Original article

Determination of Antimicrobial Susceptibility Patterns between Different Strains of Propionibacterium Acnes

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Abstract

Propionibacterium acnes (P. acnes) isolated in infections of deep tissues was originally thought to be as a result of improper sterilization of skin and hence contamination of medical processes with this commensal/ normal flora of the skin. *P. acnes* has latter been identified as the principal agent responsible for the progression of some deep tissue diseases. The antimicrobial susceptibility patterns between different strains of *P. acnes* was determined. Ten (10) *P. acnes* clinical isolates of infections were considered, two (2) from acne vulgaris and eight (8) [two (2) per *recA* types 1A1, 1B, II and III] from lumber herniation tissues. Antibiotic susceptibility of some antibiotics was also determined and resistance to tetracycline was seen in 1 isolate, trimethoprim (1 isolate) and weak activity seen in 5 isolates. Heteroresistance to rifampicin was witnessed among 6 isolates. Upon four days of incubation, no inhibition of the bacterial growth by tetracycline in isolate 1(III). Isolate 17(1B) had no inhibition from trimethoprim. There was low inhibition of *P. acnes* by this antibiotic in isolates 71(IA1), 82(IB), 55(II), 1 (III) and 64(III). Amoxicillin and rifampicin created greater zones of inhibition among *P. acnes* isolates. Erythromycin was the next most inhibiting antibiotic with greater inhibition zones. Although Rifampicin had large inhibition zones, heteroresistance was observed among isolates 82(IB), 24(II), I (III), 17(IB), 55(II) and 71(IA1) as colonies were observed growing within the zones after 4 days of incubation. The antibiotics resistance expressed in the study were seen displayed among the lumber disc herniation isolates only with none involving acne vulgaris isolates. This study supports the theory that most *P. acnes* isolates in deep tissues exhibit higher rate of antibiotic resistance. *P. acnes* hence might be named the etiological agent of foreign-body infections like infections of indwelling medical devices.

<u>*Keywords:*</u> *Propionibacterium acnes, Inhibition zones, Heteroresistance, Antibiotic susceptibility, Strains.*

Introduction

Over a century ago, a patient suffering chronic skin disease ''acne vulgaris'' was the source of the initial *Propionibacterium acnes* (*P. acnes*) isolate. *Bacillus specie* and then *Corynebacterium species* were the initial misclassification of the bacterium *P. acnes* (Bojar and Holland, 2004). *Propionibacterium acne (P. acnes)* is a slow growing, non-spore forming, aero tolerant, anaerobic, non-motile, Gram- positive, lipohilic, pleomorphic rod which belongs to the phylum *Actinobacteria* and class *Propionibacteriales* (Patrick and McDowell, 2012).

P. acnes is a primary inhabitant and colonizer of the human skin, conjunctiva, gut, intestinal tract and external ear canal along with *Streptococcus*, *Corynebacterium*, *Staphylococcus* and *Pseudomonas species. P. acnes* is usually not pathogenic (Cogen *et al*., 2008; Portillo, 2013).

P. acnes can be cultivated under anaerobic-to microaerophilic conditions using different culture media, (Patrick and McDowell, 2013) and a circular, opaque, about 1 to 2 mm in diameter and typically glistening are the characteristics of *P. acnes* colonies

grown on blood agar plates. (Jousimies-Somer *et al*., 2002), growing better in anaerobic conditions but also possessing the ability to grow in aerobic conditions. The average detection time of *P. acnes* through blood cultures are about 6.4 days anaerobically and 6.1 days aerobically (Gunthard *et al*., 1994). Tissue cultures on the other hand needs more time for *P. acnes* to grow and therefore are incubated for 10 to 14 days (Schafer *et al*., 2008: Larson *et al*., 2012). For *P. acnes* to be considered an infections etiological agent, more than a single sample from blood culture has to read positive for *P. acnes* with the same isolate when distinguishing between a bloodstream infection and a skin contamination (Achermann *et al*., 2014).

Bruggemann *et al*., in 2004 was able to sequence the complete genome of *P. acnes* using the putative haemolysin and repair and maintenance (*recA*) of DNA genes. *P. acnes* strains from different infections were grouped into three types namely I, II and III using the whole genome sequencing (McDowell *et al*., 2008). This *P. acnes recA* gene DNA sequence analysis revealed the phylogenetically unique clusters/linage between the types I and II genotypes. The phylogenetic type III exhibited a difference in cell morphology and cell surface antigen (McDowell *et al*., 2008).

