A CASE OF WOUND DUAL INFECTION WITH PASTEURELLA DAGMATUS AND PASTEURELLA CANIS RESULTING FROM A DOG BITE – LIMITATIONS OF VITEK-2 SYSTEM IN EXACT IDENTIFICATION OF PASTEURELLA SPECIES

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Abstract

Background: Pasteurella species, widely known as indigenous organisms in the oral and gastrointestinal floras of many wild and domestic animals, are important pathogens in both animals and humans. Human infections due to Pasteurella species are in most cases associated with infected injuries following animal bites. We encountered a rare case of dual infections caused by different two Pasteurella species occurred in a previously healthy 25-year-old female sustaining injury by a dog-bite.

Methodology: Exudates from the open wound of her dog-bite site, together with the saliva of the dog were submitted for bacteriological examination. Predominantly appearing grayish-white smooth colonies with almost the same colonial properties but slightly different glistening grown on chocolate and sheep blood agar plates were characterized morphologically by Gram’s stain, biochemically by automated instrument using Vitek 2 system using GN cards together with commercially available kit system, ID-Test HN-20 rapid panels, and genetically by sequencing the 16S rRNA genes of the organism using a taq dye-deoxy terminator Cycle sequencing and a model 3100 DNA sequencer instrument.

Results: The causative isolates from the dog-bite site were finally identified as P. canis and P. dagmatus from the findings of the morphological, cultural, and biochemical properties together with the comparative sequences of the 16S rRNA genes. Both the isolates were highly susceptible to many antibiotics and the patient was successfully treated with the administration of so-called the first generation cephalosporin, cefazolin followed by so-called the third generation cephalosporin, cefcapene pivoxil. The isolate from the dog was subsequently identified as P. canis, the same species as the isolate from the patient.

Conclusions: To the best of our knowledge, this was the second report of a dual infection with Pasteurella species consisting of P. dagmatus and P. canis resulting from a dog-bite, followed by the first report of dual infections due to P. dagmatus and P. multocida in 1988. Our isolate finally identified as P. dagmatus was misidentified as P. pneumotripta by means of the Vitek 2 system. The species name “P. dagmatus” was not included in the database of the system. It is also important for routine clinical microbiology laboratories to know the limitation of the automated Vitek 2 system for the accurate identification of Pasteurella species especially P. dagmatus. It should be emphasized that there still exists much room for improvement in Vitek 2 system. Significant improvement of Vitek 2 system especially in the identification of Pasteurella species is urgently desired.

Key words: dual wound infection, dog bite, Vitek 2 system, misidentification, Pasteurella dagmatus, Pasteurella canis, Pasteurella pneumotripta

INTRODUCTION

Pasteurella species are small, nonmotile, gram-negative, bipolar-staining facultative anaerobes present in the oropharynx of the majority of healthy dogs and cats, and are the causative agents of zoonotic infections in humans [1-7]. The frequent occurrences of infections due to Pasteurella species have been documented to date accompanied by the recent popularity of pets. Indeed, human pasteurellosis are most often caused by dog and cat bites, resulting in cellulitis and subcutaneous abscesses [8-10]. Pasteurella species are infrequently caused systemic infectious diseases and mostly strike in patients with underlying diseases. P. multocida is the most recurrent species in human in-
fections [11], but other species may be involved, such as *P. canis*, and *P. dagmatis* [7, 12]. Automated systems are generally used for the identification of *Pasteurella* isolates. However, the failure of commercial systems to satisfactorily identify microorganisms is of concern, and unusual identification should be correlated with patient's clinical pictures. We are reporting here a rare case of dual infections due both to *P. canis* and to *P. dagmatis*, focusing on the limitations of automated Vitek 2 system using GN cards (Nippon Symsmex bioMérieux, Co., Ltd., Tokyo, Japan) as well as commercially available kit system, ID-Test HN20 rapid panels (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) for exact identification of *Pasteurella* species.

**CASE REPORT**

A previously healthy 25-year-old female patient was admitted to the department of emergency and critical care in Azumino Red Cross Hospital, Azumino, 399-8292, Japan on March 3 in 2010. She complained of a severe inflammation accompanied by sensations of burning along the circumference of thumb root part in her left hand. She was bitten by her pet dog two days before, and her injured area was 15mm in length and 10mm in depth. An X-ray examination on her admission manifested that she had no fracture of the bones. Skin examination of her left hand revealed inflammation, swelling, and sharp pain without purulent discharges. No regional lymphadenopathy was noted. Two distinctive *Pasteurella* isolates were recovered as the causative agents. After treating the injured area with the gentamicin-ointment, she was initially administered for 3 days with cefazolin (1g) as intravenous administration but exhibited faint and faded or no-visible growth on modified Drigalski agar (Nippon Becton Dickinson) and on Chocolate agar (Nippon Becton Dickinson) plates. Incubation in the anaerobic chamber at 35°C for 72 hours yielded no detectable strictly anaerobic microorganisms. Although no-visible or dim growth was observed on modified Drigalski agar (Nippon Becton Dickinson) both the Sheep Blood agar (Nippon Becton Dickinson) and Chocolate agar (Nippon Becton Dickinson) plates exhibited distinctively positive growth for numerous bacterial cells, representing almost the homologous but discriminative two kinds of non-pigmented, opaque, and small to tiny colonies with a diameter of about 1.5 to 2mm, designated as strain-A and strain-B, respectively. Colonies of the strain-A grown after overnight incubation at 35°C on the Sheep Blood agar (Nippon Becton Dickinson) plates in an ambient air demonstrated to be smooth and slightly glistening and reminiscent of *Haemophilus* or *Aggregatibacter* species. On the other hand, colonies of the strain-B were grayish white and smooth in shape and resembled *Enterococcus* species.

