurrent VAP in adult ICUs [11]. The World Health Organization has recently designated antimicrobial resistance as one of the three most important problems facing human health [14, 17, 18, 19].

A study in the Bacteriology laboratory of the national reference hospital Manuel de Jesus Rivera, Nicaragua, in 133 laboratory records in the period from 2012 to 2016, found that multidrug-resistant A spp. infections mainly affected patients in Intensive Therapy Unit wards and multidrug-resistant A spp. strains, were sensitive only to polymyxins [22].

In Cuba, in recent years, the incidence of A spp., in ICUs has increased dramatically, becoming one of the most frequently isolated microorganisms in many hospitals [23-28]. Works carried out in several Cuban provinces showed the high prevalence of A spp. isolates, and multidrug resistance as a serious problem in medicine today [24-28]. In several studies carried out in Cuba, they found that non-fermenting Gram-negative bacilli are a frequent cause of infections in
patients hospitalized in ICUs, with *P. aeruginosa* and *A.* spp. being the most frequently isolated within this group of microorganisms [26-28].

The aim of this study was to characterize the isolates of *P. aeruginosa* and *A.* spp., in respiratory secretions of patients hospitalized in the Clinical Surgical Hospital of Santa Clara, Villa Clara, Cuba, from January 2014 to December 2018.

### 2. Material and methods

#### 2.1. Type of research

Developmental Research.

#### 2.2. General aspects of the study

A descriptive, longitudinal and retrospective study of *P. aeruginosa* and *A.* spp., isolates in respiratory secretions of patients in the ICU wards of the “Arnaldo Milián Castro” Hospital of Santa Clara, Villa Clara, from January 2014 and December 2018 was carried out. For the realization of this work, a discretionary and intentional sampling by criterion was selected. The population was constituted by 2 207 isolates and the sample by 429 *P. aeruginosa* and 705 *A.* spp.

#### 2.3. Epidemiological classification

Qualitative nominal dichotomous. Epidemiological classification of microorganisms in relation to HAIs. The following scales were considered:

- Associated
- Not associated

#### 2.4. Methods and procedures for data collection

##### 2.4.1. Empirical methods

A bibliographic review was carried out to meet the needs of the different parts of the subject under study. The data to provide answers to the proposed variables were obtained from the record books of the respiratory samples section of the Microbiology laboratory and were arranged in a documentary observation guide designed for the research, as shown below.

#### 2.4.2. Documentary observation guide for respiratory secretions

- Identification of the sample Year____ Month____ Registration number _______
- Service of origin UCI-1 _____ UCI-2 _____
- Isolated microorganisms

<table>
<thead>
<tr>
<th>S. aureus</th>
<th>Escherichia coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacter spp.</td>
<td><em>P. aeruginosa</em></td>
</tr>
<tr>
<td><em>Klebsiella</em> spp.</td>
<td><em>Acinetobacter</em> spp.</td>
</tr>
</tbody>
</table>

- Epidemiological Classification Associated _____ Not associated _____
- Antimicrobial Resistance

<table>
<thead>
<tr>
<th><em>Pseudomonas aeruginosa</em></th>
<th><em>Acinetobacter</em> spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antimicrobians</td>
<td>R Np</td>
</tr>
<tr>
<td>Ciprofloxacino</td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td></td>
</tr>
</tbody>
</table>
Ceftazidima  Ceftriax/Cefotax
Cefepime       Ceftazidima
Aztreonam      Cefepime
Meropenem      Meropenem
Piper.-Tazo    Ampi -Sulba
                         Piper.-Tazo.

**Legend:** R: resistant; Np: Not tested.

### 2.5. Statistical method

A database was created using the Microsoft Excel application version 2016, with the results of the proposed variables; extracted from the Documentary Observation Guide, processed using the SPSS (Statistical Package for the Social Sciences) computer system version 21.0 for Windows. Absolute and relative frequencies (percentages) were determined. In the analysis and interpretation of the results, the percentage method and X² of goodness of fit were used in each year of the study and if p<0.05, then significant differences existed. The text editor Microsoft Word version 2016 was used for the preparation of the final report and publication of the results, which were reflected in tables.

