Investigations on the involvement of Arcanobacterium pyogenes in various infections in productive and companion animals and sensitivity of isolates to antibacterials

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SUMMARY

Arcanobacterium pyogenes is an opportunistic pathogen that causes mastitis, abortions, arthritis, osteomyelitis, pneumonies, suppurative skin and wound infections in domestic animals. For a one-year period in 2006-2007, 238 samples obtained from different animal species were bacteriologically examined. From these specimens, 52 strains identified as arcanobacteria, have been isolated. The sensitivity of isolates to chemotherapeutic drugs was evaluated. The highest percentage of resistance was observed against trimethoprim/sulfonamide.

Keywords: Arcanobacterium pyogenes, domestic animals, antibacterial activity.

RÉSUMÉ

Rôle de Arcanobacterium Pyogenes dans diverses affections des animaux de production et des animaux de compagnie ; sensibilité des isolats aux antibactériens.

Arcanobacterium pyogenes est un pathogène opportuniste responsable de différentes pathologies chez les animaux domestiques : mammite, d’avortement, d’arthrite, d’ostéomyélite, de pneumonie, de lésions cutanées suppurées et de surinfection de plaies.

Sur une période d’une année (2006-2007), 238 échantillons issus de différentes espèces animales ont fait l’objet d’un examen bactériologique. A partir de ces échantillons, 52 souches identifiées comme des arcanobactéries ont été isolées. La sensibilité des isolats à différents agents antibactériens a été évaluée. La fréquence de résistance est apparue la plus élevée pour le couple triméthoprime/sulfonamide.

Mots clés : Arcanobacterium pyogenes, animaux domestiques, activité des antibactériens.

Introduction

Arcanobacterium pyogenes is a member of the normal microflora of mucous membranes in a number of domestic animal species – cattle, swine, goats etc. It was recently reclassified from the genus Actinomyces into a separate genus Arcanobacterium. It is an opportunistic pathogen because it is incapable to cause disease a priori.

BILLINGTON et al. [1, 2] reported its involvement in the etiogenesis of other primary bacterial infections, most commonly due to traumas, as well as to viral infections.

Arcanobacteria could cause mastitis [5], abortions and a number of suppurative infections of the skin, joints, the urogenital tract and visceral organs in domestic animals. It is acknowledged as a causative agent of osteomyelitis in turkeys and pneumonies in swine, deer and wild sheep [4].

Despite the variety existing in A. pyogenes strains isolated from domestic animals, the specific determinants of virulence factors have not been yet specified. A. pyogenes produces numerous potential virulence factors, including DNase, as well as several proteases. Furthermore, arcanobacteria produce the haemolytic protein pyolysin (PLO) that possesses cytolytic activity against erythrocytes in a number of animal species, as well as dermonecrotic and lethal effect in laboratory animal species. PLO is also cytolytic against polymorphonuclear leukocytes (PMN) and renal kangaroo cells [6].

With regard to the sensitivity of arcanobacteria to chemotherapeutics, it is believed that the higher percentage of resistant strains are isolated from cattle (in these animals, most commonly in milk samples), as compared to swine and sheep. Some authors reported 39% strains resistant to tetracycline, isolated from cows with mastitis. Also, increased resistance to erythromycin – 16%, was reported for strains isolated from milk samples [8].

In some cases the resistance to the combination trimethoprim/sulfamethoxazole was reported to be 7-8% in pig isolates. High resistance percentage (33%) was observed in sheep isolates against neomycin [8].

Materials and Methods

SAMPLES

For the period 2006-2007, a total number of 238 samples were examined with the purpose of arcanobacteria identification. The samples were distributed by species as followed: cattle - 116, swine – 55, sheep – 38, dogs – 18 and cats – 11 samples.

Bovine samples included 45 milk secretion specimens from clinical and subclinical mastitis, 52 uterine-vaginal discharges, 10 samples from hoof lesions and 9 wound discharges.

Sheep samples consisted of 22 milk secretions from cases of clinical mastitis, 11 hoof lesion specimens and 5 wound samples.

Porcine samples were 30 of pulmonary origin, 18 from uterine-vaginal secretions and 7 from joints.

The samples obtained from dogs and cats were only from wound discharges.

