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European surveillance study on the antibiotic susceptibility of *Propionibacterium acnes*

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ABSTRACT

Propionibacterium acnes strains are recovered from infections linked to surgical procedures, foreign bodies and septicaemia. This study investigated the antibiotic susceptibility patterns of *P. acnes* isolates from different systemic infections and determined the genomic diversity among resistant *P. acnes* isolates with low-frequency restriction analysis of chromosomal DNA by pulsed-field gel electrophoresis (PFGE). In total, 304 *P. acnes* isolates from 13 laboratories in 13 European countries were tested against six antimicrobial agents by the NCCLS reference agar dilution method and the breakpoints recommended by the European Committee on Antimicrobial Susceptibility Testing. Blood isolates were encountered most frequently, followed by those from skin and soft tissue infections, and abdominal infections. Of the isolates examined, 2.6% were resistant to tetracycline, 15.1% to clindamycin, and 17.1% to erythromycin. No resistance was observed to linezolid, benzylpenicillin or vancomycin. There was considerable variation between countries in the proportion of resistant strains, ranging from 83% in Croatia and 60% in Italy to 0% in The Netherlands. Isolates from blood were predominant among the resistant isolates. Seventeen clones and 78 banding patterns were identified among the resistant isolates. It was concluded that antimicrobial resistance has now emerged among *P. acnes* isolates from systemic infections.

Keywords Antimicrobial resistance, clinical isolates, *Propionibacterium acnes*, pulsed-field gel electrophoresis, resistance

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INTRODUCTION

Propionibacterium acnes is a Gram-positive anaerobic bacillus which is a member of the resident flora of the skin, nares, conjunctival flora, oral cavity, upper respiratory tract and intestinal tract [1–3]. *P. acnes* is associated with the inflammatory processes in acne lesions, and resistance of *P. acnes* to antimicrobial agents has often been encountered in acne patients in response to oral and topical antibiotic therapy [4]. It has also been found that antibiotic therapy determines the emergence of antimicrobial resistance in close contacts of such patients [5].

During recent years, the prevalence of life-threatening infections caused by *P. acnes* has increased [6]. These infections generally occur in compromised patients, in newborn infants [7] and, less frequently, in previously healthy individuals [8]. According to Brook and Frazier [9], predisposing conditions include: the presence of foreign bodies, diabetes, previous surgery, trauma, malignancy, immunodeficiency and steroid therapy. Systemic infections are associated more frequently with surgical procedures, such as cerebrospinal fluid shunts or the implantation of prosthetic devices (heart valves, orthopaedic implants, intraocular lenses) [10,11]. It is possible for these

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infections to appear either shortly after surgery (as a result of a high inoculum) or after a longer period. The second possibility is explained by the fact that *P. acnes* resides intracellularly and can remain in a dormant state for months or years [6,12]. The management of severe infections should involve a combination of parenteral antimicrobial agents and surgical procedures (e.g., removal of the device or drainage of the infected site) [6,9]. Benzylpenicillin is the drug of choice for serious infections, with clindamycin, tetracycline, erythromycin, chloramphenicol and vancomycin as alternatives in cases of allergy or concern about resistance to β-lactam agents [13]. All isolates collected from serious infections should be tested for in-vitro susceptibility, because of the possible recovery of resistant strains [13,14].

The first objective of the present study was to evaluate the activity of six antimicrobial agents against clinical isolates of *P. acnes* from 13 European countries. The second objective was to determine the genomic diversity and the epidemiological relatedness among resistant *P. acnes* isolates by using pulsed-field gel electrophoresis (PFGE).

MATERIALS AND METHODS

Bacterial isolates

Laboratories in each country included in the study (Croatia, Czech Republic, Denmark, Finland, Germany, Greece, Hungary, Italy, The Netherlands, Norway, Slovenia, Sweden and Great Britain) collected consecutive, non-duplicate isolates of *P. acnes*, together with information regarding sampling day, type of infection and antibiotic treatment. The isolates were sent to the Division of Clinical Bacteriology, Karolinska University Hospital Huddinge, Karolinska Institute, Stockholm, Sweden. The study was approved by the Ethics Committee of the hospital. The strains were identified by Gram's stain, biochemical tests (Rapid-Ana II System; REMEL Inc., Lenexa, KS, USA) [15] and gas chromatography. Following identification, the isolates were frozen (– 70°C) until required; susceptibility testing was performed simultaneously with all isolates.

