The first case of *Raoultella terrigena* infection in an infant

Tayfur Demiray[1], Mehmet Köroğlu[1,2], Ahmet Özbek[1,2], Taner Hafizoğlu[3], Mustafa Altındiş[1,2]

[1]Clinical Microbiology Laboratory, Department of [2]Medical Microbiology, and [3]Pediatrics, Sakarya University, Faculty of Medicine, Sakarya, Turkey. E-mail: maltindis@gmail.com

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***Raoultella terrigena*, formerly known as *Klebsiella terrigena* is Gram-negative, non-motile, facultative anaerobic, encapsulated bacilli and is a very rare cause of infections in humans. Until now, only two cases of actual clinical infection caused by *R. terrigena* were reported in adults. This report is the first case of neonatal infection with this microorganism, which was isolated from the urinary tract of a premature newborn followed in Neonatal Intensive Care Unit.**

Vitek 2® automated system had identified the bacteria as *R. planticola*. The result was duplicated with a new urine sample. Although Vitek 2® automated system identified the isolates as *R. planticola*, 16S rRNA sequencing and blast analysis of the bacterium had figured out that the bacterium was *R. terrigena* with 92% identicality.

The bacterium was resistant to empirically given antibiotics, ampicillin and gentamicin. The patient was successfully treated with cephaperazone/ sulbactam according to antimicrobial susceptibility test result.

Key words: Raoultella terrigena, urinary tract infection, premature, NICU, 16S rRNA sequencing.

Raoultella terrigena, formerly known as *Klebsiella terrigena,* is a very rarely isolated bacterium from clinical specimens. When it was first described by Izard et al.[1] in 1981 as *K. terrigena,* it was thought that it had originated from the environment and was then reported as environmental microorganism. Later in 2001, according to16S rDNA and *rpoB* gene sequencing studies, *K. terrigena* was reclassified under new genus as *Raoultella* spp. as *R. terrigena* in the family Enterobacteriaciae[2].

Until now, only two clinically important infections caused by *R. terrigena* were reported. First case was a 45-year-old liver transplant recipient who developed infective endocarditis due to *R. terrigena*[3]. In the second case, *R. terrigena* caused sepsis in a 69-year-old male who underwent major surgery (*Whipple's procedure*)[4].

With this case report, we have presented a patient who had urinary tract infection with *R. terrigena*. This case is the third report in literature, and first case with urinary tract infection in a premature newborn.

Case Report

The premature baby was born to a 31-year-old gravida 3 para 3 mother at 31 weeks of gestation by caesarean section due to threatened preterm labor. Mother had no history of smoking, alcohol or drug usage. There was no history of urinary tract or vaginal infection. The newborn weighed 1260 g at birth and was admitted to Neonatal Intensive Care Unit. The Apgar scores were 8 and 9 at first and fifth minutes, respectively. On admission to NICU, the infant was mechanically ventilated. Umbilical vein catheter was inserted then, and Surfactant (4 ml/kg) was administered. Ampicillin (50 mg/kg) twice a day and gentamicin 4.5 mg/kg/day once at 36 hours was started empirically.

At second week of follow-up, the infant was spontaneously ventilated and fully fed oral-enterally. Routine urinary ultrasound was performed to the patient and Grade I ectasia was determined in both renal collector systems. Right antero-posterior (AP) diameter was measured 5 mm and left AP diameter was 14 mm. Due to Grade I ectasia, urine and

blood samples were sent to microbiology laboratory. Gram-negative bacilli were detected at gram stain of urine sample. To exclude fecal contamination, another urine sample was collected by urinary catheterization. Gram-negative bacilli was also detected at gram stain of the second sample. Both urine samples yielded *R. planticola* more than 100,000 cfu/ml. Identification and antimicrobial susceptibility tests were performed by VITEK® 2 automated System (Biomerieux, France). Since *R. planticola* was an uncommon agent, 16S rRNA sequencing with an automated capillary electrophoresis system by using ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, USA) was performed in Iontek® Laboratories, Istanbul and blast analyses were carried out in our laboratory (*http://blast.ncbi.nlm.nih.gov/Blast.cgi, access date: 14.01.2015, RID: BCXYGB6T114, Query ID: lcl|31891, Description: DT14_1925 sequence exported from R-16sRNA_F.ab1, Raoultella terrigena strain ATCC 33257 16S ribosomal RNA gene, partial sequence*). The isolate was determined as *R. terrigena* with 92% identicality.

