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Case Report

Misidentification of *Streptococcus uberis* as a Human Pathogen: A Case Report and Literature Review

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SUMMARY

Streptococcus uberis is an environmental bacterium responsible for bovine mastitis. It is occasionally described as a human pathogen, though in most cases the identification was based on biochemical phenotyping techniques. This report shows that the biochemical phenotyping may incorrectly identify *Enterococcus faecium* as *S. uberis*.

1. Introduction

Streptococcus uberis is an environmental Gram-positive bacterium belonging to the *Streptococcaceae* family. It is responsible for a high percentage of mastitis in dairy cattle and it is rarely associated with human infections.[1]

In contrast to *S. uberis, E. faecium* has emerged as an important nosocomial pathogen, and the treatment of the infections caused by this bacterium poses a critical challenge due to the ability of *Enterococci* to acquire antibiotic resistance.[2] Therefore, early detection of *E. faecium* is crucial to prevent nosocomial transmission of this pathogen and to provide the appropriate treatment to the patients, particularly when it shows a broad range of antibiotic resistance in patients with impaired host defence, such as those with cancer.

In this report we demonstrate that automated biochemical phenotyping may erroneously identify *E. faecium* as *S. uberis*. Moreover, a review of the medical literature showed that the previously reported cases of *S. uberis* infections were based on biochemical identification, thus raising serious concern about the real occurrence of *S. uberis* in humans. Our data strongly suggest that the presence of this bacterium must always be supported by more specific identification procedures such as genotypic methods.[1,3]

2. Case Report

In January 2014 a 67-year-old man, presenting with abdominal pain, fever, leucocytosis and abdominal soft tissue infection was admitted to our Institution. The past medical history included hypertension and left colic resection with splenectomy for bowel obstruction due to colonic cancer with synchronous hepatic metastasis (T3N0M1).

On admission, computed tomography scan revealed necrosis of the right lobe lesion in the liver and gallbladder perforation caused

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by ischemic injury with biliary leak. A percutaneous drainage was performed and the microbiological assessment indicated the presence of gram-positive, β-Glucuronidase positive cocci, identified as *Streptococcus uberis* with a 95% probability by the VITEK 2 system (BioMérieux Inc.). According to the microbiological results and the antibiotic susceptibility profiles (Table 1), the first empirical antibiotic treatment with tazobactam/piperacillina and metronidazole was substituted with vancomycin and the patient was subjected to parenteral nutrition. Since *S. uberis* is a pathogen mainly associated with mastitis in dairy cows, this unusual bacterial identification compelled the microbiologists to repeat the test for confirmation. The second round of analysis by the VITEK 2 system confirmed the previous identification of *S. uberis*. However, a few days later, microbiological testing by VITEK 2 of newly collected samples yielded gram-positive, β-Glucuronidase negative cocci, identified as *Enterococcus faecium* with a 95% certainty. Surprisingly, further testing, performed three days later, showed again the presence of *S. uberis* with a 95% identification confidence.

No additional microorganisms were isolated. Thus, to obtain a clear bacterial identification, the samples were first analysed by matrix assisted laser desorption/ionisation – time of flight mass spectrometry (MALDI-TOF MS system – Bruker Daltonics, Bremen, Germany), which classified the bacterium as *E. faecium* and subsequently, sequence analysis (ABI PRISM 3130xl Genetic Analyzer) of the 16S rRNA gene confirmed that the bacterium was *E. faecium* (99,4% sequence similarity and 100% sequence coverage).

As the clinical conditions did not improve, the patient underwent a resection of hepatic residual metastases with cholecystectomy and abdominal surgical toilette. The postoperative course was complicated by a low-grade hepatic failure that required intensive care for five days.

The patient was then discharged in good clinical condition with abdominal drainage for a moderate biliary leakage and conservative treatment.

3. Discussion

Infections caused by *Enterococci* are continuously increasing, representing one of the leading causes of nosocomial infections, mostly affecting the the bloodstream, urinary tract, perineal skin and surgical wounds. An important cause for the increased frequency of *E. faecium* as a nosocomial pathogen is the capacity to acquire novel adaptive traits, including genetic elements encoding antibiotic resistance determinants.[2]

In routine clinical microbiology, the detection of *Enterococci* is generally performed by biochemical characterization. However, this procedure has often failed the identification and antimicrobial susceptibility profiling of enterococci.[4,5] A commercial biochemical kit used for the detection of non-human enterococcal isolates showed inconsistencies in genus and species identification.[6] A study conducted by Ligozzi et al. documented the inability of VITEK 2 to correctly identify *E. faecium* (71,4% correct).[7] Garcia-Garrote et al. reported a rate of accuracy in *E. faecium* identification of 76.3% using the VITEK 2 system.[8] In particular, half of the misidentifications were due to *E. faecium* with low-level resistance to vancomycin (LRV).[8] This is in accordance with the data reported in Abele-Horn et al. where the VITEK 2 system correctly identified all vancomycin resistant *E. faecium* strains while all isolates identified with low discrimination were *E. faecium* LRV.[9] The *E. faecium* described in the present report corresponds to an LRV strain (Table 1) and this may, at least in part, explain the incorrect identification by the VITEK 2. However, this does not explain why it was classified as *S. uberis*.

