

Antibiotic Sensitivity of *Propionibacterium acnes* Isolates from Patients with Acne Vulgaris in a Tertiary Dermatological Referral Centre in Singapore

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Abstract

Objectives: To study the minimum inhibitory concentrations (MICs) of *Propionibacterium acnes* (*P. acnes*) isolates to selected antibiotics from patients with acne vulgaris in Singapore and determine if resistance increases with prolonged use of antibiotics. **Design:** A single-centre prospective study. **Setting:** Tertiary dermatological referral centre in Singapore. **Patients:** One hundred and fifty patients with acne vulgaris seen at the National Skin Centre. **Results:** In patients who had never been on antibiotics, there were no resistant isolates of *P. acnes*. In patients who had been on short-term antibiotics (between 6 to 18 weeks), there were 2 resistant strains among the 34 isolates (6.25%); in patients who had been on antibiotics for longer periods, there were 11 resistant strains among the 51 isolates (21.6%). The differences in the rates of isolation of resistant strains between patients who had not been on antibiotics to those that had been on long-term antibiotics were statistically significant ($P = 0.015$). There was also a significant difference in isolation of resistant strains from those on short-term antibiotics compared to those who had been on long-term antibiotics ($P = 0.036$). Resistance to erythromycin was most commonly encountered. Most of the erythromycin-resistant strains also showed cross-resistance to clindamycin. The average MICs to antibiotics such as minocycline, erythromycin and clindamycin in those on long-term antibiotics were significantly higher when compared to patients who had not been on antibiotics. **Conclusions:** Antibiotic resistance in *P. acnes* isolates in Singapore follows similar patterns to studies conducted in Europe. Resistance to erythromycin was most commonly seen, and this is associated with cross-resistance to clindamycin. Among the tetracycline group of drugs, the average MICs to tetracycline was higher than that for doxycycline, which in turn was higher than that for minocycline. Antibiotic resistance can occur with short-term antibiotic courses, and the rate of resistance increases as the duration of antibiotic consumption increases.

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Key words: Bacterial resistance, Erythromycin, Tetracycline

Introduction

In Southeast Asia, there have been few studies on the emergence of antibiotic-resistant strains of *Propionibacterium acnes* on the skin of patients with acne vulgaris. We previously reported the effect of short courses of antibiotics on the minimum inhibitory concentrations (MICs) of *P. acnes*.¹ There was a trend towards higher average MICs, as well as an increase in the rate of isolation of resistant strains of *P. acnes*, in patients who had been on antibiotics for a period ranging from 6 to 18 weeks compared to acne patients who had not been previously treated with antibiotics.

In this present study, we compared the MICs of *P. acnes* with some commonly used antibiotics in 3 groups of patients with acne vulgaris: the first (group A) that had received no prior antibiotic treatment, the second (group B) that had received a short course of antibiotics ranging from

6 to 18 weeks in the past 6 months, and the third (group C) who had been on systemic antibiotics for a period ranging from 24 weeks to 52 weeks.

Patients and Methods

One hundred and fifty patients with acne vulgaris seen at a tertiary dermatological referral centre in Singapore were recruited. The skin was first wiped with 70% alcohol, and material from 20 closed comedones was extracted and cultured on Colistin Nalidixic Agar (Becton Dickinson, Cockeysville, Md.) anaerobic blood culture plates with thioglycolate-enrichment broth. Plates were incubated under anaerobic conditions and examined at 48 hours and, if there was no initial growth, reincubated up to 5 days with subcultures of the enrichment broth. *P. acnes* was identified by a commercial kit for anaerobes (API 20A; Biomerieux

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Vitek Inc, Lyon, France), which uses a system of microtubules containing dehydrated substrates that are reconstituted when a bacterial suspension is introduced. The metabolites produced are revealed by pH indicators after an incubation period of 24 to 48 hours at temperatures of 35°C to 37°C. The MIC is defined as the lowest concentration of the antibiotic that completely inhibits the growth of *P. acnes*. Isolates which show an MIC greater than 1 µg/mL are defined as resistant strains. The MICs to tetracycline, doxycycline, minocycline, erythromycin, clindamycin, cotrimoxazole and ampicillin were determined using Etest strips (AB Biodisk, Solna, Sweden), which is a diffusion method that generates MICs from a continuous gradient. Fisher's exact, χ^2 , and *t* tests were used for statistical analysis, with a *P* value of less than 0.05 considered statistically significant.

