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Isolation of *Kerstersia gyiorum* from a Patient with Cholesteatomatous Chronic Otitis Media

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We describe the first case of a *Kerstersia gyiorum* strain isolated from a patient with cholesteatomatous chronic otitis media. We emphasize the isolation of members of the family *Alcaligenaceae* in serious infections and unusual sites and the importance of polyphasic identification addressing the definitive identification.

CASE REPORT

A 16-year-old male patient was admitted to the otolaryngology service at a university hospital with pain and paresthesia in the left side of the face, left eye epiphora, and difficulties in gesturing. Hospitalization for diagnosis and treatment was indicated.

The patient had a history of left acute otitis media at the age of 12 which did not respond to antibiotics. Later, he developed a retroauricular abscess that was drained and treated with antibiotics without improvement. The patient lived in overcrowded and unhealthy housing conditions (a single-room house shared with his parents and 12 siblings).

On physical examination, we found that the patient had effacement of the left nasolabial folds, lip commissure, which was diverted down, difficulty closing his left eye, a Bell positive sign, and left hemifacial numbness. Additionally, otoscopy revealed a hypertrophic tragus occluding the external auditory canal with purulent secretion, a laterocervical and retroauricular volume increase with redness and tenderness, and cervical lymphadenopathy. His body temperature was 38°C (100.4°F).

Peripheral blood laboratory findings on admission were as follows: white blood cell count, 18,000/mm^3 (with 93% neutrophils); hematocrit, 34%; hemoglobin count, 11 g/dl; and platelet count, 239,000/mm^3.

An axial temporal bone computed tomography scan revealed the presence of an expansive mass in the left petrous that is compatible with congenital cholesteatoma.

A diagnosis of complicated cholesteatomatous chronic otitis media with left peripheral facial palsy grade IV was made. Treatment with ampicillin-sulbactam at 1 g/6 h intravenously (IV) and dexamethasone at 8 mg IV every 8 h was initiated.

On the third hospital stay, a left radical mastoidectomy with tympanoplasty was made. In addition, a facial nerve decompression with Bezold's abscess drainage was performed. The purulent discharge was sent for culture.

Gram-negative coccobacilli were observed in the Gram-stained smear. After 24 h of incubation, the growth of a nonfermentative Gram-negative bacillus was obtained in pure culture. After 48 h of incubation, the colonies of the organism grew on blood agar and on nutrient agar in air atmosphere. They were white in color, smooth, and nonadherent and had entire edges. The organism was identified using standard biochemical tests (11, 13) as *Alcaligenes faecalis*-like (oxidase and acetamide negative and no fruity odor) or as an oxidase-negative *Bordetella* species and by API 20 NE (bioMérieux, Marcy l'Etoile, France) as *Alcaligenes faecalis* 1 biocode 0000053 (with 57.6% accuracy; low discrimination). The isolate was also analyzed on a Vitek 2 Compact (bioMérieux). The bionumber obtained was 0000001001500242, giving an identification of *Acinetobacter lwoffii* with 34% probability, *Pseudomonas fluorescens* with 33% probability, and *Alcaligenes faecalis* subsp. *faecalis* with 33% probability and a low-discrimination confidence level. Biochemical tests of the isolate and related microorganisms are shown in Table 1.

PCR amplification of the 16S rRNA was performed in order to identify the species. A PCR product of the 16S rRNA gene, using the primers described by Weisburg et al. (14), was obtained with the *Taq* DNA polymerase based on the manufacturer's specifications (Promega). Sequencing of the 1.4-kb PCR product was performed on both DNA strands at the Macrogen, Inc., Seoul, South Korea, sequencing facility. The sequences were analyzed using the BLAST v2.0 software (http://www.ncbi.nlm.nih.gov/BLAST/), showing 99.9% identity with the sequences corresponding to the 16S RNA ribosomal gene of *Kerstersia gyiorum* strains LMG 5906T and HF1 (GenBank accession numbers NR_025669 and HQ407220, respectively) and 98.3% identity with the 16S RNA gene of *Kerstersia similis* strain LMG 5890 (GenBank accession number AY131212). In order to obtain a more discriminatory sequence and also confirm the obtained result, we amplified the *gyrB* gene (coding for subunit beta of DNA gyrase), which has been shown to resolve phylogenetic relationships in various bacterial groups (8). A PCR product of 331 bp was obtained using the primers described by Tayeb et al. (8). Sequence analysis revealed 100% identity with the *gyrB* sequence of the *K. gyiorum* strain LMG 5906T (GenBank accession no. HE585644) and 97.9% identity with the *gyrB* sequence of the *K. similis* strain LMG 5890 (GenBank accession number HE585647). These results confirm the species identification.

