Short Communication

Prevalence of *Providencia* Strains among Patients with Diarrhea and in Retail Meats in Thailand

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SUMMARY: In the present study, we examined the prevalence of *Providencia* spp. in retail meats and in stool samples from diarrheal patients in Thailand. *Providencia*-specific (Psp)-PCR amplification was positive for 58% (15/26) of the chicken, 64% (16/25) of the pork, and 68% (17/25) of the beef samples. Subsequently, *Providencia* strains were isolated from 65% (17/26) of the chicken, 68% (17/25) of the pork, and 72% (18/25) of the beef samples. Although *P. alcalifaciens* (40–58%) was the most prevalent *Providencia* strain, *P. rettgeri*, *P. stuartii*, and *P. rustigianii* were also isolated. Of the patient stool samples tested, 7.5% (16/214) had Psp-PCR products, of which 4 were *P. alcalifaciens* (1.9%), 4 were *P. rettgeri* (1.9%), and 1 was *P. stuartii* (0.5%). In conclusion, retail meats were substantially contaminated with *Providencia*, especially *P. alcalifaciens*. Therefore, retail meats are possibly the source of *Providencia* infections in humans.

The genus *Providencia*, belonging to the family *Enterobacteriaceae*, consists of 9 species, namely: *P. alcalifaciens*, *P. stuartii*, *P. rettgeri*, *P. rustigianii*, *P. heimbachae*, *P. vermicola*, *P. sneebia*, *P. burhodogranariea*, and *P. thailandensis* (1–4). Among these, *P. alcalifaciens* has been considered to be a causative agent of diarrhea in children and travelers from developing countries (5–7). There are reports suggesting that *P. rettgeri* and *P. heimbachae* may be associated with diarrhea (8,9). Furthermore, 2 large outbreaks of food poisoning caused by *P. alcalifaciens* have been reported in Japan and the Czech Republic. (10,11). Despite that, the increasing cases of infection have attracted public attention, the contamination routes and prevalence of *Providencia* spp. in foods are still unclear. In addition, the prevalence of *Providencia* spp. in diarrheal patients in Thailand has not yet been investigated. Therefore, we conducted a survey to determine the prevalence of *Providencia* spp. in retail meats and in stool samples from patients with diarrhea in Thailand.

Meat samples were randomly purchased at supermarkets and open markets in Bangkok and on the outskirts of Bangkok in Thailand between August and September 2011. The meat samples were kept in the refrigerator until use. From each meat sample, a 25 g portion was aseptically weighed and added to a sterile bag containing 25 mL of PBS (−). The samples were homogenized for 1 min in a masticator classic apparatus (IUL Instruments, Barcelona, Spain). One milliliter of homogenate was added to 4 mL of 1.25× tryptic soy broth (TSB) and incubated at 37°C overnight. The overnight culture was used to prepare the PCR template, examined by *Providencia*-specific (Psp)-PCR as previously described (12), and inoculated to polymyxin-mannitol-xylitol medium for *Providencia* (PMXMP) agar (9). The PMXMP agar was incubated at 37°C for 2 days and colonies suspected to belong to *Providencia* spp. were examined for ornithine decarboxylase and oxidase activities. To further identify the species of the *Providencia*-positive isolates, the API 20E biochemical strip for the family *Enterobacteriaceae* (SYSMEX bioMérieux Co., Ltd., Tokyo, Japan) and adonitol and galactose utility tests were employed as previously described (12). Psp-PCR products were obtained from 15 of the 26 (58%) chicken samples, 16 of the 25 (64%) pork samples, and 17 of the 25 (68%) beef samples (Table 1). Seventeen (65%), 17 (68%), and 18 (72%) bacteria suspected to be of genus *Providencia* were isolated from the chicken, pork, and beef samples, respectively. The number of isolates obtained by the culture method was slightly higher than that of the Psp-PCR-positive samples. *P. alcalifaciens* (40–58%) was the most prevalent in the 3 different meats samples. *P. rettgeri*, *P. stuartii*, and *P. rustigianii* were also isolated from the meat samples. There was no significant difference in isolation ratio among the 3 types of meats. However, 7 *P. stuartii* (28%) strains were isolated from the beef samples, whereas 2 (8.0%) were obtained from the chicken samples and 0 (0%) from the pork samples. It is interesting to note that *P. stuartii* has been reported to be the causative agent of neonatal diarrhea in calves (13). Further studies are needed to prove that cows are a potential source of *P. stuartii*.