BACTERIOLOGICAL STUDIES

For isolation of arcanobacteria, the materials were inoculated on trypticase-soy-blood agar base No.2 (BD–Diagnostic Systems). Cultures were incubated parallelly under aerobic and microaerophilic conditions, the latter being placed into a jar with 10% 2 (Bul Bio – National Centre of Infectious and Parasitic Diseases,Bulgaria).

The strains were identified by means of the semi-automated identification system BBL Crystal -MIND (BD – Diagnostic Systems,USA).

The behaviour of isolates to antimicrobial drugs was tested by the disk diffusion method and the results were interpreted according CLSI recommendations (National Committee for Clinical Laboratory Standards, 2002. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard.M31-A2. National Committee for Clinical and Laboratory Standards, Wayne, PA.)

Results and Discussions

The results from bacteriological examinations are presented in Table 1. Out of the studied 238 samples, 53 strains (22.26%), whose affiliation to A. pyogenes was confirmed, were isolated. The highest percentage of isolates were recovered from sheep – 28.9%, followed by cattle – 13.9%, swine – 12.7% and dogs – 11.1%. No strains from this species were isolated from feline specimens.

From cattle, 33 Arcanobacterium strains were isolated (63.5%). In this species, A. pyogenes was most commonly isolated from the discharges of infected purulent wounds – 57.1%, followed by milk secretions of dairy cows with clinical or subclinical mastitis – from the examined 40 secretion specimens, 20 strains (44.4%) were isolated. Third in occurrence were isolates from uterine-vaginal discharges – 5 strains out of 52 samples or 9.6%.

It is interesting to mention that unlike sheep, there were no strains isolated from the 34 samples of bovine hoof lesions – infected sole ulcers and severe digital dermatitis.

In sheep, A. pyogenes was the most frequent in hoof lesion samples – from the studied 11 samples, positive findings were determined in 5 (45.5%). Out of the 22 ovine milk secretion samples, this microbial species was detected in 5 samples (22.7%), and in wound discharges – one strain was found out in the five studied samples.

In swine, A. pyogenes was most commonly prevalent in uterine-vaginal discharges in sows with gynaecological problems. From the tested 18 samples, 4 or 22.2% were positive. Then followed positive findings from pulmonary samples – 3 strains from 30 samples (10%). All tested joint punctate specimens were negative for this microbial species.

In dogs, the isolated 2 strains (11.1%) were from discharges of infected gunshot wounds.

Table 2 presents the results for the sensitivity of isolates to chemotherapeutical drugs.

As per Table 2, the highest percentage of resistant isolates was determined against to trimethoprim/sulfamethoxazole

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Number of studied samples</th>
<th>Number of isolates</th>
<th>%</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>cattle</td>
<td>116</td>
<td>33</td>
<td>13.90</td>
<td>milk secretion, uterine-vaginal and wound discharges, hoof lesions</td>
</tr>
<tr>
<td>swine</td>
<td>55</td>
<td>7</td>
<td>12.70</td>
<td>lungs, uterine-vaginal discharges, joint punctates</td>
</tr>
<tr>
<td>sheep</td>
<td>38</td>
<td>11</td>
<td>28.90</td>
<td>hoof lesions, milk secretions, wound discharges</td>
</tr>
<tr>
<td>dogs</td>
<td>18</td>
<td>2</td>
<td>11.10</td>
<td>wound discharges</td>
</tr>
<tr>
<td>cats</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>238</td>
<td>53</td>
<td>22.26</td>
<td></td>
</tr>
</tbody>
</table>

Table I : A. pyogenes prevalence in samples obtained from different animal species.
URUMAVA (V.) AND COLLABORATORS

The highest sensitivity of arcanobacteria was observed to lincomycin (84.2%), as well as to gentamicin and erythromycin (68.4%).

Figures 1 and 2 present the cumulative curves of tested chemotherapeutics in *Arcanobacterium* strains isolated from domestic animals. The X-axis of these curves represents the growth inhibition zones whereas the Y-axis – the cumulative percentages of isolates, thus allowing commenting both the width of zones as determined by the disk diffusion method and to plot the respective cumulative percentages.

Fig.1. shows the cumulative curves of amoxicillin, gentamicin and erythromycin in *Arcanobacterium* isolates from all studied animals.

Half (50%) of strains were resistant to amoxicillin. Growth inhibition zones varied within a wide range from 6 mm to 30 mm for amoxicillin.