Results

One hundred and fifty consecutive patients who sought treatment or were being treated for acne vulgaris at the National Skin Centre were recruited between January 1999 to June 1999.

Age, Sex, Race and Duration of Acne

Table I shows the demographics of the patients studied. There were 40 patients in group A, 44 patients in group B and 66 patients in group C. The average age of the patients was 19.6 years (range 13 to 32 years). The average duration of acne was 24.3 months (range 2 to 84 months). There was no statistically significant difference among the three groups in terms of the average age of the patients, average duration of acne, sex ratio or racial distribution of the patients.

Isolation of *P. acnes* and Resistant Strains

Table II shows the rate of isolation of *P. acnes* and number of resistant strains from the various groups. There were no resistant strains isolated from group A. There were 2 resistant strains among the 34 isolates of *P. acnes* in group B (6.25%) and 11 resistant strains among the 51 isolates in group C (21.6%). There was no significant

difference in the rates of isolation of *P. acnes* among the 3 groups; the rate of isolation of resistant strains between group A and B was not significant, but the difference between group A and C (*P* = 0.015) and between group B and C (*P* = 0.036) was statistically significant.

Mean MICs of Susceptible Strains of *P. acnes*

Table III shows the mean MICs of susceptible strains of *P. acnes* from the various groups. The mean MICs shows a rising trend with a longer duration of treatment. The differences in the mean MICs between group A and group B were statistically significant only for erythromycin (*P* = 0.005). The differences in mean MICs between group B and group C were statistically significant for minocycline (*P* = 0.014) and clindamycin (*P* = 0.035), but the differences in mean MICs between group A and group C were statistically significant for minocycline (*P* = 0.014), erythromycin (*P* = 0.001), clindamycin (*P* = 0.027) and ampicillin (*P* = 0.005). The mean MICs to tetracycline was higher than doxycycline, which in turn was higher than that of minocycline in all groups.

TABLE I: DEMOGRAPHICS OF STUDY POPULATION

	Group A	Group B	Group C
No. of patients	40	44	66
Average duration of acne (mo)	21.9 ± 9.5	22.1 ± 14.2	28.7 ± 20.8
Male to female ratio (M:F)	26:14	32:12	55:11
Average age (y)	19.1 ± 4.2	19.8 ± 4.1	19.9 ± 2.5
Race (%)			
Chinese	35 (87.5)	36 (81.8)	57 (86.4)
Malay	3 (7.5)	7 (13.6)	6 (9.1)
Indian	1 (2.5)	2 (4.6)	1 (1.5)
Others	1 (2.5)	0 (0)	2 (3.0)

Group A: No prior antibiotic treatment

Group B: Short-term antibiotics (6 to 18 weeks)

Group C: Long-term antibiotics (24 to 52 weeks)

TABLE II: ISOLATION RATES OF *P. acnes* AND RESISTANT STRAINS

	Group A	Group B	Group C	<i>P</i> value comparing groups A to B	<i>P</i> value comparing groups B to C	<i>P</i> value comparing groups A to C
Rate of <i>P. acnes</i> isolation (%)	35/40 (87.5)	32/44 (72.7)	51/66 (77.3)	ns	ns	ns
No. of resistant strains (%)	0/35 (0)	2/32 (6.25)	11/51 (21.6)	ns	0.036	0.015

ns: not significant

Group A: No prior antibiotic treatment

Group B: Short-term antibiotics (6 to 18 weeks)

