Anaerobe 32 (2015) 57–62

Contents lists available at ScienceDirect

Anaerobe

journal homepage: www.elsevier.com/locate/anaerobe

Clinical microbiology

Antibiotic susceptibility of *Propionibacterium acnes* isolated from orthopaedic implant-associated infections

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ARTICLE INFO

Article history:
Received 18 September 2014
Received in revised form
15 December 2014
Accepted 22 December 2014
Available online 23 December 2014

Keywords:
Propionibacterium acnes
Prosthetic joint infections
Antibiotic susceptibility testing
Etest
Synergy testing

ABSTRACT

Introduction: Prosthetic joint infections (PJIs) caused by *Propionibacterium acnes* account for a larger proportion of the total number of PJIs than previously assumed and thus knowledge of the antimicrobial susceptibility patterns of *P. acnes* is of great value in everyday clinical practice.
Materials and methods: Using Etest, the present study investigated the susceptibility of 55 clinical isolates of *P. acnes*, obtained from orthopaedic implant-associated infections of the knee joint ($n = 5$), hip joint ($n = 17$), and shoulder joint ($n = 33$), to eight antimicrobial agents: benzylpenicillin, clindamycin, metronidazole, fusidic acid, doxycycline, moxifloxacin, linezolid and rifampicin. Synergy testing was also conducted, in which rifampicin was combined with each of the remaining seven antibiotics.
Results: All isolates ($n = 55$) were susceptible to most of the antibiotics tested, with the exception of 100% resistance to metronidazole, five (9.1%) isolates displaying decreased susceptibility to clindamycin, and one (1.8%) to moxifloxacin. None of the antimicrobial agents investigated were synergistic with each other when combined and nine isolates were antagonistic for various antimicrobial combinations. The majority of the antimicrobial combinations had an indifferent effect on the isolates of *P. acnes*. However, the combination of rifampicin and benzylpenicillin showed an additive effect on nearly half of the isolates.
Conclusion: Almost all *P. acnes,* isolated from orthopaedic implant-associated infections, predominantly PJIs, were susceptible to the antibiotics tested, with the exception of complete resistance to metronidazole. Synergy test could not demonstrate any synergistic effect but additive effects were found when combining various antibiotics. Antagonistic effects were rare.

1. Introduction

Propionibacterium acnes is a slow-growing, gram-positive, pleomorphic, facultative anaerobic bacillus. The bacterium is mainly found on the skin of humans as part of the normal flora. *P. acnes* is an opportunistic pathogen that is involved in the development of a number of inflammatory skin diseases such as acne vulgaris and psoriasis [1,2]. The bacterium is also associated with a number of infections and inflammatory conditions such as infective endocarditis [3], prosthetic joint infections (PJIs) [4], infections of neurosurgical shunt catheters [5], and sarcoidosis [6]. Recently, *P. acnes* has been discussed as a causative agent of the inflammatory process involved in specific back pain pathologies [7] *P. acnes* has also been found in prostate tissue from patients with benign prostatic hyperplasia and prostate cancer and is believed to play a role in the pathogenesis of these conditions [8].

P. acnes is a low-virulent microorganism and a PJI caused by this bacterium may result in symptoms and signs of infection several months after surgery [4]. A study of PJIs caused by *P. acnes* showed that the majority of the patients became symptomatic indicating an infection after 24 months or more postoperatively [9]. In recent

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http://dx.doi.org/10.1016/j.anaerobe.2014.12.006
1075-9964/

research, infections caused by *P. acnes* have accounted for a larger proportion of the total number of PJIs than previously assumed [10]. PJIs due by *P. acnes* have Probable previously been missed due to diagnostic challenges or misinterpreted as contamination [11]. Furthermore, several studies have shown the significance of *P. acnes* as a causative agent, especially in prosthetic shoulder infections [12,13].

Much remains unclear about the exact mechanisms of how *P. acnes* cause PJIs. One of the mechanisms likely to be of importance is the ability of *P. acnes* to produce biofilm [14–16].

The optimal treatment for deep PJIs caused by *P. acnes* has not yet been established [16,17]. These bacteria are usually susceptible to penicillin why beta-lactam antibiotics can be a favourable option of treatment. However, the presence of sessile, stationary bacteria in a biofilm predominantly counteracts antibiotics with the mechanisms of action targeting the bacterial cell wall (e.g. beta-lactams and glycopeptides). The bacterial formation of biofilm is a mechanism that reduces antibiotic susceptibility and represents a challenge in everyday clinical practice. Antimicrobial combinations that include rifampicin, which penetrates the biofilm and is effective also against stationary phase bacteria, have been investigated in experimental studies as well as in clinical trials regarding treatment of foreign body infections caused by staphylococci [18].

The current algorithm for treatment of PJIs is combination antibiotic treatment and early surgery with debridement and soft tissue revision in order to salvage the joint prosthesis [19].

