Emerging Non-Fermenter Gram Negative Pathogens in Paediatric Patients: Rhizobium Radiobacter Bacteremia

Çocuk Hastalarda Fırsatçı Non-Fermentatif Gram Negatif Patojenler: Rhizobium Radiobacter Bakteremisi

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Summary

Aim: We aimed investigation of *R.radioacter* may be an opportunistic/emerging Gram negatif pathogen.

Material and Methods: *Rhizobium radiobacter (R. radiobacter)* was grown from blood cultures of six patients presenting with signs and symptoms of bacteremia in the paediatric intensive care unit at Mersin University Hospital, Mersin, Turkey, between March and November 2004.

Results: One patient had two *R. radiobacter*-positive blood culture, five patients had one. Four patients survived. All isolates were resistant to tobramycin, netilmycin, aztreonam, ceftazidime, and cotrimoxazol. All isolates had the same random amplified polymorphic DNA analysis type, indicating the presence of nosocomial spread of the organism.

Conclusion: *R. radiobacter* are normally environmental bacteria but should be recognized as an opportunistic pathogen that may cause nosocomial infection. *(J Pediatr Inf 2007; 1: 143-6)*

Key words: *Rhizobium (Agrobacterium) radiobacter,* bacteremia, paediatric intensive care unit (PICU), emerging non-fermenter Gram negative rods

Özet

Amaç: *R. radiobacter*'in fırsatçı bir Gram negatif patojen olabileceğinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: Mersin Üniversitesi Tıp Fakültesi Çocuk Hastalıkları Yoğun Bakım Ünitesinde Mart-Kasım 2004 tarihleri arasında bakteriyemi tablosu gösteren 6 çocuğun kan kültürlerinden Rhizobium radiobacter izole edilmiştir.

Bulgular: Bir hastanın 2, beş hastanın birer kan kültürü *R. radiobacter* pozitifti. İzole edilen tüm suşlar tobramisin, netilmisin, aztreonam, seftazidim, ve kotrimoksazol'e dirençli bulundu. Aynı genotipik yapıya sahip olmaları hastane kaynaklı bulaşı düşündürdü. Dört hasta komplikasyonsuz iyileştiler.

Sonuç: Her ne kadar dış ortam bakterisi olsa da *R. radiobacter* hastane infeksiyonuna yol açabilen fırsatçı patojen olarak dikkate alınmalıdır. *(Çocuk Enf Derg 2007; 1: 143-6)*

Anahtar kelimeler: *Rhizobium (Agrobacterium) radiobacter,* bakteremi, çocuk yoğun bakım ünitesi (PYBU), non-fermentatif Gram negative çomaklar

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Introduction

Human disease caused by the members of Rhizobium (formerly Agrobacterium, most widely recognized as a plant pathogen) is uncommon (1). This organism has recently been reclassified in the genus Rhizobium based on comparative 16S rRNA gene analyses (2). Since the first case of human infection with *Rhizobium radiobacter* in a patient with prosthetic aortic valve endocarditis, was reported in 1980, *R. radiobacter* has been recognized as emerging organisms affecting mostly immunocompromised and debilitated hosts (3, 4).

In this report, we describe six patients with *R. radiobacter* bacteremia in the paediatric intensive care unit (PICU) and phenotypic and genotypic characteristics of the isolates. Our's is the first report that describes an mini-outbreak with 6 paediatric patients, determined the epidemiological relationships of *R. radiobacter* isolates by PCR technique.

Patients and Methods

Over a 8-month period (between March and November 2004), 6 patients with *R. radiobacter* bacteremia who were hospitalized in the PICU we-

re identified (Table 1). Relevant information on the clinical presentation of these patients were collected: age, underlying diseases, associated medical conditions (e.g., the receipt of chemotherapy, the use of indwelling catheter, mechanical ventilation, intubation), clinical syndromes, hospitalization period, antibiotic regimens received, and outcome.

Bacterial isolates

Blood cultures were performed by using the BACTEC-9120 automated culturing system (Becton-Dickinson Diagnostic Systems, UK), and 7 blood specimens from six patients were culture positive after 48 hours of incubation. Seven isolates were initially identified on the basis of phenotypic characteristics (colonial morphology, oxidase and catalase reactions, esculin and urea hydrolisation, motility, reaction on triple sugar iron agar, growth on MacConkey agar) and then also identified by ATB ID 32 GN (Bio-Mérieux, France) as *R. radiobacter*. Genotyping was performed by arbitrarily primed polymerase chain reaction (AP-PCR) method.

Environmental specimens

Consequently, different potential sources were considered and all disinfectants used in the PICU, all equipments that contact with the distilled water (the water transport containers, the water chambers of the mechanical ventilators), and nasal and hand carriage of medical staff were subjected to microbiological culture.

Epidemiological typing

Molecular epidemiological discrimination of *R. radiobacter* isolates were done by AP-PCR.

Antimicrobial susceptibility

In vitro susceptibilities were determined using disk-diffusion method according to the National Committee for Clinical Laboratory Standarts' 2004 guidelines (5).

