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First report of high levels of clindamycin-resistant *Propionibacterium acnes* carrying *erm*(X) in Japanese patients with acne vulgaris

Dear Editor,

Propionibacterium acnes and *Staphylococcus epidermidis*, which are human skin microflora, are commonly isolated from pyrogenic inflammation sites in acne vulgaris. *P. acnes* is a Gram-positive bacillus that grows under anaerobic conditions following the obstruction of the follicle.[1] In addition, *P. acnes* exacerbates acne vulgaris because this bacterium produces free fatty acids, which causes inflammation.[1] To treat inflammatory acne vulgaris, antimicrobial therapies target *P. acnes*; both oral and topical antimicrobial agents have been developed. β-Lactams, fluoroquinolones, macrolides, and tetracyclines are used as oral drugs, and clindamycin and nadifloxacin are used as topical drugs.[2]

Recently, an increased frequency of macrolide–clindamycin-resistant *P. acnes* has become a concern in Europe and in Japan.[3,4] Macrolides exert their antimicrobial effect by inhibiting bacterial protein synthesis by binding to the 23S rRNA of the 50S subunit. The macrolide resistance of *P. acnes* is caused by a mutation in domain V of the 23S rRNA or by alteration of the target site by the 23S dimethylase encoded by *erm*(X).[4,5] The *erm*(X) gene is encoded by the transposon Tn*5432* of *Corynebacterium*, which is a closely related *Propionibacterium*, and is the primary mediator of macrolide resistance.[4] In addition, macrolide-resistant strains show cross-resistance to lincosamides such as clindamycin, which bind to the same region of the 23S rRNA.[3] Macrolide–clindamycin-resistant *P. acnes* strains are classified into four types, G2057A, A2058G, and A2059 of 23S rRNA mutations and possession of *erm*(X), based on the differences between the macrolides and clindamycin susceptibilities.[4]

In Japan, a study conducted between 1994 and 1995 revealed that macrolide-resistant strains were found in only 4% (2/50) of *P. acnes* isolates.[6] The macrolide-resistant strains possessed the 23S rRNA mutation, which was identified between 2006 and 2007 in our previous study. However, no strain carrying *erm*(X) was found.[3] Here, we showed that a high level of macrolide–clindamycin-resistant *P. acnes* carrying *erm*(X) was found in 2008 in Japan for the first time.

A total of 43 *P. acnes* strains were collected from 50 patients (ratio of males to females, 2:3; mean age, 21.2 years) with acne vulgaris in 2008 in Japan. *P. acnes* was identified as previously described.[3]

Susceptibility testing was performed using an agar dilution procedure according to the criteria of the Japanese Society of Chemotherapy.[7] The minimum inhibitory concentration (MIC) was determined after 48 h of growth. Cefaclor, cefditoren, faropenem, levofloxacin, nadifloxacin, clarithromycin and clindamycin were kindly provided by their manufacturers. Amoxicillin and erythromycin were purchased from Sigma-Aldrich (Tokyo, Japan). Ciprofloxacin, josamycin and minocycline were purchased from Wako Pure Chemical Industries (Osaka, Japan).

The detection of the *erm*(X) gene was performed as previously described.[3] The sequences of the amplified *erm*(X) gene were verified by DNA sequencing.[8] The presence of mutations in the 23S rRNA was determined using DNA sequencing as previously described.[3]

Antimicrobial susceptibility testing was performed on 43 *P. acnes* isolates (Table 1). There was no difference in susceptibility to β-lactams between the strains that were isolated in 2006–2007 and 2008. Two strains were isolated in 2008 and were resistant to fluoroquinolones, ciprofloxacin and levofloxacin, and all strains were susceptible to the common topical antimicrobial agent nadifloxacin. The resistance rates of macrolides and clindamycin in *P. acnes* isolated from 2008 were greater than those isolated from 2006 to 2007. Furthermore, one strain with resistance to macrolides, clindamycin, and tetracycline was found.