### 2.6. Ethical considerations

The study complied with the World Helsinki Assembly declaration [29]. It was approved by the Hospital’s Scientific and Ethics Committees, which guaranteed the confidentiality of the data obtained and its use for scientific purposes.

### 3. Results

In relation to the microorganisms that were most frequently isolated in respiratory secretions in the period studied, it was found that *A. spp.* was the predominant microorganism with 705 (31.9%) followed by *P. aeruginosa*, with 429 for 19.4%. The rest of the microorganisms, including *Staphylococcus aureus*, *Enterobacter* spp., *Klebsiella* spp., and *Escherichia coli*, did not show percentage differences that indicate a predominance of any of them (Table 1).

**Table 1** Microorganisms most frequently isolated in respiratory secretions of hospitalized patients in ICUs

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>General insulations N=2207</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>240</td>
</tr>
<tr>
<td><em>Enterobacter</em> spp.*</td>
<td>111</td>
</tr>
<tr>
<td><em>Klebsiella</em> spp.*</td>
<td>247</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>144</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>429</td>
</tr>
<tr>
<td><em>Acinetobacter</em> spp.*</td>
<td>705</td>
</tr>
<tr>
<td>Total</td>
<td>1876</td>
</tr>
</tbody>
</table>

* Refers to a genus that includes several species; **Source:** Record book of the respiratory specimen section of the Microbiology Laboratory.

The years included in the study showed that, *P. aeruginosa* in 2017 had the highest number of isolates, with 115 (26.8%). The rest of the years ranged with minimal variations, between 16.3% and 20.7%. *A. spp.* remained around 20% in the years studied, except in 2015, when isolates decreased to 14.3% (Table 2).
Table 2 Distribution of *P. aeruginosa* and *A.* spp. isolates in respiratory secretions from ICUs hospitalized patients by years

<table>
<thead>
<tr>
<th>Years</th>
<th><em>Pseudomonas aeruginosa</em> N=429</th>
<th>%</th>
<th><em>Acinetobacter</em> spp. N=705</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td>70</td>
<td>16.3</td>
<td>141</td>
<td>20.0</td>
</tr>
<tr>
<td>2015</td>
<td>77</td>
<td>17.9</td>
<td>101</td>
<td>14.3</td>
</tr>
<tr>
<td>2016</td>
<td>89</td>
<td>20.7</td>
<td>157</td>
<td>22.3</td>
</tr>
<tr>
<td>2017</td>
<td>115</td>
<td>26.8</td>
<td>163</td>
<td>23.1</td>
</tr>
<tr>
<td>2018</td>
<td>78</td>
<td>18.2</td>
<td>143</td>
<td>20.3</td>
</tr>
</tbody>
</table>

Source: Record book of the respiratory specimen section of the Microbiology Laboratory.

Regarding the variability of monthly isolates in the years studied, it was difficult to reach general conclusions about the distribution of isolates. However, there are striking aspects, with an apparent regularity of isolates from November and December 2014, and January-February 2015. This situation is also reflected in November and December 2015 and January-February 2016. Similarly, to the above, but at a higher level of isolations, behaved November and December 2016, and January-February 2017, continuing with the same characteristics, November and December 2017 and January-February 2018. The regularity so far described of isolations in January and February and November and December is maintained in the total of the five years studied that, with the exception of September, these months described above are the high points of isolations (Table 3).

Table 3 Chronology of *P. aeruginosa* isolates in respiratory secretions in hospitalized ICUs patients by months and years

