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First Report of *Yokenella regensburgei* Isolated from the Wound Exudate after Disarticulation Due to Diabetic Foot Infection in Korea

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Yokenella regensburgei, a member of the family *Enterobacteriaceae*, is rarely isolated in humans. Here, we report a 71-year-old man with diabetic foot infection from which *Y. regensburgei* was isolated. Following debridement and disarticulation of the foot, an exudate specimen was obtained, from which Gram-negative bacilli were recovered. The organism was identified as *Y. regensburgei* using the Vitek 2 system (bioMérieux, USA) and 16S rRNA and *gyrB* gene sequencing. To our knowledge, this is the first case of *Y. regensburgei* isolation in Korea. **(Ann Clin Microbiol 2015;18:135-139)**

Key Words: Diabetic foot, DNA sequencing, *Yokenella regensburgei*

INTRODUCTION

Yokenella regensburgei is the only species of the genus *Yokenella* in the family *Enterobacteriaceae* [1]. *Y. regensburgei* has been isolated from wounds, knee fluid, blood, respiratory tract secretions, urine, and stool [1-4]. But it is unclear if the organism has been an actual cause of infection when isolated or more of a colonizer in humans. *Y. regensburgei* has been also isolated from environment such as well water, insect intestines, and salad [1,2]. The epidemiology, clinical significance, and presentation for this organism are not well established because of the rarity of infection and the limited number of literature reports. Here, we report the first Korean case of *Y. regensburgei* isolated from the wound exudate after disarticulation due to diabetic foot infection.

CASE REPORT

A 71-year-old man was admitted to the hospital with gangrene of his left fourth and fifth toes that had persisted for two months. The patient had a medical history of diabetes mellitus and chronic kidney disease. He had undergone kidney transplant in December 2003. In 2011, edema of the face and lower limbs had gradually progressed, and the patient was diagnosed with acute kidney injury in the setting of chronic kidney disease. He was started on and has continued hemodialysis since that diagnosis. Four months prior to this admission, he was diagnosed with osteomyelitis of a left lateral malleolus lesion and had undergone three rounds of irrigation and debridement, as well as defect coverage with a thoracodorsal artery perforator free flap.

Upon admission, arteriography showed calcification and stenosis of the left common femoral artery, left anterior tibia artery, and posterior tibial artery. Angioplasty was performed to restore blood flow. Two days later, debridement and disarticulation of the metatarsophalangeal joints of the fourth and fifth toes were performed. Intravenous cefotetan was administered for antibiotic prophylaxis. Samples of necrotic tissue and exudate were collected intraoperatively and inoculated onto a blood agar plate (BAP), a MacConkey agar plate (MAC), a Brucella agar plate, and thioglyollate broth. Following incubation, greyish and

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mucoid colonies were observed and were identified as *Pseudomonas aeruginosa*, *Citrobacter freundii*, and *Enterobacter cloacae* using the Vitek 2 system (bioMérieux, Hazelwood, MO, USA). Given these results, antibiotic treatment was modified to piperacillin-tazobactam.

Following disarticulation, massive irrigation and debridement were performed daily. Tissue and exudate specimens were sampled at an interval of three days and were cultured as described above. On post-operation day 4, *P. aeruginosa* and *C. freundii* were identified and on post-operation day 7, *P. aeruginosa* and *Y. regensburgei* were identified by the Vitek 2 system (bioMérieux). *Y. regensburgei* grew in small, greyish, mucoid colonies on BAP and MAC plates and was observed as Gram-negative rods on microscopy (Fig. 1).

The strain was confirmed by 16S rRNA sequencing. DNA was extracted from colonies using the MagNa Pure LC Total Nucleic Acid Isolation Kit (Roche, Mannheim, Germany). PCR testing targeting the bacterial 16S rRNA gene was carried out using the primer pairs (forward, 4F: 5'-TTG GAG AGT TTG ATC CTG GCT C-3' and reverse, 534R: 5'-TAC CGC GGC TGC TGG CAC-3' and forward, 27F: 5'-AGA GTT TGA TCM TGG CTC AG-3' and reverse, 801R: 5'-GGC GTG GAC TTC CAG GGT ATC T-3') [5]. The amplified sequences were compared to the NCBI Blast sequence database, which revealed 100.0% (697/697 bp) identity with a *Y. regensburgei*-type strain (GenBank accession no. NR_104934.1) and 99.3% (692/697 bp) identity with an *Escherichia vulneris*-type strain (GenBank accession no. NR_114080.1). The *gyr*B gene fragments were also amplified using the primer set (forward: 5'-TAARTTYGAYGA YAACTCYTAYAAAGT-3', reverse: 5'-CMCCYTCCACCARG TAMAGTTC-3'). The amplified sequences were compared with the NCBI Blast sequence database, showing 99.6% (276/277 bp) identity with a *Y. regensburgei* (GenBank accession no. JX425-088.1) and 93.8% (303/323 bp) identity with an *Enterobacter cloacae* (GenBank accession no. AB084013.1). Based on these results, we confirmed the isolated colony as *Y. regensburgei*.

