Drug Resistance and R Plasmids in Pasteurella multocida Isolates from Swine

Junya YAMAMOTO,* Tetsuya SAKANO, and Mikio SHIMIZU
Zen-noh Institute of Animal Health, Sakura, Chiba 285
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Abstract A total of 163 strains of Pasteurella multocida isolated from swine were examined for drug resistance and R plasmids. Strains resistant to sulfadimethoxine (Sa), ampicillin (Ap), streptomycin (Sm), kanamycin (Km), and chloramphenicol (Cp) were found in 93.9, 1.8, 16.6, 1.2, and 10.4%, respectively. There were two patterns of drug resistance (Sa and SaCp) in isolates from nasal cavities, and five patterns (Sa, SaSm, SaSmCp, SaSmAp, and SaSmKmCp) in isolates from pneumatic lung specimens. Two isolates studied were proved to carry a nonconjugative R plasmid pJY2 or pJY8 with other unidentified plasmids, respectively. pJY2 (3.6 megadaltons) encoding resistance to SaSm had one cleavage site for EcoRI or HindIII endonuclease and two sites for PstI endonuclease. pJY8 (5.5 megadaltons) encoding resistance to SaSmKmCp had one EcoRI site and two PstI sites.

Pasturella multocida is a causative agent of porcine pneumonia or atrophic rhinitis (13), which causes great economic loss in the swine industry. Antimicrobial treatment is still commonly used to control these diseases and has been compromised due to the emergence of drug-resistant strains. There have been only a few reports on drug susceptibility in P. multocida isolates from swine (5, 15, 19). This paper describes drug resistance and partial characterization of R plasmids in porcine P. multocida isolates.

Materials and methods

Strains and media. A total of 163 strains of P. multocida were isolated on dextrose starch agar (Difco, Detroit, Mich., U.S.A.) plates from nasal cavities of 30 swine and from 50 pneumatic lung specimens on 29 farms from 1982 to 1985. The isolates were identified according to the method described by Iwamatsu and Sawada (8). Capsular serotyping was done by the hyaluronidase decapsulation test and acriflavine flocculation test of Carter et al (3, 4) using strain Kobe 5 (serotype A) and Kobe 6 (serotype D) as the reference strains. Dermonecrotic toxin production (DNT+) was determined by the method of Nakai et al (11) as described previously (17). Escherichia coli JM109 was used as a recipient of R plasmids and E. coli V517 (10) was employed as a single source of covalently closed circular DNA molecules.
of different sizes for use as references in agarose gel electrophoresis. Brain heart infusion broth and Mueller Hinton medium (Difco) were used.

Antibiotic susceptibility test. Minimal inhibitory concentrations (MICs) were determined by the agar dilution method standardized by the Japan Society of Chemotherapy (12).

Isolation and characterization of plasmid DNA. Plasmid DNAs were detected by the method of Kado and Liu (9) and were extracted by the method of Birnboim and Doly (2). Conjugal transfer of R plasmids was performed according to the mixed cultivation method described previously (14). Transformation using \textit{E. coli} JM109 competent cells, digestion of DNA with endonucleases (Takara Shuzo, Kyoto, Japan), and agarose gel electrophoresis of the DNAs were performed according to recommendations by the supplier.

RESULTS

Isolation of \textit{P. multocida}

Of 80 isolates from nasal cavities on 9 farms, 71 and 9 belonged to capsular serotype A and D, respectively, and all 83 isolates from 50 pneumonic specimens on 20 farms belonged to serotype A. Seven of the 9 strains that belonged to serotype D were DNT+.

Drug-Resistant Strains and Resistance Patterns

There was no significant difference between isolates from nasal cavities and those from lung specimens in distribution of drug susceptibility. Table 1 shows bimodal distributions in susceptibility to sulfadimethoxine (SA), ampicillin (APC), streptomycin (SM), kanamycin (KM), and chloramphenicol (CP). Strains were recorded as resistant if they grew on plates containing the following concentrations of drug: 100 \( \mu \text{g/ml} \) for SA, SM, and KM; 12.5 \( \mu \text{g/ml} \) for APC; and 3.1 \( \mu \text{g/ml} \) for CP.