We determined the macrolide resistance mechanism based on the sequencing of the 23S rRNA gene and the detection of *erm*(X)

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Table 1. Antimicrobial susceptibilities of *P. acnes* strains that were isolated from 2006–2007 (n = 48)[3] and 2008 (n = 43)

	MIC range (μg/mL)		% Resistant isolates (n)	
Antimicrobial agent	2006–2007	2008	2006–2007	2008
Amoxicillin	≤0.063–0.25	≤0.063–0.25	0	0
Cefaclor	0.5–4	0.25–32	0	0
Cefditoren	≤0.063–0.5	≤0.063–0.25	0	0
Faropenem	≤0.063–0.25	≤0.063	0	0
Ciprofloxacin	0.5–2	0.125–16	0	4.7 (2)
Levofloxacin	0.5–2	0.125–8	0	4.7 (2)
Nadifloxacin	0.125–1	≤0.063–4	0	0
Erythromycin	≤0.063–≥256	≤0.063–≥256	10.4 (5)	20.9 (9)
Clarithromycin	2–≥256	≤0.063–≥256	10.4 (5)	20.9 (9)
Josamycin	≤0.063–≥256	≤0.063–≥256	8.3 (4)	20.9 (9)
Clindamycin	≤0.063–≥256	≤0.063–≥256	8.3 (4)	18.6 (8)
Minocycline	0.125–0.5	0.25–16	0	2.3 (1)

Propionibacterium acnes JCM6425 (ATCC6919) was used as a quality control strain.[3] Resistance breakpoints of the following antimicrobial agents were defined according to a previous study as follows: amoxicillin, ≥16 μg/mL; cefaclor, ≥16 μg/mL; cefditoren, ≥64 μg/mL; faropenem, ≥16 μg/mL; ciprofloxacin, ≥8 μg/mL; levofloxacin, ≥8 μg/mL; nadifloxacin, ≥8 μg/mL; erythromycin, ≥2 μg/mL; clarithromycin, ≥2 μg/mL; josamycin, ≥4 μg/mL; clindamycin, ≥8 μg/mL; and minocycline, ≥16 μg/mL.

Table 2. MLS_B-resistant types[4] of *P. acnes* strains used in this study

		MIC (μg/mL)				
Strain no.	Year	Clarithromycin	Josamycin	Clindamycin	*erm*(X)	23S rRNA mutation
1a	2006	≥256	4	≥256	–	A2058G
43a	2006	≥256	≥256	32	–	A2059G
62a	2007	≥256	8	≥256	–	A2058G
70a	2007	≥256	4	128	–	A2058G
72b	2007	2	0.25	0.25	–	G2057A
78a	2008	≥256	≥256	≥256	+	Wild
83a	2008	≥256	≥256	≥256	+	Wild
84a	2008	≥256	≥256	≥256	–	Wild
86a	2008	≥256	2	128	–	A2058G
88a	2008	≥256	≥256	≥256	+	Wild
95a	2008	≥256	≥256	32	–	A2059G
96b	2008	≥256	≥256	≥256	+	Wild
102a	2008	≥256	≥256	4	–	A2059G
121a	2008	≥256	8	64	–	A2058G

Propionibacterium acnes JCM6473 (ATCC11828) was used as a wild-type strain for 23S rRNA.[3]

for the strains that were isolated in 2008 (Table 2). We have previously demonstrated that the macrolide–clindamycin-resistant strains that were isolated in 2006–2007 had the 23S rRNA mutation.[3] In contrast, the *erm*(X) gene was found for the first time in four strains that were isolated in 2008. The strains carrying *erm*(X) exhibited a high level of resistance to all macrolides and clindamycin. The macrolide–clindamycin-resistant strains isolated in 2008 were classified into the following types: A2058G (2/9, 22.2%), A2059G (2/9, 22.2%) and possession of *erm*(X) (4/9, 44.4%). Furthermore, no resistance determinant was found from the strain 84a that exhibited the resistant to macrolides, clindamycin, and tetracycline.