<table>
<thead>
<tr>
<th>Months</th>
<th>2014 N=70</th>
<th>2015 N=77</th>
<th>2016 N=89</th>
<th>2017 N=115</th>
<th>2018 N=78</th>
<th>Total N=429</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>14</td>
<td>3</td>
<td>7</td>
<td>14</td>
<td>10</td>
<td>48</td>
</tr>
<tr>
<td>February</td>
<td>6</td>
<td>4</td>
<td>10</td>
<td>12</td>
<td>10</td>
<td>42</td>
</tr>
<tr>
<td>March</td>
<td>8</td>
<td>7</td>
<td>4</td>
<td>6</td>
<td>4</td>
<td>29</td>
</tr>
<tr>
<td>April</td>
<td>3</td>
<td>9</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>28</td>
</tr>
<tr>
<td>May</td>
<td>3</td>
<td>4</td>
<td>9</td>
<td>5</td>
<td>10</td>
<td>31</td>
</tr>
<tr>
<td>June</td>
<td>7</td>
<td>9</td>
<td>4</td>
<td>8</td>
<td>7</td>
<td>35</td>
</tr>
<tr>
<td>July</td>
<td>0</td>
<td>8</td>
<td>2</td>
<td>10</td>
<td>7</td>
<td>27</td>
</tr>
<tr>
<td>August</td>
<td>9</td>
<td>6</td>
<td>5</td>
<td>10</td>
<td>2</td>
<td>32</td>
</tr>
<tr>
<td>September</td>
<td>12</td>
<td>8</td>
<td>4</td>
<td>11</td>
<td>6</td>
<td>41</td>
</tr>
<tr>
<td>October</td>
<td>3</td>
<td>8</td>
<td>10</td>
<td>5</td>
<td>6</td>
<td>32</td>
</tr>
<tr>
<td>November</td>
<td>3</td>
<td>4</td>
<td>12</td>
<td>15</td>
<td>5</td>
<td>39</td>
</tr>
<tr>
<td>December</td>
<td>2</td>
<td>7</td>
<td>17</td>
<td>13</td>
<td>6</td>
<td>45</td>
</tr>
</tbody>
</table>

Source: Record book of the respiratory specimen section of the Microbiology Laboratory.

The variability of the monthly isolates of *A.* spp., in the years studied makes it difficult to establish a regularity in them. In the results of the five years of study, with the exception of the month of February, isolates ranged from 50 to 77 per month. There was a predominance of isolates between 60 and 69 per month, followed by isolates between 50 and 59, with only two months with 70 and more isolates. The quarter with the highest number of isolates was July, August and September with 203 (Table 4).
Table 4 Chronology of A. spp., isolates in respiratory secretions of hospitalized patients in ICUs by months and years

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>8</td>
<td>15</td>
<td>11</td>
<td>19</td>
<td>3</td>
<td>56</td>
</tr>
<tr>
<td>February</td>
<td>4</td>
<td>8</td>
<td>7</td>
<td>11</td>
<td>7</td>
<td>37</td>
</tr>
<tr>
<td>March</td>
<td>9</td>
<td>5</td>
<td>15</td>
<td>12</td>
<td>12</td>
<td>53</td>
</tr>
<tr>
<td>April</td>
<td>11</td>
<td>13</td>
<td>18</td>
<td>11</td>
<td>9</td>
<td>62</td>
</tr>
<tr>
<td>May</td>
<td>14</td>
<td>4</td>
<td>15</td>
<td>10</td>
<td>7</td>
<td>50</td>
</tr>
<tr>
<td>June</td>
<td>14</td>
<td>6</td>
<td>10</td>
<td>20</td>
<td>20</td>
<td>70</td>
</tr>
<tr>
<td>July</td>
<td>9</td>
<td>9</td>
<td>12</td>
<td>12</td>
<td>19</td>
<td>61</td>
</tr>
<tr>
<td>August</td>
<td>18</td>
<td>10</td>
<td>15</td>
<td>19</td>
<td>15</td>
<td>77</td>
</tr>
<tr>
<td>September</td>
<td>11</td>
<td>11</td>
<td>8</td>
<td>21</td>
<td>14</td>
<td>65</td>
</tr>
<tr>
<td>October</td>
<td>20</td>
<td>12</td>
<td>11</td>
<td>12</td>
<td>11</td>
<td>66</td>
</tr>
<tr>
<td>November</td>
<td>9</td>
<td>5</td>
<td>17</td>
<td>9</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>December</td>
<td>14</td>
<td>3</td>
<td>18</td>
<td>7</td>
<td>16</td>
<td>60</td>
</tr>
</tbody>
</table>

Source: Record book of the respiratory specimen section of the Microbiology Laboratory.