S. uberis is an opportunistic pathogen in dairy cattle, adapted to challenging and changing environmental conditions and capable of a nutritional flexibility based on multiple metabolic options.[10] *In-vitro* experiments demonstrated that *S. uberis* can readily develop penicillin resistance, and microbial analysis of bacterial population in treated milk showed that *S. uberis* can grow even in cold storage.[11,12] It has been hypothesized that the stability of this pathogen under various environmental conditions and the expression of virulence factors[11–13] may expand the pathogenicity of this bacterium from cattle to humans as described for *Streptococcus agalactiae*, a pathogen that is associated with both animal and human diseases.[14] However, few data provide support for this hypothesis, and in many cases the tests used for the identification of *S. uberis* in humans have been debated.[1,3] Additionally, *S. uberis* in combination with other bacteria was used as a probiotic in a clinical trial aimed at contrasting the growth of periodontal pathogens. The results as well as a toxicity study conducted in rats revealed no adverse-effect of the probiotic mouthwash by daily usage, which on the other hand contributed to maintaining both dental and periodontal health.[15,16]

Nevertheless, in 1988 Rabe reported a case of genital infection in a human due to *S. uberis* and in 1989 Sarkar described a pneumonia infection caused by an *S. uberis* sensitive to penicillin.[1] In 1991 Sanchez reported a single case of hepatic abscess of a cattle man and in 1999 Bouskraoui isolated an *S. uberis* strain sensitive to penicillin-G and amoxicillin from an 11 month old infant. Haffajee in 1988 and Dzink in 1989 identified *S. uberis* from periodontal tissues, suggesting that it may represent an oral commensal rather than an oral pathogen in humans.[1] Lazińska described a microbiological urinalysis of 269 patients after renal transplantation, and identified one patient with an infection of *S. uberis*.[17] Velez-Montoya reported a postoperative endophthalmitis caused by *S. uberis* that was resistant to the majority of the latest generation of antibiotics however, the method used for the identification was not mentioned and the reliability of the recognition was questioned.[1,3] Recently, Gülen and collaborators identified S. *uberis* from urine samples of seven of 148 patients by phenotypic methods.[18]

The common feature of all cases of human infection supposedly caused by *S. uberis* is that the detection was obtained by phenotypic bacterial identification systems. Therefore, it is possible that the real occurrence of this infection has been overrepresented and that the previously reported incidence of *S. uberis* in association with various human infections may depend on the inappropriate identification by conventional phenotypic methods.

In our report, the identification of the isolate as *S. uberis* relies on the presence of β-Glucuronidase positive tests, and the negative growth in 6.5% of NaCl. The growth in 6.5% NaCl may not represent a valuable discriminatory test for enterococci and streptococci. On

Table 1
Antibiotic susceptibility pattern of the isolated bacteria

Antibiotic tested	MIC	Test Result
Ampicillin	≥ **16** mcg/ml	**R**
Cefotaxime	≥ **8** mcg/ml	**R**
Ceftriaxone	≥ **8** mcg/ml	**R**
Clindamycin	≥ **1** mcg/ml	**R**
Erythromycin	≥ **8** mcg/ml	**R**
Levofloxacin	≥ **16** mcg/ml	**R**
Linezolid	**2** mcg/ml	**S**
Penicillin G	**8** mcg/ml	**R**
Tetracycline	≥ **16** mcg/ml	**R**
Vancomycin	**1** mcg/ml	**S**
Ampicillin/Sulbactam	≥ **32** mcg/ml	**R**
Chinupristin/Dalfopristin	**0.5** mcg/ml	**R**
Teicoplanin	≤ **0.5** mcg/ml	**S**
Tigecycline	≤ **0.12** mcg/ml	**S**

MIC: Minimal Inhibitory Concentration according to EUCAST 4.0 methodology; R: resistant; S: Sensitive.

the contrary, β-Glucuronidase activity is an important biochemical parameter for the identification of *S. uberis* as *E. faecium* is normally β-Glucuronidase negative.[2] Nevertheless, a comparative analysis of the genome of an emerging epidemic hospital strain of *E. faecium* (Aus0085) isolated in Australia and now widespread from Europe to Asia, predicted the presence of the β-Glucuronidase gene.[2] From this study it appears that many of the nosocomial *E. faecium* strains contain a high number of mobile genetic elements that distinguish them from community-acquired and non-pathogenic strains. It has been estimated that 24% of the *E. faecium* Aus0085 genes are located on the six plasmids, demonstrating that this element has a critical role in the transmission of specific traits to other clinical *E. faecium* isolates.[2] β-Glucuronidase gene catalyses the breakdown of complex carbohydrates, and this gene might confer a survival and growth advantage in hospital environments.

Similar concerns regarding the low discrimination of phenotypic tests have emerged previously for coagulase-negative staphylococci, suggesting that ambiguous test results might lead to misidentification of bovine pathogens as human pathogens.[19]

In addition to the biochemical phenotyping, different molecular methods have been developed to characterise *S. uberis* and sequence-based identification emerging as a powerful diagnostic technique that can be applied to routine clinical isolates. Indeed, the Clinical and Laboratory Standards Institute (CLSI) published a document to specifically address the criteria used for identification of bacteria and fungi establishing that genotypic methods have to be preferred over phenotypic systems, encouraging the implementation of such techniques by diagnostic laboratories.[20]

In summary, this report describes a case of erroneous identification of *E. faecium* with *S. uberis* by biochemical phenotyping. This finding reinforces the doubts previously reported in the literature concerning the possible pathogenicity of *S. uberis* in humans and points to the limitation of phenotypic tests, suggesting that the identification of this pathogen must always be supported by more specific procedures such as genotypic methods. This is particularly important in immunocompromised patients, such as those affected by cancer and undergoing intensive chemotherapy where the appropriate antibiotic therapy should be started as soon as the pathogen is detected.

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