<table>
<thead>
<tr>
<th>Drug(^{2)}</th>
<th>0.2</th>
<th>0.4</th>
<th>0.8</th>
<th>1.6</th>
<th>3.1</th>
<th>6.3</th>
<th>12.5</th>
<th>25</th>
<th>50</th>
<th>100</th>
<th>&gt;100</th>
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<tbody>
<tr>
<td>SA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10( ^{b)})</td>
<td>153</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APC</td>
<td>79*</td>
<td>39</td>
<td>36</td>
<td>6</td>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM</td>
<td>1</td>
<td>2</td>
<td>16</td>
<td>28*</td>
<td>51</td>
<td>38</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KM</td>
<td>1</td>
<td>24*</td>
<td>57</td>
<td>71</td>
<td>8</td>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SPC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>51*</td>
<td>102</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL</td>
<td>16*</td>
<td>28</td>
<td>43</td>
<td>53</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>CP</td>
<td>35</td>
<td>110*</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OTC</td>
<td>8*</td>
<td>48</td>
<td>62</td>
<td>13</td>
<td>32</td>
<td></td>
<td></td>
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</tbody>
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\(^{2)}\text{SA, sulfadimethoxine; APC, ampicillin; SM, streptomycin; KM, kanamycin; SPC, spectinomycin; CL, colistin; CP, chloramphenicol; OTC, oxytetracycline.}\n
\(^{b)}\text{Number of strains, *: A position of MIC value against a reference strain Kobe 6.}\n

Strains resistant to Sa (Sa<sup>r</sup>), APC (Ap<sup>r</sup>), SM (Sm<sup>r</sup>), KM (Km<sup>r</sup>), and CP (Cp<sup>r</sup>) were found in 93.9, 1.8, 16.6, 1.2, and 10.4%, respectively. There were two patterns of drug resistance (Sar and SarCpr) in nasal cavity isolates, and five patterns (Sar, SarSmr, SarSmrApr, SarSmrApr, and SarSmrKmrCpr) in lung isolates (Table 2).

### Isolation and Characterization of R Plasmids

Two *P. multocida* strains from lung specimens were studied for R plasmids. Strain Z102 and Z108 had a resistance pattern of SarSmrAp<sup>r</sup> and SarSmrKmrCpr.
Several plasmid bands were detected in DNA extracts from both strains by agarose gel electrophoresis (Fig. 1). Attempts to transfer drug resistance from Z102 or Z108 to E. coli JM109 by conjugation were not successful. However, several transformants of JM109 with the DNA extracts were successfully isolated. A transformant from Z102 gave SarSmr and carried a 3.6 megadalton (MDa) plasmid (designated pJY2), and a transformant from Z108 gave SarSmrKmCpr and carried a 5.5 MDa plasmid (designated pJY8) (Table 3, Fig. 1). Both plasmid pJY2 and pJY8 were very unstable in JM109 during cultivating, and elimination of these plasmids from the host bacteria resulted in the

### Table 3. Drug susceptibility of *P. multocida* and *E. coli* derivatives

<table>
<thead>
<tr>
<th>Strains</th>
<th>Minimal inhibitory concentration (μg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>SA</td>
</tr>
<tr>
<td><em>P. multocida</em></td>
<td></td>
</tr>
<tr>
<td>Z102 (pJY2)a)</td>
<td>1,600</td>
</tr>
<tr>
<td>Z108 (pJY8)</td>
<td>1,600</td>
</tr>
<tr>
<td>Kobe 6</td>
<td>12.5</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td></td>
</tr>
<tr>
<td>JM109</td>
<td>100</td>
</tr>
<tr>
<td>JM109 (pJY2)</td>
<td>&gt;3,200</td>
</tr>
<tr>
<td>JM109 (pJY8)</td>
<td>&gt;3,200</td>
</tr>
</tbody>
</table>

