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Susceptibility profiles of *Propionibacterium acnes* isolated from patients with acne vulgaris

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ABSTRACT

The wide prescription of antibiotics in patients with acne vulgaris has generated the concern of emergence of antibiotic-resistant *Propionibacterium acnes*. To study the susceptibility profiles of *P. acnes* isolated from patients with acne vulgaris, 90 isolates were isolated from sebum collected from lesions of 191 patients. Susceptibilities to amoxicillin, minocycline, erythromycin and clindamycin were studied by the Etest method. Thirty-four isolates (37.8%) were resistant to both erythromycin and clindamycin, whereas another four isolates (4.4%) were resistant to all four tested antimicrobials. All resistant isolates to any of the tested antimicrobials had very high minimum inhibitory concentrations (>256 μg/mL). Among all analysed host factors, only history of oral treatment with macrolides and/or clindamycin within the last 2 years was independently associated with the acquisition of resistant *P. acnes* (odds ratio = 5.573; $P = 0.001$). The present results provide useful information to guide antimicrobial prescribing strategies in acne. Any information for past exposure to macrolides or clindamycin should suggest avoidance of prescription of these antibiotics. Tetracyclines and amoxicillin are the suggested solutions for these patients. In contrast, lack of history of exposure allows the safe prescription of macrolides and clindamycin.

1. Introduction

Acne vulgaris is a common skin disorder; 15–20% of people present moderate to severe acne between the ages of 15–20 years [1]. This often leads to severe psychological problems and the need to alleviate symptoms.

The pathogenesis of acne is a multifactorial process; dietary habits, genetic factors, hormonal disturbances and alterations of the normal skin flora contribute to the development of moderate to severe disease [2]. Colonisation of the skin by *Propionibacterium acnes* is largely recognised as a salient factor in the pathogenesis of acne. *P. acnes* is a Gram-positive anaerobic rod that stimulates keratinocytes for the production of pro-inflammatory mediators resulting in the development of the inflammatory macules and pustules of acne. To this end, long-term antibiotic treatment either with topical preparations or with orally administered antimicrobials is part of the therapy of moderate to severe acne.

Traditionally used antimicrobial agents for the management of acne are tetracyclines, macrolides and clindamycin. One major concern is whether long-term treatment may lead to the emergence of resistance by *P. acnes* and to subsequent treatment failures. More and more recent reports appear to reveal this problem [3–5].

The present study was conducted in Greek patients. Greece is a country with significant emergence of resistance owing to the less severe restrictions of antibiotic prescription compared with other European countries. As a consequence, resistance to broadly prescribed antimicrobials such as macrolides is steadily increasing [6]. To this end, a study of the susceptibility profiles of *P. acnes* in Greek patients with acne vulgaris was conducted in order to unravel the problem of emerging resistance. Moreover, any linkage between resistance emergence and the case history of the patient that may provide helpful guidance for future therapies was investigated.

2. Patients and methods

In a prospective study conducted during the period September 2009 to January 2011, the relationship between colonisation by *P. acnes* and carriage of single nucleotide polymorphisms of Toll-like

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receptor-4 in a population of 191 patients with acne vulgaris was investigated [7]. Sebum was collected from the lesions of these patients with acne vulgaris, and *P. acnes* was isolated from 90 patients; these isolates were used for the present study. The study protocol was reviewed and approved by the Ethics Committee of ATTIKON University General Hospital (Athens, Greece). Patients provided written informed consent before study enrolment. Acne vulgaris was defined by the following criteria [8]: (a) disease onset by early adolescence and (b) typical comedones, papules, nodules, cysts or sinus tracts involving the face, neck, chest or back. When these lesions coalesced to form sinus tracts and to involve the entire face, acne conglobata was diagnosed.

For every patient, a complete questionnaire was completed including the following information: (a) age and sex; (b) presence of acne conglobata; (c) history of isotretinoin administration; and (d) history of topical or oral antibiotic intake the last 2 years.

To isolate *P. acnes*, one sample of sebum was collected over at least three affected skin areas with separate wet sterile swabs. For patients with acne conglobata, the sample was collected from the deepest area possible from at least three lesions. The swabs were transported immediately for culture under anaerobic conditions on Columbia sheep blood agar (Becton Dickinson, Cockeysville, MD) at 37 °C (Thermo Scientific Forma Incubator; Thermo Scientific, London, UK). Anaerobic bacteria were identified by colony morphology, Gram stain and positive indole spot test (DMACA-Indole; Becton Dickinson). Further identification was done by the Rapid ID 32A gallery (bioMérieux, Paris, France).

Susceptibilities of the isolates to amoxicillin, minocycline, erythromycin and clindamycin was determined by the Etest method following incubation of each isolate on *Brucella* agar plates supplemented with 5% horse blood with antibiotic-enriched strips (BBL Microbiology Systems, Cockeysville, MD). Incubation was done under an anaerobic atmosphere in jars (BBL GasPak™ System; BBL Microbiology Systems) for 48 h at 37 °C. The minimum inhibitory concentration (MIC) was considered as the lowest concentration around which a visible rim of inhibition of bacterial growth was achieved. Resistance to the studied antibiotics was defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints [9].

