Antibiotics for the eradication of *Propionibacterium acnes* biofilms in surgical infection

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**Objectives:** *Propionibacterium acnes* is increasingly recognized as a cause of delayed infection after spinal instrumentation or shunting for hydrocephalus. Biofilm development by this organism has recently been demonstrated. We therefore investigated the effect of two different courses of three antibiotics (penicillin, rifampicin and linezolid) on mature *P. acnes* biofilms *in vitro*. Outcomes were eradication or regrowth after withdrawal of antibiotics, simulating successful treatment and relapse.

**Methods:** *P. acnes* biofilms were grown on titanium discs for 6 days until mature, then exposed to the antibiotics for either 7 or 14 days before sonication and culture. Further, discs were similarly exposed, but after each course, they were reincubated for a further 9 days to check for regrowth.

**Results:** Penicillin, linezolid and linezolid plus rifampicin eradicated *P. acnes* biofilms after 14 days, but only penicillin had this effect after 7 days. ‘Relapse’ was prevented only by 14 day courses of penicillin or linezolid plus rifampicin, but not by linezolid alone.

**Conclusions:** For *P. acnes* spinal instrumentation infections, either penicillin or linezolid plus rifampicin might be equally effective. For shunt infections, as penicillin does not give therapeutic cerebrospinal fluid concentrations, rifampicin plus linezolid might be the treatment of choice. Linezolid alone appears not to be as effective as penicillin against *P. acnes* biofilms.

Keywords: orthopaedic implants, spinal instrumentation, hydrocephalus shunts, *P. acnes*

**Introduction**

*Propionibacterium acnes*, a major component of the skin commensal flora, is implicated in an increasing number of cases of biomaterials-related infection (BRI) involving arthroplasty, cerebrospinal fluid (CSF) shunts, spinal instrumentation and others.¹⁻⁵ This is partly due to the greater use of implantable devices and also to wider recognition of its involvement in infections associated with them. Such infections are notable for their delayed presentation after surgery, and Richards and Emara⁶ reported *P. acnes* from 52% of their delayed spinal infection cases with an average time from operation to presentation of 25 months (range 11–79). CSF shunt infections due to *P. acnes* are also usually delayed, typically presenting several years after shunt insertion.⁵⁻⁷ In those occurring in ventriculoatrial shunts, the delay can give rise to immune complex nephritis.⁸⁻¹⁰ There is now clear evidence that biofilm development by *P. acnes* is a key factor in both orthopaedic and neurosurgical infections.⁷,¹¹ In order to eradicate the infections, device removal is recommended. *P. acnes* is usually susceptible to a wide range of common antibiotics, although some, particularly in acne patients, have been found to be resistant to clindamycin, erythromycin and tetracyclines.¹² Despite their susceptibility, they are sometimes remarkably difficult to eradicate, and long courses are often recommended. In addition, where the implant is used as a structural stress-bearing component as in fracture repair or spinal instrumentation, it may be highly desirable to retain the implant until fusion is complete and to administer suppressive therapy until such time that the implant can be safely removed. In view of the biofilm formation and difficulty in eradicating the infections, we decided to investigate the effect of three antibiotics on mature *P. acnes* biofilms *in vitro* and to determine which gave the most promising results that might guide clinical practice. Benzylpenicillin is currently the drug of choice for *P. acnes* BRI.

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but we considered that linezolid, to which \( P.\) \( \text{acnes} \) strains have been shown to be susceptible,\(^{13}\) might be a more effective alternative, especially for neurosurgical infections in view of its effective CSF penetration. Rifampicin has been reported to have good anti-biofilm activity,\(^{14}\) but should not be used alone; therefore, it was tested in combination with linezolid to determine whether faster eradication could be achieved by this combination than with either penicillin or linezolid alone. Also, as a common feature of biofilm infections is that they relapse after the course of treatment has finished, we therefore determined which of the drugs/combinations gave the lowest ‘relapse’ rate.

**Methods**

A clinical isolate of \( P.\) \( \text{acnes} \) (NB692) from a hip arthroplasty infection was chosen as the test strain. This strain had previously been shown to be representative of the isolates in our collection. It was resurrected from frozen by culturing anaerobically on sheep blood agar (Oxoid, Basingstoke, UK) and was characterized by conventional methods including Gram stain and API Rapid ID 32A (bioMérieux, Marcy-l’Étoile, France). Antibiotic susceptibility testing by agar incorporation on Brucella blood agar (Oxoid)\(^{15}\) showed full susceptibility to all agents tested including penicillin (MIC 0.03 mg/L), rifampicin (MIC 0.0075 mg/L) and linezolid (MIC 0.94 mg/L).