a) *P. multocida* Z102 carrying plasmid pJY2.
loss of their drug resistance. Thus, pJY2 and pJY8 were proved to be associated with Sa'Sm\(^r\) and Sa'Sm\(^r\)Km\(^r\)Cp\(^r\), respectively. Digestion of pJY2 with endonucleases followed by gel electrophoresis produced one fragment for EcoRI or HindIII, and two fragments for PstI (Fig. 2). Digestion of pJY8 produced one fragment for EcoRI, and two fragments for PstI (Fig. 3).

**DISCUSSION**

There have been only a few reports \((5, 15, 19)\) on drug susceptibility in porcine *P. multocida* isolates. We previously reported that most of *P. multocida* isolates in 1979 were antibiotic-susceptible because of poor drug selection pressure in bacteria in respiratory tracts of swine \((14)\) whereas most of *Escherichia coli* isolates were multiple drug-resistant and R plasmid-bearing \((16)\). However, the present study demonstrated six drug-resistance patterns—Sa\(^r\), Sa'Cp\(^r\), Sa'Sm\(^r\), Sa'Sm'Cp\(^r\), Sa'Sm'Ap\(^r\), and Sa'Sm'Km'Cp\(^r\)—in porcine *P. multocida* isolates from 1982 to 1985. Very recently, Takahashi et al \((19)\) reported porcine isolates of Sa\(^r\), Sa'Sm\(^r\), Sa'Tc\(^r\), and Sa'Sm'Ap\(^r\). These results indicate that the number of multiple drug-resistant strains of *P. multocida* have recently increased in swine farms in Japan, suggesting that extensive use of antibiotics in intensive swine husbandry might have brought about the appearance and selection of these resistant strains. Chang and Carter \((5)\) reported that 84\% of porcine isolates in the United States were resistant to at least one antibiotic with resistance patterns of Sm\(^r\), Sm'Tc\(^r\) (tetracycline resistance), Sm'Pc\(^r\) (benzylpenicillin resistance), or Sm'Tc'Pc\(^r\). Drug-resistant
isolates from cattle (5, 18) and turkeys (1, 6, 7) were also reported. Table 3 shows that CP resistance level of pJY8 was expressed higher in E. coli JM109 (MIC: over 400 µg/ml) than in P. multocida Z108 (MIC: 12.5 µg/ml). Silver et al (18) also described that a Tc\(^r\) plasmid from a P. multocida isolate from cattle conferred resistance to 32 µg/ml of TC in P. multocida but to 256 µg/ml in E. coli, whereas a Sa\(^r\) Sm\(^r\) plasmid mediated resistance to over 256 µg/ml of SM in P. multocida but to only 64 µg/ml in E. coli.

There have been few reports of R plasmids in porcine P. multocida isolates, although nonconjugative plasmids (1, 7, 18) and conjugative plasmids (6) have been reported in P. multocida of cattle and turkey origin. We partially characterized two nonconjugative R plasmids: pJY2 (3.6 MDa) coding for Sa\(^r\)Sm\(^r\) and pJY8 (5.5 MDa) coding for Sa\(^r\)Sm\(^r\)Km\(^r\)Cp\(^r\) from porcine isolates. Our isolates Z102, Z108 (Fig. 1), and some other isolates (unpublished data) were observed to carry small unidentified cryptic plasmids in agarose electrophoresis. Hirsh et al (6) also described that cryptic plasmids (2.6 MDa) were observed in approximately 22% of isolates from turkeys. Further study is now in progress to identify the function of these cryptic plasmids in P. multocida.

Note added in proof. After this work was submitted for publication, an article by Ishii et al (Jpn. J. Vet. Sci. 52: 399-409 (1990)) was published that also demonstrated a P. multocida isolate resistant to SA, SM, KM, and CP.

REFERENCES


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