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Case Report

Staphylococcus Hyicus, A Natural Skin Colonizer In Pigs And Causative Agent Of Exudative Epidermitis In Piglets, Associated With Periprosthetic Knee Infection In A Pig Farmer. First Case and Review of the Literature

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Abstract

We report on the first case of a periprosthetic joint infection with *Staphylococcus hyicus* as the causative agent. The bacterium is a natural skin colonizer in pigs and predominantly associated with exudative epidermitis in piglets. The strain was recovered in a pig farmer.

However, infections in humans are uncommon. Only three cases have been reported so far, among them wound infection after a donkey bite, bacteremia, and a case with spondylodiscitis. Nevertheless, laboratories, which focus on the diagnostics of human pathogens, should expect to have to identify zoonotic microorganisms. Furthermore, the case also shows the importance of suitable culture media in order to reliably recover the causative agents. Clinical evaluation six months after surgery showed a patient free of local pain and without any signs of functional limitation.

Keywords: Periprosthetic joint infection; Animals; Pathogens.

Introduction

Periprosthetic joint infection (PJI) is a serious complication in joint replacement surgery. Reliable identification of the causative agent is the prerequisite for targeted antimicrobial therapy. However, the identification of animal pathogenic germs as the cause of infection in a human is a challenge for every laboratory that focuses on human pathogen diagnostics. Eight years ago, a PJI with *Robinsoniella peoriensis*, originally described in pig manure, could only be identified in our laboratory using molecular approaches. Mass spectrometry incorrectly proposed *Lactobacilli* [1]. Meanwhile, databases now contain a large number of animal pathogens so that in the current case, although there have so far only been three other reports describing a proven human infection, the causative pathogen was reliably identified using mass spectrometry.

Here we report on the first case of a PJI associated with *Staphylococcus hyicus,* a natural skin colonizer in pigs, that was identified from synovial fluid, periprosthetic tissue samples and prosthetic components from a pig farmer.

Case Presentation

A 65-year-old man suffering from osteoarthritis of the knee underwent cemented (gentamicin-loaded) total joint replacement surgery on the right knee in November 2022 at the LVR-Klinik for Orthopaedic Surgery, Viersen, Germany. In August 2024, the patient consulted the hospital again because of chronic pain in his right knee during weight-bearing which had persisted over a period of about five months. Radiological assessment showed no signs of loosening. The serum C-reactive protein (CRP) was elevated at 64 mg/l. Synovial fluid cytological analysis revealed a white blood cell count of 4.890/µl with 88% polymorphonuclear cells (PMN). Empiric antimicrobial therapy was started intravenously with Vancomycin 1 g/12 h prior to revision. Two days later, the mobile component was exchanged, and extensive debridement was carried out.

Histopathological examination of periprosthetic tissue specimens showed signs of inflammation with a variable proportion of PMN. Synovial fluid taken preoperatively, four homogenized periprosthetic tissue samples taken intraoperatively and the processing fluid from sonication were applied for cultivation onto sheep-blood agar and chocolate agar (Oxoid, Basingstoke, Hampshire, United Kingdom) using 0.1 ml aliquots and incubated aerobically at 36 +- 1°C in 5-7% carbon dioxide for fourteen days.

Additionally, 10 ml of the processing fluid from sonication was transferred to an aerobic and 10 ml to an anaerobic BD BACTEC™ blood culture bottle (Becton Dickinson, Sparks, USA) and incubated automatically for fourteen days. 0.5 ml aliquots were inoculated using brain-heart infusion broth (BHI, Oxoid, Basingstoke, Hampshire, United Kingdom), schaedler broth with vitamin K (BD, Sparks, USA) and a modified semifluid thioglycollate broth (LT) containing beef-liver extract and a mixture of horse serum and hemin (SIFIN, Berlin, Germany). For detailed information about anaerobic culture conditions and the composition of the ingredients of the LT broth, see the literature [2]. Antimicrobial activity in sonicate fluid and tissue samples were screened in vitro by a phenotypical inhibition test (PIT). For information about this method, please see the literature [2]. There was no evidence of antimicrobial agents in the samples. This finding was not in accordance with the protocol that antibiotics were administered prior to sample collection.

Regardless of origin, bacterial growth on the samples first became detectable in LT broth within two days of incubation, whereas blood culture bottles, BHI broth and schaedler broth each became positive after four days. Colonies, however, were recovered from cultured plates only within five days and appeared whitish and non-hemolytic. The clumping factor test, a marker for coagulase-positive staphylococci, was negative.

Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics, Bremen, Germany) identified *Staphyloccocus hyicus* with a high identification score value of 2.24 as the only possible species. Molecular 16S rRNA partial gene sequence analysis was performed showing a similarity of 99.62% (520/522 nucleotides) compared to the reference sequence of the type strain *Staphylococcus hyicus* (GenBank accession no. CP103964).

Antimicrobial susceptibility was tested by the broth microdilution technique and Etest method (bioMérieux, Marcy I'Etoile, France) using Mueller-Hinton agar. The results were interpreted according to the application sheets and guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) using the minimal inhibitory concentration (MIC) breakpoints for Staphylococci [3].