The isolates of P. aeruginosa in respiratory secretions by ICU rooms are shown in table 5. 429 strains were obtained, of which in ICU-1, 64.7% (156) obtained the epidemiological classification of HCAI. In ICU-2, 65.9% of the strains were HCAI, resulting from 188 identifications. It was evident that, for both wards, the highest number corresponded to HAIs with a total of 280 (65.3%).

Table 5 Distribution of isolates according to epidemiological classification of P. aeruginosa by ICUs rooms

<table>
<thead>
<tr>
<th>Epidemiological classification</th>
<th>UCI-1 N=241</th>
<th>UCI-2 N=188</th>
<th>Total N=429</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nº</td>
<td>Nº</td>
<td>Nº</td>
</tr>
<tr>
<td>IAAS</td>
<td>156</td>
<td>124</td>
<td>280</td>
</tr>
<tr>
<td>No IAAS</td>
<td>85</td>
<td>64</td>
<td>149</td>
</tr>
</tbody>
</table>

Source: Record book of the respiratory specimen section of the Microbiology Laboratory.

Table 6 shows the isolates of A. spp., in relation to the epidemiological classification of HAIs. In ICU-1, 60.3% of the 320 strains identified were HAIs, while in ICU-2, 245 of 385 microorganisms, 63.6% obtained the same designation. Thus, 438 of 705 A. spp., were classified as HAIs, 62.1%.

Table 6 Distribution of isolates according to epidemiological classification of A. spp., by ICUs rooms

<table>
<thead>
<tr>
<th>Epidemiological classification</th>
<th>UCI-1 N=320</th>
<th>UCI-2 N=385</th>
<th>Total N=705</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nº</td>
<td>Nº</td>
<td>Nº</td>
</tr>
<tr>
<td>IAAS</td>
<td>193</td>
<td>245</td>
<td>438</td>
</tr>
<tr>
<td>No IAAS</td>
<td>127</td>
<td>140</td>
<td>267</td>
</tr>
</tbody>
</table>

Source: Record book of the respiratory specimen section of the Microbiology Laboratory.
The distribution of antimicrobial resistance by years according to *P. aeruginosa* isolates is reflected in table 7. It was observed that they showed higher resistance to meropenem, ceftazidime and aztreonam. In 2015, 2016 and 2017 they had an increase in resistance with respect to the rest of the antimicrobials tested, highlighting meropenem in 2015 with 29.2%. It was evidenced that there was a significant difference in favor of sensitivity in all antimicrobials tested in the isolates of *P. aeruginosa*, being ciprofloxacin the most sensitive, presenting in the years studied low percentages of resistance of no more than 10.0%.

Table 7 Chronology of antimicrobial resistance of *P. aeruginosa* in respiratory secretions of hospitalized patients in ICUs by years (X² goodness of fit; p<0.05)

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>Resistance</th>
<th>Years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2014</td>
</tr>
<tr>
<td>Ciprofloxacina</td>
<td>Total</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>7.7</td>
</tr>
<tr>
<td>Gentamicina</td>
<td>Total</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>6.1</td>
</tr>
<tr>
<td>Amikacina</td>
<td>Total</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>9.1</td>
</tr>
<tr>
<td>Ceftazidima</td>
<td>Total</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>19.3</td>
</tr>
<tr>
<td>Cefepime</td>
<td>Total</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>13.8</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>Total</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>16.1</td>
</tr>
<tr>
<td>Meropenem</td>
<td>Total</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>12</td>
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<tr>
<td></td>
<td>%</td>
<td>18.5</td>
</tr>
<tr>
<td>Piperacilina-Tazobactam</td>
<td>Total</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>16.1</td>
</tr>
</tbody>
</table>

Source: Logbook of the respiratory specimen section of the Microbiology Laboratory.