The antimicrobial susceptibility test (AST) was performed using the Vitek 2 system (bioMérieux) and showed susceptibility to piperacillin-tazobactam, cefepime, ertapenem, imipenem, amikacin, gentamicin, and trimethoprim-sulfamethoxazole. Resistance was demonstrated to ampicillin, amoxicillin-clavulanate, cefoxitin, cefotaxime, and cefuroxime. The AST results were interpreted according to CLSI M100-S25 breakpoints [6]. The AST using E-test strips (bioMérieux) was also performed and minimum inhibitory concentrations (MICs) were as follows: ampicillin ≥256 mg/L, cefotaxime ≥32 mg/dL, levofloxacin 0.064

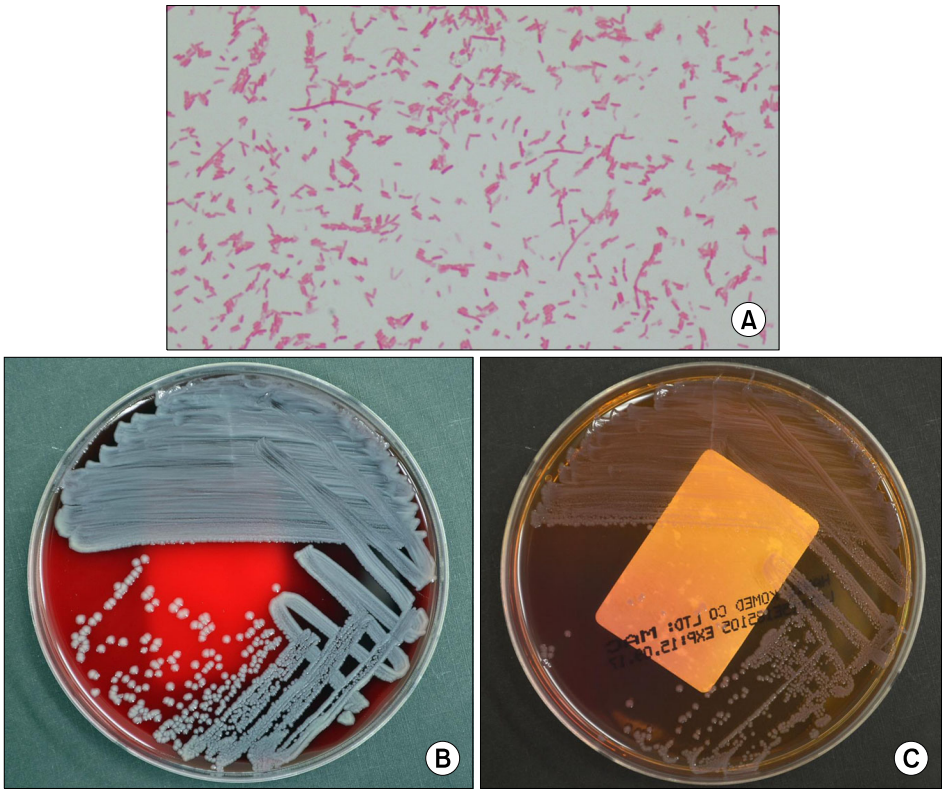

Fig. 1. (A) Gram stain microscopy of a colony grown on a blood agar plate. Gram-negative rods are shown (Gram stain, ×1,000). (B) Small, grayish, mucoid colonies on a blood agar plate, 2 days. (C) Small, colorless, mucoid colonies on a MacConkey agar plate, 2 days.

mg/dL, imipenem 0.25 mg/dL, and meropenem 0.016 mg/dL. On post-operation day 20, subsequent exudate cultures revealed *P. aeruginosas*. The patient continued antibiotic treatment with piperacillin-tazobactam. However, the necrotic lesions progressed, and the patient underwent left leg below-knee amputation.

DISCUSSION

Y. regensburgei was first identified as NIH biogroup 9 by the National Institutes of Health in Japan and as enteric group 45 by the US Centers for Disease Control and Prevention (CDC). In 1984, this new genus and species was renamed "*Yokenella regensburgei*" by Kosako et al., and the CDC proposed the name "*Kosenella trabulsii*" for enteric group 45 [1,2]. In 1991, it was recognized that *Y. regensburgei* and *K. trabulsii* were synonymous and represented the same organism [7]. The name *Y. regensburgei* had priority upon the basis of prior publication and so was retained [7].

Y. regensburgei resembles *Hafnia alvei* biochemically. Due to this similarity, it had been historically difficult to distinguish the two species using commercial systems [8]. Stock et al. [4] studied biochemical parameters to differentiate the species. Strains of *Y. regensburgei* are weakly catalase positive and cannot produce hydroxyproline amidase, tripeptidase, proline deaminase, and acid from glycerol. *Y. regensburgei* can ferment melibose and myo-inositol and is negative for the Voges-Proskauer test. A database of commercial identification systems for identification of *Y. regensburgei* is now available.