These different types of *P. acnes* express different inflammatory properties and virulence factors (McDowell *et al*., 2005; Mc Dowell *et al*., 2008). A study by McDowell *et al*. (2008) used immunofluorescence microscopy and monoclonal antibodies (QUBPa1 and QUBPa2) which are precise to types I and II *P. acnes* to associate these two *recA* types with different infections. An approximately equal type I and type II strains population was recorded from detailed examination of isolates (*P. acnes*) found directly from prosthetic hip implants. Type I predominated isolates from related tissue and bone samples. Patient's surgical incision sites (skin), acne vulgaris and dental infection *P. acnes* isolates were found also to be predominated by type I. It was observed that the prosthetic hip implant had obvious type II isolates population (McDowell *et al*., 2008). Type III is associated with surgical excised spinal disc material (McDowell *et al*., 2008). The types IA and IB sub-strains within *rec A* type I was identified using the *P. acnes recA* sequence analysis (Valanne *et al*., 2005). The whole genome sequencing and multilocus sequence typing (MLST) has been used to expand the above classification of *P. acnes* to types: IA_1, IA_2, IB, IC, II and III which the clinical and phenotypic levels of *P. acnes* back up (Mc Dowell *et al*., 2012).

The pathogenesis of the chronic skin infection acne vulgaris was thought to be associated directly to *P. acnes* but this has brought so much controversies in the science community due to its inability to satisfy Koch's postulate (Bojar and Holland, 2004: Segre, 2013).

As early as the 1980's, studies had suggested a relationship between the development of resistance by *P. acnes* to tetracycline, clindamycin, erythromycin and trimethoprim-sulfamethoxazole and the use of these antibiotics therapeutically (Eady *et al*., 1988: Eady *et al*., 1993: Cunliffe, 1995). The antibiotics resistance of *P. acnes* has been slowly rising from about 35% in 1991 to 60% in 1996 and this peaked up to 64% in 1997 (Eady, 1998: Coates *et al*., 2002). Acne vulgaris is a chronic multifactorial disorder of the skin connected to a lot of pilosebaceous follicle factors and of the lipid rich sebaceous gland (Grange *et al*., 2009). Treatments of acne vulgaris acts through a mechanism of interference with the growth and metabolism of *Propionibacterium* (Oprica *et al*., 2004). A characteristics of acne vulgaris that questions *P. acnes* role as the disease's etiological agent is the incomplete response derived from the therapeutic options used which includes topical or systemic antimicrobial therapy to reduce bacteria load (Achermann *et al*., 2014). This antibiotic treatment failure has been related to the development of resistant strains in clinical isolates among patients (Leyden *et al*., 1983). This may be also due to biofilm intervening tolerance by bacteria (Ross *et al*., 2003). Analysis of some clinical trials carried out in the last two decades with the greatest evidence for the relationship between *P. acnes* and acne vulgaris showed decrease in the efficacy of erythromycin and clindamycin topical treatment in acne vulgaris. The production of resistant *P. acnes* strains was preceded by the decline in efficacy (Eady *et al*., 1989: Simonart and Dramaix, 2005). After treatment failure, recurrence of inflammation is witnessed supporting the theory of *P. acnes* not being the lone player in the pathogenicity of acne vulgaris since the specific antibiotic in therapeutic use might not be effective against other species of bacteria (Leyden, 2001).

Materials and Methods

Eleven (11) *P. acnes* isolates (10 clinical infection isolates and NCTC 737 a type I reference strain, {biotype 3}) were selected at random for this experiment, with no two (2) samples coming from the same source (patient). The sample size covered two (2) isolates per *recA* genotype ($1A_1$, 1B, II, III). Lumber disc herniation provided eight (8) of the isolates while two (2) came from acne lesions. The used isolates were 71($1A_1$), 84($1A_1$), 17(IB), 82(IB), 24(II), 55(II), 1(III), 64(III), acne lesion 1, acne lesion 7 and NCTC 737.

Chemicals/Reagents: Mueller- Hinton agar, Brain heart infusion agar and broth, purchased from Oxoid, United Kingdom. Antibiotics used were Ciprofloxacin 5 µg, Rifampicin 5 µg, Trimethoprim 5µg, Erythromycin 30 µg, Amoxicillin 25µg, Vancomycin 5 µg, Tetracycline 30 µg and Fusidic acid 10µg (Eucast 2016).

Materials: petri dishes, anaerobic incubator, spreader, centrifuge, Elisa auto reader (Tecan, sunrise plate reader, digital vernier caliper.

Overnight cultures preparation
Brain heart infusion agar (BHI) and broth (Oxoid) was prepared using the Manufacturer's prescription. Plates of BHI were used to anaerobically grow *P. acnes* cultures (by streaking) and incubated at 37°C for 4 days (96 hours). Overnight cultures were made from these pure *P. acnes* colonies by anaerobically growing them in sterile broth at 37°C.

