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Two Cases of *Kerstersia gyiorum* Isolated from Sites of Chronic Infection

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***Kerstersia gyiorum* is infrequently associated with human infection. We report the isolation of *Kerstersia gyiorum* from two patients: the first, a patient with chronic ear infections, and the second, a patient with a chronic leg wound. Both isolates were resistant to ciprofloxacin, which has not been previously reported.**

CASE REPORTS

Case 1. A 55-year-old man with a past medical history of chronic ear disease, alcoholism, and smoking (2 packs/day) was seen in the Barnes-Jewish Hospital otolaryngology clinic with a chief complaint of bilateral ear drainage. At the ages of 13 and 16, he had undergone canal wall-down mastoidectomies of the right and left ears, respectively. Since that time, he had reported some hearing loss and bilateral ear drainage. One month prior to his current encounter, the patient complained of increasing drainage from his left ear, which reportedly exhibited a reddish hue and an odor of “dead fish.” At that time, the patient was prescribed 0.3% ciprofloxacin–0.1% dexamethasone otic solution (four drops, twice daily). At a follow-up visit 1 month later, he admitted to being only partially compliant with his prescribed regimen. During the same visit, the left mastoid cavity was suctioned and cleaned and a specimen was taken from the posterior pocket at the sinodural angle and submitted for aerobic bacterial culture. The patient was instructed to continue using ciprofloxacin-dexamethasone drops and expressed that he would make an effort to be more compliant.

The direct Gram stain of the specimen submitted from the mastoid cavity showed no polymorphonuclear cells, moderate numbers of Gram-positive bacilli, and moderate numbers of Gram-negative bacilli. The culture grew abundant amounts of *Corynebacterium amycolatum*, as well as an abundant amount of a Gram-negative coccobacillus, which appeared in singles, pairs, and short chains on Gram stain (Fig. 1A). The isolate formed flat, opaque, gray colonies with spreading edges on blood (Fig. 1B) and chocolate agar, with a colony morphology somewhat resembling that of *Alcaligenes* spp. but lacking the characteristic “fruity” odor associated with this genus. On MacConkey agar, the isolate was non-lactose fermenting, but colonies had a slight lavender hue (Fig. 1C), which was especially evident when the colonies were picked up using a swab (Fig. 1D). The isolate was oxidase negative, spot indole negative, catalase positive, and nonmotile. An oxidation/fermentation (OF) glucose test was performed; the isolate was found to be a nonutilizer of glucose. Disks containing vancomycin and penicillin were added to subculture plates to obtain additional information about the isolate; there was no inhibition around the vancomycin disk, and a zone size of 16 mm was measured around the penicillin disk. A Vitek 2 Gram-negative identification (GNI) card (bioMérieux, Durham, NC) resulted in no identification. A RapID NF plus assay (Thermo Fisher Scientific, Lenexa, KS) was performed and gave a biocode of 010200, which

resulted in an identification of *Pseudomonas oryzihabitans* (64.6% confidence), *Burkholderia cepacia* (34.5% confidence), or *Acinetobacter* spp. (0.85% confidence). The isolate was subsequently analyzed by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MS) and identified as *Kerstersia gyiorum* on the BioTyper system (software version 3.0; Bruker-Daltonics, Billerica, MA). BioTyper scores of 2.3 and 2.4 (excellent identification) were obtained with and without a formic acid overlay, respectively (1). The isolate was unidentified on the Vitek MS (database version 2.0; bioMérieux). However, *K. gyiorum* is not present in the Vitek MS database. The identification of *K. gyiorum* by MALDI-TOF was confirmed by 16S rRNA gene sequencing using previously described methodology (2, 3). The sequence was 100% identical to *K. gyiorum* using the NCBI nr/nt database; the next nearest matches were *Bordetella* spp. (96% sequence homology) and *Achromobacter* spp. (95% sequence homology).

