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First isolation of *Dysgonomonas mossii* from intestinal juice of a patient with pancreatic cancer

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Background. *Dysgonomonas* species were first designated in 2000. However, clinical infections due to this microorganism have rarely been described. Our aim was to present the first isolation of *Dysgonomonas mossii* from intestinal juice of a patient with pancreatic cancer.

Methods. Predominantly appearing grayish-white colonies grown on Chocolate and Sheep Blood agar plates were characterized by morphologically by Gram's stain, biochemically by automated instrument using Vitek II ID-GNB (Nippon bioMérieux, Co., Ltd., Tokyo, Japan.) card together with commercially available kit systems, ID-Test HN-20 (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) and API rapid ID 32A (Nippon bioMérieux, Co., Ltd., Tokyo, Japan), and genetically by sequncing the 16S rRNA gene of the organism using a Taq DyeDeoxy Terminator Cycle Sequencing and a model 3100 DNA sequencer instrument (Applied Biosystems, Foster City, CA, USA). The isolate was further characterized by antimicrobial susceptibility using MicroFast 4J (Dade Behring Co., Ltd., Tokyo, Japan) Panels, and additional biochemical and phisiological properties.

Results. The isolate was finally identified as *D. mossii* from the findings of the morphological, cultural, and biochemical properties together with the comparative sequence of the 16S rRNA genes. The isolate was highly susceptible to many antibiotics but resistant to penicillins and cephems.

Conclusions. *D. mossii*, rarely encountered in clinical microbiology laboratory, was found that misidentification as X-factor dependent *Haemophilus* species may occur because of its negative result for porphyrin test. Accumulation of the case reports with the isolation of this species is expected to elucidate the infections due to *D. mossii*. The pathogenicity of our isolate was ambiguous despite of its repeated isolations, as the patient had no conspicuous abdominal complaints. However, our report is a noteworthy and useful piece of information.

Introduction.

Dysgonomonas species were first designated in 2000 (1) as the new genus consisting of two species such as *D. gadei* and *D. capnocytophagoides*. In 2002, *D. mossii* was additionally proposed (2) in this genus.

The species belonging to the genus *Dysgonomonas* are gram-negative, facultatively anaerobic coccobacillus-shaped organisms. The genus *Dysgonomonas* constitutes a phylogenetic cluster within the *Bacteoides - Prevotella - Porphyromonas* groups. Among the 3 species in the genus *Dysgonomonas*, *D. capnocytophagoides* was reported to cause bacteremia in an immunocompromised host (3). Moreover, *D. gadei* and *D. mossii* were isolated from infected gall bladders to date (1,2), and unspeciated *Dysgonomonas* species were also recovered from stool in the immunocompromised hosts and the patients with severe underlying diseases (4,5), although their pathogenicities have not been comprehensible.

In this paper, we reported the first isolation in Japan of *D. mossii* from intestinal juice of an inpatient in our hospital.

Case report:

A 64-year-old male patient was admitted to Shinshu University Hospital on December 12 in 2003 for the surgical resection of pancreatic cancer. On January 9 in 2004, he had undergone the operation without any troubles. No clinical specimen for microbiological examination was submitted prior to the operation. After his successful operation, intestinal juice through drainage tube was submitted for bacteriological examination on January 13. On the same day, peripheral blood culture and catheterized urine samples were also submitted to our Department of laboratory Medicine for bacteriological examination, although the patient had no apparent complaints suggestive of intestinal infection such as abdominal pain, fever and/or diarrhea. Blood and urine cultures exhibited negative for bacterial growth. While the intestinal juice yielded numerous uniform growth of non-pigmented, opaque, smooth, and tiny colonies with a diameter of about 1mm, together with a small quantity of *Morganella morganii* colonies on both Sheep Blood agar plates (Nippon Becton Dickinson Co., Ltd., Tokyo, Japan.) and Chocolate agar plates (Nippon Becton Dickinson Co., Ltd., Tokyo, Japan.) but not on modified Drigalski agar plates (Nippon Becton Dickinson Co., Ltd., Tokyo, Japan). Moreover, coincident with its isolation, small quantities of *Bacteroides thetaiotaomicron* and *B. uniformis* were grown on Anaero Columbia agar plates (Nippon Becton Dickinson Co., Ltd., Tokyo, Japan.). The intestinal juice specimens were submitted repeatedly to our laboratoory, that is eleven times, for bacteriological examination and almost exactly the same quantity of *D. mossii* colonies were successfully cultivated at every time.

