The Sensitivity of Psittacine Isolated Pseudomonas Strains towards Antibiotics

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Abstract. Avian Pseudomonas disease evolves in an acute or chronic form on cage birds and involves difficulties concerning establishing an optimal therapeutical conduct. Due to the risk of transmission to other individuals, and also to the personnel involved directly in care and treatment of ill animals, it is very important to rapidly institute an adequate therapy in order to assure the rapid limitation of pseudomonads transmission to humans or other animals. These facts constitute the base of the initialization of different studies on some different Pseudomonas spp. strains sensitivity, these being isolated from several clinical aviary cases (especially psittacines), in comparison to the different groups and types of antibiotics used currently in the treatment scheme of birds. Considering the investigations performed, the conclusion is that, even though the latest cephalosporins and fluoroquinolons have a maximum efficiency towards some of the strains, these registered also the antibiotic resistance phenomenon, in a strong proportion (41,8 to 94,12 % from the tested strains). There have been obtained constant results considering streptomycin, azithromycin, amoxicillin, associated with clavulanic acid, doxicycline and tetracycline.

Keywords: Pseudomonas, psittacines, antibiotics, therapy

INTRODUCTION

From opportunist Gram-negative bacteria, Pseudomonas aeruginosa represents a very important concern for pathology, due to its ubiquitary distribution, and also the various and numerous mechanisms of pathogenity, which allow it to produce a large gamut of primary and secondary infections, with anathomical and clinical manifestations of high diversity in humans and animals. The fact that this bacterium is present in the water, soil, on the surface of the plants, in the organisms of insects or human one and of different animals species in considerable quantities, under the shape of a colony included in a carbohydrates mass, with fibrillar aspect, explains why, especially in stress conditions or after the long application of antibiotic treatments, this bacterium is rapidly multiplying, determining pathological processes in minor resistance spots of different tissues. There has been demonstrated that this bacterium possesses several structures or it elaborates during its metabolism numerous substances able to produce the deep alteration of the normal functions of the organism (tissues). The structures on the surface of the bacterial cell (pili, surface carbohydrates, lipopolyglucids from the cell wall) are the main determining agents of virulence. But the bacterium elaborates also some toxic substances as: exotoxin A, proteases, lecitinases and endotoxins, which determine irreversible lesions in different types of tissues. If it is considered the limited possibilities to treat the infections that this bacterial species determines in humans and animals, due to a small number of active antibiotics for this bacterium, the relative high rate of mutation towards the antibiotic resistance and of rapid selection

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possibilities for resistant mutants, the result is a precise image considering the causes which explain the implications that this bacterium has in pathology field.

Considering the aspects presented until this point and also the fact that in the birds population there are many situations in which Pseudomonas aeruginosa can be incriminated on the disease production to cage and volery birds, the investigations concerning the morphological properties, the pathogenic potential of isolated Pseudomonas aeruginosa strains, and also the prophylactic measures which are implied in this paper, are fully justified. In most of the cases, the etiological agent of avairy pseudomonad is Pseudomonas aeruginosa, but there have been described also embryos’ mortality and respiratory diseases in birds, caused nu Pseudomonas fluorescens and also respiratory infections due to Pseudomonas stutzeri. Pseudomonas aeruginosa is representative for the main genus considered in avairy pathology, producing systemic infections and localized ones. Pseudomonas spp. infections, either systemic or localized, are frequent in numerous species of birds, being either domestic or savage, and they produce high losses to intensive aviculture, through their large spreading and their high prevalency.

The behavior of Pseudomonas aeruginosa towards the antibiotic action remains one of the main problems, every day being an up-to-date one, in pathology. In this sense, there has been proven the natural resistance of this bacterium towards the action of available antibiotics, the frequent earning of antibiotic resistance towards the bacterium was initially sensitive, and also to the high toxicity of efficient antibiotics for the organisms subjected to treatment. The antibiotic activity towards Pseudomonas aeruginosa in comparison with their capacity of penetrating the cell wall, of their resistance to inactivating action of bacterial enzymes and the affinity of the antibiotic for some of the cell structures. In turn, Pseudomonas aeruginosa can thwart the antibiotic action through one of the following mechanisms: the elaboration of some alternative systems which can replace or substitute the one which is inhibited; the reduction of target enzyme activity, which keeps its normal structure and the substitution of its activity through other metabolic routes; the exclusion of the inhibiting agent from the target spots by reducing the permeability of cell wall or the production of several macromolecules on the surface; the destruction of the antibiotic through hydrolysis, the substitution of some chemical groups of the antibiotic. The resistance of Pseudomonas aeruginosa species towards the antibiotic action has a genetic correspondent; usually, more than 95 % of genetic information is contained in the chromosomes and only 1-5 % in plasmids. There has also been observed that, generally, there is no full correspondence between the “in vitro” sensitivity of Pseudomonas aeruginosa and the one “in vivo”.

MATERIALS AND METHODS

In this study, there have been used 17 strains of Pseudomonas aeruginosa and 12 strains of Pseudomonas fluorescens, isolated from different species of psittacines, being tested the sensitivity towards: nalidixic acid, ampicillin, amoxicillin, amoxicillin associated with clavulanic acid, doxycycline, tetracycline, rifampicin, erythromycin, azithromycin, streptomycin, gentamicin, kanamycin, cephalothin, cefaclor, cefotaxime, cefoperazone, ceftriaxone, enrofloxacine, ciprofloxacine, ofloxacine.