The isolate was resistant to ampicilin, trimethoprim/sulphamethoxasole, amoxicillin/clavulanic acid, cefixime, cefuroxime, cefuroxime axetil, and gentamicin. Cephaperazone/sulbactam, cefoxitin, piperacillin/tazobactam, ertapenem, meropenem, imipenem, ciprofloxacin, fosfomycin, nitrofurantoin and amikacin were found to be susceptible. Since the bacterium was resistant to empirically given antibiotics, antimicrobial therapy was switched to cephaperazone/sulbactam (50 mg/kg/day) at treatment dose. After 10 days of treatment, with negative urine culture, the infant was discharged with nitrofurantoin prophylaxis (3 mg/kg/day). Nitrofurantoin was the only oral agent available according to the susceptibility testing results. After two days, the patient had undergone voiding cystourethrogram. There were no dilations detected, therefore prophylaxis was discontinued.

Discussion

Raoultella terrigena was first described in 1981 as *K. terrigena*. It was primarily reported as environmental bacteria, found in water and soil[1]. According to 16S rDNA and *rpoB* gene sequencing studies in 2001; it was renamed as *R. terrigena* in new *Raoultella* genus, together with *R. planticola, R.* ornithinolytica[2]. It is a Gram-negative, non-motile, oxidase-negative, facultative anaerobic, capsulated bacillus.

Until now, there are only two reported cases of *R. terrigena* infections. The first case of human infection of *R. terrigena* was detected in a 45-year-old male liver transplant patient. In that case, the patient was colonized with extended spectrum beta-lactamase (ESBL) producing *R. terrigena*. Piperacillin-tazobactam was given to patient but it was unsuccessful to eradicate the ESBL producing microorganism. After liver

Table I. Clinical and Microbiological Features of the Present, and the Previously Reported cases of *R. terrigena* Infections.

	Present case	Shaikh[4]	Goegele[3]
Patient	Premature newborn	69-year-old male	45-year-old male
Special feature	Prematurity	Surgery (Whipple's procedure)	Transplant patient
Sampling site	Urine	Blood, drain fluid	Bronchial secretions
Identification method	Vitek 2®, 16 S rRNA Sequencing	API20E, VITEK 2®, specific PCR, 16S sequencing	Not available
Susceptibility testing method	Vitek 2®	Vitek 2®	E-test, double disk
ESBL production	Negative	Negative	Positive
Treatment	Cephaperazone-sulbactam	Piperacillin-tazobactam	Piperacillin-tazobactam, meropenem, ertapenem

Vitek 2: A commercial automated bacterial identification system, API20E: A commercial manual bacterial identification system, rRNA: ribosomal ribonucleic acid, PCR: polymerase chain reaction

transplantation, ESBL producing *R. terrigena* was isolated from bronchial secretions of patient. It caused severe infective endocarditis and pneumonia. Besides the use of carbapenems, the patient died due to multi-organ failure[5]. The second case was reported as sepsis in a 69-year-old male, who underwent pancreatic resection (*Whipple's procedure*). Blood culture and drain fluid yielded *R. terrigena* and he was successfully treated with piperacillin-tazobactam[4]. Clinical and microbiological properties of the reported cases of *R. terrigena* were listed in Tables I and II.

Raoultella spp. are naturally resistant to several antimicrobial agents due to a chromosomal beta-lactamase[6]. There are very few studies about the antimicrobial susceptibilities of *R. terrigena*. In one study, Podschun examined colonization *R. terrigena* in intestinal systems of healthy individuals. In that study, out of 5377 stool samples, *R. terrigena* was isolated in only 10 samples, and all the *R. terrigena* isolates were susceptible to amoxicillin/clavulonate, gentamicin, ciprofloxacin, doxycycline, cefotaxime, and imipenem[6]. Castanheira et al.[7] stated that acquisition of antibiotic-resistant plasmid genes transfer could take place in *Raoultella spp.* as in *Klebsiella spp.* The isolate presented here, is rather more resistant compared to previously reported isolates (Table I). This might be due to transfer of resistance genes among bacteria in intensive care unit, since drug resistance is high due to vigorous usage of antibiotics.

Many putative virulence factors such as: capsular polysaccharides, type 1 fimbriae and mannose sensitive hemagglutination, ability to resist human serum and enterobactin

Table II. Antimicrobial Susceptibilies of the Reported Cases of *R. terrigena* Infections.