DNA preparation

A rapid DNA extraction procedure for the direct testing of Rhizobium isolates on Muller-Hinton agar (Oxoid, UK) was performed. A loopful of organisms was suspended in 1 ml of sterile water and bacteria were lysed by boiling for 20 min at 80°C. The cells were centrifuged (12.000 x g for 5 min) and supernatant was discharged. Pellet was mixed with 200 ml of chloroform and 200 ml of sterile water. Then mixture was centrifuged at 12.000 x g for 10 min. The supernatant was used as a template for amplification.

PCR amplification

The oligonucleotide primer, M13 (5'-GAGGGTGGCG-GTTCT-3'), was chosen from a method book of Durmaz and Ayan (6). Two ml of DNA solution were amplified in a 50 ml reaction containing 75 mM Tris-HCl pH 8.8, 0.2 mM dNTPs (Sigma, DNTP-100), 1.5 mM MgCl2 (Promega, A3513), 0.5 mM of universal primer M13, and 1 U Taq polymerase (Sigma, D1806). The PCR conditions were 2 cycles of 5 min at 94°C, 5 min at 40°C and 72°C followed by 40 cycles of 60 s at 94°C, 60 s at 40°C, and 60 s at 72°C. PCR products were separated on a 2% agarose gel.

Results

Clinical features

The clinical characteristics of 6 patients with infections are provided in table 1. *R. radiobacter* was recovered from 7 blood cultures from six PICU patients (one female, and five male, aged 6 months-11 years). All patients had some underlying conditions; one had haemotological malignancy, one had malnutrition, one had prematurity, one had undergone tonsillectomy one week before admission. All patients acquired *R. radiobacter* infections during hospital stays or via therapy-related indwelling catheters (Intravas-

Table 1. Characteristics of reported patients

Patient	Sex /Age	Clinical manifestations	Underlying conditions	Drug Indwelling Device	Period of stay in the PICU	Hospitalization period (days)	Positive cultures (no.)	Antibiotic therapy	Outcome
1	F/8 years	tonsillectomy	VUR	IV	29.3/5.4.2004	6	Blood (1)	Ampicillin-sulbactam	Cure
2	M/11 years	Febril neutropenia	ALL	Subclavian catheter, LP	20.4/28.4.2004	7	Blood (2)	Chlaritromycin Amikacin	Cure
3	M/9 years	FMF	Collageneus tissue disease	IV, LP	9.6/16.6.2004	7	Blood (1)	None	Cure
4	M/6 months	Septicemia	CVC	IV, NI, Foley, MV	17.10/21.10.2004	4	Blood (1)	Meropenem	ex
5	M/6 months	Septicemia	CVC+ Prematurity	IV, PD, MV	19.10/30.10.2004	11	Blood (1)	Ampicillin-sulbactam, Meropenem	ex
6	M/18 months	necrotize enterocolitis + malabsorption	Malnutrition	IV, NI	25.10/4.11.2004	10	Blood (1)	None	Cure

ALL, acute lymphocytic leukemia; CVC, central venous catheter; FMF, Familial Mediterranean Fever; MV, mechanical ventilation; NI, nasogastric intubation; PD, peritoneal dialysis; IV, intravenous drug perfusion; LP, lumber punction; VUR, Vesicoureteral reflux

cular and urinary catheterization, mechanical ventilation, nasogastric intubation, intravenous drug perfusion, intubation, lumbal punction). Four patients were treated with appropriate antibiotics. Four patients survived (Table 1).

Bacteriological features

Gram negative, non-fermentative, oxidase and catalase positive, hydrolised urea and esculin, indole-negative, motile bacteria had grown with mucoid colonies on 5% sheep blood Columbia agar and MacConkey agar after 48 hours of incubation, at 35-37ºC, were isolated from 7 culture-positive blood specimens from 6 patients, and two environmental specimens: (i) a ventilator humidifier, (ii) surface of the water-chamber of the ventilator. The reaction profiles generated with API ID 32 GN (bio-Merieux, France) were identical (the profile number was 77077776173, excellent identification, 99,9% id).

Antimicrobial susceptibilities

The susceptibility was done by Kirby-Bauer disk-diffusion method according to NCCLS. All isolates had the same susceptibility patterns: resistant to tobramycin, netilmycin, aztreonam, ceftazidime, cefoperazone, and cotrimoxazol; susceptible to cefepime, ceftriaxone, cefuroxim, cefoxitin, ampicillin+sulbactam, amikacin, gentamicin, tetracycline, levofloxacin, imipenem, piperacilline-tazobactam, and ciprofloxacin (Table 2).

Epidemiological features

Epidemiological typing of all *R. radiobacter* strains by AP-PCR showed the unique genotype (Figure 1, Table 1).

Discussion

Radiobacter is a Gram-negative, non-fermentative rod found in soils and is distributed world-wide (7). This organism traditionally has been associated with plant rather than human disease. Human infections due to this organism tend to occur in debilitated patients who may acquire the organism from contaminated equipment or water supplies in hospitals (8). So the isolation of Rhizobium spp. from clinical sources was considered incidental and likely indication of environmental contamination, as similar as Pseudomonas, Burkholderia, Alcaligenes, Serratia and Stenotrophomonas (9-13).