Disc diffusion assay (EUCAST)
Mueller-Hinton agar and Brain Heart infusion agar were inoculated with suspended *P. acnes* isolates from overnight culture standardised to an optical density of 0.1 at a wave length of 590nm with a colorimeter (Corning 253) by centrifugation and addition of sterile broth using the spread plate technique. The desired antibiotics disc was placed on the agar plates within 15mins of inoculation and incubated within 15mins. NCTC 737 was used as positive control. Plates were inverted and incubated anaerobically at 37°C for 4 days (96 hours). The diameter of zones of inhibition formed was measured to the nearest millimetre using a digital vernier calliper.

Statistics
Completely Randomized Design (CRD) as described by Gomez and Gomez (1984) was used with three replication and data obtained were analyzed statistically by Analysis of Variance (ANOVA) according to Gomez and Gomez (1984).`

Result

Antibiotics disc diffusion assay
After four (4) days of incubation, the result obtained showed that there was no inhibition of the bacterial growth by tetracycline in isolate 1(III). Isolate 17(1B) had no inhibition from trimethoprim. There was low inhibition of *P. acnes* by this antibiotic in isolates 71(IA1), 82(IB), 55(II), 1 (III) and 64(III). Greater zones of inhibition found among isolates of *P. acnes* were from the antibiotics amoxicillin and rifampicin. Erythromycin was the next most inhibiting antibiotic with greater inhibition zones (figure 1). Although Rifampicin had large inhibition zones, heteroresistance was observed among isolates 82(IB), 24(II), I (III), 17(IB), 55(II) and 71(IA1) as colonies were observed growing within the zones after 4 days of incubation.
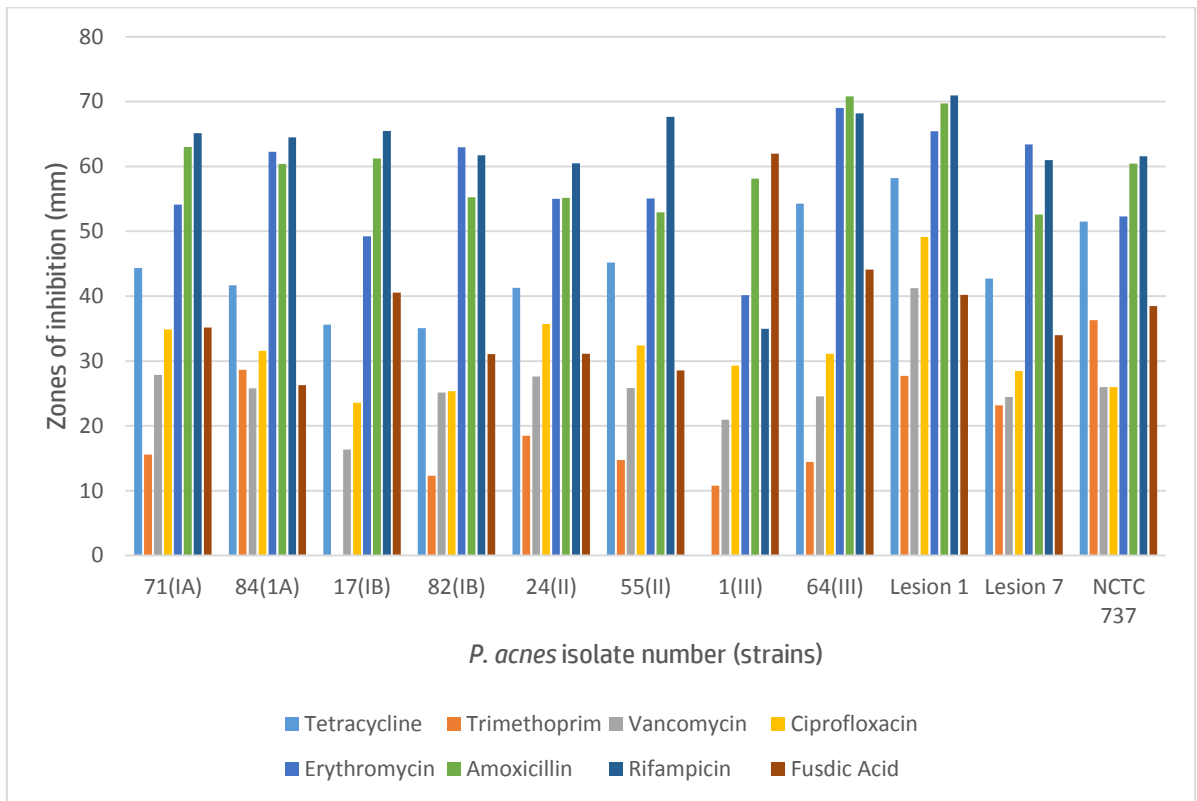
Figure 1: Zones of inhibition of different P. acnes isolates to a range of antibiotics on incubation for 4 days.