The distribution of antimicrobial resistance by year according to *A. spp.*, isolates is shown in table 8. As the most significant data, it was evidenced that the resistance profile shown ceftaxima-ceftriaxon presented resistance above 90.0% in all years of the study, and the lowest resistance was shown against ampicillin-sulbactam disks in the years 2014 and 2015, with resistance rates below 60.0% in those years, subsequently increasing resistance, with 87.5% in the years 2017 and 2018. It was evident that there was a significant difference in favor of resistance in all antimicrobials tested.
Table 8 Chronology of antimicrobial resistance of A. spp., in respiratory secretions of hospitalized patients in ICUs by years (X² goodness of fit; p<0.05)

<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td>Total</td>
<td>134</td>
<td>79</td>
<td>91</td>
<td>114</td>
<td>70</td>
<td>488</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>110</td>
<td>66</td>
<td>84</td>
<td>100</td>
<td>55</td>
<td>415</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>82.1</td>
<td>83.5</td>
<td>92.3</td>
<td>87.7</td>
<td>78.6</td>
<td>85.0</td>
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<tr>
<td>Ciprofloxacina</td>
<td>Total</td>
<td>137</td>
<td>100</td>
<td>153</td>
<td>154</td>
<td>38</td>
<td>582</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>124</td>
<td>87</td>
<td>143</td>
<td>136</td>
<td>37</td>
<td>527</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>90.5</td>
<td>87.0</td>
<td>93.5</td>
<td>88.3</td>
<td>97.4</td>
<td>90.5</td>
</tr>
<tr>
<td>Gentamicina</td>
<td>Total</td>
<td>136</td>
<td>99</td>
<td>152</td>
<td>140</td>
<td>99</td>
<td>626</td>
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<tr>
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<td>Resistant</td>
<td>116</td>
<td>81</td>
<td>130</td>
<td>115</td>
<td>83</td>
<td>525</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>85.3</td>
<td>81.8</td>
<td>85.5</td>
<td>82.1</td>
<td>83.8</td>
<td>83.9</td>
</tr>
<tr>
<td>Amikacina</td>
<td>Total</td>
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<td>97</td>
<td>147</td>
<td>153</td>
<td>137</td>
<td>642</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>104</td>
<td>95</td>
<td>145</td>
<td>149</td>
<td>130</td>
<td>623</td>
</tr>
<tr>
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<td>97.9</td>
<td>98.6</td>
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<td>97.0</td>
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<tr>
<td>Cefotaxima-Ceftriaona</td>
<td>Total</td>
<td>136</td>
<td>100</td>
<td>153</td>
<td>154</td>
<td>135</td>
<td>678</td>
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<tr>
<td></td>
<td>Resistant</td>
<td>104</td>
<td>82</td>
<td>142</td>
<td>140</td>
<td>123</td>
<td>591</td>
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<tr>
<td></td>
<td>%</td>
<td>76.5</td>
<td>82.0</td>
<td>92.8</td>
<td>90.9</td>
<td>91.1</td>
<td>87.2</td>
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<tr>
<td>Cefepime</td>
<td>Total</td>
<td>135</td>
<td>99</td>
<td>152</td>
<td>147</td>
<td>56</td>
<td>589</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>109</td>
<td>78</td>
<td>130</td>
<td>129</td>
<td>53</td>
<td>499</td>
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<tr>
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<td>85.5</td>
<td>87.8</td>
<td>94.6</td>
<td>84.7</td>
</tr>
<tr>
<td>Meropenem</td>
<td>Total</td>
<td>119</td>
<td>94</td>
<td>121</td>
<td>128</td>
<td>112</td>
<td>574</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>70</td>
<td>56</td>
<td>88</td>
<td>112</td>
<td>98</td>
<td>424</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>58.8</td>
<td>59.6</td>
<td>72.7</td>
<td>87.5</td>
<td>87.5</td>
<td>73.9</td>
</tr>
<tr>
<td>Ampicillin-sulbactam</td>
<td>Total</td>
<td>129</td>
<td>38</td>
<td>108</td>
<td>150</td>
<td>82</td>
<td>507</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>106</td>
<td>31</td>
<td>97</td>
<td>136</td>
<td>73</td>
<td>443</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>82.2</td>
<td>81.6</td>
<td>89.8</td>
<td>90.7</td>
<td>89.0</td>
<td>87.4</td>
</tr>
</tbody>
</table>

The research conducted provided microbiological, epidemiological and clinical information for better diagnosis, treatment and containment of healthcare associated Infections (HAI) in ICU ward patients.