Group C: Long-term antibiotics (24 to 52 weeks)

TABLE III: MEAN MICs OF SENSITIVE STRAINS OF *P. acnes* IN THE VARIOUS GROUPS

	Group A MIC (ug/mL)	Group B MIC (ug/mL)	Group C MIC (ug/mL)	<i>P</i> value comparing groups A to B	<i>P</i> value comparing groups B to C	<i>P</i> value comparing groups A to C
Tetracycline	0.209 ± 0.109	0.309 ± 0.250	0.394 ± 0.191	0.330	0.242	0.061
Doxycycline	0.192 ± 0.175	0.202 ± 0.148	0.345 ± 0.275	0.727	0.055	0.066
Minocycline	0.108 ± 0.101	0.120 ± 0.113	0.159 ± 0.103	0.801	0.034	0.014
Erythromycin	0.018 ± 0.005	0.037 ± 0.018	0.049 ± 0.028	0.005	0.293	0.001
Clindamycin	0.029 ± 0.024	0.039 ± 0.033	0.033 ± 0.031	0.131	0.035	0.027
Ampicillin	0.035 ± 0.033	0.029 ± 0.019	0.025 ± 0.013	0.495	0.501	0.532
Cotrimoxazole	0.136 ± 0.078	0.151 ± 0.111	0.155 ± 0.122	0.220	0.352	0.252

MIC: minimum inhibitory concentration

Group A: No prior antibiotic treatment

Group B: Short-term antibiotics (6 to 18 weeks)

Group C: Long-term antibiotics (24 to 52 weeks)

Antibiotic Resistance in *P. acnes*

Table IV shows the profile of the 13 antibiotic-resistant strains. Resistance to erythromycin was the commonest (11 out of 13 strains), followed by clindamycin (10 out of 13 strains). These included cases of concurrent resistance to both erythromycin and clindamycin, which was common and was seen in 10 of the 11 strains showing erythromycin resistance. All strains, which were resistant to clindamycin, were simultaneously resistant to erythromycin. There was one isolate that showed resistance to both tetracycline and doxycycline, and one isolate that showed low-level resistance to cotrimoxazole.

Discussion

Antibiotics are the most commonly used drugs for the treatment of acne vulgaris. Isolates resistant to one or more anti-acne antibiotics have been reported in the USA,² United Kingdom³ and Japan,⁴ but to the best of our knowledge, there has not been a previous study concerning this problem in Southeast Asia. The emergence of antibiotic resistance may have resulted from the widespread use of topical antibiotics or from decades of long-term systemic administration. As antibiotic therapy in acne vulgaris is often continued for months, there is a strong likelihood that resistant strains begin to emerge due to selective pressure. The possibility that sequential or concurrent use of different antibiotics may result in multiply resistant strains of *P. acnes* is very real. Poor compliance probably encourages the overgrowth of resistant strains as well, but this is not easily demonstrable.

Our finding that resistant strains of *P. acnes* can be isolated from patients who have been on short-term antibiotics concurs with a British study, which showed that in previously untreated patients, resistant strains begin to emerge between 12 and 24 weeks of therapy.⁵ Although the difference between the rate of isolation of resistant strains was not statistically significant between the group

TABLE IV: MICs OF ANTIBIOTIC-RESISTANT ISOLATES OF *P. acnes*

S/N	Group/Duration of treatment (weeks)	Antibiotic resistance	MIC (ug/mL)
1	B/16	Erythromycin Clindamycin	>256 >256
2	B/14	Erythromycin Clindamycin	>256 >256
3	C/26	Erythromycin Clindamycin	>256 >256
4	C/32	Erythromycin Clindamycin	>256 >256
5	C/24	Erythromycin Clindamycin	>256 24
6	C/40	Erythromycin Clindamycin	>256 >256
7	C/52	Erythromycin Clindamycin	>256 >256
8	C/26	Erythromycin	>256
9	C/36	Erythromycin Clindamycin	256 256
10	C/26	Erythromycin Clindamycin	256 48
11	C/36	Erythromycin Clindamycin	>256 >256
12	C/38	Tetracycline Doxycycline	8 6
13	C/40	Cotrimoxazole	1.5