Determination of MICs

MICs of six antimicrobial agents were determined by the NCCLS agar dilution technique, with 10^5 CFU/spot and brucella base sheep blood agar [16]. The plates were incubated in anaerobic jars (GasPak Anaerobic System; BBL, Cockeysville, MD, USA) for 48 h at 37°C. The MIC was defined as the lowest concentration of antimicrobial agent that resulted in a marked change in the appearance of growth in comparison with the control plate, as described in the NCCLS protocol. *P. acnes* ATCC 6919, *P. acnes* ATCC 11828, *Bacteroides fragilis* ATCC 25285, *Bacteroides thetaiotaomicron* ATCC 29741 and *Enterococcus faecalis* ATCC 29212 were used as control strains. Tetracycline was obtained from Wyeth Ayerst (Pearl River, NY, USA), clindamycin from Pharmacia Upjohn (Kalamazoo, MI, USA), erythromycin and benzylpenicillin from AstraZeneca (Södertälje, Sweden), linezolid from Pharmacia Upjohn (Milan, Italy), and vancomycin from Abbott Scandinavia (Solna, Sweden).

Genotypic analysis with PFGE

Genomic DNA was prepared in agarose plugs, digested with *Spe*I restriction endonuclease (Promega, Madison, WI, USA) and electrophoresed using a contour-clamped homogeneous electric field apparatus (Gene Path System; Bio-Rad Laboratories, Hercules, CA, USA) as described previously [17]. All antibiotic-resistant isolates (n = 89) and two *P. acnes* reference strains (ATCC 6919 and ATCC 11828) were typed by PFGE [18].

Computerised numerical analysis of PFGE data

Calculation of similarity matrices and creation of dendrograms were done with the Molecular Analyst Software program (Bio-Rad), using the unweighted pair-group method with arithmetic averages (UPGMA). The similarity coefficients were calculated according to the method of Dice [19]. *P. acnes* ATCC 6919 was used as a control for each gel experiment to allow comparisons within the Molecular Analyst Software program. The clonal group level was set at ≥80% similarity. Capital letters (A–Q) were used to designate the main genetic lineages observed [17]. The preparation and digestion of DNA from isolates chosen randomly were repeated under the same conditions to assess the reproducibility of the method.

Analysis of results

For comparison of results from different regions of Europe, the countries were grouped as follows: north—Denmark, Finland, Norway and Sweden; south—Greece and Italy; east—Croatia, Czech Republic, Hungary and Slovenia; and west—Germany, Great Britain and The Netherlands.

The results were registered in a single database, and analysed and validated with WHONET 5 (World Health Organization, Geneva, Switzerland) and Statistica v. 6.0 software (StatSoft, Tulsa, OK, USA).

Susceptibility breakpoints used were those recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [17,20] (tetracycline, MIC ≥ 2 mg/L; clindamycin, MIC ≥ 0.25 mg/L; erythromycin, MIC ≥ 0.5 mg/L; linezolid, MIC ≥ 8 mg/L; benzylpenicillin, MIC ≥ 0.25 mg/L; and vancomycin, MIC ≥ 4 mg/L).

The correlations between antibiotic resistance rates and the reported sales of antibiotics in eight European countries were analysed with a linear regression model, with a value of $p < 0.05$ set as the level of significance.

RESULTS

Distribution and source of *P. acnes* isolates

Each participating country was originally supposed to contribute a maximum of 50 isolates to

the study, but some participants had difficulty in collecting the agreed number of isolates (Table 1). In total, 328 clinical isolates collected between 1996 and 2002 were received, but 24 isolates not belonging to *P. acnes* were excluded from the study. The distribution of isolates according to the country and type of infection is shown in Table 1. Most (18.7%) isolates were received from Italy, and blood isolates (n = 105), followed by those from skin and soft tissue infections (n = 77), were encountered most frequently.