The isolates resistant to gentamicin and erythromycin (R+I) were 31.5%. Inhibition zones varied within a wide range too – 6 – 32 mm (erythromycin) and 6 – 26 mm (gentamicin).

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The widest range of growth inhibition zones was observed for enrofloxacin: 6 to 35 mm, followed by doxycycline and lincomycin with 6 – 28 mm and trimethoprim/sulfamethoxazole with 6-17 mm.

The highest resistance in this study - 81.6% occurred against the combination trimethoprim/sulfamethoxazole. LIU et al. [11] established resistance at one hundred percentages to sulfonamides and bacitracin zinc among arcanobacteria isolates in bovine endometritis. In a survey on *Arcanobacterium* isolates from swine and sheep in Norway in 1998-2003, a predominant resistance was observed against the same combination as well, but at considerably lower percentages: 15-18% for sheep and 7-10% for swine [8]. The same survey also reported the behaviour of isolates to tetracycline and erythromycin. These data are particularly important for strains isolated from cattle with mastitis. The authors established resistance to tetracycline of 29 to 39% for the period of survey, that were lower compared to our own data (47.4%) but tetracycline is discussed as one of the chemotherapeutics with highest resistance percentages from the part of mastitis isolates. The reported resistance to erythromycin in bovine isolates (10-16%) was similar, to that of ovine strains (10-20%). In our study, 31.5% of bovine isolates were resistant to erythromycin. In a survey on Arcanobacterium isolates from cattle and pigs in Japan, resistance was observed against oxytetracycline at 85.7% in porcine isolates and 57.1% among bovine isolates [10]. GUÈRIN-FAUBLÈE et al. [3] established resistance to tetracycline at 67% against tetracycline, doxycycline and minocycline among arcanobacteria isolates of animal origin. Very high percentages was established against amoxicillin 50%, significantly different from the data of TEALE et al. [8] reporting 3% - to ampicillin. LIU et al. [11] established resistance to penicillins at

<table>
<thead>
<tr>
<th>Chemotherapeutics</th>
<th>S (%)</th>
<th>CL</th>
<th>R+I (%)</th>
<th>CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>50.0</td>
<td>36.3+63.7</td>
<td>50.0</td>
<td>36.3+63.7</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>68.4</td>
<td>55.0+80.4</td>
<td>31.5</td>
<td>19.5+44.9</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>68.4</td>
<td>55.0+80.4</td>
<td>31.5</td>
<td>19.5+44.9</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>47.4</td>
<td>33.8+61.2</td>
<td>52.6</td>
<td>38.8+66.2</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>47.4</td>
<td>33.8+61.2</td>
<td>52.6</td>
<td>38.8+66.2</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>84.2</td>
<td>72.9+92.8</td>
<td>15.8</td>
<td>7.1+27.1</td>
</tr>
<tr>
<td>Trimethoprim/Sulfamethoxazole</td>
<td>18.4</td>
<td>9.0+30.2</td>
<td>81.6</td>
<td>69.8+91.0</td>
</tr>
</tbody>
</table>

TABLE II: Percentages of sensitive, intermediate and resistant *Arcanobacterium pyogenes* strains isolated from various animal species.

FIGURE 1: Cumulative curves of amoxicillin, gentamicin and erythromycin in *Arcanobacterium* isolates.

FIGURE 2: Cumulative curves of enrofloxacin, doxycycline, lincomycin and trimethoprim/sulfamethoxazole in *Arcanobacterium pyogenes* isolates.
high percentages (50-65.6%) in bovine endometritis Arcanobacterium pyogenes isolates. Another major discrepancy is the percentage of resistance to enrofloxacin in our study - 47.4% compared to the lack of resistance in the cited survey [8].

TRINH et al. [9] has investigated the resistance of Arcanobacterium isolates from cattle and swine with emphasis on resistance to macrolides and lincosamides, i.e. the so-called MLSB resistance. In this study, 12.5% of isolates were resistance to erythromycin and 25% to clindamycin. YOSHIMURA et al. [10] was observed the resistance of Arcanobacterium porcine isolates against erythromycin, tilmicosin and lincomycin at 28.6%. We have included lincomycin in our investigation as a member of the lincosamides group and established a resistance of 15.78%. MLSB resistance is a very interesting mechanism concerning for a number of gram-positive pathogens and deserves further attention with regard to more impartial evaluation of resistance to these two groups of chemotherapeutic drugs.

References