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A Case of Wound Dual Infection with *Pasteurella dagmatis* 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 DyeDeoxy 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. dagmatis* 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. dagmatis* and *P. canis* resulting from a dog-bite, followed by the first report of dual infections due to *P. dagmatis* and *P. multocida* in 1988. Our isolate finally identified as *P. dagmatis* was misidentified as *P. pneumotripica* by means of the Vitek 2 system. The species name "*P. dagmatis*" 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. dagmatis*. 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 dagmatis*, *Pasteurella canis*, *Pasteurella pneumotropica*

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 sysmex 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 drip infusion, and then switched to oral administration of cefcapene pivoxil (300mg/day) therapy for additional 5 days. Her skin inflammation, swelling, and tenderness disappeared, but she felt a slight sensation at the injured site on 9 March in 2010, and she continued the oral administration of cefcapene pivoxil (300mg/day) until her last consultation on 12 March in 2010, when she was confirmed the complete recovery.

Cultural Findings of Each Medium.

The exudates from the open wound of her dog-bite site submitted for bacteriological examination were cultivated at 35 °C for 24 hours under an ambient air on Sheep Blood agar (Nippon Becton Dickinson Co., Ltd., Tokyo, Japan.), on Chocolate agar (Nippon Becton Dickinson Co., Ltd., Tokyo, Japan.), and on modified Drigalski agar (Nippon Becton Dickinson Co., Ltd., Tokyo, Japan) 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 distinctly 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 sysmex bioMérieux) together with commercially available kit system, ID-Test HN-20 rapid panels (Nissui Pharmaceutical), and geneticall-ly 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% H_2O_2 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 sysmex bioMérieux), 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 manufactures. As shown in Table 2, Vitek 2 GN cards (Nippon Sysmex 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 sysmex 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* *Pasteurella dagmatis*	Strain-B* *Pasteurella canis*	Strain-C* *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.

Table 2. Identification results obtained with Vitek 2 GN cards and ID-Test HN20 rapid panels compared with those by 16S rRNA analyses.

	16S rRNA	Vitek 2 (GN card)	ID-test(HN-20rapid)
Strain-A*	*Pasteurella dagmatis* (559 / 559bp; Homology 100%)	*Pasteurella pneumotropica* (Probability of 91.3%, 8h) Good Identification	*Pasteurella dagmatis* Profile No. 7517552 100%
Strain-B*	*Pasteurella canis* (540 / 541bp ; Homology 99.8%)	*Pasteurella canis* (Probability 99.0%, 7h) Excellent Identification	*Pasteurella multocida* Profile No. 7605152 100%
Strain-C	*Pasteurella canis* (409 / 409bp ; Homology 100%)	*Pasteurella canis* (Probability 99.0%, 7h) Excellent Identification	*Pasteurella multocida* Profile No. 7615552 100%

*: See text in Microbiological Properties of the Isolates for the origins and the backgrounds of respective isolate.

However, as shown in Table 2, ID-Test HN20 rapid panels (Nissui Pharmaceutical) conduced to the different identification results; strain-A as 100% *P. dagmatis* with the biochemical profile of 7517552, strain-B as 100% *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 the 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 bioMérieux, 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.

	Strain-A* *Pasteurella dagmatis*		Strain-B* *Pasteurella canis*		Strain-C* *Pasteurella canis*	
Antimicrobial agents	MIC#(μg/ml)	Category	MIC#(μg/ml)	Category	MIC(#μg/ml)	Category
Ampicillin	≤ 2	S*	≤ 2	S	≤ 2	S
Sulbactam・Ampicillin	≤ 2	S	≤ 2	S	≤ 2	S
Clavulanic・Amoxicillin	≤ 2	S	≤ 2	S	≤ 2	S
Piperacillin	≤ 4	S	≤ 4	S	≤ 4	S
Cefazolin	≤ 4	S	≤ 4	S	≤ 4	S
Cefotaxime	≤ 1	S	≤ 1	S	≤ 1	S
Ceftazidime	≤ 1	S	≤ 1	S	≤ 1	S
Cefpime	≤ 1	S	≤ 1	S	≤ 1	S
Imipenem	≤ 1	S	≤ 1	S	≤ 1	S
Meropenem	≤ 0.25	S	≤ 0.2	S	≤ 0.25	S
Aztreonam	≤ 1	S	≤ 1	S	≤ 1	S
Gentamicin	≤ 1	S	≤ 1	S	≤ 1	S
Amikacin	4	S	≤ 2	S	≤ 2	S
Minocycline	≤ 1	S	≤ 1	S	≤ 1	S
Ciprofloxacin	≤ 0.25	S	≤ 0.25	S	≤ 0.25	S
Levofloxacin	≤ 0.25	S	≤ 0.251	S	≤ 0.12	S
Sulfamethoxazole・Trimethoprim	≤ 20	S	20	S	≤ 20	S

