Wound infection by multiresistant Staphylococcus sciuri identified by molecular methods

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SUMMARY

We describe a case of wound infection by multidrug-resistant *Staphylococcus sciuri* in a patient admitted to hospital for injuries in Agreste Alagoas, Brazil, identified through broad-spectrum PCR and sequencing of 16S rDNA gene. Due to its high resistance profile, the infection was characterized as methicillin-resistant *Staphylococcus* presenting sensitive only to vancomycin and chloramphenicol. The injury resulting from trauma associated with infection resulted in amputation of the infected limb.

KEY WORDS: Staphylococcus sciuri, Wound infection, 16S rDNA

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CASE REPORT

Wound infection is the most common infectious disease in hospitals caring for trauma cases. These infections can be caused by several types of gram-positive and negative bacterial agents. The incidence of coagulase-negative Staphylococcus involved in hospital infections has increased substantially with the advent of more invasive technologies in the hospital environment and the development of resistance by microorganisms.

Herein we describe a case of a 30-year-old man run over by a motorcycle and admitted the Agreste Emergency Unit - Alagoas, Brazil. He suffered multiple injuries, with two major injuries in the posterior and lateral regions of the right leg. He underwent vascular and intestinal surgeries on the day of hospital admission. He received empirical therapy with ciprofloxacin (400 mg 12-12 h) and metronidazole (500 mg 8-8 h). Haematological analyses showed 4.3 g/dL, hemoglobin level, 12.5 x 10^9/L leukocytes, 10.6 x 10^9 neutrophil and platelet counts at normal levels. Two red blood cells concentrates were used to compensate the anemia. Two days after admission he had fever and complained of intense pain in his right leg.

The presence of pus secretion in his leg was indicative of infection. Surgical debridement was indicated for removal of necrotic lesions. Before surgical procedures, samples of pus secretion were collected for laboratory analysis. The clinical reports associated with necrosis reaching the fascia, and the surgical findings of low adhesion of subcutaneous tissue allowed the establishment of the diagnosis of necrotizing fasciitis. Even after the debridement, the patient remained with fever, infected wound and still complaining of severe pain. The extensive muscle involvement resulting from the traumatic injury associated with the infection resulted in limb amputation. Amikacin (1g 24/24 h), oxacillin (2g 6/6 h) and ceftriaxone (1g 12/12 h) were used as prophylactic therapy to surgery.

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Samples collected were plated on Columbia agar (Himedia) with 5% sheep blood and incubated at 35°C with 5% CO_2. They were also inoculated into BHI (Himedia) and MacConkey agar (Acumedia), with incubation at 35°C. Bacterioscopy of the secretion showed many gram-positive cocci and rare gram-negative bacilli. Preliminary tests were performed on the basis of colonial morphology and characteristics of the culture, Gram reaction, catalase, DNase and coagulase for the identification of the isolate, characterizing it as a coagulase-negative and DNase-positive *Staphylococcus*. Aiming to make a molecular diagnosis, the bacterial DNA was extracted from colonies in culture by phenol/chloroform extraction. Universal primers for the 16S rDNA gene parcial amplification were used (forward: 5'-AGAGTTTGATCATGGCTCA-3', reverse: 5'-ACGGCGACTGCTGCTGGCAC-3') (Gatselis, Malli *et al.,* 2006). The amplicon of approximately 480bp was subjected to electrophoresis on 1.5% agarose gel and visualized under ultraviolet light.

The sequencing was performed on ABI PRISM 310 automated equipment (Applied Biosystems) and FASTA sequence was analyzed against sequences deposited in the GenBank (http://www.ncbi.nlm.nih.gov/BLAST) and the Ribosomal Database Project II (http://rdp.cme.msu.edu/seqmatch/seqmatch_intro.jsp). In both banks, sequence analysis showed *Staphylococcus sciuri* subsp. *sciuri* as a species with a high degree of similarity (*E-value:* 0.0, *Max Ident:* 99%; *S_ab score:* 0.894). (GenBank accession number BankIt1435724 Seq1 JF748725).

DISCUSSION

The clinical importance of *S. sciuri* result rom the fact that it has been associated with infections such as endocarditis (Wallet, Stuit *et al.,* 2000), urinary tract infection (Stepanovic, Jezek *et al.* 2003) and wound infections (Shittu, Lin *et al.,* 2004). Initially described by Kloos *et al.*, it is considered the ancestral species and one of the most abundant in the genus (Kloos, *et al.,* 1976). Widely distributed in nature, it is found as a commensal of rodent species, marsupials and was isolated from pet and farm animals (Hauschild and Schwarz 2003).

The method used to define the resistance profile to antimicrobial was Disc Diffusion. After 24h of incubation, the inhibition zones were measured and interpreted according to criteria recommended by the Clinical and Laboratory Standards Institute (CLSI, 2010). The resistance to cefoxitin categorized it as methicillin-resistant *Staphylococcus*, showing the resistance to beta-lactam antibiotics. Most strains of *S.sciuri* are quite sensitive to this group of antimicrobials (Couto, *et al.,* 2003). However, increasing levels of minimum inhibitory concentration have recently been found (Stepanovic, *et al.* 2005). The isolate was resistant to aminoglycosides, quinolones, macrolides and lincosamides tested. The methicillin-resistant *Staphylococcus* is often resistant to other antibiotics. However, resistance to aminoglycosides and macrolides in *S. sciuri* has shown low prevalence in other studies (Stepanovic, *et al.* 2006; Hauschild, Vukovic *et al.,* 2007). The results obtained by Stepanovic *et al.* indicate that *S.sciuri* may be naturally resistant to lincosamides.

Only vancomycin and chloramphenicol antibiotics exhibited sensitivity in vitro. Vancomycin remains the last-choice antibiotic in the fight against serious staphylococcal infections due to its high sensitivity. The occurrence of resistance to chloramphenicol was not detected in our study indicating it to be an important therapeutic alternative.

Correct identification of *S. sciuri* is essential for the interpretation of sensitivity testing. Laboratories with few resources use DNAse agar as diagnostic test for *S. aureus* rather than the coagulase test. The DNase test shows positive for species of *S. aureus* and other rare cocci gram-positive, including *S. sciuri*, therefore it should only be used as a screening test. The reading of the inhibition zone for the cefoxitin 30 mg (DME) disk showed 23 mm in diameter in our analysis. The misidentification of the isolate as *S. aureus* could lead to a misinterpretation of the inhibition zone of the cefoxitin disk, classifying it as sensitive to methicillin. This misunderstanding could have important implications such as the probable failure of antimicrobial therapy in the patient, the need for more potent antibiotics and prolonged hospitalization.

Our observations highlight the importance of evolution and resistance of isolates of *S. sciuri* in clin-

ical infections and the need for anappropriate diagnostic protocol for proper identification of this species.

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