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Antimicrobial susceptibility of strains of *Propionibacterium acnes* isolated from inflammatory acne

Walter Gübelin,*,** M. Angélica Martínez,*** M. Teresa Molina,* Solange Zapata,* M. Eugenia Valenzuela****

ABSTRACT. In the last decade, a significant increase in the antimicrobial resistance of clinical specimens of *Propionibacterium acnes* to first line antibiotics used for acne treatment, has been informed in Europe and in the USA. No information about strains isolated from Latin-American countries is available. The antimicrobial susceptibility of 53 strains of *P. acnes* isolated from skin specimens of inflammatory acne patients, at the clinical Hospital University of Chile was tested. All isolates were susceptible to penicillin, minocycline, and nadifloxacin. Erythromycin and clindamycin resistance was found in 3.8 and 1.9% isolates respectively. Resistance to lymecycline was observed in one isolate, which was intermediate to tetracycline and doxycycline.

Key words: Antimicrobial susceptibility, *Propionibacterium acnes*, inflammatory acne.

RESUMEN. En la última década se ha informado un aumento significativo en la resistencia de *Propionibacterium acnes* a antimicrobianos de primera línea para el tratamiento del acné, en Europa y Estados Unidos. No existe información sobre la susceptibilidad antimicrobiana de cepas de este microorganismo aisladas en Latinoamérica. Se determinó la susceptibilidad antimicrobiana de 53 cepas de *P. acnes* aisladas de lesiones de piel de pacientes con acné inflamatorio, atendidos en el Hospital clínico de la Universidad de Chile. Todas las cepas fueron susceptibles a penicilina, minociclina y nadifloxacina, observándose resistencia a eritromicina y clindamicina en 3.8 y 1.9% cepas respectivamente. Una cepa fue resistente a limeciclina, pero intermedia a tetraciclina y doxiciclina.

Palabras clave: Susceptibilidad, *Propionibacterium acnes*, acné inflamatorio.

Propionibacterium acnes is an anaerobic, non-motile, non-sporulating Gram-positive, bacillus, found as part of the cutaneus comensal microbiota.[1] It is frequently isolated as contaminant in clinical specimens, but it is also found as primary pathogen in patients with predisposing factors, specially foreign-body implants, diabetes, and previous surgery.[2,3] Severe clinical syndromes include sepsis, neurosurgical infections, endocarditis, arthritis and endophtalmitis.[2,3] The role of *P. acnes* in the etiology of inflammatory acne is widely accepted.[1,4] Acne is a common skin disease, affecting mainly adolescents and young adults, worldwide. The disfiguring skin sequelae which sometimes accompany this disease may have an important impact in the psychological health of young people.[5] The treatment of acne involves the empirical use of antimicrobials. However, in the last years several studies have documented the increase in the prevalence of antimicrobial resistance, particularly to macrolides, clindamycin and to the tetracyclines.[6-8] The objective of this study was to determine the antibiotic susceptibility of strains of *P. acnes* strains isolated from patients with inflammatory acne.

* Department of Dermatology, Faculty of Medicine, University of Chile.
** Department of Dermatology, Faculty of Medicine, University of Los Andes.
*** Program of Microbiology, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile.
**** National Health Institute of Chile.

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Between March and October 2001, 53 strains of *P. acnes* obtained from skin specimens of patients with inflammatory acne, presenting at the clinical hospital of the University of Chile were studied. Patients had not received antibiotics in the previous 3 weeks, nor have been treated for acne before enrolment, and gave informed consent for taking clinical specimens. Strains were grown on sheep blood agar plates supplemented with hemin (5 μg/ml) and vitamin K (0.5 μg/ml), in an anaerobic system (Oxoid, Ltd., United Kingdom) at 36ºC for 96 h. Strains were identified based on conventional criteria including cell and colonial morphologies, production of catalase and indole, and nitrate reduction. The organisms did not hydrolyzed esculin or urea, and did not fermented maltose or sucrose.

Antimicrobial susceptibility testing was performed by the NCCLS reference agar dilution method by using brucella agar (Difco Laboratories, Detroit, Mich.) supplemented with 5% lysed horse blood.[9] Standard powders were obtained from the following manufacturers: penicillin G, Laboratorio Chile, erythromycin and tetracycline, Sigma Chemical (St. Louis, Mo.), doxycycline and minocycline, Pfizer Inc., clindamycin, Pharmacia Upjohn, nadifloxacin and lymecycline, Laboratorio Galderma, Chile. The inocula were prepared by suspending colonies from 48 h culture plates in brucella broth to achieve a density equivalent to a No. 0.5 McFarland standard. A Steers replicator was used to deliver a final inoculum of 10^5 cfu per spot. The plates were incubated in an anaerobic jar at 36ºC for 48 h. The MIC values were read visual-

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ly as the lowest concentration of the antimicrobial agent that prevented visible growth. Control strains included *Bacteroides fragilis* ATCC 25285 and *Eubacterium lentum* ATCC 43055.