4. Discussion

Table 1 shows the results of the isolations in respiratory secretions from the ICUs, where A. spp. came in first place, with 31.9% of 2,207 general isolates, followed by P. aeruginosa (19.4%), whose results agree with those obtained by other authors in this regard, both in Cuba and in other countries [30-35], mainly in patients with infections associated with mechanical ventilation in critical care services; although other species of microorganisms have also been isolated, including Staphylococcus aureus, Escherichia coli, Klebsiella spp., and Enterobacter spp. [33-35].

Table 2 analyzed the distribution of isolates of P. aeruginosa and A. spp, in respiratory secretions of hospitalized patients in ICUs by year, where it was also found, in studies carried out in Colombia in 2010 [36,37] on bacterial sensitivity
profiles provided by 14 tertiary level hospitals, that among the Gram-negative bacilli, *A. baumannii* as the most isolated in intensive care units in the first year of the study, with 381 isolates (7%), followed by *P. aeruginosa*, with 344 (6%), coinciding in terms of the frequency of isolation with our research, although not in the hundreds found, where the remaining years did not coincide, neither in the position, nor in the frequency of isolations. On the other hand, in a study conducted in the intensive care service of the Guillermo Almenara Irigoyen National Hospital, Lima, Peru (2008) [34], during the period 2004-2006, it was found that *P. aeruginosa* was the most frequent in the three years (15.9%, 15.0% and 13.5%) compared to *A. spp.*, isolates, with 6.7%, 10.8% and 13.5%, these data not coinciding with those obtained in our research.

In a study carried out in Spain, Chile and Brazil during the years 2008-2019 [37-41] on the epidemiology and impact of nosocomial infections, the results obtained do not coincide with those obtained in our work in terms of position, where *P. aeruginosa* was the predominant microorganism in pneumonia associated with mechanical ventilation in ICUs, with a mean isolation rate of 17.8%, which is similar to ours. In the case of *A. baumannii* isolates, the mean was 10%, which does not coincide with our results (20.0%). Despite an exhaustive review of the subject in the literature, no national studies were found that analyzed the behavior of these two microorganisms by year.

Table 3 shows the chronology of *P. aeruginosa* isolations in respiratory secretions in ICUs hospitalized patients by months and years, with a predominance of isolations in January, February, November and December. In a study by Zúñiga & Miliar [38] on seasonal cycles, heat and humidity, and factors for the increase of nosocomial infections in Mexico in 2019, they state that the monthly incidence of *A. spp.*, infections increased in the months of July to October, more than in November and June. In addition, they determine an increase in *P. aeruginosa* infections in the period from November to June, and the regularity of isolations does not coincide with ours. It is not possible to go deeper into the seasonality of *P. aeruginosa* isolates because we have not found more bibliographies related to the subject.

Table 4 analyzed the chronology of *A. spp.*, isolations in respiratory secretions of hospitalized patients in ICUs by months and years, where the results obtained coincide with those of the CDC and other countries in the region, which since 1974 have reported higher rates of nosocomial infections by *A. spp.*, in summer than in other seasons, in summer more than in other seasons, and among the most convincing explanations is: hotter and more humid environments in the ICUs, which could be preventable by condensation in the units with air conditioning equipment [41-43]. HVAC systems help maintain a relatively constant indoor temperature, however, changes in outdoor humidity can affect humidity in the hospital environment [42,43]. *Acinetobacter spp.* have been isolated from hospital air and are suspected to play a role in nosocomial transmission. In general, air-conditioned areas are not monitored, internal and/or external temperature and humidity are not taken into account, and humidity is often not well controlled in ICUs [38,41,42,43].

All retrospective analyses carried out confirm that the incidence of *A. baumannii* is higher in summer in climates with seasonal temperature variation. The incidence between the months of July to October is 50% higher than that observed during the rest of the year. This is not only due to more favorable temperature and humidity conditions, but also to condensation in cooling systems [40-42].

