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Molecular Epidemiologic Analysis of an Outbreak of *Flavobacterium odoratum* by Infrequent Restriction Site Polymerase Chain Reaction : The First Report in Korea

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= **Abstract** =

저자들은 1996년 9월부터 11월 사이에 가톨릭대학교 성가병원 신경외과 중환자실에서 다약제 내성을 보이는 *F. odoratum* 4 예가 다발성으로 분리되었음을 인지하였다. *Flavobacterium odoratum* 은 그람 음성 간균으로 이 균에 의한 임상적으로 유의한 감염증례는 전세계적으로 보고된 예가 매우 드물며 국내에서도 현재까지 보고된 예가 없었기 때문에, 이에 저자들은 국내 최초로 보고하는 바이다. 저자들은 역학적 조사를 실시함과 동시에 분자역학적 형별로서 infrequent restriction site polymerase chain reaction (IRS-PCR)을 사용하여 분자수준에서 이 균주들의 일치성 여부를 분석하였다. IRS-PCR 결과 이들 네 균주 모두 동일한 균주임을 증명할 수 있었다. 역학적 조사상 감염원을 규명하지는 못했으나 형별 성적을 토대로 볼 때, 한 균주가 여러 환자들에게 다발적으로 전파되었음을 추정할 수 있었다. *F. odoratum* 에 의한 감염질환은 발생 빈도가 드물지만 다약제 내성의 경향을 보이는 균주라는 점에서 앞으로 주요한 병원 감염균 중 하나로서 주목해야 할 대상이라고 생각한다.

Key Words : *Flavobacterium odoratum*, PCR, typing, outbreak

INTRODUCTION

From September to October 1996, we recognized unusual episodes of isolation of *Flavobacterium odoratum* from patients in the neurosurgical unit at Holy Family Hospital. As reported earlier, clinically significant cases of infection by *F. odoratum* were extremely rare[1,2] and there has been no report of an outbreak by this organism in Korea. In order to discriminate the strain-to-strain clonal relationship, we examined the genotypic pattern of these isolates by using infrequent restriction site polymerase reaction (IRS-PCR). We also performed MEDLINE search from 1966 to 1997 by using keywords "*Flavobacterium odoratum*" and also searched Korean Index Medicus.

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Table 1. Characteristics of cases and resistance profile in this study

Patient No.	Date isolated	Specimen	Underlying disease	AMK	GM	CAZ	CRO
1	'96. 9.17	urine	traumatic C-spine fracture	R	R	S	R
2	'96. 9.25	catheter tip	Intracerebral hemorrhage	R	R	R	R
3	'96.10.31	urine	brain abscess	R	R	R	R
4	'96.11. 1	urine	Intracerebral hemorrhage	R	R	R	R

R : Resistant, S : Susceptible
AMK : amikacin, GM : gentamicin, CAZ : ceftazidime
CRO : ceftriaxone

MATERIALS AND METHODS

1. Clinical setting, Patients and Surveillance

Holy Family Hospital is a community-based and 500-bed hospital in Bucheon city that serves a diverse patient population. It includes a 12-bed neurosurgical intensive care unit (NSICU). From September to November 1996, four clinical isolates of *F. odoratum* were recovered in this unit.

Characteristics of patients are summarized as in Table 1. These four patients were in the NSICU. The underlying disease of two patients were intracerebral hemorrhage (patient No. 2 & 4), one patient (No. 3) had brain abscess, the other patient (No. 1) was in a quadriplegic state due to traumatic cervical spine fracture.

All patients were under long-term catheterization of urine. Among them, one patient (No. 1) showed an overt manifestation of urinary tract infection such as fever and pyuria, while others were asymptomatic. All isolates showed resistance to nearly all antimicrobials.

Extensive surveillance cultures were performed after the outbreak of *F. odoratum* and samples were obtained from inanimate sources and health care personnels' hands.

2. Identification of Organisms

Species identification of *F. odoratum* was confirmed by growth characteristics of various agars, chemical reaction[3,4], motility test and API 20NE system (BioMérieux, France).