The strain was susceptible to oxacillin, cefoxitin, cefazolin, cefuroxime, and imipenem. The phenotypic measurement data for the ß-lactams were confirmed by the absence of the resistance genes *mecA* and *mecC*. Furthermore, gentamicin, levofloxacin, trimethoprim-sulfamethoxazole, clindamycin, erythromycin, fusidic acid, linezolid and daptomycin were also susceptible. But there was resistance to penicillin and tetracycline.

The therapy was changed according to the susceptibility pattern and continued with cefazolin 2 g/6 h for 10 days. As the wound healed normally, the patient was discharged from hospital and the treatment was continued by oral administration of levofloxacin 0.5 g/12 h and rifampicin 0.45 g/12 h for four weeks. Six weeks after surgery the patient was seen for follow-up. Since the healing process remained without side effects the existing therapy was continued orally for another six weeks. Clinical evaluation three months after the end of treatment (six months after surgery) showed a patient free of local pain and without any signs of functional limitation.

Discussion

Staphylococcus hyicus, a Gram-positive facultatively anaerobic bacterium, was initially named *Micrococcus hyicus* and later classified among the genera *Staphylococcus*. It belongs to the coagulase-positive animal adapted species, with variable strain-dependent expression.

It is known to be part of the commensal skin flora of various animals including pigs, cows and chickens [4]. However, this bacterium can differ in its pathogenic potential depending on its ability to produce numerous exfoliative toxins designated (ExhA, ExhB, ExhC, ExhD and SHETB) as the main virulence factors responsible for dermatological lesions. In piglets, in particular, the bacterium's ability to cleave desmoglein-1 in the epidermis of porcine skin can lead to severe exudative epidermitis which often ends fatally [5,6]. Furthermore, this bacterium is also involved in skin infections in donkeys, horses, osteoarticular infections in pigs and in a variety of birds, like turkeys and falcons [7-11].

On the other hand, infections in humans are uncommon, but it is not unexpected that livestock farmers are affected. Three cases have been reported so far, among them wound infection after a donkey bite, bacteremia, and a case with spondylodiscitis [12-14]. To our knowledge, this is the first device-associated infection with *S. hyicus* to be described.

According to the medical history of our patient, close contact with his piglets and recurring skin injuries are the suspected source of infection. Nevertheless, preoperatively, the skin in the surgical area showed no signs of any lesion. Experimental evidence has shown that the exfoliative toxins of *S. hyicus* cannot cleave human desmoglein-1 and therefore cannot damage human epidermis [15].

While the literature describes variable antimicrobial resistance profiles of *S. hyicus* strains isolated from swine herds in different countries, for example for lincosamides, macrolides, fluoroquinolones and tetracyclines, only resistance to tetracyclines was found in our strain [16]. However, the applied breakpoints for the interpretation of results were obtained from the EUCAST dataset for *Staphylococcus* spp., since no reference is made for veterinary pathogens there.

In addition, the patient had also been known to be a nasal carrier with a livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) for two years. This strain was identified as the spa type t034 typically isolated from pigs.

At first, clinicians were therefore worried that the patient might have a polymicrobial infection and decided to continue the therapy with Vancomycin. When it was clear that the LA-MRSA strain was not detected in the tissue samples and the measured phenotypic susceptibility to oxacillin in *S. hyicus* was confirmed by absence of the resistance genes *mecA* and *mecC*, the therapy was reassessed and continued using cefazolin as the therapeutic antimicrobial agent for the period of intravenous administration. The subsequent interval with the orally administered levofloxacin and rifampicin showed no complications.

Despite the inconspicuous course, a residual uncertainty remains as only the mobile parts of the prosthesis were replaced. Due to the remaining fixed femoral and tibial components, it cannot be ruled out that a biofilm has also become established there which could not be removed even with targeted antimicrobial therapy.

Although the time of infection cannot be determined due to the daily contact with the piglets, a chronic course cannot be ruled out and even appears probable due to the constant joint-dependent symptoms over several months without any acute deterioration. Looking at the three other case reports describing an infection due to *S. hycius*, there are also indications of a chronic course in the patient with spondylodiscitis. However, the fact that there is no evidence of a persistent or relapsed infection six months after surgery is at least promising.

It should be noted that PJI are often complicated, both in clinical and microbiological diagnostics, if chronic courses are suspected. This poses particular challenges for the laboratory, as these infections are very often accompanied by a variable bacterial load at the infection site. Additionally, due to changes in the requirements for nutrient supply these organisms are frequently slow-growing or sessile in vivo and using conventional culture methods in vitro, too. This might explain the different time to positivity before the bacterium was detected in the individual cultivation media.

Therefore, until appropriate processing and cultivation standards for this entity of infections have been established, every laboratory that wants to overcome this problem needs to compile its own additional modified and supplemented culture media to promote growing conditions as well as using conventional culture approaches. As a consequence, evaluations on this subject remain laboratory-specific. We have extensively investigated the influence of the different culture media on the detection rate in diagnosis of PJI, see the literature [17,18].

Conclusion

Here we report a case with PJI that provides convincing evidence that the animal adapted species *Staphylococcus hyicus* is the causative agent. We should be aware that organisms associated with livestock and pet animals might act more frequently as facultative human pathogens. That makes it all the more important to establish generally accepted standards for the cultivation of samples that allow reliable detection of PJI-causing microorganisms.

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