Antimicrobial susceptibilities

MIC values are shown in Table 2. The MICs for the control strains were always within recommended limits.

Clindamycin
Of the clinical isolates, 15% were resistant to clindamycin. Most resistant isolates were associated with meningitis (21.4%), prosthetic devices (20%) and blood (20%).

Erythromycin
Resistance to erythromycin was shown by 17% of the isolates. MICs ranged between < 0.064 and > 128 mg/L. Most resistant isolates were associated with blood (31.4%), meningitis (21.4%), abdominal infections (18.2%) and lung and pleural fluids (16.7%).

Tetracycline
Most *P. acnes* isolates were susceptible to tetracycline, with both the MIC_{50} and MIC_{90} values below the breakpoint for tetracycline, and < 3% of all isolates showing resistance. Tetracycline-resistant isolates were associated with biliary, bone, head and neck, skin/soft tissue and blood infections.

Linezolid
Linezolid was active against all *P. acnes* isolates, with MICs between 0.25 and 2 mg/L.

Benzylpenicillin
All the clinical isolates were uniformly susceptible to low concentrations of penicillin, with MICs between 0.008 and 0.125 mg/L.

Vancomycin
All the isolates were susceptible to vancomycin.

Resistance profiles

Overall, 29% of *P. acnes* isolates were resistant to at least one of the tested antimicrobial agents. Most (83%) of these isolates were resistant to only one antibiotic, and most of the erythromycin-resistant or clindamycin-resistant isolates were from Italy (Table 3).

The MICs of erythromycin and clindamycin for isolates resistant to both antimicrobial agents, as well as the MICs for isolates resistant to each of these antimicrobial agents individually, are presented in Fig. 1. In the case of double-resistance (clindamycin and erythromycin), the MICs were higher for most isolates than for isolates with single antibiotic resistance.

Table 1. Distribution and source of isolates of *Propionibacterium acnes*

	Country (no. of isolates)												
Source of isolate (no.)	**DNK**	**FIN**	**NOR**	**SWE**	**GRE**	**ITA**	**CRO**	**CZE**	**HUN**	**SVN**	**GER**	**GBR**	**NLD**
Blood (105)	18	3	7			38	5		16	11	3	3	1
Skin/soft tissue (77)				8	9	4		35	11	8	1	1	
Abdominal infection (22)			1	1		3		5	6	2	1	3	
Head/neck (19)		13								2	1	3	
Bone (15)				11								4	
Meningitis (14)						6	1			3		3	1
Eye infection (13)						3			3	2		5	
Endocarditis (13)						1			3		4		5
Prosthetic device (10)				6						3		1	
Lung/pleural fluid (6)						2			2			2	
Bile (4)								3			1		
Lymph node (2)												2	
Female genital tract (2)													2
Arthritis (1)												1	
Faeces (1)		1											
Total (304)	18	17	8	26	9	57	6	43	41	31	11	30	7
%	5.9	5.5	2.6	8.5	2.9	18.7	1.9	14.1	13.4	10.1	3.6	9.8	2.3

DNK, Denmark; FIN, Finland; NOR, Norway; SWE, Sweden; GRE, Greece; ITA, Italy; CRO, Croatia; CZE, Czech Republic; HUN, Hungary; SVN, Slovenia; GER, Germany; GBR, Great Britain; NLD, the Netherlands.

Table 2. In-vitro activity of six antimicrobial agents against 304 isolates of *Propionibacterium acnes*

Antibiotic	Breakpoint (mg/L)[a]	MIC (mg/L)			Resistant isolates (%)
		MIC_{50}	MIC_{90}	Range	
Clindamycin	0.25	< 0.064	0.25	< 0.064–64	15.1
Erythromycin	0.5	0.25	0.5	< 0.064 to > 128	17.1
Tetracycline	2	0.5	1	< 0.064–32	2.6
Linezolid	8	0.5	1	0.25–2	0
Benzylpenicillin	0.25	0.032	0.064	0.008–0.125	0
Vancomycin	4	0.5	1	0.25–2	0

[a]Breakpoints recommended by the European Committee on Antibiotic Susceptibility Testing (EUCAST) [17,20].