3. IRS-PCR

IRS-PCR was performed as previously described by Mazurek *et al.* with some modification[5]. In brief, the *HhaI* adaptor (AH) consist of a 22-base oligonucleotide (AH1 : 5'-AGA ACT GAC CTC GAC TCG CAC G-3') with a 7-base oligonucleotide (AH2 : 5'-TGC GAG T-3') annealed to bases 14 through 20 from the 5' end leaving a CG-3' overhang. AH1 and AH2 were mixed in equal molar amounts (10 pmol/μl each). They were annealed as the mixture cooled from 80 to 4℃ over 1h in a thermal cycler. The mixture was centrifuged briefly and was stored at -20℃ until use. *XbaI* adaptor (AX) which consist of a phosphorylated 18-base oligonucleotide (AX1 : 5'-PO_4-CTA GTA CTG GCA GAC TCT-3') with a 7-base oligonucleotide (AX2 : 5'-GCC AGT A-3') annealed to bases 5 through 11 from the 5' end leaving a 5'-CTAG overhang. AX1 was phosphorylated by T4-polynucleotide kinase, 3'-phosphatase free (Boehringer Mann-

Table 2. Summary of the identification profile in this study

Test	Result
Gram stain	negative rod
Motility test	immotile
Growth on oxidation-fermentation agar^{+}	no growth
Growth on MacConkey agar	no growth
Growth on blood agar	growth
DNase^{+}	positive
Catalase	positive
Oxidase	positive
Nitrate	negative
Esculin^{+}	negative
Indole^{+}	negative
Urea^{+}	positive

$^{+}$: Factors which can differentiate *F. odoratum* from other *Flavobactererium* species such as *F. meningosepticum* or *F. indologenes.*

heim, Germany) for 1 h at 37℃. AX1 and AX2 were mixed and were annealed under the same condition as that of the AH. Chromosomal DNAs of clinical isolates of *F. odoratum* were isolated by using QIAamp tissue kit (QIAGEN, Germany). The isolated DNAs were digested with *HhaI* and *XbaI* for 1 h at 37℃. Then they were ligated to AH and AX by using Rapid DNA ligation kit (Boehringer Mannheim, Germany) and were digested again with the same restriction enzymes in order to cleave any restriction sites reformed by ligation. In amplification procedure, AH1 and PX (5'-AGA GTC TGC CAG TAC TAG A-3') were used as primers. PX is complementary to AX and one base left on the 3'-end of the native DNA following *XbaI* digestion. Amplification was performed in a DNA thermal cycler (Perkin Elmer, Branchburg, N. J., USA) with an initial denaturation step at 95℃ for 5 min and then 30 cycles with denaturation at 94℃ for 1 min, annealing at 60℃, and extension at 72℃ for 1.5 min. The PCR products were separated on a polyacrylamide gel [6.5% T (total monomer concentration), 2.7 % C (crosslinker concentration)] in 0.5×TBE buffer at a constant voltage of 100 for 3 h. Then the gel was stained with ethidium bromide and photographed with UV illumination.

4. Search of Related Literature

The literature on *Flavobacterium odoratum* was reviewed through a MEDLINE search under the key words "*Flavobacterium odoratum.*" The eight articles that identified the *Flavobacterium odoratum* were included. The data collected for these groups were used in the review of literature. We also searched Korean Index Medicus in order to find any cases of *F. odoratum* reported in Korea.

RESULTS

1. Identification of Flavobacterium odoratum

After step-by-step identification procedure, we confirmed that all the causative organisms were *F. odoratum.* All strains were gram-negative rods and produced greenish-yellow colonies with a characteristically strong and fruity odor. They did not grow on the oxidation fer-

