

ISOLATION OF RAHNELLA AQUATILIS FROM BONE AND SOFT TISSUE OF A FOOT OF A PATIENT WITH DIABETES (CASE REPORT)

DİYABETLİ BİR HASTANIN AYAĞINA AİT KEMİK VE YUMUŞAK DOKUDAN RAHNELLA AQUATILIS İZOLASYONU (OLGU SUNUMU)

Elif AKTAŞ¹, Canan KÜLAH¹, Bülent TEKEREKOĞLU², Füsun Beğendik CÖMERT¹, Zahide Doyuk BEKTAŞ¹, Eksal KARGI²

Zonguldak Karaelmas University, ¹ Faculty of Medicine, Department of Microbiology and Clinical Microbiology, ² Department of Plastic and Reconstructive Surgery, Zonguldak

İletişim / Correspondence:

Elif AKTAŞ

Zonguldak Karaelmas University, Faculty of Medicine, Department of Microbiology and Clinical Microbiology, Zonguldak
Tel: +30 372 261 02 43 / 4441 - Fax: +90 372 261 01 55
E-mail: drelifaktas@yahoo.com

SUMMARY

This is a case report on the first isolation of *Rahnella aquatilis*, a very rare enteric Gram negative rod, from bone and soft tissue of a foot of a patient with diabetes. Previous reports of isolation of *R. aquatilis* from patients are also summarized. It is concluded that clinical microbiologists must be aware of the differential characteristics of this rare microorganism, which is likely to be resistant to ampicillin and cephalothin, particularly in immunocompromised patients.

Key words: *R. aquatilis*, bone tissue, soft tissue, diabetes

ÖZET

Çok nadir rastlanan bir Gram negatif enterik çomakın diyabetli bir hastanın ayak kemik ve yumuşak dokusundan izolasyonuna dair ilk vaka bildirisidir. Hastalardan *R. aquatilis* izolasyonunu bildiren önceki yayınlar da bu bildiriye özetlenmiştir. Ampisilin ve sefalotine dirençli olan bu nadir rastlanan etkenin ayırıcı özellikleri açısından klinik mikrobiyologlar, özellikle immünkompres hastalarda, dikkatli olmalıdırlar.

Anahtar kelimeler: *R. aquatilis*, kemik dokusu, yumuşak doku, diabet

INTRODUCTION

Rahnella aquatilis, a facultative anaerobic Gram negative rod, was first described by Gavini et al. in 1976 [1] as a group of *Enterobacteriaceae* designated as H2. DNA hybridization studies conducted by Izard et al. [2] revealed that the group was a previously unknown member of the family *Enterobacteriaceae* and the name *Rahnella* was given in the honor of German-American microbiologist Otto Rahn. Today, *R. aquatilis* is considered to be made up of at least three DNA-DNA hybridization groups [3]. Though all initially described isolates were recovered from water, the organism can serve as reservoir for hu-

man infections [4]. However, it remains infrequent in human infections.

Here we report, to our knowledge, the first documented case of isolation of *R. aquatilis* from bone and soft tissue of a foot of a patient with diabetes.

CASE

A 57-year-old female referred to plastic surgery clinic with the complaint of foot wound with ulceration. The patient had type II diabetes mellitus for 20 years and physical examination revealed a diabetic foot ulceration and hyperemia around the ulcerated

site and total necrosis of the left toe extending from the first metatars to the phalanx tip. The ulceration had begun about two months before admission to the hospital and as a lesion with a diameter of 1 cm in the medial part of the metatarsal bone and it had spread to the distal sites in time. Systemic examination showed no abnormalities except left sided hemiplegia due to cerebrovascular event that developed two years ago. Significant laboratory results were as follows: leukocyte count, 11400/mm³; hemoglobin, 7.4 mg/dl; and hematocrit, 22.2% with 71% neutrophils, 18.8% lymphocytes, 8.6% monocytes, and with 404.000 platelets per mm³. She had normal electrolytes, renal and liver function, urinalysis, and electrocardiography. The patient was not given immunosuppressive or antibiotic therapy. The blood culture performed by BACTEC 9120 blood culture system (Becton Dickinson, Maryland, USA) was negative. Deep tissue and bone biopsy was performed and *R. aquatilis* was isolated from both cultures. Upon this finding, the patient was questioned again, however no history of contact with contaminated water was found.