**Antibiotics tested**

Penicillin G (Crystapen, Britannia Pharmaceuticals, Redhill, UK), linezolid ( Zyvox, Pharmacia, Milton Keynes, UK) and rifampicin (Rifadin, Aventis Pharma, West Malling, UK) were used to make stock solutions that were then stored at \( \sim 20^\circ \text{C} \). Final concentrations used were 12, 20 and 8 mg/L, respectively.

**Conditioning of biomaterial**

Titanium discs (6 mm diameter) were cut from a 0.25 mm sheet (Goodfellow, Cambridge, UK) and sterilized by autoclaving. They were then placed in thawed fresh frozen human plasma (National Blood Service, Sheffield, UK) on a rocker at \( 37^\circ \text{C} \) for 30 min for a plasma conditioning film to form.

**Bacterial adherence and biofilm development**

Each disc was then rinsed in sterile PBS and placed in a 2 mL microtube containing 1 mL of bacterial suspension made from an overnight culture of NB692 in anaerobe basal broth (ABB, Oxoid), diluted in ABB to give \( \sim 10^7 \) cfu/mL. The microtubes were then incubated at \( 37^\circ \text{C} \) for 1 h. Sixty tubes for each drug and 60 for control were prepared. Each disc was then aseptically rinsed in sterile 2% ABB to remove surplus bacteria and incubated for 6 days in 1.5 mL of full-strength ABB on a roller drum (LEEC, Cardiff, UK) at \( 37^\circ \text{C} \). \( P.\) \( \text{acnes} \) had previously been shown by experiment to develop mature biofilms under these conditions.\(^{7}\)

**‘Treatment’ phase**

After 6 days of incubation on the roller, each disc was aseptically transferred to a microtube containing 1.5 mL of fresh ABB, and the antibiotics were added to give the final concentrations required. The control discs were transferred to tubes containing ABB without antibiotic. All tubes were incubated at \( 37^\circ \text{C} \) on the roller drum. Each day, the ABB was aspirated and replaced with fresh ABB/antibiotic solution. One set of 4 \( \times \) 30 discs was ‘treated’ for 7 days and a second set for 14 days. After each ‘treatment’ period, the discs were transferred to 1 mL of 2% ABB and sonicated to detach any remaining biofilm. A 100 \( \mu \)L aliquot of each sonicate was spread onto sheep blood agar and incubated at \( 37^\circ \text{C} \) anaerobically for 6 days. Any growth was recorded quantitatively by colony counting. The detection limit of the assay was determined to be \( \sim 10 \) cfu/mL by experiment. The MIC for any bacteria surviving the exposure to antibiotics was determined anaerobically on Brucella blood agar (Oxoid) by agar incorporation.\(^{15}\)

**‘Relapse’ phase**

Two further sets of 30 discs for each antibiotic/combination and control, as in the ‘treatment’ phase, were prepared and exposed to antibiotics as above, for either 7 or 14 days. After this, they were transferred to 1.5 mL of ABB in microtubes and incubated in the absence of antibiotic on the roller drum for a further 9 days to check for regrowth (‘relapse’). They were then sonicated and cultured as described earlier.

**Statistical analysis**

Using SPSS V14 software, paired sample \( t \)-test was used to determine statistical significance.

**Results**

The results are shown in Table 1 and Figure 1. Culture of the samples following 7 days of ‘treatment’ with penicillin G showed that none of the samples grew \( P.\) \( \text{acnes} \), whereas 15 and 20 out of 30 samples grew \( P.\) \( \text{acnes} \) following 7 days of ‘treatment’ with the combination and linezolid alone, respectively, and the average counts were 970 and 2010 cfu/mL, respectively. None of the samples showed growth following 14 days of ‘treatment’ for each of the three regimens. However, in the ‘relapse’ series, 14 days of exposure to penicillin alone and to the combination of rifampicin and linezolid was necessary to prevent regrowth, whereas linezolid alone did not prevent regrowth even after 14 days of exposure. No change in MIC of any of the three antibiotics was seen after exposure as biofilms.