Table 1: Anova: Single Factor

SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Row 1	8	340.02	42.5025	303.3514		
Row 2	8	341.1	42.6375	292.6958		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.0729	1	0.0729	0.000245	0.987742	4.60011
Within Groups	4172.331	14	298.0236			
Total	**4172.403**	**15**				

P is 0.988 which is greater than 0.05 this shows there is a significant difference in the effect of the different antibiotic treatments given to the samples.

Discussion

The statistical analysis used showed that there was a significant difference between the results obtained from the antibiotics used for this study. The study showed resistance in trimethoprim and tetracycline, which was only observed among lumber disc herniation and not an acne lesion isolates. These are commonly used antibiotics. Amoxicillin/ penicillin, clindamycin or linezolid/rifampicin are the most available current treatments for postsurgical or prosthetic *P. acnes* infections (Jakeb *et al*., 1996; Bayston *et al*., 2007: Nisbet *et al*., 2008). The role of antibiotics in the future therapy of back pain (Albert *et al*., 2008; Albert *et al*., 2013) was suggested by the isolation of *P. acnes* at the site of raptured disc as shown by Rollason *et al*., 2013. Although present back pain treatments focuses on controlling pain, physiotherapy and surgery (Rollason *et al*., 2013). Relating to the intensity of low back pain, a positive effect was observed in chronic back condition patients upon treatment with amoxicillin-clavulanate (Albert *et al*., 2008: Albert *et al*., 2013). This is similar to the result of this study, although amoxicillin was not used in combination, it exhibited a very high level of bacterial inhibition. The inhibition zones created by Amoxicillin can be questioned because it was 4 to 5 times more

concentrated than the other antibiotics. The demonstration that anaerobic bacteria infected lumber disc have a higher possibility of developing modic changes and low grade discitis (Albert *et al*., 2013), and the evidence of the isolation of *P. acnes* from excised disc herniation tissue (Rollason *et al*., 2013) supports the theory of bacterial infection having an important role to play in this condition's development. Prolonged use of erythromycin and tetracycline for the treatment of acne vulgaris has led to the emergence of resistant *P. acnes* strains (Leyden *et al*., 1983; Eady *et al*., 1988; Kurokawa *et al*., 1999; Ross *et al*., 2003: Oprica *et al*., 2004). Although resistance to erythromycin was not witnessed in this study, which might be due to the fact that most of the clinical isolates used were from lumber disc herniation. Resistance to tetracycline was observed in one *P. acnes* isolate studied, which was also witnessed by Oprica *et al*. (2004). Resistance to trimethoprim was also observed and low/weak inhibition was seen in some isolates, this was also reported by Dessinioti and Dreno, 2015 and is similar to the findings of Oprica *et al*., 2004. Over the years, multiresistance has been observed to originate from isolates of acneic skin (McDowell *et al*., 2012). In contrast, all type IA1 isolates of *P. acnes* from disc tissues used in this study were sensitive to both erythromycin and tetracycline.

Development of resistance by *P. acnes* to the antibiotic rifampicin has been reported by many researchers (Zappe *et al*., 2008; Furustrand *et al*., 2015). Furustrand, Trampuz and Corvec, 2013 described the molecular mechanism of resistance by rifampicin in *P. acnes* mutants which is due to point mutation in the *rpoB* gene. Antimicrobial combinations which includes rifampicin is known to penetrate bacteria biofilms in *Staphylococci* implant associated infections and also has a positive effect on the bacteria in stationary phase (Zimmerli *et al*., 1998: Zimmerli *et al*., 2004). In 2012, in vitro studies a foreign-body infection animal model were used to test the activity of rifampicin against *P. acnes* biofilms (Furustrand *et al*., 2012). Heteroresistance of *P. acnes* to antibiotics was also seen in this study in rifampicin with bacteria colonies growing in cleared zones although it was only observed in isolates from lumber disc herniation tissues only. There have been great studies done in the combination therapy of rifampicin with other antimicrobial agents to prevent emergence of rifampicin resistant strains due to monotherapy (Forrest and Tamura, 2010: Khassebaf *et al*., 2015). The inhibition zones created by rifampicin in the study can also be questioned because it was 4 to 5 times more concentrated than other antibiotics. The antibiotics resistance expressed in the study were seen displayed among the lumber disc herniation isolates only with none involving acne vulgaris isolates. The susceptibility of *P. acnes* planktonic cells is higher than sessile cells to antimicrobial agents and this has been reported (Ramage *et al*., 2003).

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