The patient was hospitalized and intravenous ampicillin-sulbactam treatment was begun. The soft tissue culture was repeated on the third day of treatment and germ tube negative *Candida* species was isolated while the culture was negative for *R. aquatilis*. Voriconazole treatment was added to ampicillin-sulbactam treatment and continued for 10 days. On the fourth day of hospitalization, the toe and the second digit of the foot were amputated along the metatars upon the finding of extending necrosis to the other digits. The remainder tissue was closed with flat flap. The patient was discharged with oral ampicillin-sulbactam treatment.

BACTERIOLOGY

Quantitative cultures of bone and soft tissue samples were performed on blood agar and Eosin Methylene Blue agar plate. Colony count was done on blood agar plate. After 24 hours of incubation at 36°C, 1000 and 1100 CFU/g of oxidase negative Gram negative rods grew for bone and soft tissue cultures, respectively, which yielded similar biochemical reactions. The cultures were pure. The colonies were grey, smooth and nonhemolytic on blood agar plate. There was

no yellow pigment production. The microorganisms were lactose fermenting and gas production was observed in triple sugar iron medium. They yielded negative urea, indole and lysine decarboxylase reactions while citrate utilization and Voges-Proskauer reactions were positive. The organism was negative for arginine dihydrolase and ornithine decarboxylase reactions. The organisms were nonmotile at 36 °C while motile at 25°C. The identification was performed with API 20E system (bioMerieux, France). After 24 hours of incubation, the system yielded excellent identification with the identification level of 99.9% as *R. aquatilis* with the numerical code being 10055731. The isolates were concurrently identified by BD Phoenix (Becton Dickinson, USA) as *R. aquatilis* with the confidence value of 99%. The biochemical reactions and percentages of strains with a positive reaction previously reported for *R. aquatilis* [3] were similar to those for our case.

When the isolates were tested by disc diffusion method for susceptibility to several antibiotics according to CLSI criteria (formerly NCCLS) for the *Enterobacteriaceae* [5], they were found to be susceptible to amikacin, gentamicin, amoxicillin-clavulanate, ampicillin-sulbactam, levofloxacin,

Table 1. The minimal inhibitory concentrations of antibiotics tested for *Rahnella aquatilis* isolates of the present case.

Antimicrobial tested	MIC
Amikacin	<=8
Amoxicillin/clavulanate	<=4/2
Ampicillin	>16
Aztreonam	<=2
Cefazolin	>16
Cefepime	<=2
Cefoperazone/sulbactam	<=0,5/8
Cefotaxime	<= 4
Cefoxitin	<= 4
Ceftazidime	<=1
Chloramphenicol	<=4
Ciprofloxacin	<=0,5
Colistin	1
Gentamicin	<=2
Imipenem	<=1
Levofloxacin	<=1
Meropenem	<=1
Piperacillin	8
Piperacillin/tazobactam	<=4/4
Tetracycline	4
Trimethoprim/sulfamethoxazole	<=0,5/9,5

ciprofloxacin, trimethoprim-sulfamethoxazole, meropenem, cefepime, ceftazidime, ceftazidime, aztroenam, piperacillin, piperacillin-tazobactam, tetracycline and cefoperazone-sulbactam while they were resistant to ampicillin and cephalothin. The minimal inhibitory concentrations obtained by the BD Phoenix system are shown in Table 1.

The isolates were found to produce beta lactamase, the presence of which was confirmed by nitrocefin test (Becton Dickinson, USA). The two isolates were indistinguishable by pulsed-field gel electrophoresis using *Xba*I.

DISCUSSION

R. aquatilis is widely recognized in environmental samples, particularly water [4, 7]. Though infrequent, it has also been reported in literature from human samples ie. respiratory samples, burn and surgical wounds, urine, feces and blood [7-23]. The

major features of the present case and the patients and clinically significant isolates reported in the literature are reviewed and summarized in Table 2.

It is interesting to notice that most of the patients from whom *R. aquatilis* was recovered were immunocompromised [9-13], though there are few reports declaring isolation from patients without major immunosuppression [14, 21]. In our case the patient had diabetes mellitus and hypertension as the underlying diseases.