To our knowledge, only seven cases of *Y. regensburgei* isolated from clinical specimens have been reported. Report details of these cases are summarized in Table 1. A literature review of cases revealed an association of *Y. regensburgei* infection with immunocompromised condition, including alcohol abuse, chronic kidney disease, diabetes mellitus, and use of steroids or other immunosuppressive drugs. All cases except one were adult or elderly patients and were treated successfully with antibiotics. This suggests that *Y. regensburgei* infection is responsive to treatment. The source of infection and the transmission route are unclear in the documented cases.

In our case, the patient was an elderly man in an immunocompromised state with chronic kidney disease secondary to type 2 diabetes mellitus. *Y. regensburgei* was isolated from the wound exudate after disarticulation due to diabetic foot infection. For accurate identification, we confirmed *Y. regensburgei* using 16S rRNA sequencing in addition to the commercial identification system. We could not prove that *Y. regensburgei* was the pathogenic organism in this patient because *Y. regensburgei* was isolated from only one exudate specimen, and other organisms were isolated at the same time. But the possibility remains that *Y. regensburgei* was the opportunisitc pathogen, based on the details of similar case reports including four patients with diabetes mellitus and/or chronic kidney disease and four patients with lower limb infection including septic knee, perimalleolar ulcer, and cellulitis in which *Y. regensburgei* was the likely pathogenic agent.

Stock et al. [4] reported that *Y. regensburgei* was susceptible

Table 1. Reports of *Yokenella regensburgei* isolation in clinical specimens

Year	Age/Sex	Clinical diagnosis	Specimen	Underlying condition	Treatment	Outcome
1994 [3]	74/M	Septic arthritis	Knee wound	Alcohol abuse	AMK	Unknown
1994 [3]	35/F	Transient bacteremia	Blood	Alcohol abuse, liver disease, pancreatitis	CIP	Improved
2005 [9]	82/M	Perimalleolar ulcer	Wound	Chronic kidney disease, venous thrombosis	CIP (oral)	Improved
2009 [10]	77/M	Septic shock, abdominal abscess, pneumonia	Blood, abdominal aspirate, sputum	DM, esophageal adenocarcinoma, renal cancer	P/T, LVF	Transferred, no follow-up
2011 [11]	42/M	Soft tissue infection with bacteremia	Blood	DM, steroids, immunosuppressants	CTR	Improved
2013 [12]	48/M	Cellulitis	Blood, bulla aspirate	Multiple myeloma, autologous stem cell transplant, liver failure, chronic kidney disease, steroids	IPM/CS, CLI, GEN	Died
2013 [13]	5/M	Enteric fever	Blood	None	CIP	Improved
This case	71/M	Diabetic foot infection	Wound	DM, chronic kidney disease	P/T below-knee amputation	Improved

Abbreviations: DM, diabetes mellitus; AMK, amikacin; CIP, ciprofloxacin; P/T, piperacillin/tazobactam; LVF, levofloxacin; CTR, ceftriaxone; IPM/CS, mipenem/cilastatin; CLI, clindamycin; GEN, gentamicin.

to several β-lactams (e.g. piperacillin, ticarcillin, and mezlocillin), chloramphenicol, folate-pathway inhibitors (e.g., trimethoprim-sulfamethoxazole), fosfomycin, nitrofurantoin, quinolones, tetracyclines, and all tested aminoglycosides. *Y. regensburgei* demonstrated intermediate susceptibility or resistance to penicillin G, oxacillin, amoxicillin, amoxicillin-clavulanate, cefaclor, cefazoline, cefoxitin, all tested macrolides, lincosamides, streptogramins, ketolides, fusidic acid, glycopeptides, linezolid, and rifampicin [4]. *Y. regensburgei* had a similar antimicrobial susceptibility test pattern in our case.

We present the first Korean report of *Y. regensburgei* isolation confirmed by biochemical and molecular identification from wound exudate after disarticulation due to diabetic foot infection. Although *Y. regensburgei* is an uncommon pathogen in humans and is rarely isolated from clinical samples, it can be an opportunistic pathogen in immunocompromised patients. Historically, the clinical significance of *Y. regensburgei* might have been underestimated because of difficult identification using commercially available diagnostic systems. With advances in identification system, we expect the epidemiological characteristics and clinical implication of *Y. regensburgei* infection in humans to be better understood.

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=국문초록=

당뇨병성 족부감염으로 관절이단술을 받은 후 삼출액에서 분리된 *Yokenella regensburgei*

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*Yokenella regensburgei*는 장내세균 중 하나로 사람에게서 드물게 동정되는 것으로 알려져 있다. 저자들은 감염부위 삼출액에서 분리된 *Y. regensburgei* 1예를 경험하였기에 보고하는 바이다. 당뇨병성 족부감염으로 입원하여 괴사조직제거와 관절이단술을 시행받은 71세 남자의 수술부위 삼출액에서 그람음성 막대균이 분리되었다. 이 균종은 Vitek 2 (bioMérieux, USA)와 16S rRNA 및 *gyrB* 유전자 염기서열 분석에서 모두 *Y. regensburgei*로 동정되었다. 본 증례는 국내에서 *Y. regensburgei*를 동정한 첫 증례보고이다. [Ann Clin Microbiol 2015;18:135-139]

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