There has been used the diffusimetric quantitative antibiogram on solid media (Stokes method modified by Bals), which involves:
- obtaining a bacterial culture, after 18 hours, with a concentration of $10^7$ cells/ml;
- it is poured a quantity of 16 ml of Müeller-Hinton gelose in each Petri plate, afterwards the plates being dried for 15-20 minutes in the thermostat;
- the surface of the gelose with bacterial culture prepared before is flooded, the excess being discarded;
- the Petri plates are incubated after the inoculation, with half open lid, for 25-30 minutes;
- the microcomprimates with antibiotics are distributed afterwards, 6/plate;
- after 48 hours, the inhibition areas are read, being measured their diameter with the slide rule.

RESULTS AND DISCUSSIONS

By comparison analysis of the results obtained after performing the antibiograms, there has been observed that the Pseudomonas strains are generally resistant to nalidixic acid (inhibition area with an average of 1,48 mm), ampicillin (inhibition area with an average of 4,58 mm), tetracycline (inhibition area with an average of 1,27 mm) and streptomycin (inhibition area with an average of 2,66 mm). There has also been observed the antibiotic resistance of some strains to nalidixic acid and tetracycline.

The maximum sensitivity has been observed to the latest cephalosporins (cefaclor, cefotaxime, cefoperazone) and to quinolones (enrofloxacin, ciprofloxacin), in which the inhibition areas were of 12 to 14 mm.

The statistical results of antibiogram interpreting by applying the Balş method are presented in Table no. 1. There are considered:
- resistant strains: the ones with an inhibition area of 0-1 mm;
- medium sensitivity strains: the ones with an inhibition area of 10-14 mm;
- maximum sensitivity strains: the ones with an inhibition area of 14-16 mm.

<table>
<thead>
<tr>
<th>Tested antibiotic</th>
<th>The average inhibition area (mm)</th>
<th>Resistant strains (%)</th>
<th>Medium sensitiveness strains (%)</th>
<th>Maximum sensitiveness strains (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nalidixic acid</td>
<td>0,48</td>
<td>94,12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>4,58</td>
<td>35,29</td>
<td>41,18</td>
<td>11,76</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>4,18</td>
<td>17,65</td>
<td>35,29</td>
<td>29,41</td>
</tr>
<tr>
<td>Amoxicillin + clavulanic acid</td>
<td>13,38</td>
<td>11,76</td>
<td>58,82</td>
<td>29,41</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>10,11</td>
<td>17,65</td>
<td>35,29</td>
<td>35,29</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>9,27</td>
<td>17,65</td>
<td>35,29</td>
<td>29,41</td>
</tr>
<tr>
<td>Rifampycin</td>
<td>13,05</td>
<td>23,53</td>
<td>29,41</td>
<td>35,29</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>6,30</td>
<td>29,41</td>
<td>17,65</td>
<td>11,76</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>13,11</td>
<td>11,76</td>
<td>35,29</td>
<td>29,41</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>14,05</td>
<td>11,76</td>
<td>17,65</td>
<td>58,82</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>9,11</td>
<td>17,65</td>
<td>29,41</td>
<td>11,76</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>6,83</td>
<td>11,76</td>
<td>17,65</td>
<td>29,41</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>7,27</td>
<td>94,12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cefaclor</td>
<td>2,16</td>
<td>64,71</td>
<td>11,76</td>
<td>0</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>3,12</td>
<td>88,23</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>1,16</td>
<td>64,71</td>
<td>29,41</td>
<td>11,76</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>2,17</td>
<td>58,82</td>
<td>11,76</td>
<td>0</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>3,14</td>
<td>70,59</td>
<td>17,65</td>
<td>11,76</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>4,12</td>
<td>41,18</td>
<td>35,29</td>
<td>0</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>1,19</td>
<td>88,23</td>
<td>11,76</td>
<td>0</td>
</tr>
</tbody>
</table>
Considering this interpreting, we observed that the maximum efficiency was obtained with the latest cephalosporins, and from quinolones, there can be used in therapy enrofloxacin, ciprofloxacin, although with an average inhibition area of 11.94 mm it has also strains with small inhibitions areas. The tetracycline and the aminoglicosids (gentamicin, kanamycin, streptomycin) had small inhibition areas in almost all the strains, this being the reason for which we do not recommend them in therapy, even using them in association with another antibiotics, having a synergic activity.

Taking into account the obtained results, although the scientific literature recommends the usage of cyclines and quinolones, it can be concluded that the most efficient therapy is obtained in antibiotic therapy with streptomycin, amoxicillin associated with clavulanic acid, rifampycin, doxycycline or tetracycline.

**CONCLUSIONS**

1. The antibiotics with the most intense action on Pseudomonas strains, tested “in vivo” are streptomycin, rifampycin, amoxicillin associated with clavulanic acid, azithromycin and doxycycline.
2. The antibiotics from cephalosporins group or fluoroquinolones group have proven not to be efficient in treating the tested Pseudomonas spp. strains.
3. The antibiotics from β-lactamin and some macrolids (erythromycin) had a reduced action on the tested strains.
4. The highest percentage of resistance to the antibiotic activity has been observed in cephalosporins and fluoroquinolones.

**REFERENCES**