It should be noted, however, the values of C-reactive protein and white blood cell counts were within the normal range. In addition, other laboratory examinations in our Department of Laboratory Medicine disclosed no infection-indicative findings of intestinal infection. Hence, he discharged on March 1, without administration of any antibiotics during the period when the patient had been hospitalized.

Microbiology of the isolate.

Relatively tiny colonies were grown both on Chocolate agar and Sheep Blood agar plates in 5%CO_2 incubator, and on Anaero Columbia agar plates in an anaerobic chamber. Although the size of an appearing colony was extremely small after overnight incubation, the isolate was able to grow on Chocolate agar and Sheep Blood agar plates in an ambient air. The isolate was revealed to be facultativelly anaerobic Gram-negative coccobacilli to short-rod shaped morphology, reminiscent, at a glance, of *Haemophilus* species, as shown in Figure 1.

Biochemical characterization of the isolate was investigated with the VITEK 2 ID-GNB card (Nippon bioMérieux, Co., Ltd., Tokyo, Japan.), together with ID-Test HN20 (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) and with API rapid ID 32A (Nippon bioMérieux, Co., Ltd., Tokyo, Japan) kit systems. Inoculated cards and kit systems were kept at 35C in the atmosphere, and final readings were carried out according to the instructions of the manufactures, giving the profile number of

4737640222 for API ID32A, and the profile number of 2417571 for ID-Test HN-20. The isolate reduced nitrate to nitrite and demonstrated negative catalase reaction with no formation of oxygen gas bubbles after emulsifying a fresh colony in a drop of 5% H_2O_2 on a slide-glass, and was oxidase negative with the paper strip (Wako Pure Chemical Industry Co., Ltd., Tokyo, Japan) method. In addition, the isolate was able to grow only around the X-, and XV-discs (Eiken Chemical Co., Ltd., Tokyo, Japan.) on Heart Infusion agar plates (Eiken Chemical Co., Ltd., Tokyo, Japan.) but failed to grow around V-discs (Eiken Chemical Co., Ltd., Tokyo, Japan.), suggesting that the isolate was X-factor dependent. These phenomena were confirmed with the negative result of porphyrin test performed as described previously (6). The isolate proved to be a β-lactamase producer when examined by the chromogenic cephalosporin by Cefinase disk (Nippon Becton Dickinson Co., Ltd., Tokyo, Japan). MICs determined with MicroFast 4J (Dade Behring Co., Ltd., Tokyo, Japan) were shown in Table 1. On the basis of the morphological, cultural, and biochemical properties as shown in Table 1, we tried to identify the isolate, but in vain. Therefore, for more information to approach the accurate identification of the isolate, 16S rRNA gene of the organism was 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 sequence was retrieved from the Ribosomal Database Project databases. Comparative sequence analysis showed 99% 16S rRNA sequence similarity to that of *D. mossii*. Based on the phenotypic and genetic properties, we identified the isolate as *D. mossii*.

Discussion:

We experienced the first case of an inpatient yielding *D. mossii* from the clinical specimen. In recent years, the species belonging to the genus *Dysgonomonas* other than *D. mossii* were sometimes recovered from the stool and/or blood of the immunocompromised hosts. In fact, a case of bacteremia due to *D. capnocytophagoides* was reported in an immunocompromised host (5). In addition, *D. gadei* as well as *D. mossi* were found to be isolated from human gall bladder (1,2). In this case, abundant *D. mossii* was repeatedly cultivated from the intestinal juice of an inpatient with pancreatic cancer after the successful resection operation, although the patient demonstrated no apparent symptoms such as diarrhea and fever.

