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CASE REPORT

Enterococcus hirae-related acute pyelonephritis and cholangitis with bacteremia: An unusual infection in humans

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Abstract Very few reports are available from the literature related to *Enterococcus hirae* infection in humans, which is more frequently seen in animals and birds. We report two patients with *E hirae* bacteremia caused by acute pyelonephritis and acute cholangitis. The clinical courses have been smooth on use of sensitive antibiotic therapy. In both cases, the primary sources and portals of entry are clearly identified.

Introduction

The genus *Enterococcus* was separated from the genus *Streptococcus* after being first described by Schleifer and Kilpper-Bälz in 1984. *Enterococcus faecalis* and *E faecium*, as other members of the genus that form part of human autochthonous microflora [1], are the most frequently encountered pathogens causing severe infections, such as urinary tract infections (UTIs) and infective endocarditis. The incidence of *E hirae* among human clinical isolates is between 1% [2] and 3% [3], which is one of the lowest for the genus *Enterococcus*. We herein report two cases of *E hirae*-related bacteremic infection. In both cases, the bacterial species were confirmed by molecular and genetic methods.

Case presentation

Case 1

The patient was a 62-year-old female senior high-school teacher who was single and lived alone in Taipei. She had

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constipation for which she occasionally took laxatives. She had never been hospitalized before and she had not been taking any antibiotic agent before admission. She had suffered a 5-day fever and chills that persisted despite having taken over-the-counter medications. Symptoms of urinary irritation developed later and she visited our hospital. At the hospital, the patient was found febrile (39.1°C). The physical examination was unremarkable but there was prominent punch tenderness over the right costovertebral angle. A urinalysis disclosed the presence of 80—90 white blood cells (WBCs) per high-power field. The urine protein and leukocyte esterase reaction were both strongly positive. A hemogram showed leukocytosis (WBC: 14,140/mL) with left shift. The patient was admitted and cultures were obtained from blood and urine. She was empirically treated with intravenous cefazolin and gentamicin first. The urine and blood culture data showed *Enterococcus* sp, which was further identified as *E hirae* with BD Phoenix ID/AST Panel Inoculation System; BD (Becton Drive), Franklin Lakes, NJ, USA. The antibiotic sensitivity profiles were identical, showing sensitivity to ampicillin. Ampicillin was then subsequently given. An abdominal sonogram showed gallbladder stones but unremarkable kidneys. Her fecal analysis was normal. As her condition improved, the patient was discharged on Day 5 of hospitalization and continued with oral amoxicillin for 7 days, totalizing a 12-day treatment. For microbiological identification, strains from blood and urine were subcultured on blood agar plates/eosin methylene blue agar and named as EH-1. The polymerase chain reaction (PCR) with *muramidase-2* (*mur-2*) gene gave a product of 521 base pairs, which was not visible on amplified *E faecium* DNA (Fig. 1A). To genetically confirm the identity of the bacteria, the amplified *mur-2* gene sequence was compared with the publicly available BLAST sequence database (http://www.ncbi.nlm.nih.gov/blast) [4], showing a 98—99% similarity. To the best of our knowledge, this is the first reported case of an *E hirae*-related acute pyelonephritis (AP) with bacteremia in humans.

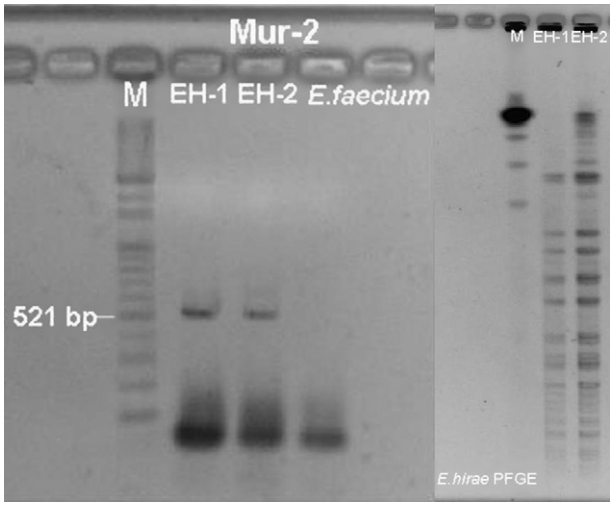

Figure 1. (A) Polymerase chain reaction of the two *Enterococcus hirae* strains (EH-1 and EH-2) together with *E faecium* using the *mur-2* primers. Amplification products of 521 base pairs can only be seen for the *E hirae* DNA but not for the *E faecium* DNA. (B) Pulsed-field gel electrophoresis of *Apa*I-digested genomic DNA from EH-1 and EH-2 showed a close kinship between the two isolates. *mur-2* = *muramidase-2*; PFGE = pulsed-field gel electrophoresis.

