DOI: 10.23925/1984-4840.2019v21i2a8 **Original Article /** *Artigo Original*

Paenibacillus stellifer: a new cause of human infections

Paenibacillus stellifer: uma nova causa de infecções humanas

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ABSTRACT

Introduction: *Paenibacillus stellifer* is widely distributed in nature, but its pathogenicity has not been reported since it was first identified in 2003. **Objectives:** This work aimed to identify Gram-variable rods isolated from cases of health care-associated infections in a hospital in a mountainous region of Rio de Janeiro, Brazil, between September/2015 and August/2016, and analyze their sensitivity to antibiotics commonly used in clinical practice. **Methods:** Initially, microorganisms were identified with conventional methods and confirmed by the Matrix-Assisted Laser Desorption/Ionization — Time of Flight (MALDI-TOF) technique. The sensitivity to antimicrobials test was performed according to recommendations from the Clinical and Laboratory Standards Institute. **Results:** We analyzed 105 samples: 59 surgical wound secretions and 46 blood cultures. Gram-variable rods were identified in two surgical wound secretion samples (3.39%) and two blood cultures (4.35%). *Paenibacillus stellifer* was the microorganism isolated from the four samples, showing sensitivity to all tested drugs. **Conclusion:** *P. stellifer* is a microorganism that is not in the make-up of human microbiota and has an environmental origin. According to current knowledge, this is the first identification of *P. stellifer* as the etiological agent of surgical wound infections in the world, and bacteremia in Brazil. Lastly, we highlight that microorganisms normally found in the environment are able to cause infections in a hospital.
Keywords: *Paenibacillus stellifer*; cross infection; bacteremia; surgical wound infection.

RESUMO

Introdução: *Paenibacillus stellifer* está amplamente distribuído na natureza, mas sua patogenicidade não foi relatada, desde que foi identificado pela primeira vez em 2003. **Objetivos:** Este trabalho objetivou identificar bacilos Gram-variáveis, isolados em casos de infecções relacionadas à assistência à saúde, no período de Setembro/2015 a Agosto/2016, em um hospital da região serrana do Rio de Janeiro, Brasil e avaliar o perfil de sensibilidade destes microrganismos a antibióticos comumente empregados na prática clínica. **Métodos:** Inicialmente, os microrganismos foram identificados a partir de testes bioquímicos convencionais e o resultado foi confirmado através da técnica de *Matrix-Assisted Laser Desorption/Ionization — Time of Flight* (MALDI-TOF). O teste de sensibilidade aos antimicrobianos foi realizado de acordo com as recomendações do *Clinical and Laboratory Standards Institute*. **Resultados:** Foram analisadas 105 amostras: 59 secreções de feridas cirúrgicas e 46 hemoculturas. Bacilos Gram-variáveis foram identificados em duas amostras de secreção de ferida cirúrgica (3,39%) e em duas hemoculturas (4,35%). *Paenibacillus stellifer* foi o microrganismo isolado nas quatro amostras e apresentou sensibilidade perante todas as drogas testadas. **Conclusão:** *P. stellifer* é um microrganismo de origem ambiental e não compõe a microbiota humana. De acordo com o conhecimento atual, esta é a primeira identificação de *P. stellifer*, como agente etiológico de infecções de ferida cirúrgica no mundo, e bacteremia no Brasil. Por fim, destaca-se o fato de que microrganismos normalmente encontrados no ambiente são capazes de causar infecções, quando presentes no ambiente hospitalar.
Palavras-chave: *Paenibacillus stellifer*; infecção hospitalar; bacteriemia; infecção da ferida cirúrgica.

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Received on 03/14/2018 – Accepted on 10/29/2018

INTRODUCTION

The *Paenibacillus* genus is characterized as a rod-shaped Gram-positive, facultatively anaerobic, and spore-forming bacteria. These microorganisms are motile by means of peritrichous flagella, abundant in nature, and closely related to the *Bacillus* genus.[1] Despite the structure of Gram-positive bacteria, phenotypically, *Paenibacillus* spp. reacts weakly with the Gram stain, and young colonies may even seem Gram-negative.[2]

Currently, the genus *Paenibacillus* consists of over 240 species (http://www.bacterio.net/paenibacillus.html). The *Paenibacillus* species are mainly environmental in origin. This genus has been found in a variety of sources such as: soil, water, tree roots, and foods.[3,4] Members of the *Paenibacillus* genus are often recognized as environmental contaminants when isolated in a clinical microbiology laboratory.[5] However, their potential to cause systemic human diseases has been documented.[6-8]