**Discussion**

In a laboratory study of 24 isolates of \( P.\) \( \text{acnes} \) from non-implant-associated intracranial infections, Moray et al.\(^ {13}\) showed that all were susceptible to penicillin, rifampicin and linezolid on conventional testing, but did not test them as biofilms. Our results show that penicillin is at least as effective as linezolid plus rifampicin and much more effective than linezolid alone against \( P.\) \( \text{acnes} \) biofilms. However, there are some limitations to such a study. The large standard deviations betray the considerable between-sample variation in controls and tests. This is inherent in studies of biofilms attached to biomaterials, mainly due to the ease with which parts of the biofilm detach during manipulation and rinsing. Despite this, the results are clear. In addition, the exposure to antibiotics was constant rather than pulsed, as would be found during clinical treatment. The concentrations used were based on the serum \( C_{\text{max}} \).\(^ {16,17}\) The results can therefore serve as a guide to choice of antibiotic for \( P.\) \( \text{acnes} \) biofilm infections, and they also...
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Table 1. Results of ‘treatment’ and the rate of ‘relapse’

<table>
<thead>
<tr>
<th>Control mean cfu/mL (±SD) [no. of samples showing growth]</th>
<th>Treatment mean cfu/mL (±SD) [no. of samples showing growth]</th>
<th>Relapse mean cfu/mL (±SD) [no. of samples showing growth]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>L+R</td>
</tr>
<tr>
<td></td>
<td>7 days</td>
<td>9 days</td>
</tr>
<tr>
<td>2.3 × 10^7 (±3.3 × 10^7) [30/30]</td>
<td>0 [0/30]</td>
<td>970* (±1800) [15/30]</td>
</tr>
<tr>
<td>3.3 × 10^7 (±4.1 × 10^7) [30/30]</td>
<td>0 [0/30]</td>
<td>0 [0/30]</td>
</tr>
</tbody>
</table>

Two terms of ‘treatment’ were used (7 and 14 days), each followed by 9 days of incubation to detect regrowth (‘relapse’). P, penicillin; L+R, linezolid plus rifampicin; L, linezolid alone. The mean count of bacteria (cfu/mL) growing from each group (control, P, L+R and L) is shown along with ± SD. The numbers of samples out of 30 in each case in which growth occurred are shown in square brackets. All assays were carried out in triplicate.

*Indicates statistically significant difference (P < 0.001) compared with relevant controls.

Suggest that a course of at least 2 weeks might be necessary to avoid relapse, although in practice, this might need to be lengthened further. However, from our results, it appears that penicillin alone is superior to linezolid and is equal to a combination of linezolid and rifampicin, leading to eradication without regrowth. The diagnosis of P. acnes BRI is usually delayed, often by at least several months, by which time the infection has become chronic. From what is known of BRI in other settings, it is highly likely that mature biofilms are well established by then. The published antimicrobial regimens for P. acnes BRI vary considerably. Hahn et al.1 writing of infection in spinal instrumentation recommended ‘non-specific wide-ranging parenteral therapy’ for a few days, followed by culture-directed oral therapy for up to 9 weeks. They used ciprofloxacin alone or combined with rifampicin or teicoplanin, or amoxicillin. Richards et al.6 used intravenous cephalosporin (usually cefazolin) for a mean of 6.5 days (2–14) followed by oral cephalosporin for a mean of 20 days (9–42). Clark and Shufflebarger18 recommended much shorter courses, but all recommendations of choice of antibiotic and length of course were linked to the need to remove all the spinal instrumentation. However, management of spinal instrumentation infections differs depending on whether they present early or late.19 In late infections, presenting more than ~18 months after surgery, where bony fusion has occurred, the instrumentation can be safely removed in most cases (although some loss of correction might occur). In early infections where fusion is incomplete, infection is managed by surgical debridement, with or without irrigation, and antibiotics. In such cases, the antibiotic therapy is often termed ‘suppressive’ as the hope is that the patient will remain asymptomatic for long enough for fusion to progress so that, if necessary, instrumentation can be removed. However, our results suggest that, where P. acnes is the cause, a shorter course might actually eradicate the infection. Either penicillin or linezolid plus rifampicin could be used, and the former is obviously preferable on grounds of cost and potential toxicity. In the case of CSF shunts, penicillin does not penetrate into the CSF in therapeutic quantities in low-grade infections, and the combination of linezolid plus rifampicin, both of which give therapeutic CSF concentrations in conditions of low inflammation, would be preferable. It is also interesting to note that the risk of thrombocytopenia from linezolid can be reduced by the addition of rifampicin.20 To the best of our knowledge, no attempt has yet been made to treat P. acnes shunt infections without shunt removal, although Thompson and Albright5 suggested that, in such cases, antibiotic courses might be considerably curtailed. If this were attempted, then our results suggest that a combination of rifampicin and linezolid (but not linezolid alone) could be used, with the option of oral administration.

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Transparency declarations
None to declare.

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