Comparison of qualitative characteristics between carriers of susceptible and resistant isolates was done by Fisher's exact test. Age was expressed as the mean ± standard deviation and comparison between carriers of susceptible and resistant isolates was done by Student's *t*-test. Clinical factors with a statistically significant difference between non-carriers and carriers of resistant isolates were entered into stepwise forward logistic regression analysis. Odds ratios and 95% confidence intervals were determined. Any *P*-value < 0.05 was considered statistically significant.

3. Results

The susceptibility profiles of the 90 *P. acnes* isolates to the four tested antimicrobials are shown in Table 1. Thirty-four (37.8%) of the isolates were resistant to both erythromycin and clindamycin, whereas another four isolates (4.4%) were resistant to all four tested antimicrobials. The detailed distribution of MICs of each of the four tested antibiotics is shown in Fig. 1. It is striking that all resistant isolates to any of the tested antimicrobials have very high MICs (>256 μg/mL).

Table 1
Susceptibility profile of 90 *Propionibacterium acnes* isolates to four antimicrobials.

Antimicrobial	MIC (μg/mL)			% inhibited[a]
	Range	MIC_{50}	MIC_{90}	
Amoxicillin	≤0.016 to >256	≤0.016	0.047	95.6
Minocycline	≤0.016 to >256	≤0.016	≤0.016	95.6
Erythromycin	≤0.016 to >256	0.047	>256	57.8
Clindamycin	≤0.016 to >256	0.032	>256	57.8

MIC, minimum inhibitory concentration, $MIC_{50/90}$, MIC required to inhibit 50% and 90% of the isolates, respectively.
[a] At the following breakpoints: amoxicillin, erythromycin and clindamycin, 4 μg/mL; and minocycline, 2 μg/mL.

Since the major finding was that 38 of all studied isolates were resistant to at least erythromycin and clindamycin, it was investigated which host-associated factors may be related to acquisition of resistance. A comparison of clinical characteristics between carriers of susceptible isolates and carriers of resistant isolates is shown in Table 2. Only the presence of acne conglobata and a history of oral intake of macrolides or clindamycin in the last 2 years were more frequent among carriers of resistant isolates than carriers of susceptible isolates. These two variables were then entered into logistic regression analysis revealing that only a history of oral treatment with macrolides and/or clindamycin was independently associated with the acquisition of resistant *P. acnes* (Table 3).

4. Discussion

The current findings confer clinically very useful information on the susceptibility profiles of *P. acnes* that can guide the management of patients. Isolates remain extremely susceptible to amoxicillin and minocycline. In contrast, 42.2% of all isolates are highly resistant to both erythromycin and clindamycin. Analysis indicated that the only factor related to resistance to any of these two antimicrobials was oral intake of macrolides and/or clindamycin during the last 2 years. Isolates from patients not previously exposed to macrolides or clindamycin remain fully susceptible.

Several studies have been published over the last years describing the susceptibility patterns of *P. acnes* [3–5]. The number of studied isolates varied between 80 and 90. Originally, *P. acnes* is highly susceptible to penicillins, macrolides, tetracyclines and clindamycin [10]. Emergence of resistance has been reported at the end of a treatment course of 2–6 months with tetracyclines, macrolides and clindamycin. In these cases, isolates usually become resistant to one single antibiotic or cross-resistant to several antibiotics. Resistance patterns comprise both isolates with moderately increased MICs and isolates with very high MICs [5–8]. The current findings suggest that the situation in Greece differs in many aspects: (a) resistance may emerge even as long as 2 years after the end of a treatment course and (b) isolates resistant to one single antimicrobial agent do not exist. The existing resistance pattern involves cross-resistance to erythromycin and clindamycin. This predominating cross-resistant phenotype is characterised by very high MICs of both erythromycin and clindamycin. This is indicative of the macrolide–lincosamide–and streptogramin B (MLS_B) resistance phenotype usually linked with ribosomal mutations [11]. This probably explains why past exposure to macrolides or clindamycin confers high-level resistance to both compounds.

Statistical analysis of factors related to the emergence of resistance indicates a lack of association between topical exposure to clindamycin and the emergence of resistance. This is in contrast to a recent survey on 80 isolates of *P. acnes* showing that topical application of erythromycin and clindamycin is a risk factor for carriage of *P. acnes* resistant to erythromycin and clindamycin, respectively [11]. However, the current finding of past history of oral antibiotic intake as the only risk factor related to carriage of resistant isolates derived from a stepwise approach ending in logistic regression analysis. This approach has never been described by any other author.

The present results provide useful information to guide antimicrobial prescribing strategies in acne. It is evident that