P. acnes is highly susceptible to a wide spectrum of antibiotics, e.g. rifampicin, beta-lactams and quinolones [17–20]. However, treatment of implant-associated infections with rifampicin in monotherapy results in emergence of resistance in other bacterial species such as methicillin-resistant *Staphylococcus aureus* [19]. However, administration of rifampicin in combination with levofloxacin, could prevent emergence of rifampicin-resistance in methicillin-resistant *S. aureus* in an animal model of foreign-body infection [17]. The same concept of study [17] was adopted in order to investigate if the emergence of rifampicin-resistance of *P. acnes* in vitro was prevented or reduced when combining rifampicin with levofloxacin, clindamycin and penicillin G, respectively. None of these antimicrobial agents prevented resistance if the bacterial concentration was high (10^8 cfu/mL). However, in lower bacterial concentrations (10^6 cfu/mL) the addition of these antibiotics to rifampicin prevented high-level rifampicin resistance [17].

Thus, depending on the bacterial agent and the size of the bacterial load, the combination of different antimicrobial agents can prevent the emergence of resistance by various mechanisms of action and also conduces additive or synergistic antibiotic interactions. Finding synergistic drug combinations provide important benefits such as maintenance of drug efficacy while toxicity can be decreased, or alternatively, increasing the efficacy without any rise in toxicity [17].

The aims of the present study were to investigate the antibiotic susceptibility of *P. acnes* isolated from deep orthopaedic implant-associated infections and to evaluate antibiotic combinations in synergy testing, information valuable for optimization of the antibiotic treatment of PJIs caused by *P. acnes*.

2. Materials and methods

2.1. Patients and bacterial isolates

Fifty-eight clinical isolates of suspected *P. acnes* from patients with infections associated with orthopaedic implant devices were obtained from the Departments of Clinical Microbiology, University hospital of Örebro and Linköping from 1999 to 2013 and frm 2008 to 2013, respectively. The *P. acnes* isolates were characterised according to routine laboratory procedures, such as indole and catalase tests and microscopic examination. Final species determination was performed by MALDI-TOF MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) using a Microflex LT (Bruker Daltonic GmbH, Bremen, Germany) and MALDI Biotyper software (Bruker Daltonic), resulting in 55 *P. acnes* strains. The remaining isolated were *Propionibacterium granulosum*, *Propionibacterium avidum* and *Propionibacterium* spp. The 55 clinical *P. acnes* isolates were recovered from infections of knee joints ($n = 5$), hip joints ($n = 17$) and shoulder joints ($n = 33$). Forty-two isolates were related to PJIs and 13 associated with other implant devices obtained from shoulder joint infections.

2.2. Phylogenetic typing

Furthermore, the *tly* gene was sequenced, as previously described [21], in order to categorize the isolates into the phylogenetic types; IA, IB, II, and III.

2.3. Antibiotic susceptibility testing

The antimicrobial susceptibility testing was performed on FAA plates (4.6% LAB 90 Fastidious Anaerobe Agar, LAB M, Heywood, United Kingdom) supplemented with 5% horse blood (v/v) with an inoculum of 1.0 McFarland suspension using Etest (bioMérieux, Marcy l'Etoile, France) and incubation at 36 °C in anaerobic conditions (10% H_2, 10% CO_2, 80% N_2) for 40–48 h [22–25].

2.4. Synergy testing

The minimum inhibitory concentration (MIC) was established for each of the eight antibiotics (see below) and each antibiotic was also tested in combination with rifampicin. This method for synergy testing, the fixed ratio method [23–26], can briefly be described as follows: an Etest strip of a first antimicrobial agent was placed on an FAA plate and left for 1 h at room temperature in aerobic atmosphere. Then the first Etest strip was removed and an Etest strip of a second antimicrobial agent was placed on the exact location on the agar plate as the first strip [22–26] and incubated at 36 °C in anaerobic conditions for 40–48 h.

The synergistic, additive, indifferent or antagonistic effect of the various antimicrobial combinations was determined through calculation of Fractional Inhibitory Concentration (FIC) index using the MIC values for each combination, as previously described [23].

Benzylpenicillin, clindamycin, metronidazole, fusidic acid, doxycycline, moxifloxacin, linezolid and rifampicin were investigated. These specific antimicrobial agents (besides metronidazole which is an antibiotic primarily used for anaerobic infections) were chosen, as their mechanisms of action are likely to be effective against *P. acnes*. In addition, the antibiotics investigated can all be administrated orally, which is a great advantage compared to intravenous administration because of the extended treatment in this type of infection. In the synergy testing all of the antibiotics were combined with rifampicin, as this agent is active in biofilm and against stationary phase or sessile bacteria such as staphylococci [18].

The concentrations tested of the antimicrobial agents were 0.016–256 μg/ml for clindamycin, metronidazole, fusidic acid, doxycycline and linezolid. As for benzylpenicillin, moxifloxacin and rifampicin the range was 0.002–32 μg/ml. The calculations determining the FIC index and thereby the combined antimicrobial effect required conversions between these who concentration ranges. The FIC index values were interpreted as follows; a FIC index $\leq$0.5 indicated a synergistic effect, FIC index >0.5 and $\leq$1.0 an additive effect, FIC index >1.0 and $\leq$4.0 indifferent effect, and FIC index >4.0