Our isolate, *D. mossii* exhibited non-motile Gram-negative coccobacilli, grew slowly on chocorate and blood agars, and formed grayish to white colonies with diameters of 1-2mm after 48 hours of incubation at 35C in CO_2 incubator. Our *D. mossii* isolate demonstrated to be X-factor dependent, thus confirming negative result in porphyrin test (6). These findings in routine clinical microbiology laboratory may lead to the misidentification of the isolate as X-factor dependent *Haemophilus* species. *D. mossii* may, however, be differentiated by means of critical observation of the appearing colonies. This was the first step to the exact identification of this species. Analytic results for the 16S rRNA sequences in combination with API ID32A system are the most favorable method to identify this species.

Intensive cares and strong chemotherapies have undoubtedly increased

immunocompromised hosts, and opportunistic pathogens will have had potentials as causative agents year by year. *D. mossii* may be one of the opportunistic pathogens, despite the fact that our case report disclosed no obvious pathogenicity of *D. mossii* and that this species may be one of the members of normal intestinal flora. Implication of this microorganism from intestinal juice is an important finding, and our rare case report in Japan indicated that clinical microbiologist should pay more attention to the isolation of this species and its possible role in various infections.

Accumulation of the case reports with the isolation of this species is inevitable for clarifying the infections due to *D. mossii*. Our case report is a noteworthy and useful piece of information.

References.

1. Hofstad T, Olsen I, Eribe ER, Falsen E, Collins MD, Lawson PA. *Dysgonomonas* gen. nov. to accommodate *Dysgonomonas gadei* sp. nov., an organism isolated from a human gall bladder, and *Dysgonomonas capnocytophagoides* (formerly CDC group DF-3). Int J Syst Evol Microbiol 2000; 50: 2189-2195.

2. Lawson PA, Falsen E, Inganas E, Weyant RS, Collins MD. *Dysgonomonas mossii* sp. nov., from human sources. Syst Appl Microbiol 2002; 25: 194-197.

3. Hansen PS, Jensen TG, Gahrn-Hansen B. *Dysgonomonas capnocytophagoides* bacteraemia in a neutropenic patient treated for acute myeloid leukaemia. APMIS 2005; 113: 229-231.

4. Blum RN, Berry CD, Phillips MG, Hamilos DL, Koneman EW. Clinical illnesses associated with isolation of dysgonic fermenter 3 from stool samples. J Clin Microbiol 1992; 30: 396-400.

5. Heiner AM, DiSario JA, Carroll K, Cohen S, Evans TG, Shigeoka AO. Dysgonic fermenter-3: a bacterium associated with diarrhea in immunocompromised hosts. Am J Gastroenterol 1992; 87: 1629-1630.

6. Kilian M. A rapid method for the differentiation of *Haemophilus* strains. The porphyrin test;. Acta Pathol Microbiol Scand [B] Microbiol Immunol 1974; 82: 835-842.

7. Neilan BA, Jacobs D, Del Dot T, Blackall LL, Hawkins PR, Cox PT, Goodman AE. rRNA sequences and evolutionary relationships among toxic and nontoxic cyanobacteria of the genus Microcystis. Int J Syst Bacteriol 1997; 47: 693-697.

Table 1.
Antimicrobial susceptibility results
of *Dysgonomonas mossii* isolate

Antimicrobial agent	MF4J* (μg/ml)
Ampicillin	>=8[#]
Cefaclor	32
Cefditoren	2
Cefepime	>=4
Cefixime	>=2
Cefotaxime	>=4
Cefotiam	>=16
Cefozopran	>=16
Ceftriaxone	>=4
Chloramphenicol	2
Clarithromycin	<=2
Clavulanic acid / Amoxicillin	<=1
Levofloxacin	>=4
Meropenem	<=0.12
Rifampicin	<=0.5
Sulbactam/Ampicillin	1
Tetracycline	<=0.5
Trimethoprim-sulfamethoxazole	0.5

*: Antimicrobial susceptibility (Dade Behring Co., Ltd., Tokyo, Japan) Panels
[#]: The number indicates the values of minimum inhibitory concentrations (MIC).

Figure 1.
Gram's stain of *D. mossii* isolate (×1,000; Olympus, Tokyo, Japan.)