Despite the exhaustive search about the subject in the national and international literature, we did not find enough studies that address this issue. Nevertheless, we believe that within the Gram-negative bacilli, *A. spp.*, and *P. aeruginosa* species remain important HAI pathogens in hospitals, and appear to be associated with a unique and persistent seasonal variation in infection rates [40-45].

In table 5 the results obtained in our study about the distribution of *P. aeruginosa* HAI isolates according to ICUs, do not agree with those obtained by Necla et al. (2013) [41] in Brazil, instead, they do agree with the results reached by Lopez, 2015 [40] and Basulto et al. (2009) [45]. We also found differences with the results obtained by Munive et al. (2013) in Colombia, as well as with those achieved by Abdo and Castellanos (2015), and Basulto et al. (2009) in Cuba. These data do not coincide with our research in terms of isolation percentages.

In relation to the distribution of *A. spp.* HAI isolates according to ICUs (Table 6), there is full agreement with our study in terms of position, but not in percentage of isolation, finding in our study 42.1% more, in terms of the results obtained by Necla et al. (2013) in a three-year study of evaluation of nosocomial infection rates in ICUs in Turkey.

In a study conducted in Cuba, in 2017 on the incidence of healthcare-associated infections in ICUs, they found *A. baumannii* as the microorganism with the most in hospital isolations in patients with ventilator associated pneumonia, with 19 (14.1%), coinciding with the most isolated microorganism, but not in percentage of isolation, which may be a biased sample due to the number of isolations in the sample studied (19 of 135).
Table 7 shows the results obtained on the chronology of antimicrobial resistance of *P. aeruginosa* in respiratory secretions of hospitalized patients in the ICUs by year, where in a multicenter study of bacterial resistances nosocomiales en México en el año 2017 [46], in relación con las bacterias del grupo ESKAPE causantes de infecciones IAAS importantes en las UCIs, encontraron perfiles de resistencia más elevados que los nuestros en los aislamientos de *P. aeruginosa*, cefepime, con un (38.1%), cefazidima (38.1%), Amikacina (30.9%), gentamicina (29.3%), piperacilina- tazobactam (26.6%), ciprofloxacino (25%), y meropenem (25%), el último con una proporción similar a la nuestra, alcanzando el 22.2%. Sin embargo, en un estudio de 2018 sobre la incidencia y microbiología de ventilador asociadas neumonías en el centro de cuidado inmunodeprimido de un hospital de referencia en México, no concuerda con lo que se observó en nuestro estudio, encontrando perfiles de resistencia en aislados de *P. aeruginosa* en respiratorios secretiones, con 69.2% a meropenem, cefazidima 64.0%, piperacilina-tazobactam 48%, cefepima 44%, amikacina 52% y ciprofloxacino, con 65.4% [46,47].

In Cuba, in a study carried out in Havana, year 2014 about microorganisms isolated from patients hospitalized in ICUs, they found percentages of resistance not higher than 50% in *P. aeruginosa* against most antimicrobials, cefazidima 40%, and cefepime, amikacina, gentamicin and ciprofloxacino, with 20%, differing with the resistance profile shown in our research [27]. Another study, also in Havana during 2015-2016 [48] on *P. aeruginosa* in critically ill patients found resistance to meropenem (33.3%), cefazidima (54.7%), aztreonam (49.5%), cefepime y amikacina (42.7%), gentamicina (41.8%) y ciprofloxacino (48.7%); no coinciding with the study of Garcia et al. (2014) [27], nor with our research in terms of percentage of resistance; however, we agree on the progressive increase over time of the same, mainly against meropenem, cefazidima and aztreonam.