Macrolides and clindamycin are frequently used to target *P. acnes* because these drugs have antimicrobial and anti-inflammatory activities.[2] Recently, *P. acnes* carrying *erm*(X) was found in additional regions of Europe.[9] In the present study, a high level of macrolide–clindamycin-resistant *P. acnes* carrying *erm*(X) was found in Japan for the first time.

erm(X) is known as a gene that confers a high level of macrolide–clindamycin resistance in *P. acnes* although *erm*(X) was first isolated from *Corynebacterium*.[4] The isolation rate of macrolide–clindamycin-resistant *P. acnes* has still been lower in Japan and Korea than that in Europe.[3,6] In Europe, although the detection rate of *erm*(X) was less than 10% in macrolide–clindamycin-resistant *P. acnes*, 80% of all isolates were resistant to macrolide and clindamycin.[4] In our study, the number of 23S rRNA mutant strains detected was approximately the same for 2006–2007 and 2008. However, strains carrying *erm*(X) were found for the first time

in samples from 2008 and comprised half of the macrolide–clindamycin-resistant strains. Therefore, our results indicate that the increased macrolide–clindamycin resistance rates in *P. acnes* in 2008 are related to the prevalence of *erm*(X).

The *erm*(X) gene can be transferred within a species or among species. Therefore, the dissemination of a high level of macrolide–clindamycin-resistant *P. acnes* is expected in the future. In the current study, we demonstrated the increase in macrolide–clindamycin resistance among *P. acnes* strains in Japan. However, no significant differences were seen with regard to therapeutic effect, regardless of the presence or absence of resistant strains, suggesting that an anti-inflammatory effect is involved as well.[6] In addition, combination therapy of antimicrobial agents with adapalene or benzoyl peroxide was recommended to reduce antibiotic use and antimicrobial-resistant *P. acnes*.[10] Therefore, the adequate use of antimicrobial agents is important to prevent the spread of antimicrobial-resistant strains of *P. acnes*.

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Sporotrichosis of the face by autoinoculation in a patient undergoing tacrolimus treatment

Dear Editor,

Sporotrichosis is a fungal infection caused by the dimorphic fungal pathogen *Sporothrix schenckii* that is inoculated into the skin by trauma. The immunological response of the host likely determines the clinical form that the infection assumes. The most common type of clinical form is the localized lymphatic form that follows implantation into the skin, where it usually appears on the upper extremities as nodules or pustules. The less common type is the fixed cutaneous form that typically occurs on the arm or face where the fungal pathogen remains localized at the point of inoculation. It is thought that these forms may reflect a high degree of immunity by patients. In patients with immunodeficiency or topical steroid use, however, there have been a few unusual cases reported.[1–3] In the current study, we describe a patient undergoing tacrolimus treatment who developed multiple skin eruptions on the face and neck in a short period of time.

A 73-year-old woman presented in March 2008 with a 5-month history of rapidly growing eruptions on the face. The patient had hypothyroidism and was medicated with levothyroxine sodium hydrate for 12 years. Five months previously, the patient had been diagnosed with lupus nephritis and treated with 2 mg tacrolimus daily. Two weeks after the dose of tacrolimus was increased to 3 mg daily, the patient noticed an erythematous nodule on the left cheek. The number of eruptions rapidly increased and spread to the right cheek and anterior neck within a few weeks. The presumptive diagnosis was impetigo contagiosa. Despite antibiotic therapy and withdrawal of tacrolimus, no clinical improvement was achieved.

The patient's husband farmed in Chiba Prefecture in eastern Japan and the patient did not attend to farm work herself, neither did she have a history of trauma on the face. Physical examination revealed a number of erythematous nodules and pustules on the bilateral cheeks and the anterior neck with crusty or erosive surfaces (Fig. 1); some of these were adhesive and the left lacrimal gland was swollen. Laboratory studies disclosed the following: white blood cell count, 12000 cells/μL (differential count, 52.1% neutrophils, 4.7% eosinophils, 0.4% basophils, 8.4% monocytes, 34.4% lymphocytes); $CD4^+$ cell count, 1055 cells/μL; serum immunoglobulin (Ig)G, IgA, IgM and IgD levels, normal; C-reactive protein level, normal; serum urea concentration, 13.4 mg/dL; and creatinine, 0.86 mg/dL. Other data including full blood count and liver dysfunction tests were within normal ranges. Serological tests for HIV were negative and skin cultures for bacteria were negative. The

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