Table 3. Resistance profiles of 304 clinical isolates of *Propionibacterium acnes* from 13 European countries

Profile	No. of isolates (%)	Country (no. of isolates)
Susceptible	215 (70.7)	
Resistant	89 (29.2)	
TCY	5 (5.6)	CZE (3); FIN (2)
EM	37 (41.5)	ITA (22); SVN (6); DNK (4); SWE (2); CZE, CRO, GBR(1)
CL	32 (35.9)	ITA (10); SVN (7); HUN (6); CZE, SWE (3); CRO, DNK, GBR (1)
EM; TCY	1 (1.1)	SWE (1)
CL; EM	12 (13.4)	SVN (5); CRO (3); ITA (2); GER (1); GRE (1)
CL; EM; TCY	2 (2.2)	NOR (1); GRE (1)

CL, clindamycin; EM, erythromycin; TCY, tetracycline; DNK, Denmark; FIN, Finland; NOR, Norway; SWE, Sweden; GER, Germany; GBR, Great Britain; NLD, The Netherlands; GRE, Greece; ITA, Italy; CRO, Croatia; CZE, Czech Republic; HUN, Hungary; SVN, Slovenia.

Prevalence of resistant bacteria from different countries

There was considerable variation between countries in the proportion of resistant isolates, ranging from 83% in Croatia and 60% in Italy, to 7% in Great Britain and 0% in The Netherlands. All resistant isolates collected from Denmark, Croatia and Norway, and also many of the resistant isolates from Italy, Slovenia and Hungary, were obtained from blood (Table 4).

The clindamycin MIC_{90} value was highest for Greek isolates (32 mg/L), while the highest values for erythromycin were found in isolates from Greece, Croatia and Slovenia (≥ 128 mg/L). One isolate from Norway had the highest tetracycline MIC (8 mg/L) (Table 5).

Antibiotic resistant rates from different countries/regions

The highest prevalence of resistance was detected in isolates from the southern European regions (Greece and Italy), where 54.5% of isolates were resistant. The lowest prevalence (6.2%) was found in the western European countries (Germany, Great Britain and The Netherlands). In the eastern regions (Croatia, Czech Republic, Hungary and Slovenia), the situation did not differ from the rest of Europe, although there was considerable variation from country to country (Table 6).

Some correlations (Table 6) were noted when the results from this study were compared with published data [21] on outpatient antibiotic sales in countries of the European Union. In Finland, the country with the highest use of tetracycline, a higher rate (11.8%) of tetracycline resistance among *P. acnes* isolates was found. In Italy, currently with the lowest tetracycline consumption, no isolates were tetracycline-resistant. Similar correlations were observed between the highest sales of macrolides and lincosamides (MLS) in Italy and high rates of resistance to erythromycin (42.1%) and clindamycin (21.1%). Sweden has reported a large reduction in antibiotic use, but the rates of resistance to clindamycin and erythromycin were 11.5% for both antimicrobial agents. The very low rate of non-hospital antibiotic use in The Netherlands (Table 6) was reflected in the present results by the absence of resistant isolates.

The antibiotic resistance rates were found to correlate with the sale of MLS agents for the eight countries from the European Union that reported these data (Table 6) for clindamycin ($p < 0.05$, $r = 0.753$; Fig. 2a) and erythromycin ($p < 0.05$, $r = 0.713$; Fig. 2b). No correlation was found between tetracycline-resistant rates and the sale of tetracycline in these countries.

PFGE typing

All resistant isolates were distinguishable by PFGE. Seventy-eight different patterns of *Spe*I DNA digests were detected. Among the 91 isolates studied, a band-based cluster analysis revealed 17 clones (A–Q) with a cut-off value of 80% similarity. Visual analysis revealed that isolates from the same clone had fewer than three bands different, while the number of band differences between isolates belonging to different clones was greater than four. Each clone included between one and 32 isolates. The largest cluster of isolates ($n = 32$; 35.1%) was grouped into clone F. Ten clones comprised single isolates (Table 7).