In addition, the oral swabs and the saliva juice specimens from her pet dog were also submitted to our laboratory for bacteriological examination and successfully yielded numerous colonies designated as strain-C with almost exactly the same colonial types as those of strain-B from her injured site were cultivated on both the Sheep Blood agar (Nippon Becton Dickinson) and the Chocolate agar (Nippon Becton Dickinson) plates.

The isolates of strain-A, strain-B, and strain-C were characterized morphologically by Gram's stain, biochemically by automated instrument, Vitek 2 system using GN cards (Nippon Becton Dickinson) together with commercially available kit system, ID-Test HN20 rapid panels (Nissui Pharmaceutical), and genetically by sequencing the 16S rRNA genes of the organism [13] using a Taq DyeDeoxy Terminator Cycle Sequencing and a model 3100 DNA sequencer instrument [14].

**MICROBIOLOGICAL PROPERTIES OF THE ISOLATES.**

The causative agents of two isolates, strain-A and strain-B, from exudates of her injured area, with discriminative colonial morphology were subjected to microbiological examinations. Both the isolates displayed good growths on Sheep blood agar (Nippon Becton Dickinson) and on Chocolate agar (Nippon Becton Dickinson) plates, but exhibited faint and faded or no-visible growth on modified Drigalski agar (Nippon Becton Dickinson) plates. They exhibited facultatively anaerobic Gram-negative coccobacilli to short rod-shaped morphology, demonstrating positive catalase reactions with formation of oxygen gas bubbles after emulsifying a fresh colony in a drop of 5% H2O2 on a slide-glass, and were also oxidase positive with the paper strip (Wako Pure Chemical Industry Co., Ltd., Tokyo, Japan) method. Biochemical characterizations of the isolates were carried out with the Vitek 2 system using GN cards (Nippon Becton Dickinson), together with ID-Test HN20 rapid (Nissui Pharmaceutical) kit (Table 1) panels. Inoculated cards and kit panels were kept at 35°C in the atmosphere, and final readings were carried out according to the instructions of the manufacturers. As shown in Table 2, Vitek 2 GN cards (Nippon Symsmex bioMérieux) identified both the causative isolates as 91.3% *P. pneumotropica* for strain-A with good identification confidence level, and 99.0% *P. canis* for strain-B with excellent identification confidence level, after incubation for 8 and 7 hours, respectively. In addition, the isolate of strain-C from her pet dog was identified by the Vitek 2 GN cards (Nippon Symsmex bioMérieux) as 99.0% *P. canis* with excellent identification confidence level, after incubation for 7 hours.
Table 1. Differential biochemical characteristics of 3 Pasteurella isolates, Strain-A*, Strain-B*, and Strain-C*, obtained with ID-Test HN20 Rapid panels.

|                | Strain-A* | Strain-B* | Strain-C* |
|----------------|-----------|-----------|-----------|
|                | Pasteurella dagmatis | Pasteurella canis | Pasteurella canis |
| Acid from:     |           |           |           |
| glucose        | +         | +         | +         |
| maltose        | +         | -         | -         |
| fructose       | +         | +         | +         |
| mannose        | +         | +         | +         |
| mannitol       | -         | -         | -         |
| trehalose      | +         | -         | -         |
| sucrose        | +         | +         | +         |
| lactose        | -         | -         | -         |
| xylose         | +         | -         | -         |
| Nitrate to nitrite | +     | +         | +         |
| Catalase       | +         | +         | +         |
| Oxidase        | +         | +         | +         |
| Indole production | +     | +         | +         |
| Urease activity | +     | -         | -         |
| Ornithine decarboxylase | -     | +         | +         |
| ONPG* reaction | –        | –         | –         |

*: See text in Microbiological Properties of the Isolates for the origins and the backgrounds of respective isolate. #: ortho-nitrophenyl-β-D-galactopyranoside.