To determine the prevalence of *Providencia* spp. in diarrheal patients in Thailand, rectal swabs were randomly collected from 214 patients with diarrhea (0 to 88 years old) who were admitted to the Thammasat Univer-
strains isolated from meat and patient samples, respectively, the \( cdtB \) gene was detected in only 1 \( P. \) \( \text{alcalifaciens} \) strain isolated from a patient with diarrhea, but not in any other \( P. \) \( \text{alcalifaciens} \) strains, including 39 \( P. \) \( \text{alcalifaciens} \) strains isolated from meat and patient samples. The result indicates that \( cdt \) genes are not prevalent in \( P. \) \( \text{alcalifaciens} \) and it was limited to the particular strain isolated from the patient in our survey. It has also been reported that 59\% of \( P. \) \( \text{alcalifaciens} \) strains show invasion against culture cells (17). The virulence mechanisms of \( P. \) \( \text{alcalifaciens} \), such as CDT production and invasion, might be varied among strains. Some of the \( P. \) \( \text{alcalifaciens} \) strains may be pathogenic for humans, but not all are. Further study is required to elucidate the pathogenicity of \( P. \) \( \text{alcalifaciens} \).

In conclusion, this is the first report showing the prevalence of \( P. \) \( \text{alcalifaciens} \) in retail meats in Thailand. Our results indicate that retail meats in Thailand are substantially contaminated with \( P. \) \( \text{alcalifaciens} \). The fact that \( P. \) \( \text{alcalifaciens} \), \( P. \) \( \text{rettgeri} \), and \( P. \) \( \text{stuartii} \) were isolated from both diarrheal patients and meat samples indicates that food animals, in particular meats, might be a source of \( P. \) \( \text{alcalifaciens} \) infection to humans; thus, further studies are required to confirm this theory.

**Conflict of interest** None to declare.

**REFERENCES**

11. Murata T, Iida T, Shiomi Y, et al. A large outbreak of foodborne \( P. \) \( \text{alcalifaciens} \) infection to humans; thus, further studies are required to confirm this theory.

**Table 1.** Isolation of \( P. \) \( \text{alcalifaciens} \) species from meat samples in Thailand

<table>
<thead>
<tr>
<th>Meat</th>
<th>No. of samples</th>
<th>No. of Psp-PCR positive (%)</th>
<th>Total</th>
<th>No. of culture positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>( P. ) ( \text{alcalifaciens} )</td>
</tr>
<tr>
<td>Chicken</td>
<td>26</td>
<td>15 (58)</td>
<td>17 (65)</td>
<td>15 (58)</td>
</tr>
<tr>
<td>Pork</td>
<td>25</td>
<td>16 (64)</td>
<td>17 (68)</td>
<td>14 (56)</td>
</tr>
<tr>
<td>Beef</td>
<td>25</td>
<td>17 (68)</td>
<td>18 (72)</td>
<td>10 (40)*</td>
</tr>
</tbody>
</table>

* One strain each of \( P. \) \( \text{stuartii} \) and \( P. \) \( \text{alcalifaciens} \) was isolated from a beef sample.

**Table 2.** Isolation of \( P. \) \( \text{alcalifaciens} \) species from patient with diarrhea in Thailand

<table>
<thead>
<tr>
<th>No. of Psp-PCR positives* (%)</th>
<th>Total</th>
<th>No. of culture positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( P. ) ( \text{alcalifaciens} )</td>
</tr>
<tr>
<td>16 (7.5)</td>
<td>9 (4.2)</td>
<td>4 (1.9)</td>
</tr>
</tbody>
</table>

* A total of 214 stool specimen of diarrhea was examined.