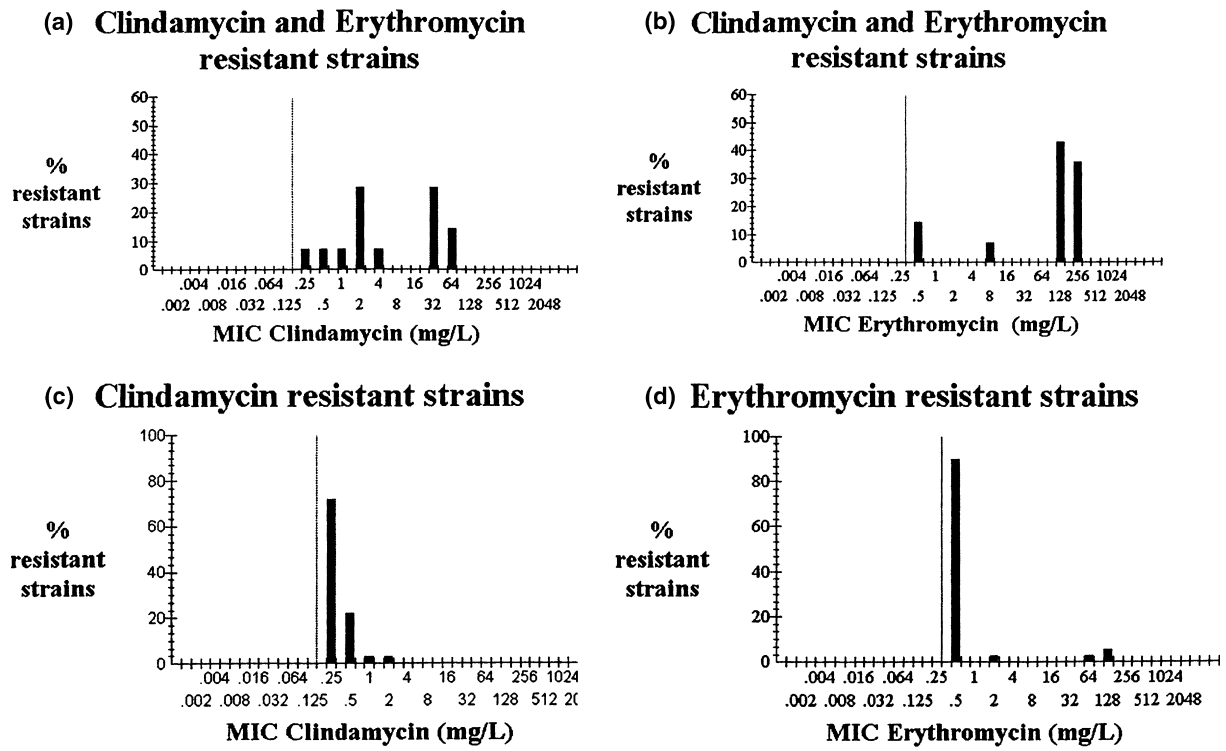

Fig. 1. (a) Clindamycin MICs for the isolates resistant to both clindamycin and erythromycin; MIC range = 0.25–64 mg/L, 73% of strains have MIC ≥ 2 mg/L. (b) Erythromycin MICs for the isolates resistant to both clindamycin and erythromycin; MIC range = 0.5–128 mg/L, 84% of strains have MIC ≥ 2 mg/L. (c) Clindamycin MICs for the clindamycin-resistant isolates; MIC range = 0.25–2 mg/L, 3% of strains have MIC ≥ 2 mg/L. (d) Erythromycin MICs for the erythromycin-resistant isolates; MIC range = 0.5–128 mg/L, 11% of strains have MIC ≥ 2 mg/L.