Case 2

The second case involves an 86-year-old woman with congestive heart failure, hypertension, valvular heart disease, parkinsonism, dementia, and a recent history of hospitalization because of *Pseudomonas aeruginosa*-related UTI. Fever recurred 3 weeks after her latest discharge. On arrival, the patient was hypotensive, febrile, tachycardiac, and tachypneic. The conjunctivae were pink but the sclera and skin were yellowish. The abdomen was soft and showed tenderness over the right upper quadrant. The hemogram showed leukocytosis (WBC 13,620/mL) with left shift. The urinalysis was normal. The laboratory examination disclosed the following: aspartate aminotransferase/alanine aminotransferase: 882/1,105 U/L, alkaline phosphatase/gamma-glutamyl transferase: 335/1,305 U/L, and total bilirubin/direct bilirubin: 5.14/3.13 mg/dL. A sonogram revealed dilations of bilateral intrahepatic ducts and common bile duct. She was provided with cefmetazole empirically under the clinical suspicion of acute cholangitis. An endoscopic retrograde cholagiopancreatography showed common bile duct stones, which were retrieved successfully after endoscopic papillotomy. On the subsequent days, her blood culture yielded *E hirae* (named as EH-2), with pertinent confirmation as with the Case 1. The serology tests were negative for hepatitis B and hepatitis C. Her condition improved and the patient was discharged on Day 16 of hospitalization. She was kept with oral antibiotic for 23 days in total.

Discussion

Enterococci are gram-positive, catalase-negative, non-spore-forming, facultative anaerobic bacteria. The *Enterococcus* genus comprises 37 different species. *E faecalis* and *E faecium* are the most important ones for their frequency of occurrence and pathogenic role. Five groups have been identified in the genus, namely the *E faecalis* sp group; the *E faecium* sp group, which include *E hirae*; the *E avium* sp group; the *E gallinarum* sp group; and the ungrouped enterococci [5]. *Enterococcus* sp are an essential part of the microflora of both humans and animals. They inhabit the alimentary tract [6], subsequently, enterococci are found mainly in the feces of healthy adults. Enterococci can also be found in vaginal and oral specimens. These bacteria were used to be considered innocuous and were used in the preparation of alimentary products, such as cheese or sausage. However, the importance of enterococcal infections has been appealing attention of epidemiologists and microbiologists. First, an unobserved rising incidence was reported recently by the Health Protection Agency of the United Kingdom in 2007. Second, the generation of drug-resistant *Enterococcus* sp has been increasing in humans.

E hirae was first described by Farrow and Collins [7] in 1985, when they found that these bacteria caused growth

Table 1 The primers used for the PCR identification of the *Enterococcus hirae* and *E faecium* isolates.

Gene	PCR product (base pairs)	Primer name	Primer sequence
mur-2	521	mur-2-F	+CGTCAGTACCCTTCTTTTGCAGAGTC
		mur-2-R	−GCATTATTACCAGTGTTAGTGGTTG
van-A	732	van-A-F	+GGGAAAACGACAATTGC
		van-A-R	−GTACAATGCGCCTTA
van-B	635	van-B-F	+ATGGGAAGCCGATAGTC
		van-B-R	−GATTTCGTTCCTCGACC

depression in young chickens. Since then, there have been numerous reports in animals and birds, but very limited cases were found in humans. The incidences of *E hirae* were reported to be around 1% (0.2—3%) of all enterococcal infections, probably underestimated because of inadequate identification [2,3,8,9]. Interestingly, *E hirae* has a higher isolation rate in particular regions of Europe, such as Sweden and Denmark [10].

Enterococcal infections include UTI, hepatobiliary infection, endocarditis, surgical wound infection, bacteremia, and neonatal sepsis [11]. *E hirae* has been reported to cause wound infection [12] and gastritis [13] in humans. There have been only three cases of *E hirae* bacteremic infection reported in the literature. These were found in patients with end-stage renal disease [14], native valve endocarditis [15], and spondylodiscitis [16]. The first case was reported in 1998 when a 49-year-old male undergoing renal replacement therapy was found to have a positive blood culture. The second case was a 72-year-old male with aortic valve endocarditis-related bacteremia, which required surgical intervention after a failed antibiotic treatment course. The third case was a 55-year-old diabetic patient with spondylodiscitis. A culture of the surgical specimen showed *E hirae*. The actual portals of entry for *E hirae* remained unclear in these patients.

AP refers to an upper UTI with the involvement of renal parenchyma and renal pelvis. AP has a wide spectrum of clinical presentations, ranging from mild disease to fatal septicemia. The diagnosis of AP is clinical, based on evidence of UTI from urinary analysis and culture, along with signs and symptoms suggesting upper UTI, such as fever, chills, flank pain, nausea, vomiting, and costovertebral angle tenderness [17,18]. Enterococci are important causative agents of nosocomial UTI. When endocarditis is not present, the urinary tract represents the most frequent sources of enterococcal bacteremia. So far, there have been no reports of *E hirae*-related AP or biliary tract infection in the literature.