The antimicrobial susceptibilities of 53 isolates of *P. acnes* to 8 antibiotics is shown in Table 1. In the present study, which is the first to report of data on the antimicrobial susceptibility of *P. acnes* in Chile, all of the isolates were susceptible to penicillin, minocycline, and nadifloxacin. Two (3.8%) strains were resistant to erythromycin, and one (1.9%) to clindamycin. One isolate was resistant to lymecycline, and intermediate to tetracycline and doxycycline.

P. acnes is naturally susceptible to various antimicrobial classes including, β-lactams, macrolide, lincosamide, quinolone, tetracycline's and aminoglycoside.[1,4,7] Furthermore, there are numerous topical and or systemic options available for the treatment of acne, being macrolide and tetracycline's usually the first-line antimicrobials[1,4,7] Nevertheless, since the 1970s antimicrobial resistance has gradually accumulated in cutaneus isolates of this organism, especially in European countries, with 51 to 94% of strains showing some antibiotic resistance.[8] Erythromycin resistance is the most common antimicrobial resistance detected in *P. acnes,* with rates ranging between 17.1 to 52%.[6-8] Furthermore, up 91% of the macrolide resistant strains present combined resistance with clindamycin.[8] Antimicrobial resistance to tetracyclines is lower than that to macrolides, affecting 0 to 26% of isolates.[7,8] In this study, minocycline was as active *in vitro* as tetracycline and doxycycline, but no isolates resistant to minocycline were found. Minocycline has also been rarely associated with *P. acnes* resistance in other studies, but can occasionally lead to potentially serious adverse effects.[4] Lymecycline is a new tetracycline as effective as minocycline for the treatment of moderately severe acne, and which has been associated with fewer adverse gastrointestinal and dermatological effects than minocycline.[11]

Table 1. Susceptibility of 53 strains of *Propionibacterium acnes* to 8 antimicrobial agents.

Antimicrobial Agent	MIC_{50} (mg/l)	MIC_{90} (mg/l)	Range (mg/l)	% Susceptible
Penicillin G	0.03	0.03	≤ 0.03-2	100
Erythromycin	0.03	0.03	≤ 0.03-32	96.2
Clindamycin	0.03	0.03	≤ 0.03-32	98.1
Lymecycline	0.25	1	0.06-16	98.1
Tetracycline	0.06	0.06	0.03-8	98.1
Doxycycline	0.06	0.06	0.06-8	98.1
Minocycline	0.03	0.03	0.03-1	100
Nadifloxacin	0.03	0.06	≤ 0.03-0.12	100

MIC_{50} and MIC_{90}, MICs at which 50 and 90% of strains, respectively, are inhibited.

Nadifloxacin is a topical fluorquinolone, which acts through its bactericidal action as well as by the suppression of neutrophil chemotaxis and superoxide formation.[10] In our study, nadifloxacin demonstrated to be highly active *in vitro* against skin isolates of *P. acnes,* with MICs ≤ 0.12 μg/ml. Other study has also noted a good activity of this quinolone against this organism.[12] However, our MIC values were lower than that reported by these authors.[12]

Several strategies have been proposed to prevent the development of antibiotic resistance that accompanies antimicrobial treatment of *P. acnes.*[1,4,7] They include restrictions in the overall use of antibiotics, avoiding use of systemic antibiotics, and long term therapies. It is highly advisable not to use concomitantly topical and systemic antibiotic therapies, but instead to combine use of oral antibiotics with local retinoids.[1,4,7] Last drugs improve the vascularization and benefits further therapy with their anti-inflammatory effect.[4] Topical antibiotics are not intended as first line therapy, and if used should be prescribed with a topical nonantibiotic medication, for example benzoyl peroxides in alternation with retinoids.[4,7] Along with, patients may be sufficiently informed about the dose, duration and form of administration of the treatment, like also the importance of therapy adherence to prevent the selection of resistant strains may be emphasized. Considering the antimicrobial pattern of our *P. acnes* isolates, as well as the pharmacokinetic characteristics of the drugs, the antibiotics of election for the oral treatment of acne are in order doxycycline, minocycline, and tetracycline. Lymecycline is a good therapy choice for acne, but it is not available in our country. Erythromycin is a good alternative for pregnant women and for patients that does not respond or that present or display allergy to former antibiotics. We do not recommend the prescription of quinolone for topical nor systemic use. Nevertheless, we have included them in our study since they are used in some countries.

In conclusion, our results indicate that most *P. acnes* strains isolated in our country remain susceptible to antimicrobial agents commonly used in the treatment of inflammatory acne, as well as to new alternatives.

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Correspondence to:

M. Angélica Martínez
Suecia 1524 Departamento 403
Santiago, Chile
Phone:(562)9786639-9786296
Fax: (562)7355855
E-mail:mamartin@med.uchile.cl