Suominen et al.[1] were the first ones to describe *Paenibacillus stellifer*. Since then, no article regarding the pathogenicity of this species has been published in major databases such as: MEDLINE, COCHRANE, and LILACS. On the other hand, human infections by a number of *Paenibacillus* species have recently been reported, such as: *P. alvei* — isolated in prosthetic joint infection;[9] *P. polymyxa*, *P. konsidensis*, *P. thiaminolyticus, P. larvae*, and *P. pasadenensis* — bacteremia;[6,8,10-12]; *P. pasadenensis* — wound infection in humans;[13] and *P. glucanolyticus* — endocarditis.[14] Furthermore, *P. provencensis* was isolated from human cerebrospinal fluid, *P. urinalis* from human urine, and *P. massiliensis*, *P. sanguinis*, and *P. timonensis* from blood cultures.[7,15]

Thus, this study aimed to identify Gram-variable rods involved in cases of health care-associated infections in a hospital in the mountainous region of Rio de Janeiro, Brazil, between September 2015 and August 2016, and analyze their sensitivity to antibiotics commonly used in clinical practice.

METHODS

Ethics

The ethics committee of Universidade Federal Fluminense properly approved this study, under the number 00887812.1.0000.5243, process 146.816. This investigation complied with the rules in Resolution number 466/12 of the National Health Council.

Bacterial strain

The Gram-variable rods used in this study were isolated from cases of health care-associated infections. This hospital provides care to several locations in the state of Rio de Janeiro and is the referral center for cardiac surgery in the Brazilian health care program.

Culture

The samples were collected according to the Clinician Guide for Collecting Cultures.[16]

Pus samples were processed according to the UK Standards for Microbiology Investigations: Investigation of Pus and Exudates.[17] The aspirated pus samples obtained from the surgery site were transported in a syringe for microbiological analysis. Pus samples were inoculated in thioglycolate broth and aerobically incubated for 24 and 48 hours at 37ºC. After, an aliquot of the broth was inoculated in Columbia blood agar, MacConkey agar, and Brain Heart Infusion agar (BD Difco™) for 24 and 48 hours at 37ºC.

An automated system (BACTEC® 9050) was used to detect and monitor microbial growth from blood specimens. The blood culture incubation time and temperature followed the UK Standards for Microbiology Investigations: Investigation of Blood Cultures.[18] An aliquot of positive blood culture samples was inoculated on Columbia blood agar, MacConkey agar, and Brain Heart Infusion agar (BD Difco™) and aerobically incubated for 24 to 48 hours at 37ºC.

Microorganism identification

The methods to identify the microorganisms consisted of an initial description of the colony morphology, colony details after 24 and 48 hours of incubation, no hemolytic activity, observation of Gram stain, catalase, and biochemical characteristics. Conventional biochemical tests were performed according to the method described by Hong et al.[19] Lastly, species identification was confirmed by matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Wissembourg, France).

Antimicrobial susceptibility

The sensitivity test to antimicrobials was performed using the disk diffusion method according to recommendations from the Clinical and Laboratory Standards Institute, 2016. We tested tetracycline (30 µg), rifampin (5 µg), gentamicin (10 µg), imipenem (10 µg), ciprofloxacin (5 µg), vancomycin (30 µg), cephalothin (30 µg), trimethoprim-sulfamethoxazole (25 µg), amoxicillin-clavulanate (30 µg), and ampicillin-sulbactam (10 µg). The zone diameters of each drug were interpreted using the criteria published by the Clinical and Laboratory Standards Institute, 2017.

RESULTS

During this study period, the hospital lab conducted bacteriological tests on 105 clinical samples from suspected cases of health care-associated infections. Out of the 105 cultivated samples, 59 (56.20%) were surgical wound secretions and 46 (43.80%) were blood cultures.

This study found Gram-variable rods in two blood cultures (4.35%) and two wound secretion samples (3.39%).

Among the positive cultures, 100% (4 samples) were from male patients, aged 64–96 years, who had been hospitalized for a long period. The four patients had comorbidities, and three of them (75%) had heart disease and hypertension (Table 1).

The microorganism isolated from positive pus samples and positive blood culture samples collected from the surgical wound showed white convex colonies with transparent borders, were non-hemolytic, catalase positive, and had thin and long Gram-variable rods. Species identification was confirmed as *Paenibacillus stellifer* (Sample 194, score: 2,001 and Sample 224, score: 2,055) by MALDI-TOF MS and the corresponding database.

Paenibacillus stellifer was the microorganism isolated from the four samples, all of them susceptible to the tested antibiotics.

DISCUSSION

Health care-associated infections affect patients in a hospital or other health care facility and are not present or incubating at the time of admission. Several factors can cause health care-associated infections, such as prolonged and inappropriate use of invasive devices and antibiotics, high-risk and sophisticated procedures, and immunosuppression.[20] In this study, all patients who had positive cultures for *P. stellifer* were exposed to the above risks factors. When a patient undergoes surgical procedures, comorbidities and the use of invasive devices (such as catheters) and antibiotics for a long period can cause the individual to become susceptible to infections. The skin defense barrier is broken, and the microbiota is compromised. A long length of stay favors this process, and environmental microorganisms, such as *P. stellifer*, can act as etiologic agents of opportunistic infections when present in the hospital.