The predisposing risk factors for enterococcal bacteremic infection have been reported to previous antibiotic treatment, diabetes mellitus, neurological conditions, heart disease, renal disease, pressure sores, and malignancy. Medical instrumentations, such as urinary catheterization, vascular access, endoscocopy, mechanical ventilation, and surgical operation, were found to be associated with enterococcal infections as well. In biliary tract infection, bacteria gain access to the biliary tree from the duodenum or from portal venous blood. Any conditions that promote bile stasis, for example cholelithiasis, or any damage occurring to intestinal barrier may predispose to cholangitis. There is no bile culture to demonstrate the presence of *E hirae* from the second patient. However, in view of patient's clinical presentations, cholangitis still remains the most probable original site of *E hirae* bacteremia.

Because of phenotypic and biochemical similarities, many genetic diagnostic methods have been developed to specifically identify the different components of the Enterococcus genus. Most of them were to differentiate between *E faecalis* and *E faecium*. The *mur-2* gene codes for a 74-kDa peptidoglycan hydrolase that plays a role in the cell wall growth and division [19]. The gene was cloned, sequenced, and used to specifically identify *E hirae* from other enterococcus. We successfully identified EH-1 and EH-2 with *mur-2*. The similar restriction pattern obtained from pulsed-field gel electrophoresis with restriction enzyme *Apa*I hints for genomic similarity and relatedness between them (Fig. 1B). The primers used for the PCR identification of *E hirae* and *E faecium* are listed in Table 1.

Independent of the virulence and the pathogenicity, an important feature of *Enterococcus* sp resides in their acquisition of drug resistance, especially to glycopeptide agents, such as vancomycin or teicoplanin. Robredo et al.

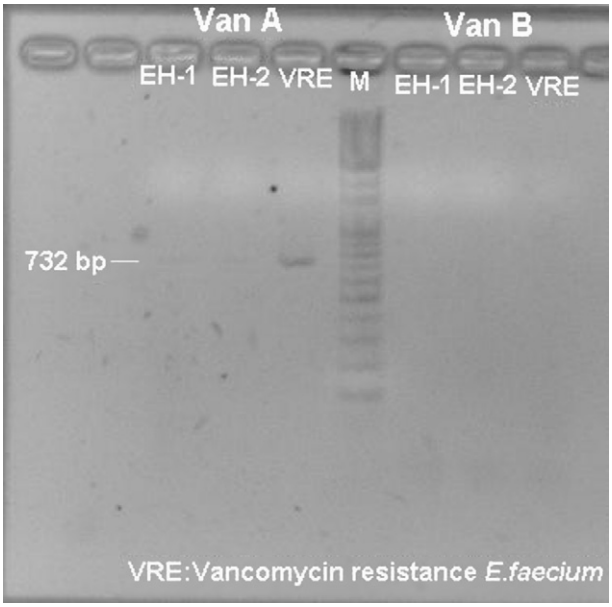

Figure 2. Polymerase chain reaction of EH-1, EH2, and vancomycin-resistant enterococcus (VRE). The products of 732 base pairs was visible only in VRE, corroborating with the antibiotic sensitivity test indicating that the isolated EH-1 and EH-2 are not intrinsically vancomycin resistant. The VRE used are *E faecium* carrying *vanA* only.

[20] had demonstrated that *E hirae* may be a significant source of vancomycin resistant gene, *vanA*, and may carry the potential to spread the gene to other enterococcal species that infect humans or animals. In the first case, the antibiotic sensitivity profiles were identical for the bacteria got from blood and urine. Meanwhile, the antibiotic sensitivity profiles between EH-1 and EH-2 were very similar: sensitive to teicoplanin, vancomycin, and high-dose gentamicin but resistant to oxacillin and gentamicin. To look inside if the isolated *E hirae* are intrinsically vancomycin resistant, a PCR was performed among EH-1, EH-2, and vancomycin-resistant enterococcus. The vancomycin-resistant enterococcus used is a *vanA* gene carrying *E faecium*. Neither EH-1 nor EH-2 carries the resistant gene, corroborating the antibiotic sensitivity test (Fig. 2).

In summary, our cases constitute the first descriptions of *E hirae*-related human diseases, specifically AP and acute cholangitis, both of which are complicated with bacteremia. The clinical courses have been smooth on use of sensitive antibiotic therapy. These two cases further expand the spectrum of diseases caused by *Enterococcus* sp. More importantly, they are the first reports of *E hirae*-related infections in which the primary sources of infection and portals of entry can be clearly identified.

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