The patient was scheduled for follow-up in the otolaryngology clinic 2, 4, and 6 weeks later, but he did not present for his appointments. During a telephone consultation 6 weeks postculture, he reported continued ear drainage and was prescribed 2 weeks of trimethoprim-sulfamethoxazole based on antimicrobial susceptibility data. During a subsequent clinic visit 1 week later, the patient reported that drainage had stopped.

Case 2. A 54-year-old morbidly obese woman was admitted to the University of Iowa Hospitals and Clinics (UIHC) in July 2006 for lower leg cellulitis, for which she was treated with ceftriaxone and vancomycin with clinical improvement. Wound cultures were not collected prior to the initiation of antibiotics, and superficial swabs of the wound collected post-treatment were negative for bacterial growth. After being lost to follow-up in August 2006, the patient presented to the UIHC burn clinic in January 2013, at age 61, with a nonhealing, gradually enlarging, 10-cm ulcer on her left lower leg. During the intervening years, the wound had been managed with topical

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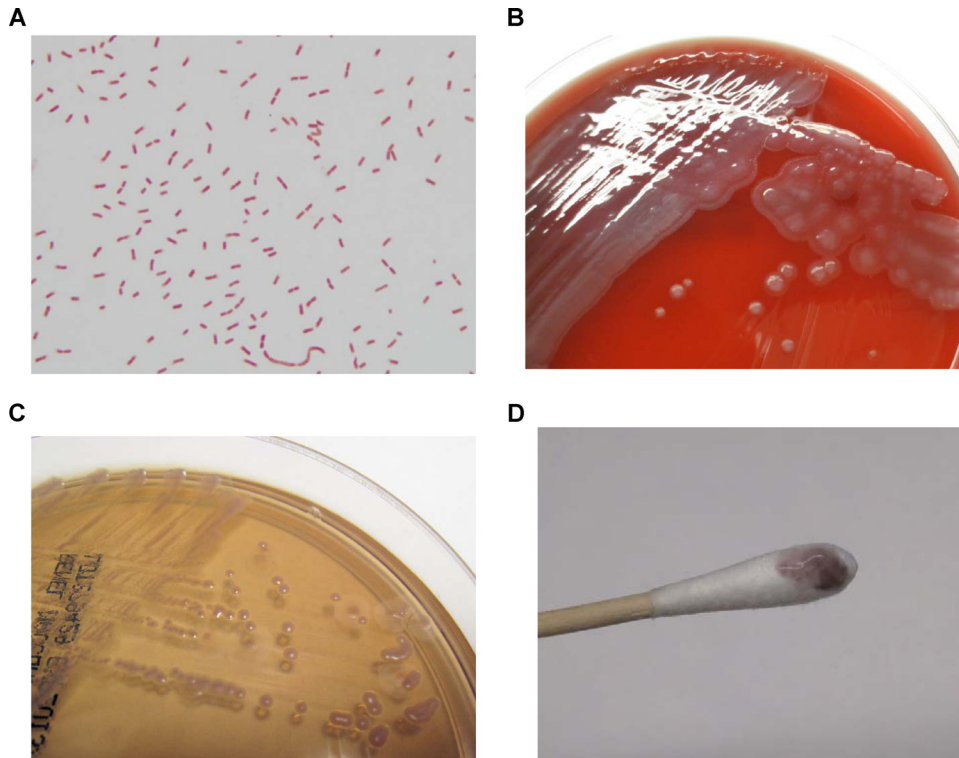


FIG 1 Phenotypic characteristics of *Kerstersia gyiorum*. (A) *K. gyiorum* appears as Gram-negative coccobacilli in singles, pairs, and short chains on Gram stain. (B) On blood agar, the *K. gyiorum* isolates appeared as gray colonies with spreading edges. (C, D) Both isolates showed a slight lavender hue on MacConkey agar (C), which was prominent when the colonies were picked up on a swab (D).

nifedipine, skin ointment, hydrocortisone, and according to the patient, a number of different types of systemic antibiotics (not further specified).

