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Genetic homogeneity/heterogeneity of *Propionibacterium acnes* isolated from patients during cardiothoracic reoperation

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Abstract

Following cardiothoracic surgery, deep sternal wound infection (SWI) remains one of the most severe complications. Recently, *Propionibacterium acnes* has been suspected as an etiological agent of deep SWI. However, this bacterium constitutes part of the resident micro-flora of the human skin. Consequently, findings of *P. acnes* in invasive samples are difficult to value. The aims of this study were to develop and optimize a pulsed-field gel electrophoresis (PFGE) protocol for *P. acnes*, in order to investigate the genetic homogeneity/heterogeneity of *P. acnes* isolates from multiple tissue samples (predominantly biopsies), collected at different locations, from 12 patients during cardiothoracic reoperation. There were 24 distinguishable PFGE fingerprints identified among the *P. acnes* isolates ($n = 54$). Five (42%) of the patients carried only isolates that were interpreted as presumably clonally related. From the remaining seven patients, two or three different *P. acnes* clones were cultured, however, from six of them, the clones were identified in multiple samples. *P. acnes* may be a relatively frequent etiological agent of postoperative cardiothoracic infections. Existence of several clonally related *P. acnes* isolates derived from multiple samples from patients suffering from deep SWI after cardiothoracic surgery has not previously been shown.

Keywords: *Propionibacterium acnes*; Cardiothoracic surgery; Sternal wound infection; Pulsed-field gel electrophoresis (PFGE); Molecular epidemiology

1. Introduction

Following cardiothoracic surgery, deep sternal wound infection (SWI) remains one of the most severe complications [1–4]. In our previous study, i.e. the LOGIP trial [1], cardiac surgery patients ($n = 2000$) were randomized to routine prophylaxis with intravenous isoxazolyl-penicillin or to routine prophylaxis combined with local application of collagen-gentamicin sponges. Follow-up regarding development of SWIs continued for 2 months after operation. The incidence of postoperative SWIs declined from 9.0% to 4.3% when using the collagen-gentamicin sponges [1]. Coagulase-negative staphylococci (CoNS) followed by *Staphylococcus aureus* were identified as the most common causative agents, which is in concordance with previous studies [4–7]. Unexpectedly, in the LOGIP trial [1], presumed *P. acnes* were also isolated from multiple deep tissue samples obtained at different locations during reoperation of several patients and, thus, were identified as the third most prevalent agent. *P. acnes* has not commonly been recognized as a significant pathogen in SWIs although it has been reported as a primary pathogen of deep SWI and mediastinitis after cardiothoracic surgery [8] and in postoperative infections in association with foreign devices [9–11]. However, *P. acnes* constitutes part of the resident micro-flora of the human skin, nares, conjunctivae, oral cavity, upper respiratory tract, and intestinal tract. Therefore, the finding of *P. acnes* in samples from mediastinal tissue or fluid is difficult to value. In our previous study [1], the SWIs presumably caused by *P. acnes* were characterized by a high proportion of sternal dehiscence and an early onset despite low CRP levels [12]. Consequently, *P. acnes* as a secondary cause of SWI after primary mechanical sternal instability and several other host factors need also to be considered and further examined.

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During recent decades, several different DNA-based methods for molecular typing of mainly dairy Propionibacterium species have been developed [13–16]. For *P. acnes*, randomly amplified polymorphic DNA (RAPD) [17], different protocols for pulsed-field gel electrophoresis (PFGE) [11,18–20], and sequencing of the *16S rRNA* gene, *recA* gene, and the putative hemolysin gene (*tly*) [21] have been used. For many bacterial species, PFGE has been regarded as the gold standard for investigation of nosocomial infections, local outbreaks and micro-epidemiology [22]. This is mainly due to the high discriminatory ability of the method and, in fact, PFGE potentially indexes the entire genome.

The aims of the present study were to develop and optimize a PFGE protocol for *P. acnes*, in order to investigate the genetic homogeneity/heterogeneity and micro-epidemiology of *P. acnes* isolates obtained from patients during cardiothoracic reoperation.