However, as shown in Table 2, ID-Test HN20 rapid panels (Nissui Pharmaceutical) conducted to the different identification results; strain-A as 100% *P. dagmatis* with the biochemical profile of 7517552, strain-B as 99.8% *P. multocida* with the biochemical profile of 7605152, and strain-C as 100% *P. multocida* with the biochemical profile of 7615552, respectively. These discrepant identification results led us to approach the accurate identification of the isolates by genetic examinations. Therefore, the 16S rRNA genes of the isolates were directly sequenced as described previously [7] using a Taq DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) and a model 3100 DNA sequencer instrument (Applied Biosystems, Foster City, CA, USA). The sequences were retrieved from the Ribosomal Database Project databases [14]. As clearly shown in Table 2, comparative sequence analyses disclosed strain-A with 100% 16S rRNA sequence similarity to that of *P. dagmatis*, strain-B with 99.8% 16S rRNA sequence similarity to that of *P. canis*, and strain-C with 100% 16S rRNA sequence similarity to that of *P. canis*, respectively. Based on the phenotypic and genetic properties, we finally identified the isolate as *P. dagmatis* for strain-A, as *P. canis* for strain-B, and as *P. canis* for strain-C, respectively.

In addition, the minimum inhibitory concentrations (MICs) determined with the Vitek 2 AST-N025 panels (Nippon bioMerieux, Co., Ltd., Tokyo, Japan.) were shown in Table 3. Three isolates of strain-A, strain-B, and strain-C were exceptionally highly susceptible to all of the antimicrobial agents provided by the cards.
Table 3. Antimicrobial susceptibility of 3 Pasteurella Isolates against 17 agents provided by the Vitek 2 GN cards.

| Antimicrobial agents | MIC(µg/ml) Category | MIC(µg/ml) Category | MIC(µg/ml) Category |
|----------------------|---------------------|---------------------|---------------------|
| Amoxicillin          | ≤ 0.25 S            | ≤ 0.25 S            | ≤ 0.25 S            |
| Clavulanic-Amoxicillin | S                  | S                   | S                   |
| Gentamicin           | ≤ 1 S               | ≤ 1 S               | ≤ 1 S               |
| Meropenem            | S                   | S                   | S                   |
| Piperacillin         | S                   | S                   | S                   |
| Sulfacetam           | ≤ 0.12 S            | ≤ 0.12 S            | ≤ 0.12 S            |
| Sulfamethoxazole     | ≤ 0.12 S            | ≤ 0.12 S            | ≤ 0.12 S            |
| Sulfamethoxazole     | ≤ 0.12 S            | ≤ 0.12 S            | ≤ 0.12 S            |
| Sulfacetam           | ≤ 0.12 S            | ≤ 0.12 S            | ≤ 0.12 S            |
| Sulfamethoxazole     | ≤ 0.12 S            | ≤ 0.12 S            | ≤ 0.12 S            |
| Sulfacetam           | ≤ 0.12 S            | ≤ 0.12 S            | ≤ 0.12 S            |
| Sulfamethoxazole     | ≤ 0.12 S            | ≤ 0.12 S            | ≤ 0.12 S            |
| Sulfacetam           | ≤ 0.12 S            | ≤ 0.12 S            | ≤ 0.12 S            |
| Sulfamethoxazole     | ≤ 0.12 S            | ≤ 0.12 S            | ≤ 0.12 S            |
| Sulfacetam           | ≤ 0.12 S            | ≤ 0.12 S            | ≤ 0.12 S            |
| Sulfamethoxazole     | ≤ 0.12 S            | ≤ 0.12 S            | ≤ 0.12 S            |
| Sulfacetam           | ≤ 0.12 S            | ≤ 0.12 S            | ≤ 0.12 S            |
| Sulfamethoxazole     | ≤ 0.12 S            | ≤ 0.12 S            | ≤ 0.12 S            |
| Sulfacetam           | ≤ 0.12 S            | ≤ 0.12 S            | ≤ 0.12 S            |
| Sulfamethoxazole     | ≤ 0.12 S            | ≤ 0.12 S            | ≤ 0.12 S            |
| Sulfacetam           | ≤ 0.12 S            | ≤ 0.12 S            | ≤ 0.12 S            |
| Sulfamethoxazole     | ≤ 0.12 S            | ≤ 0.12 S            | ≤ 0.12 S            |
| Sulfacetam           | ≤ 0.12 S            | ≤ 0.12 S            | ≤ 0.12 S            |
| Sulfamethoxazole     | ≤ 0.12 S            | ≤ 0.12 S            | ≤ 0.12 S            |
| Sulfacetam           | ≤ 0.12 S            | ≤ 0.12 S            | ≤ 0.12 S            |
| Sulfamethoxazole     | ≤ 0.12 S            | ≤ 0.12 S            | ≤ 0.12 S            |
| Sulfacetam           | ≤ 0.12 S            | ≤ 0.12 S            | ≤ 0.12 S            |
| Sulfamethoxazole     | ≤ 0.12 S            | ≤ 0.12 S            | ≤ 0.12 S            |
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