The results reported in 2016 by the National Antimicrobial Resistance Surveillance System in Cuba in HAIs [49], of the resistance profile in non-fermenting Gram-negative bacillus in ICUs, showed the percentages of resistance of *P. aeruginosa* to piperacilina/tazobactam, with 19.3%, cefazidima 22.3%, cefepime 18.5%, aztreonam 30.8%, doripenem 18.5%, imipenem 31.6%, meropenem 25.4%, amikacin 13.7%, gentamicin 19.2%, ciprofloxacino 19.2% and colistina, con 3.9%. These data do not coincide completely with the results obtained in our research, since it shows higher percentages of resistance, although the high resistance to meropenem is similar. This is very similar to the results obtained in Mexico and Colombia [49,51,52].

Despite the fact that our isolates showed low resistance rates, it is important to highlight the gradual increase of resistance at international level over the years, with *P. aeruginosa* becoming a microorganism highly resistant to available antimicrobials, especially in ICUs, where there are all factors conducive to the spread of multidrug-resistant strains, especially carbapenemas producing ones, which requires extreme vigilance.

Table 8 shows the results of the chronology of antimicrobial resistance of *A. spp.*, in respiratory secretions of hospitalized patients in ICUs by years, where in a study on infections by *A. spp.*, in critical patients in ICUs in Spain in 2005 [38,45], resistance percentages of 80.7% in cefepime, 65.3% amikacin, 81.2% piperacilina/tazobactam, and 87.3% in ciprofloxacino were found. These data, like our research, show high percentages of resistance. Similarly, in Colombia, in a study in 2017 on the profile of bacterial resistance in hospitals and clinics in the department of Cesar, they found in isolates of *A. spp.*, in ICUs to ceftriaxone and cefepime, as those with the highest percentages of resistance, with 51.1%, followed by gentamicin, meropenem and ampicilina/sulbactam, with 46.7%, ciprofloxacino with 44.6% and amikacina con 43.5%, showing our study higher percentages of resistance [51-53]. In Mexico, in 2017 [46] found high percentages of resistance to antimicrobials such as cefepime and ceftriaxone, with 100% and 81.8% respectively, followed by gentamicin, with 50%, amikacina 31.5%, piperacilina/tazobactam 27.2%, ciprofloxacino 26.3%, and meropenem 15.7%; not coinciding because of the high percentage of resistance found in our research.

In Cuba, studies have been carried out in different provinces [54-57] on *Acinetobacter* isolates in patients admitted to ICUs and high rates of resistance to all the antimicrobials tested were found; in order of frequency: 95.1% to ceftriaxone, followed by cefepime, ciprofloxacino, meropenem, amikacina, gentamicin, piperacilina/tazobactam and ampicilina/sulbactam, with 91.7%, 91.0%, 88.0%, 88.0% and 88.0%, respectively. 7%, 91.0%, 88.9%, 88.2%, 85.4%, 85.4 and 65.4%, respectively. These results coincide with our research in relation to the percentage of resistance, which highlights the high resistance shown by *A. spp.*, both internationally and in our country, we emphasize the proper use of these antimicrobials in ICUs, demonstrating in our research and in correlation with international and national studies, the low usefulness of cephalosporins, especially third generation (cefotaxime/ceftriaxone) against this microorganism.

On the other hand, it was demonstrated with our results and with the studies reviewed at international level, the importance of *A. spp.*, as a multiresistant microorganism, especially in the ICUs, highlighting the vigilance to have in them, where our research was conducted, showing the high rate of resistance of *A. spp.*, to cephalosporins, and the high
resistance of *A.* spp., to cephalosporins, especially cefotaxime-ceftiraxone, losing usefulness in relation to these results obtained to treat these microorganisms, and remaining at international level as an ineffective antimicrobial against it.

5. Conclusion

*A.* spp. was the most frequently isolated microorganism in the ICUs, with a uniform behavior throughout the series studied, while *P. aeruginosa* predominated in the first and fourth trimesters. Both microorganisms prevailed in the HAI isolates, with *A.* spp., predominating, showing almost absolute levels of resistance throughout the study, while *P. aeruginosa* showed low percentages of resistance with a propensity to increase it. This demonstrates the need to reinforce epidemiological surveillance at the hospital level by taking control measures to reduce the indiscriminate use of antimicrobials, the main cause of bacterial resistance in hospital environments and the transmission of HAIs by multiresistant bacteria.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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