The isolation of *R. aquatilis* twice in pure culture, one from bone and one from soft tissue cultures which were obtained during the surgical operation and which were further typed as the same type by PFGE, and the response of infection to antibiotic therapy suggest clinical significance of the organism in our case. However, the actual source of *R. aquatilis* isolated from our patient is not clear. There was no obvious history of contact with contaminated water and no screening study was performed to evaluate the source in the plastic surgery department.

Table 2. Characteristics of the patients and clinically significant *Rahnelia aquatilis* isolates reported in the literature and the present case.

Publication (reference)	Age/Sex	Site of Isolation	Underlying Condition	Additional Information
Goubau et al. 1988 (10)	42 y/F	Blood	Acute lymphocytic leukemia Diabetes mellitus Bronchial asthma	Possibly related to Hickman catheter
Alballaa et al. 1992 (12)	40 y/M	Urine	Renal failure Use of immunosuppressive agents Diabetes mellitus Miliary tuberculosis	Suprapubic aspirate
Hoppe et al. 1993 (13)	7 y/M	Blood	Neuroblastoma Chemotherapy and irradiation	Possibly related to Hickman catheter
Maraki et al.1994 (14)	63 y/F	Surgical wound	Osteoporosis Fracture Internal fixation Skin necrosis	No major immunosuppression Beta lactamase production induced by ceftazidime Suspected nosocomial infection
Funke et al. 1995 (15)	21 y/M	Blood	HIV infection	Intravenous drug abuse
Matsakura et al. 1996 (17)	11 m	Blood	Atrial septal defect	Infective endocarditis No major immunosuppression
Caroff et al. 1998 (19)	31 y/F 61 y/M	Blood Blood	Ingestion of caustic agent (firstcase) Renal carcinoma (second case)	Epidemiologically related cases Suspected source: total parenteral nutrition solution
Chang et al. 1999 (21)	26 y/M	Blood	-	Source: intravenous fluid Immunocompetent patient
Carinder et al. 2001 (23)	46 y/M	Blood	Acute lymphoblastic leukemia	
Tash et al. 2005 (7)	76 y/M	Blood	Acute pyelonephritis	Suspected urinary source
Present case	57 y/F	Bone Soft tissue	Diabetes mellitus Hypertension	

y; years, m; month, M; male, F; female.

In a study by Stock et al. [24], all current isolates were indicated to be susceptible to carbapenems, trimethoprim-sulfamethoxazole and quinolones. The isolates in the present case had also similar susceptibility patterns. As concluded from the review of the literature, *Rahnella aquatilis* is mostly resistant to ampicillin and cephalothin.

In routine clinical microbiology laboratory, the microbiologists very rarely encounter *Rahnella* species and it is likely that most of them have limited information about this genus. As it is difficult to distinguish *R. aquatilis* from other *Enterobacteriaceae*, the clinical microbiologists must be aware of the differential characteristics of this rare microorganism. When an oxidase negative Gram negative rod with the features of weakly positive phenylalanine deaminase reaction, absence of yellow pigment, temperature dependent motility, growth at 4-10°C, negative lysine decarboxylase, ornithine decarboxylase and arginine dihydrolase reactions is encountered, particularly in an immunocompromised patient, presence of *R. aquatilis* should be suspected and identification with the automated systems should be performed, as most of the automated systems have recently included this genus in their databases.