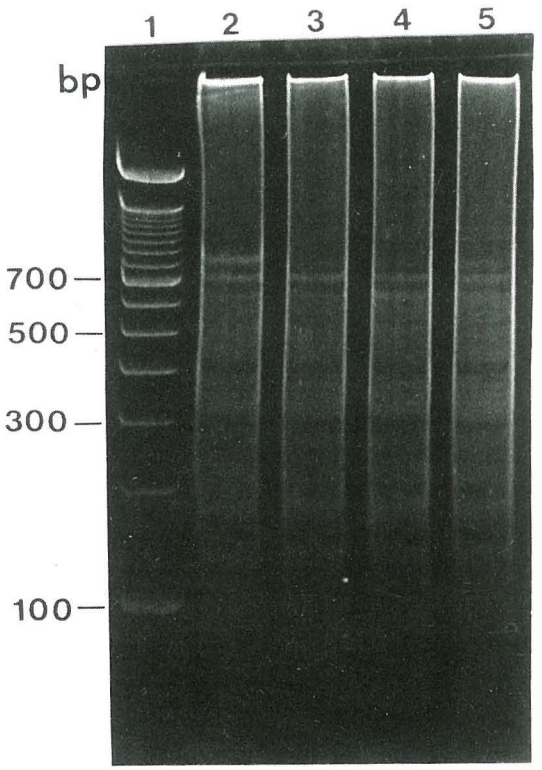

Fig. 1. Result of IRS-PCR in four clinical isolates of *F. odoratum* in this study. Lane 1:molecular weight marker, lane 2:*F. odoratum* isolated from the patient No. 1, lane 3:from patient No. 2, lane 4:from patient No. 3, lane 5:from patient No. 4. bp:basepair.

mentation agar and MacConkey agar, while they grew in the blood agar. They were immotile and were positive to DNase, catalase, and oxidase, but they were negative to nitrate and esculin. Further identification by API 20NE system (code number : 0210004) finally confirmed *F. odoratum*. The results of identification profile are summarized in Table 2.

2. Surveillance culture

Although surveillance culture was performed extensively, it revealed no further isolates from any human or environmental reservoir.

3. IRS-PCR

IRS-PCR yielded completely identical band patterns between strains (Fig. 1), which indicated that all stains were from a single clone.

4. Search of Related Literature

From 1966 to 1980, there had been twenty-five cases in the world. Among them, only five cases were clinically significant[1,6]. Since 1980, there were only three cases reported until now. These were ventriculitis[2], endocarditis[7], and necrotizing fascitis[8]. Though we searched Korean Index Medicus, we could not find any report of an outbreak by *F. odoratum* in Korea.

DISCUSSION

F. odoratum is a gram-negative, nonfermentable and immotile aerobic rod which shows multi-drug resistance to various antimicrobials [1,9]. Significant infections by this organism is rarely identified in clinical field[1,2] and there has been only a few reports in recent 30 years[1,2,6-8]. In Korea, we could not find any report of an outbreak by this organism.

As described above, we experienced four cases of isolation of *F. odoratum* in neurosurgical unit from September to November 1996. Only one patient showed symptoms of urinary tract infection. Although the organism in this patient was resistant to all antimicrobials except ceftazidime, his symptom and sign were disappeared within a few days after receiving ceftazidime and amikacin. In case of the other three patients, we could not find any significant relationship between clinical manifestation and the organisms. We thought that isolation of the organisms from the other patients should be regarded as colonization rather than clinically significant infection.

As the organisms were isolated in the same place during the brief period, we performed typing for the purpose of investigating the clonal relationship between these isolates by using IRS-PCR. IRS-PCR is a new PCR-based typing method proposed by Mazurek *et al.*[5]. The main strategy of this method is the selective amplification of DNA sequences located between a frequently occurring restriction site and an infrequent restriction site by using adaptors and primers based on these two enzymes. By using frequent- and infrequent-cutting enzyme, it produces band patterns concordant with those of pulsed field gel electrophoresis[5]. Moreover, it is less time-consuming and less laborious than any other typing scheme ever developed.

As the results of IRS-PCR indicated in Fig 1, we could think that the outbreak was a single clonal origin. Although we could not find the reservoir of infection in spite of extensive surveillance study, we thought that patient to patient trasmission could have played a role via health care personnels' hands or inanimate sources. After this epidemiologic study, we renewed all the environmental equipments in this unit and stressed personal hygiene of all the health care workers at that time. Since then, no further *F. odoratum* was isolated.

In conclusion, the outbreak of *F. odoratum* was a single-clonal origin as verified by molecular epidemiologic analysis - IRS-PCR. Though no fatality occurred in these cases, we think that we should pay attention to this organism as a newly emerging nosocomial pathogen. To our knowledge, this is the report of an outbreak of *F. odoratum* and application of IRS-PCR to this organism for the first time in Korea.

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