2. Materials and methods

2.1. Patients and bacterial isolates

In the present study, 61 presumed *P. acnes* clinical isolates, i.e. typical Gram-positive polymorphic rods showing characteristic colony morphology after facultative anaerobic growth, were included. These presumed *P. acnes* isolates were cultured from multiple tissue samples (predominantly biopsies) obtained from different locations during cardiothoracic reoperation of 13 patients, which were examined between September 2000 and September 2002 at the Department of Cardiothoracic Surgery, Örebro University Hospital, Sweden and included in the LOGIP trial [1]. The indications for reoperation of these patients ($n = 13$) were clinically suspected infection ($n = 8$) or other postoperative complications ($n = 5$) such as tamponade or bleeding. The number of samples per patient ranged between two and seven. Two patients were treated with cefotaxime prior to reoperation and all eight patients with clinically confirmed infection were treated with various antibiotics following reoperation. The outcome was favourable in all cases, although one patient underwent additional revision and one patient developed a mechanical instability. In the LOGIP trial [1], *P. acnes* isolates were cultured from deep tissue samples from one additional patient; however, in the present study this patient was excluded because the isolates were not available. In addition, five international *P. acnes* reference strains, i.e. CCUG 35749, CCUG 1794, CCUG 36609, CCUG 38584, and CCUG 48370, were included to evaluate the discriminatory ability of the PFGE protocol developed in the present study.

2.2. Culture diagnostics

The culture diagnostics and species verification of *P. acnes* was performed at the Department of Clinical Microbiology, Örebro University Hospital, Sweden. In concordance with routine diagnostic procedures, the species verification was based on presence of facultative anaerobic growth on FAA plates (4.6% LAB 90 Fastidious Anaerobe Agar (LAB M, Lancashire, United Kingdom) supplemented with 5% defibrinated horse blood) after 48–120 h at 37 °C, characteristic colony morphology, Gram-positive polymorphic rods, resistance to metronidazole, susceptibility to penicillin G, positive catalase as well as indole tests. Isolates that matched all criteria with the exception of positive indole assay were further examined by API 20 A (bioMérieux, Marcy l'Etoile, France) for species designation. All the primary isolates were preserved at −70 °C.

2.3. Antibiotic susceptibility testing

The susceptibility to metronidazole, penicillin G, clindamycin, imipenem, and piperacillin/tazobactam was initially analysed by disc diffusion method and subsequently confirmed using Etest method (AB Biodisk, Solna, Sweden) in an anaerobic atmosphere according to the instructions of the manufacturer.

2.4. Pulsed-field gel electrophoresis

All isolates that were confirmed as *P. acnes* ($n = 54$) were genotypically characterized with PFGE of *Spe* I-digested genomic DNA. The digests were prepared in agarose plugs using GenePath® Group 3 Reagent Kit according to the instructions of the manufacturer (Bio-Rad Laboratories, Hercules, CA, USA). However, some optimizations were essential; approximately 1.3×10^9 bacterial cells were embedded in the agarose plugs and in the lysis step, an addition of 185 U/ml mutanolysin (Sigma-Aldrich Chemie Gmbh, Steinheim, Germany) per plug and a prolonged incubation time (four hours) were crucial for optimal lysis. PFGE fingerprints were obtained by separation of the digested DNA on 1.4% agarose gels (Molecular biology certified agarose, Bio-Rad) in a GenePath™ System (Bio-Rad). The electrophoresis was performed in a 0.5 × TBE buffer (45 mM Tris-borate [45 mM Tris base, 45 mM boric acid] and 1 mM EDTA) equilibrated at 14 °C. A constant voltage of 6 V/cm with an initial linear pulse time ramping of 1–8 s for 12.7 h at a 120° angle followed by a linear ramping of 0.1–2 s for 7 h at a 120° angle was utilized. Initially, to confirm the resolution of also the many smaller DNA fragments, an additional PFGE program was utilized. The parameters of this program were as follows: a constant voltage of 6 V/cm with an initial linear pulse time ramping of 1–3 s for 12 h at a 120° angle followed by a linear ramping of 0.1–2 s for 7 h at a 120° angle. The gels were stained with ethidium bromide and the separated DNA fragments were visualized under UV-transillumination and digitized using Gel Doc™ 2000 system (Bio-Rad). The fingerprints were normalized and analysed using the software Molecular Analyst® Fingerprinting version