REFERENCES

- Gavini F, Ferragut C, Lefebvre B et al. Taxonomic study of enterobacteria belonging or related to the genus *Enterobacter*. *Ann Microbiol* 1976; 127B:317-335
- Izard D, Gavini F, Trinel PA et al. *Rahnella aquatilis*, a new member of the *Enterobacteriaceae*. *Ann Microbiol* 1979;130:163-177
- Farmer JJ (III). *Enterobacteriaceae*: Introduction and identification. In: Murray PR, Baron EJ, Pfaller MA, Jorgensen JH, Tenover FC, Tenover FC (eds) *Manual of Clinical Microbiology*. American Society for Microbiology, Washington, DC 2003, pp 636-653.
- Abbott SL. Gram-negative enteric bacilli. In: Murray PR, Baron EJ, Pfaller MA, Jorgensen JH, Tenover FC (eds) *Manual of Clinical Microbiology*. American Society for Microbiology, Washington, DC 2003, pp 684-700.
- Clinical and Laboratory Standards Institute/NCCLS. *Performance Standards for Antimicrobial Susceptibility Testing: Fifteenth Informational Supplement*. CLSI/NCCLS document M100-S15. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2005.
- Maslow JN, Slutsky AM, Arbeit RD. Application of pulsed-field gel electrophoresis to molecular epidemiology. In: Persing HD, Smith TF, Tenover FC, White TJ (eds) *Diagnostic molecular microbiology: principles and applications*. American Society for Microbiology, Washington, DC 1993, pp 563-572.
- Tash K. *Rahnella aquatilis* bacteremia from a suspected urinary source. *J Clin Microbiol* 2005; 43(5): 2526-2528.
- Farmer JJ III, Davis BR, Hickman-Brenner FW et al. Biochemical identification of new species and biogroups of enterobacteriaceae isolated from clinical specimens. *J Clin Microbiol* 1985; 21(1): 46- 76.
- Christiaens E, Hansen W, Moinet J. Isolement des *expectorations* d'un patient atteint de leucemie lymphoide ehronique et de broncho-emphys~me d'une *Enterobacteriaceae* nouveUement d~crite: *Rahnella aquatilis*. *Med Mal Infect* 1987; 17: 732-734.
- Goubau P, Van Aelst F, Verhaegen J et al. Septicaemia caused by *Rahnella aquatilis* in an immunocompromised patient. *Eur J Clin Microbiol Infect Dis* 1988; 7(5):697-699.
- Harrell LJ, Cameron ML, O'hara CM. *Rahnella aquatilis*, an unusual gram-negative rod isolated from the bronchial washing of a patient with acquired immunodeficiency syndrome. *J Clin Microbiol* 1989; 27(7): 1671-1672.
- Alballaa SR, Qadri SMH, Al-Furayh O et al. Urinary tract infection due to *Rahnella aquatilis* in a renal transplant patient. *J Clin Microbiol* 1992; 30(11): 2948-2950.
- Hoppe JE, Merter M, Aleksic S et al. Catheter-related *Rahnella aquatilis* bacteremia in a pediatric bone marrow transplant recipient. *J Clin Microbiol* 1993; 31(7):1911-1912.
- Maraki S, Samonis G, Marnelakis E et al. Surgical wound infection caused by *Rahnella aquatilis*. *J Clin Microbiol* 1994; 32(11): 2706-2708.
- Funke G, Rosner H. *Rahnella aquatilis* bacteremia in an HIV-infected intravenous drug abuser. *Diagn Microbiol Infect Dis* 1995; 22(3):293-296.
- Oh HM, Tay L. Bacteraemia caused by *Rahnella aquatilis*: report of two cases and review. *Scand J Infect Dis* 1995; 27(1):79-80.
- Matsukura H, Katayama K, Kitano N et al. Infective endocarditis caused by an unusual gram-negative rod, *Rahnella aquatilis*. *Pediatr Cardiol* 1996;17:108-111.
- Reina J, Lopez A: Clinical and microbiological characteristics of *Rahnella aquatilis* strains isolated from children. *J Infect* 1996;33: 135-137.
- Caroff N, Chamoux C, Le Gallou F et al. Two epidemiologically related cases of *Rahnella aquatilis* bacteremia. *Eur J Clin Microbiol Infect Dis* 1998; 17 :349-352.
- O'Hara K, Chen J, Shigenobu F et al. Appearance of fosfomycin resistant *Rahnella aquatilis* clinically isolated in Japan. *Microbios* 1998; 95(381):109-115.
- Chang CL, Jeong J, Shin JH et al. *Rahnella aquatilis* sepsis in an immunocompetent adult. *J Clin Microbiol* 1999; 37(12): 4161-4162.
- Fajardo M, Bueno MJ. Isolation of *Rahnella aquatilis* in the tracheostomy exudate from a patient with laryngeal cancer. *Enferm Infecc Microbiol Clin* 2000; 18(5):251 (In Spanish).
- Carinder JE, Chua JD, Corales RB et al. *Rahnella aquatilis* bacteremia in a patient with relapsed acute lymphoblastic leukemia. *Scand J Infect Dis* 2001; 33(6):471-473.
- Stock I, Gruger T, Wiedemann B. Natural antibiotic susceptibility of *Rahnella aquatilis* and *R. aquatilis*-related strains. *J Chemother* 2000; 12(1):30-39.