Antimicrobial susceptibility testing	Present case		Shaikh[4]	Goegele[3]
	Result	MIC value	Result	Result
Ampicillin	Resistant	≥32	*Not available*	*Not available*
Amoxicillin/clavulanic acid	Resistant	≥32	Susceptible	Not available
Cefixime	Resistant	≥4	*Not available*	*Not available*
Cefoxitin	Susceptible	≤4	*Not available*	*Not available*
Ceftriaxone	Resistant	≥64	*Not available*	*Not available*
Cefotaxime	*Not available*		Susceptible	Not available
Cefradine	*Not available*		Susceptible	*Not available*
Doxycycline	*Not available*		Susceptible	*Not available*
Cefuroxime	Resistant	≥64	*Not available*	*Not available*
Cefuroxime axetil	Resistant	≥64	*Not available*	Not available
Cefoperazone/sulbactam	Susceptible	≤8	*Not available*	*Not available*
Ceftazidime	Resistant	≥64	*Not available*	*Not available*
Ertapenem	Susceptible	≤0.5	*Not available*	*Not available*
Meropenem	Susceptible	≤0.25	*Not available*	Susceptible
Imipenem	Susceptible	0.5	Susceptible	Susceptible
Ciprofloxacin	Susceptible	≤0.25	Susceptible	*Not available*
Fosfomycin	Susceptible	≤16	*Not available*	*Not available*
Nitrofurantoin	Susceptible	≤16	*Not available*	Not available
Piperacillin/tazobactam	Susceptible	16	Susceptible	Susceptible
Gentamicin	Resistant	≥16	Susceptible	*Not available*
Amikacin	Susceptible	≤2	*Not available*	*Not available*
Trimethoprim/Sufamethoxazole	Resistant	≥320	*Not available*	*Not available*

MIC: minimal inhibitory concentration

production demonstrated for *K. pneumoniae* were also detected in *R. terrigena*[8]. These similarities may help to predict clinical outcome and cannot explain the rarity of *R. terrigena* infections.

Conventional tests and commercial kits are inadequate to discriminate between *Raoultella* spp. and *Klebsiella* spp.[9-11]. This may briefly explain the rarity of the infections due to this agent. It was stated that commercial kits such as API 20E® (bioMerieux, France) usually failed to correctly identify these two species[10]. For identification of *Klebsiella* and *Raoultella spp*., a combination of conventional indole and ornithine decarboxylase tests with four carbon substrate assimilation tests (ethanolamine, histamine, D-melezitose, and DL-3-hydroxybutyrate tests) has been suggested. Alternatively histamine assimilation testing after indole and ornithine decarboxylase tests to detect *Raoultella spp*. among *Klebsiella* isolates has been used[10]. However routine use of these tests is very limited due to increasing use of semi and fully automated systems. Park et al.[11] evaluated 3 commercial phenotypic systems to differentiate *Klebsiella* and *Raoultella*. VITEK 2® automatized system (Biomerieux, France) correctly identified *R. planticola* where as MicroScan Walk-Away® (Dade Behring Inc., USA) and API (Biomerieux, France) identified incorrectly as *K. pneumoniae*. However in this case, VITEK 2® determined the isolate correctly at genus level as *Raoultella* but at species level it was not *R. planticola* but *R. terrigena* as determined by 16 S rRNA Sequencing. Limitations of the conventional testing may be overcome by newly developed molecular based tests. ERIC-1R PCR and qPCR assays have been recently proposed for identification of *Raoultella spp*. (9, 12, 13). With increasing use of VITEK 2®, PCR methods for identification and newly developed laboratory tests such as matrix-assisted laser desorption/ionization-time of flight mass spectrometry, more cases of *R. terrigena* can be reported in the near future[14].

R. terrigena is a rare pathogen and has never been isolated from a newborn infection until this case. Infections with rare bacteria should always be kept in mind when dealing with premature. Every time a rare microorganism is isolated from a clinical specimen, laboratory and clinical staff should pay special attention to treatment of isolate as well as the source of microorganism, way of transmission, identification and antimicrobial susceptibility, and setting up appropriate empirical treatment. Yet, with very few number of clinically isolated *R. terrigena,* antimicrobial susceptibility of the bacteria cannot be estimated. Also, limited data cannot help to predict clinical courses of *R. terrigena* infections.

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