Table 4. Resistant isolates of *Propionibacterium acnes* from 13 European countries and their clinical sources

Country (no. of isolates)	No. of resistant (% resistant)	Source of isolation (no. of isolates)
DNK (18)	5 (28%)	Blood (5)
FIN (17)	2 (12%)	Head/neck, faeces (1)
NOR (8)	1 (13%)	Blood (1)
SWE (26)	6 (23%)	Bone, soft tissue (2); abdominal abscess, skin (1)
GER (11)	1 (9%)	Soft tissue (1)
GBR (30)	2 (7%)	Meningitis, head/neck (1)
NLD (7)	–	–
GRE (9)	2 (22%)	Skin (2)
ITA (57)	34 (60%)	Blood (23); meningitis, abdominal abscess, soft tissue (3); eye, pleuresia (1)
CRO (6)	5 (83%)	Blood (5)
CZE (43)	7 (16%)	Skin (4); soft tissue (2); bile (1)
HUN (41)	6 (15%)	Blood (3); abdominal infection (2); skin (1)
SVN (31)	18 (58%)	Blood (8); meningitis, abdominal abscess, soft tissue, prosthetic device (2); eye, head/neck (1)

DNK, Denmark; FIN, Finland; NOR, Norway; SWE, Sweden; GRE, Greece; ITA, Italy; CRO, Croatia; CZE, Czech Republic; HUN, Hungary; SVN, Slovenia; GER, Germany; GBR, Great Britain; NLD, the Netherlands.

Country distribution of different PFGE clones

Clonal type F was composed of isolates from ten different countries (Table 7). Clone H was composed mainly of isolates from Italy. Some *P. acnes* clones (A, B, G, H) were spread over distant areas, but other clones comprised isolates from neighbouring countries only (clones D and I were found only in Italy and Slovenia). Some isolates from the same country (Denmark, Italy and Slovenia) or from the same geographical area (Croatia and Italy), but also isolates from very different regions (Denmark and Slovenia), showed 100% similarity. In contrast, some isolates from Denmark, Italy, Czech Republic, Slovenia, Sweden and Great Britain were unlinked to any other clones (Table 7). There was no relationship between the distribution of clones and the infection type (Table 8), but 38% of resistant isolates from blood and 60% of those from soft tissue infections belonged to clone F.

PFGE and resistance phenotype

Clone F contained 69% of the clindamycin-resistant isolates, with MICs of 0.25–1 mg/L, while isolates with other PFGE patterns had

Table 5. In-vitro activities of six antimicrobial agents against *Propionibacterium acnes* isolates from different European countries

Country (no. of isolates/ no. of resistant isolates)	MIC_{50}/MIC_{90} (mg/L)					
	CL (≥ 0.25)[a]	EM (≥ 0.5)[a]	TCY (≥ 2)[a]	LNZ (≥ 8)[a]	PEN (≥ 0.25)[a]	VAN (≥ 4)[a]
DNK (18/5)	< 0.064/0.125	0.125/0.5	0.5/1	0.5/1	0.016/0.064	1/1
FIN (17/2)	< 0.064/< 0.064	0.25/0.25	1/2	1/1	0.032/0.064	1/1
NOR (8/1)	< 0.064/2	0.125/8	1/8	0.5/2	0.016/0.064	1/1
SWE (26/6)	< 0.064/0.25	0.125/64	0.5/1	0.5/1	0.032/0.125	1/1
GER (11/1)	< 0.064/< 0.064	0.25/0.25	1/1	0.5/0.5	0.016/0.032	1/1
GBR (30/2)	< 0.064/0.125	0.25/0.25	0.5/1	1/1	0.032/0.064	0.5/0.5
NLD (7/0)	< 0.064/< 0.064	0.25/0.25	1/1	0.5/0.5	0.016/0.064	0.5/1
GRE (9/2)	< 0.064/32	0.25/> 128	1/4	0.5/0.5	0.032/0.125	0.5/0.5
ITA (57/34)	< 0.064/0.5	0.25/0.5	0.5/1	0.5/0.5	0.064/0.125	0.5/1
CRO (6/5)	0.25/2	0.5/> 128	1/1	0.5/0.5	0.016/0.032	1/1
CZE (43/7)	< 0.064/< 0.064	0.25/0.25	0.5/1	1/1	0.016/0.064	0.5/1
HUN (41/6)	< 0.064/0.25	< 0.064/0.25	0.25/0.5	0.5/1	0.016/0.032	0.5/1
SVN (31/18)	0.125/4	0.25/128	1/1	0.5/1	0.016/0.064	1/1

CL, clindamycin; EM, erythromycin; TCY, tetracycline; LNZ, linezolid; PEN, penicillin; VAN, vancomycin; DNK, Denmark; FIN, Finland; NOR, Norway; SWE, Sweden; GRE, Greece; ITA, Italy; CRO, Croatia; CZE, Czech Republic; HUN, Hungary; SVN, Slovenia; GER, Germany; GBR, Great Britain; NLD, the Netherlands.
[a]Breakpoint recommended by the European Committee on Antibiotic Susceptibility Testing (EUCAST) [17,20].