A direct Gram stain of the swab specimen revealed a few polymorphonuclear cells and moderate numbers of Gram-negative bacilli. The culture of the wound grew abundant amounts of *Morganella morganii* and a second Gram-negative rod, which formed colonies with spreading edges on blood and chocolate agar. The isolate was oxidase negative and motile, and colonies on MacConkey agar displayed a lavender pigment when picked up on a swab. An API 20NE (bioMérieux) was performed and gave a biocode of 0000053, which provided an identification of *Alcaligenes faecalis* (58% confidence) or *Acinetobacter baumannii/calcoaceticus* (37% confidence). The isolate was subsequently analyzed as previously described for non-fermenting Gram-negative bacilli (1) by MALDI-TOF MS using the Bruker MALDI BioTyper system (software version 3.1), which resulted in an identification of *Kerstersia gyiorum* with scores of 2.3 and 2.24 (excellent identification) with a formic acid overlay. As with the isolate in the first case, the identification of *K. gyiorum* by MALDI-TOF was confirmed by 16S rRNA gene sequencing; the IDNS SmartGene system (version 3.6.10; SmartGene GmbH, Lausanne, Switzerland) was used to identify the isolate as *K. gyiorum* according to the standards published in the Clinical and Laboratory Standards Institute (CLSI) document MM18-A (4). The sequence was 99.8% identical to *K. gyiorum* type strain LMG 5906 using the NCBI 16S rRNA gene database. The next-nearest matches were *Bordetella*, *Alcaligenes*, *Pusillimonas*, and *Castellaniella* spp. with 93% homology.

Susceptibility testing on this isolate was performed using the Sensititre GN3F panel (Thermo Scientific). The isolate tested as susceptible to cefepime (≤ 4 $\mu\text{g/ml}$), gentamicin (≤ 2 $\mu\text{g/ml}$), meropenem (≤ 1 $\mu\text{g/ml}$), piperacillin-tazobactam (≤ 16 $\mu\text{g/ml}$), and trimethoprim-sulfamethoxazole (≤ 0.5 $\mu\text{g/ml}$) and intermediate to ciprofloxacin (2 $\mu\text{g/ml}$). The patient was treated with 500 mg ciprofloxacin orally, twice daily for 10 days, based on case reports demonstrating susceptibility of *K. gyiorum* isolates to ciprofloxacin (5, 6). Subsequently, an Etest battery was performed on both isolates for comparison between the two institutions (7) (Table 1). Follow-up information regarding the patient's antimicrobial regimen was not available.

The novel genus *Kerstersia*, first proposed in 2003 by Coenye et al., is a member of the family *Alcaligenaceae* and is closely related to *Alcaligenes*, *Bordetella*, and *Achromobacter* spp., although it is oxidase negative, in contrast to the other genera (5). The initial publication describing this genus included nine isolates recovered from leg wounds, sputum, and feces. Because most strains had been isolated from leg wounds, *gyiorum*, meaning "from the limbs," was selected as the species name. Since that time, there have been only two additional publications describing *Kerstersia* isolated from human clinical specimens (6, 8). The first isolate (*Kerstersia similis*) was isolated from a neck abscess of a 54-year-old male, and the second (*K. gyiorum*) was isolated from a 16-year-old male with cholesteatomatous chronic otitis media. As our first patient also suffers from chronic ear disease, it is plausible that *K. gyiorum* may

TABLE 1 Antimicrobial susceptibility profiles of the two *Kerstersia gyiorum* isolates using Etest

Antimicrobial agent	Isolate 1 (St. Louis) ^a		Isolate 2 (Iowa City) ^a	
	MIC (μg/ml)	Interpretation	MIC (μg/ml)	Interpretation
Cefepime	8	Susceptible	8	Susceptible
Ciprofloxacin	>32	Resistant	4	Resistant
Gentamicin	0.5	Susceptible	1	Susceptible
Meropenem	0.125	Susceptible	0.064	Susceptible
Piperacillin-tazobactam	4	Susceptible	0.5	Susceptible
Trimethoprim-sulfamethoxazole	0.125	Susceptible	0.25	Susceptible

^a Interpretive criteria were assigned according to the Clinical and Laboratory Standards Institute (CLSI) guidelines for non-*Enterobacteriaceae* (7).

have a predilection or tropism for causing infections in patients with chronic otitis media. However, we do not know the contribution of this patient's alcoholism and/or smoking history in this infection.