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TABLE 1 Biochemical identification of the *Kerstersia gyiorum* isolate and related microorganisms

Test	Result for[a]:						
	Our isolate	*Kerstersia gyiorum*	*Kerstersia similis*	*Alcaligenes faecalis*	*Bordetella trematum*	*Bordetella ansorpii*	*Bordetella petrii*
Oxidase activity	−	−	−	+	−	−	+
Motility	+	V	−	+	+	+	−
Nitrate reduction	−	−	−	−	V	−	−
Nitrite reduction	−	−	−	+	V	ND	+
Growth on acetamide	−	−	ND	+	V	ND	ND
Trypsin	−	−	ND	V	−	ND	−
Susceptibility to desferrioxamine	S	S	ND	S	R	ND	R
Assimilation of:							
Glucose	−	−	−	−	−	−	−
Caprate	+	+	+	+	−	−	−
Adipate	−	−	V	−	+	+	+
L-Malate	+	+	+	+	+	+	+
Phenylacetate	+	+	+	V	+	+	−

[a] +, positive; −, negative; V, variable; ND, not done; S, susceptible; R, resistant. Data for *Kerstersia gyiorum*, *Alcaligenes faecalis*, *Bordetella trematum*, and *Bordetella petrii* are from references 3, 11, and 13. Data for *Kerstersia similis* are from reference 9. Data for *Bordetella ansorpii* are from reference 6.

Susceptibility to 15 antimicrobial agents was determined by the Etest technique (bioMérieux) on Mueller-Hinton agar in accordance with the manufacturer's specifications. The MIC breakpoints used in this study were those established by the Clinical and Laboratory Standards Institute (2) for other non-*Enterobacteriaceae*. The MICs for the *Kerstersia gyiorum* isolate are shown in Table 2.

With the preliminary report of nonfermenting Gram-negative bacilli with sensitivity to expanded-spectrum cephalosporins, the antibiotic therapy was changed to 2 g ceftriaxone (IV). After 3 days of treatment with this antimicrobial agent, the patient was afebrile and recovered well. He was discharged on 500 mg ciprofloxacin every 12 h orally (p.o.) and 500 mg amoxicillin-clavulanic acid every 12 h p.o.

While *Kerstersia gyiorum* was isolated from human feces, leg wounds, and sputum (3), this case represents the first documented isolation of this species from a patient with cholesteatomatous chronic otitis media complicated with mastoiditis. A closely related genus of the family *Alcaligenaceae*, *Bordetella* spp. have been implicated in ear infections. *Bordetella petrii*, described by Friedrich von Wintzingerode (12) and initially isolated from an anaerobic bioreactor, has been isolated from a patient with chronic suppurative mastoiditis and from a patient with mandibular osteomyelitis (4, 7). *Bordetella trematum*, first described in 1996 by Vandamme et al. (10), was also isolated from patients with chronic otitis media. However, there are no reports of the isolation of *Kerstersia gyiorum* in such infections in the literature. *Pseudomonas aeruginosa* has been the most frequently identified nonfermenting Gram-negative bacillus in cholesteatomatous otitis (1).

TABLE 2 Antibiotic susceptibility of the *Kerstersia gyiorum* clinical isolate

Antimicrobial agent	MIC (μg/ml)[a]
Amoxicillin	0.25
Ceftriaxone	0.5
Ceftazidime	1
Cefepime	3
Imipenem	1
Trimethoprim-sulfamethoxazole	0.047
Gentamicin	0.75
Amikacin	3
Ciprofloxacin	1
Levofloxacin	0.5
Minocycline	0.5

[a] The isolate was susceptible to all antimicrobial agents.

K. gyiorum was initially described by Coenye et al. in 2003 (3). It is part of the family *Alcaligenaceae* along with the genera *Achromobacter*, *Alcaligenes*, and *Bordetella*, among others. It phenotypically resembled *Alcaligenes faecalis*, but the latter is oxidase positive and has a fruity odor. Additionally, members of the genus *Kerstersia* are difficult to separate from other members of *Alcaligenaceae*, like *Bordetella* spp. (mainly from oxidase-negative *Bordetella* species), but in contrast to *Kerstersia* spp., *Bordetella trematum* and *Bordetella ansorpii* do not assimilate caprate but do assimilate adipate (3, 6, 10) (Table 1). Additionally, *B. trematum* presents desferrioxamine resistance (13) (Table 1).

Recently, a new species of *Kerstersia*, *Kerstersia similis*, isolated from human clinical specimens (neck abscess and leg wounds), has been described by Vandamme et al. (9). In agreement with the results obtained by these authors, the analysis of nucleotide sequences of the genes for 16S rRNA and for the DNA gyrase beta subunit (*gyrB*) allowed discrimination of both species, illustrating the usefulness of this method for species level identification (9).

It should be mentioned that failure to identify *K. gyiorum* using commercial identification systems (API 20 NE and Vitek 2 Compact) may be attributed to the fact that this organism is not found in the database of such systems.

With relation to the antibiotic sensitivity, our isolate was susceptible to aminoglycosides, to ciprofloxacin, and to broad-spectrum cephalosporins, results which are in agreement with those published by Coenye et al. (3). This antibiotic susceptibility pat-

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tern allows differentiation from another related genus, *Achromobacter* (11).

Similarly to *Bordetella* species, we may infer that *Kerstersia* may have colonized the upper respiratory tract of this patient, since the latter has been isolated from human sputum samples. In addition, *Kerstersia gyiorum* has been isolated from the gut of house flies (*Musca domestica*) (5).

Kerstersia gyiorum isolation from the infectious location mentioned represents the first case described in the literature.

We emphasize the isolation of members of the family *Alcaligenaceae* from serious infections and unusual sites and the importance of polyphasic identification addressing the definitive identification.

Nucleotide sequence accession number. The obtained sequences for the *Kerstersia gyiorum*16S rRNA and *gyrB* genes have been submitted to GenBank under accession numbers JX316030 and JX316029, respectively.

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