The initial reports of Rhizobium infection found no evidence implicating them in human infection. Since the first case of human infection with *Rhizobium radiobacter* in a patient with prosthetic aortic valve endocarditis, was reported in 1980, several sporadic cases have been described (15). Dune et all. (19) summarized the 13 reports, describing 19 adult cases, Amaya et all. (17) summarized 8 reports, describing paediatrics patients with CVC-associated *R. radiobacter* bacteremia, up to that time. Than Lai et all. (4) reported only one paediatric patient with ALL. Our's is the first report that describes an outbreak with 6 paediatric patients, determined the epidemiological relationships of *R. radiobacter* isolates by PCR technique. Underlying conditions presents in these patients included immunosuppression and debilitation due to chronic disease. The presence of medical devices has also been associated with Rhizobium infection, and among them four patients had such a device in place (central venous catheter, nasogastric intubation, mechanical ventilation, peritoneal catheter), and all they had intravenous drug infusion.

The most frequent sources of bloodstream infection in ICUs are long-term indwelling venous catheters, intubation/mechanical ventilation. In descending order of frequency, catheter-related bacteremias (CRB) are caused by Gram-positive, Gram-negative, or fungal organisms (14). The presence of a biomedical implant and/or immunosuppression appears to be a strong risk factor for the development of Rhizobium infection in humans. One patient had an immunosupression. Most of the reported cases were patients with *R. radiobacter* bacteremia frequently associated with the presence of an intravascular catheter

Table 2. Antimicrobial susceptibility of Rhizobium radiobacter isolates

Drug	Result
Amikacin	S
Ampicillin+Sulbactam	S
Aztreonam	R
Cephepim	S
Ceftazidime	R
Ceftriaxone	S
Ciprofloxacin	S
Cotrimoxazol	R
Gentamicin	S
Imipenem	S
Levofloxacin	S
Netilmycin	R
Piperacillin+Tazobactam	S
Tetracycline	S
Tobramycin	R

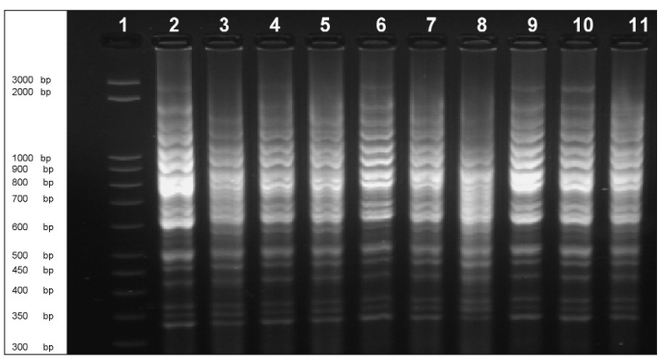

Figure 1. AP-PCR profiles obtained with the primer M13 of Rhizobium radiobacter isolates. Lane 1, Step ladder (Sigma-S 7025); 2, ventilator humidifier; 3, P1; 4, and 5, P2; 6, surface of water-chamber of ventilator; 7, P3; 8, P4; 9, P5; 10, P6; 11, wound specimen. (P: patient)

(3, 4, 8, 9, 15-19). Other reported infections due to *R. radiobacter* include endocarditis, peritonitis, urinary tract infections, endophtalmitis, cellulitis (11, 20-24).

In our study, environmental epidemiological investigation in the PICU demonstrated that distilled water used as a ventilator humidifier and the surface of water-chamber of ventilator were contaminated with *R. radiobacter*. However, all of the isolates showed the same genotype and similar antimicrobial susceptibility test results.

The present study describes *R. radiobacter* as a bloodstream pathogen. Clinical symptoms in two patients indicate genuine bloodstream infection and make pseudobacteremia highly improbable. The bacteremia persisted ending with fatal outcome despite treatment with meropenem and ampicillin/sulbactam (to which the organism were susceptible in vitro). Four patients were treated with appropriate antibiotics.

The epidemiological pattern suggest that this outbreak was hospital acquired, however environmental contamination of blood cultures with a single strain resulting in all cases of pseudobacteremia. After the sanitation of caregivers' hands and sterilization of equipment (water distillers, water transporters, water chambers of ventilators), no further cases of infection were registered in the following months. As a possible route of *R. radiobacter* transmission in the PICU, indirect transmission, presumably via medical personnel, was mainly suspected, since no medical instruments, including respiratory-therapy machines, were shared by the patients. Moreover, they stayed apart from each other within the same ward, although their hospitalization periods overlapped. We cannot exclude the possibility, however, that these two patients became infected with *R. radiobacter* from a common, but unknown hospital source. Fortunately, there was no further isolation of *R. radiobacter*.

In conclusion, though infections due to *R. radiobacter* are rare but usually occur in patients with the presence of medical devices and their removal may be essential for cure in some cases. *R. radiobacter* should be considered as an emerging potential community-acquired or hospital-acquired pathogen, with low virulence, in immunocompromised and/or debilitated patients.

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