Wound infections created by an invasive surgical procedure are generally referred to as surgical site infections. They are a common cause of health care-associated infections in Brazil, ranking third among all infections in health services, with 14 to 16% of them being found in hospitalized patients.[20]

Staphylococcus aureus, coagulase-negative staphylococci, and Gram-negative bacilli are commonly associated with surgical site infections.[20] This was the first time that *P. stellifer* was isolated as a cause of surgical wound infection. Furthermore, this was the first time that *P. stellifer* was reported as a bacteremic infection in Brazil. *Paenibacillus* spp. are ubiquitous and found in the environment.[2] According to the literature, blood is the most common source for *Paenibacillus* isolation. Several *Paenibacillus* species have been reported as the cause of bacteremic infections in humans, such as *P. thiaminolyticus* (bacteremia in a patient undergoing hemodialysis),[11] *P. konsidensis* (bacteremia in a patient with hematemesis),[6] *P. alvei* (prosthetic joint infection with bacteremia),[9] and *P. polymyxa* (bacteremia in a patient with cerebral infection).[10] *P. timonensis*, *P. massiliensis*, and *P. sanguinis* were isolated from blood cultures of patients with carcinoma, interstitial nephropathy, and leukemia, respectively.[7]

This microorganism is not detectable on human skin, but, if the skin is disturbed, infections by pathogens normally found in the environment become more likely. Usually, these bacteria are not a risk to healthy subjects due to their low virulence. However, they can cause infection in individuals with compromised clinical conditions, like opportunistic bacteria.[21,22]

Cerebrospinal fluid was the second most common source for *Paenibacillus* spp. isolation.[11,15] Furthermore, other species have been isolated from pleural fluid, urine, brain abscess, wound infection, and endophthalmitis.[23,24] Nevertheless, *P. stellifer* has never been reported as a human pathogen of surgical wound infections.

In this study, *P. stellifer* was isolated in four different patients. All of them had symptoms of infection, such as fever, chills, or purulent discharge. The hospital tried to identify and eliminate possible sources of infection. The *Paenibacillus* genus has an environmental origin; therefore, samples from different new batches of blood culture bottles were sent for microbiological analysis. No microorganism was isolated. This fact confirms that the blood culture bottles were not environmentally contaminated before use. The investigation revealed that these cases were not pseudobacteremia or environmental contamination of the rubber stoppers in the blood culture bottles. Afterward, samples from strategic points of the surgical center and Intensive Care Unit were collected and sent for microbiological analysis. Hospital analysis identified *P. stellifer* spores in the air conditioning and shelf drugs, which allowed the focus of infection to be contained.

Table 1. Epidemiological profile of microorganisms isolated from health care-associated infections.

Samples	Clinical specimen	Gender	Age	Comorbidities	MALDI-TOF Score
191	Blood culture	M	96	heart disease + hypertension	2,129
194	Surgical wound secretion	M	65	diabetes mellitus + heart disease + hypertension	2,001
197	Blood culture	M	70	diabetes mellitus + Parkinson's disease	1,985
224	Surgical wound secretion	M	64	heart disease + hypertension	2,055

MALDI-TOF: Matrix Associated Laser Desorption Ionization — Time of Flight.

Programs on effective prophylactic measures are among the main challenges for the management of hospitals.[21] The correct hand hygiene is the most important, simple, and effective measure to avoid contamination. Cleaning air conditioning filters, shelves, and surfaces are also simple actions to control these infections, as direct or indirect contact by hands accounts for 80% of transmission of infectious agents. The proper implementation of this practice contributes to control and prevent infections in the health care setting,[22] including infections caused by environmental microorganisms.

CONCLUSION

According to current knowledge, this is the first identification of *P. stellifer* as the etiological agent of surgical wound infections in the world, and bacteremia in Brazil. Misinterpretation of blood culture or wound secretion findings can have tragic consequences. Early identification is important to provide rapid treatment.

Although this microorganism is Gram-variable, MALDI-TOF MS is a reliable alternative to identify the *Paenibacillus* genus in the microbiology routine because, in this situation, the Gram staining reaction might be misleading.

Lastly, this study can provide health professionals with useful insight on the pathogenicity of this microorganism, highlighting that microorganisms normally found in the environment can cause infections in a hospital.

ACKNOWLEDGMENTS

The authors thank the National Council for Scientific and Technological Development (CNPq) for the scholarship received via the National Postgraduate program.

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How to cite this article:
Heggendornn LH, Gomes SWC, Longo LGA, Cunha GA, Póvoa HCC. *Paenibacillus stellifer*: a new cause of human infections. Rev Fac Ciênc Méd Sorocaba. 2019;21(2):83-7. http://doi.org/10.23925/1984-4840.2019v21i2a8