The biochemical characteristics of the isolates were in accordance with those previously published: oxidase negative, catalase positive, and glucose negative (5, 6). Variability in motility was seen between the two isolates; this has also been previously reported (5). Because of the unusual nature of these isolates, it is unlikely that we would have been able to assign an identification solely using biochemical methods. However, it should be noted that the second isolate generated an API 20NE biocode identical to that of the isolate described by Almuzara et al. (6). *K. gyiorum* is not included in the databases of most commercial systems, and thus, the possibility exists that oxidase-negative organisms with the same biocode may be identifiable as *K. gyiorum*.

There are no prior descriptions of this species exhibiting a spreading edge morphology on blood and chocolate agar or the presence of lavender pigment on MacConkey agar, but based on these two cases, it seems that these phenotypes may be characteristic of *K. gyiorum*. The spreading morphology distinguishes *K. gyiorum* from *Acinetobacter* spp., which are also oxidase-negative, nonfermenting Gram-negative bacilli that are able to take up crystal violet from MacConkey agar.

In the era of molecular diagnostics in the clinical microbiology laboratory, technologies such as 16S rRNA gene sequencing and MALDI-TOF MS are important contributors to the identification of new and rare taxa from clinical specimens. Identification of these novel taxa can highlight two important questions: (i) are these isolates truly rare causes of human disease, or have they historically been unidentified and/or misidentified, and (ii) how are clinical laboratories to perform susceptibility testing on these isolates to help guide clinicians in the selection of appropriate antimicrobial therapy? As identification methods such as MALDI-TOF and 16S rRNA gene sequencing become more widely adopted in clinical laboratories, atypical taxa will continue to be identified. While more frequent identification of these isolates may help to solidify the epidemiological context for such organisms, it is unlikely that standardized CLSI criteria for antimicrobial susceptibility testing and interpretation of results will be established in the near future. Thus, the approach in the Barnes-Jewish laboratory for susceptibility testing of unusual, clinically important, nonfermenting Gram-negative bacilli has been to establish a susceptibility battery using Etest (bioMérieux) on Mueller-Hinton agar following the CLSI interpretive standards for non-*Enter-*

obacteriaceae (7). The battery consists of six different antimicrobials: cefepime, ciprofloxacin, gentamicin, meropenem, piperacillin-tazobactam, and trimethoprim-sulfamethoxazole. This method was used to determine the antimicrobial susceptibility of both *K. gyiorum* isolates, which were found to be susceptible to all antimicrobials except ciprofloxacin (Table 1). The first isolate was completely resistant by ciprofloxacin Etest, with a MIC of >32 μg/ml, while the second isolate tested as resistant, with a MIC of 4 μg/ml. Case reports of *K. gyiorum* report susceptibility to ciprofloxacin, with MICs of approximately 1 μg/ml, which is in contrast to our findings (5, 6). We speculate that lack of compliance of the first patient with his prescribed antimicrobial regimen may have selected for resistance to ciprofloxacin in this isolate.

Due to the fact that a second bacterial species was isolated in each of these two cases and because of the paucity of reports on this organism in the literature, it is difficult to know how much of the disease process can specifically be attributed to *K. gyiorum*. However, the first patient experienced progression of symptoms while on an antimicrobial agent to which the isolate was resistant; he then improved when treated with an agent to which the organism was susceptible. This information provides some evidence for the contribution of this organism to this patient's disease state.

In summary, we report two cases of *K. gyiorum* isolated from human clinical specimens, from two different medical centers, over a 2-month period. Although this organism has only previously been reported twice in the literature, this current report suggests that it may be misidentified by laboratories that rely exclusively on automated identification systems. With the emergence of MALDI-TOF MS for microbial identification from clinical specimens, it is possible that the reported frequency of isolation of this organism may increase in the future.

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