*: See text in Microbiological Properties of the Isolates for the origins and the backgrounds of respective isolate. #: Minimum inhibitory concentration ※: Susceptible

Discussion

Pasteurella species, causative agents of zoonotic infections in humans, are the inhabitants in the oropharynx of the majority of healthy dogs and cats [1-7]. Indeed, they have been isolated from 20 to 30% of dog-bite wounds and more than 50% of cat-bite wounds [15]. Most *Pasteurella* infections occur in people who have frequent contact with pet animals [7]. Among the *Pasteurella* species, recently described species of the organism, called *P. dagmatis*, has previously been known as *Pasteurella* "gas", *Pasteurella* new species 1 or *Pasteurella pneumotropica* type Henriksen that is rarely implicated in human pathology [7, 16]. However, *P. dagmatis* is often isolated simultaneously with other bacteria [17], and misidentification may have contributed to the slightly underestimated frequency of its isolation [18]. *Pasteurella* acquired from pets may cause a variety of infections, including tonsillitis, sinusitis, and epiglottiditis [19, 20]. We report a rare case of *P. dagmatis* infection together with *P. canis* resulting from a dog bite.

To the best of our knowledge, this is the sixth human case report of *P. dagmatis* isolation. The incidence of *P. dagmatis* infection has been increasing in many countries. The previously described five cases were as follows; one case was *P. dagmatis* endocarditis, occurred in a healthy man after a cat-bite [21], the second case was complicated by vertebral osteomyelitis, involved the native mitral valve of a cirrhotic woman with a known history of animal contact [22], the third case was spondylodiscitis in a diabetic patient [23], the fourth case was a septicemia due to *P. dagmatis* in a diabetic patient [12], and the fifth case was a wound infection together with *P. multocida* resulting from a cat bite [24]. The above five cases demonstrated that continuous contact with small animals such as dogs or cats might be a risk factor for transmission of *Pasteurella* species for humans. Our sixth case was also apparently associated with a dog bite.

As far as we can predict, the dual or simultaneous infectious diseases due to two different *Pasteurella* species have been documented only once in 1988, just descrived above as the fifth case [24]). That is to say, our case report caused by both *P. canis* and *P. dagmatis* was the second to be documented, to the best of our knowledge.

The three isolates of Gram-negative coccobacilli with positive catalase and oxidase reactions were finally identified as *P. dagmatis* for strain-A, *P. canis* for strain-B, and *P. canis* for strain-C, respectively. ID-Test HN20 panels correctly identified strain-A as 100% *P. dagmatis*, but misidentified both the strain-B and strain-C as 100% *P. multocida*. On the contrary, Vitek 2 GN cards (Nippon sysmex bioMérieux) misidentified the strain-A as *P. pneumotropica*, but correctly identified both the strain-B and strain-C as *P. canis*.

The species name *P. pneumotropica* in the Vitek 2 system database (Nippon sysmex bioMérieux) might be *P. dagmatis*, formerly known as *P. pneumotropica* type Henricksen. It is with this fact that the Vitek 2 system database (Nippon sysmex bioMérieux) contained only *P. multocida*, *P. canis*, *P. aerogenes*, and *P. pneumotropica* for identification, and *P. dagmatis* was not included in the database until now. Suggestions should be made to manufacturers to improve their database; however, it is also important for routine clinical microbiology laboratories to know the limitation of the commercial identification systems, such as Vitek 2 (Nippon sysmex bioMérieux) and ID-Test HN20 rapid systems (Nissui Pharmaceutical). As not every laboratory has equipped to handle molecular assays, it will certainly help clinical microbiologists to remember that differential biochemical properties of

Table 4. Differential phenotypic characteristics of *Pasteurella dagmatis* and *Pasteurella pneumotropica*.

	Pasteurella dagmatis	*Pasteurella pneumotropica*
Catalase	+	+
Oxidase	+	+
Ornithine decarboxylase	–	+
Indole production	+	+
Urease activity	+	+
ONPG# reaction	-	+

#: ortho-nitrophenyl-β-D-galactopyranoside

oxidase, catalase, ornithine decarboxylase, urease activity, and indole production as shown in Table 4, fermentation of maltose, mannose, sucrose, and glucose are given by ID-Test HN20 panels (Nissui Pharmaceutical) or Vitek 2 GN cards (Nippon sysmex bioMérieux).

In addition, although ID-Test HN20 Rapid panels (Nissui Pharmaceutical) actually contained *P. canis* in the database, strain-B and strain-C were both misidentified as *P. multocida*. These findings apparently indicated that widely used identification systems prove to be unreliable in the accurate identification of *Pasteurella* species, especially *P. dagmatis*. Therefore, the remarkably underestimated frequency of isolation of *P. dagmatis* may possibly be ascribed to the misidentification of the species.

Conflicts of interest: The authors have declared that no conflict of interest exists.

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