MIC: minimum inhibitory concentration

Group B: Short-term antibiotics (6 to 18 weeks)

Group C: Long-term antibiotics (24 to 52 weeks)

without prior antibiotic treatment and the group on short-term antibiotics, there was a significant difference in isolation of resistant strains when we compared the untreated group with the group on long-term antibiotics ($P = 0.0015$) and between short-term and long-term antibiotics treated groups ($P = 0.036$). Antibiotic resistance thus increases significantly with long-term systemic antibiotics. In our study, resistance to erythromycin was the most common.

This finding is also seen overseas, where 25% of acne patients attending the outpatient clinic at Leeds General Infirmary were found to carry erythromycin-resistant propionibacteria on their facial skin.⁶ According to their cross-resistance patterns to a panel of macrolide-lincosamide-streptogramin type B (MLS) antibiotics, Eady et al⁷ were able to assign strains resistant to erythromycin into one of four phenotypic classes. The majority of isolates have a constitutive resistance to erythromycin (a 14-membered ring macrolide), clindamycin (a lincosamide) and to all other MLS antibiotics. This is consistent with our findings that all isolates which show resistance to clindamycin were also simultaneously resistant to erythromycin, while 10 out of the 11 erythromycin-resistant strains were also concurrently resistant to clindamycin. The level of resistance to erythromycin was high-level, with all isolates showing a MIC of at least 256 µg/mL. This resistance is due to a single specific base change within the peptidyl transferase centre of 23S rRNA, which forms part of the binding site for MLS antibiotics including erythromycin and clindamycin.⁸ Clindamycin-resistant strains had MICs ranging from 24 µg/mL to >256 µg/mL. This is of particular concern as clindamycin is often used in the treatment of severe anaerobic infections and in severe methicillin-resistant *Staphylococcus aureus* infections.

Only one isolate was resistant to tetracycline and doxycycline. Despite resistance to both tetracycline and doxycycline, it remained sensitive to minocycline. The average MIC to tetracycline was higher than that for doxycycline, which in turn was higher than that for minocycline in all groups. This finding is similar to a British study which showed that tetracycline-resistant propionibacteria from acne patients were cross-resistant to doxycycline but sensitive to minocycline.⁹ The low incidence of resistance to the tetracyclines is surprising, considering that they are the most commonly used antibiotics for the treatment of acne at our Centre.

Cotrimoxazole is a drug that generally has poor activity against anaerobic bacteria *in vitro*; however, it is clinically effective for the treatment of acne.¹⁰ Only one isolate showed resistance (low-level) to the drug. Although cotrimoxazole is not as commonly used in the treatment of acne, it remains a useful drug in the treatment of urinary tract infections and the possibility of resistance to cotrimoxazole increasing in other bacteria due to its use in acne vulgaris is a worrying thought.

It is likely that patients who exhibit high-level resistance to antibiotics will not show therapeutic benefit if these antibiotics are continued. The question of whether low-

level resistance is likely to give rise to therapeutic failures remains unanswered.

In conclusion, we have shown that acne patients in Singapore that are likely to carry antibiotic-resistant strains are those treated with long-term antibiotics. In addition to this, it is probable that patients who are poor responders, relapsers and poorly compliant to treatment are also more likely to harbour antibiotic-resistant strains.¹¹ The methodology we have used to isolate *P. acnes* is simpler than the quantitative detergent-scrub method developed by Williamson and Kligman,¹² and may lead to larger studies to obtain a better idea of the scope of the problem of antibiotic resistance in *P. acnes* strains in this part of the world, and its possible clinical implications. We would then be able to formulate better recommendations for the use of antibiotics in acne therapy to help prevent the emergence of resistance in *P. acnes*.

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