Antimicrobial Susceptibility of *Corynebacterium pseudotuberculosis* Isolated from Lesions of Caseous Lymphadenitis in Sheep in Hokkaido, Japan

Hong-Kun ZHAO, Hiroiuki MORIMURA*, Naoya KIKUCHI, Ryo YANAGAWA, and Shin SERIKAWA†

Department of Epizootiology, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069, and †Takikawa Animal Husbandry Experiment Station, Takikawa, Hokkaido 073, Japan

(Received 27 July 1990/Accepted 5 December 1990)


**KEY WORDS:** caseous lymphadenitis, *Corynebacterium pseudotuberculosis*, drug susceptibility.

*Corynebacterium pseudotuberculosis* is a causative agent of caseous lymphadenitis in sheep and goats. The authors recently detected the presence of the disease in sheep in the vicinity of Sapporo by an abattoir survey [6], and the spreading of the infection in sheep by a serological survey using enzyme-linked immunosorbent assay and immunodiffusion [2].

Antimicrobial susceptibility of *C. pseudotuberculosis* has been reported by Ashfaq and Campbell [1], and Muckle and Gyles [5]; the former used 10 antimicrobial sensitivity discs to show susceptibility of 71 strains isolated from goats in the U.S.A., while the latter used 10 antimicrobial agents to determine the minimum inhibitory concentration (MIC) of 25 strains isolated from sheep in Canada. On the other hand, antimicrobial susceptibility of Japanese strains of *C. pseudotuberculosis* has not been tested.

The present study was made to determine the MICs of 23 antimicrobials to *C. pseudotuberculosis* strains isolated from sheep in Hokkaido.

Eighty-six strains were used; 85 were isolated by using Trypticase soy agar (BBL, Cockeysville, U.S.A.), to which 5% bovine blood was added, from lesions of caseous lymphadenitis in slaughtered sheep in Hokkaido during 1988 to 1989, and 1 was the reference strain ATCC 19410. The 23 antimicrobial agents used and their abbreviations are as follows: penicillin G (PCG), ampicillin (ABPC), methicillin (MDPPC), cefazolin (CEZ), streptomycin (SM), gentamicin (GM), kanamycin (KM), amikacin (AMK), neomycin (NM), erythromycin (EM), tetracycline (TC), oxytetracycline (OTC), polymyxin B (PL), colistin (CL), bacitracin (BC), chloramphenicol (CP), clindamycin (CLDM), novobiocin (NB), vancomycin (VCM), nitrofurantoin (NFT), sulfisoxazole (SIX), and trimethoprim (TMP). MIC was determined by agar dilution method standardized by the Japan Society of Chemotherapy [4]. Trypto-soya broth (Nissui, Tokyo, Japan) was used for preincubation and sensitivity test agar (Nissui), to which 5% bovine blood was added, was employed as assay media, and they were incubated aerobically at 37°C for 48 hr, respectively.

The results of antimicrobial susceptibility test are shown in Table 1. Penicillin G, ABPC, EM, and BC exhibited marked antimicrobial activities against almost all *C. pseudotuberculosis* strains used, with the MICs ranging from ≤0.1 to 0.78 μg/ml. The strains were also susceptible to the 11 other drugs (DMPPC, CET, CEZ, GM, NM, TC, OTC, CP, CLDM, VCM, and TMP); almost all of the strains were inhibited at a concentration of less than 6.25 μg/ml. Four drugs (SM, KM, AMK, and NB) were less active; the MICs of these drugs ranged mostly from 12.5 to 50 μg/ml. All strains were found to be insensitive to PL, CL, NFT, and SIX; the MICs of these 4 drugs to most of the strains ranged from 200 to 1,600 μg/ml or more.

MIC values of 17 drugs (PCG, ABPC, DMPPC, CET, SM, GM, AMK, NM, EM, TC, PL, CL, BC, CP, NB, VCM, and NFT) were within a limited range; the strains were rather uniform in sensitivity to those drugs. The MIC values of the remaining 6 drugs (CEZ, KM, OTC, CLDM, SIX, and TMP), on the other hand, showed a broad range. These drugs exhibited a two-peak MIC, and it was especially obvious in CEZ and KM. As these 6 drugs have practically not been used for the sheep, the reason why these drugs exhibited the two-peak MIC is unknown.

Thus the results of the antimicrobial susceptibility test on the Japanese strains of *C. pseudotuberculosis* to the drugs generally agreed with those reported previously in the U.S.A. [1] and Canada [5] as far as the drugs used were the same. Of the drugs newly examined in the present study, BC showed marked activity, CEZ, OTC, and VCM showed moderate activity, KM, AMK, and NB showed less activity, and PL, NFT, and SIX showed no activity.

The disease caused by *C. pseudotuberculosis* has been considered to be refractory to antibiotic therapy because the caseous abscesses have a thick capsule [3]. However, an application of antibiotics such as PCG, ABPC and EM, which are effective against the organisms in vitro, may also be effective against the organisms in vivo if applied in the early stage of the infection. Our previous work revealed that the positive ratio of anti-*C. pseudotuberculosis* antibodies was low in sheep aged less than 1 year, and the ratio increased significantly in those aged 1 year [2], suggesting that *C. pseudotuberculosis* infection occurred mostly in sheep at 1 year of age. A study whether the occurrence of caseous lymphadenitis is reduced by application of the antibiotics to one-year-old sheep, particularly immediately after the first shearing, is now in progress.

*Correspondence to:* HIRAMUNE, T., Department of Epizootiology, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069, Japan.
Table 1. Antimicrobial susceptibility of 86 _C. pseudotuberculosis_ strains isolated in Hokkaido, Japan

<table>
<thead>
<tr>
<th>Drugs</th>
<th>≤0.1</th>
<th>0.2</th>
<th>0.39</th>
<th>0.78</th>
<th>1.56</th>
<th>3.13</th>
<th>6.25</th>
<th>12.5</th>
<th>25</th>
<th>50</th>
<th>100</th>
<th>200</th>
<th>400</th>
<th>800</th>
<th>≥1600</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3</td>
<td>53</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABPC</td>
<td>39</td>
<td>47</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMPPC</td>
<td>1</td>
<td>1</td>
<td>82</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CET</td>
<td>55</td>
<td>30</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEZ</td>
<td>4</td>
<td>39</td>
<td>17</td>
<td>1</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM</td>
<td></td>
<td></td>
<td>1</td>
<td>17</td>
<td>67</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM</td>
<td>1</td>
<td>1</td>
<td>52</td>
<td>32</td>
<td>39</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KM</td>
<td>1</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMK</td>
<td>1</td>
<td>1</td>
<td>82</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NM</td>
<td>1</td>
<td>1</td>
<td>83</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EM</td>
<td>83</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>26</td>
<td>59</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OTC</td>
<td>22</td>
<td>3</td>
<td>50</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PL&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>86</td>
<td></td>
<td>84</td>
</tr>
<tr>
<td>CL&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>BC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td></td>
<td></td>
<td>20</td>
<td>65</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLDM</td>
<td>5</td>
<td>23</td>
<td>33</td>
<td>1</td>
<td>23</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NB</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>59</td>
<td>25</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VCM</td>
<td>2</td>
<td>83</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NFT</td>
<td></td>
<td></td>
<td>13</td>
<td>28</td>
<td>44</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIX</td>
<td>1</td>
<td>6</td>
<td>55</td>
<td>7</td>
<td>16</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMP</td>
<td>1</td>
<td>23</td>
<td>6</td>
<td>55</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Units per ml.

REFERENCES