Table 6. Antibiotic resistance rates among *Propionibacterium acnes* isolates from different countries/ regions of Europe and comparison with reported non-hospital antibiotic sales in European Union countries (data available for only eight of the countries participating in the study); the countries were divided into four main geographical regions

Region/country (no. of isolates/ no. of resistant isolates)	% of isolates resistant to antibiotic							Antibiotic sales[a]	
	CL ≥ 0.25[b]	EM ≥ 0.5[b]	TCY ≥ 2[b]	LNZ ≥ 8[b]	PEN ≥ 0.25[b]	VAN ≥ 4[b]	% Resistant isolates	Macrolides/ lincosamides	Tetracycline
North (69/14)	7.2	11.6	5.8	0	0	0	20.2		
DNK (18/5)	5.6	22.2	0	0	0	0		1.97	0.98
FIN (17/2)	0	0	11.8	0	0	0		1.86	5.50[c]
NOR (8/1)	12.5	12.5	12.5	0	0	0			
SWE (26/6)	11.5	11.5	3.8	0	0	0		0.97[d]	2.97
West (48/3)	4.2	4.2	0	0	0	0	6.2		
GER (11/1)	9.1	9.1	0	0	0	0		2.54	3.26
GBR (30/2)	3.3	3.3	0	0	0	0		3.22	3.66
NLD (7/0)	0	0	0	0	0	0		1.24	2.35
South (66/36)	21.2	39.4	1.5	0	0	0	54.5		
GRE (9/2)	22.2	22.2	11.1	0	0	0		4.5	2.69
ITA (57/34)	21.1	42.1	0	0	0	0		5.07[c]	0.56[d]
East (121/36)	20.7	13.2	2.5	0	0	0	29.7		
CRO (6/5)	66.7	66.7	0	0	0	0			
CZE (43/7)	7	2.3	7	0	0	0			
HUN (41/6)	14.6	0	0	0	0	0			
SVN (31/18)	38.7	35.5	0	0	0	0			

CL, clindamycin; EM, erythromycin; TCY, tetracycline; LNZ, linezolid; PEN, benzylpenicillin; VAN, vancomycin; DNK, Denmark; FIN, Finland; NOR, Norway; SWE, Sweden; GRE, Greece; ITA, Italy; CRO, Croatia; CZE, Czech Republic; HUN, Hungary; SVN, Slovenia; GER, Germany; GBR, Great Britain; NLD, The Netherlands.
[a]Daily dose/1000 inhabitants/day.
[b]Breakpoints.
[c]Highest defined daily dose (data available from eight countries).
[d]Lowest defined daily dose (data available from eight countries).

clindamycin MICs of < 0.064–2 mg/L (Fig. 3). Clone H contained 46% of the erythromycin-resistant isolates, with MICs of 0.5–1 mg/L, while other erythromycin-resistant isolates had MICs of 0.5–128 mg/L.

DISCUSSION

P. acnes, which is considered to be a microorganism with low virulence, has an important role in acne. There is not always a correlation between *Propionibacterium* levels and the severity of acne [22], but it is well known that *P. acnes* triggers inflammatory reactions in this common skin disease. Generally, however, propionibacteria have a poorly defined role in severe infections. Even when the bacterium was cultured, it was considered to be a contaminant, especially if isolated following percutaneous punctures or biopsies [6,12]. However, a positive culture for *P. acnes* cannot be dismissed as a contaminant without considering the clinical circumstances. Thus, although *P. acnes* originates from the normal flora, it can act as a primary pathogen [6,9,13].

Susceptibility testing of anaerobic bacteria does not normally influence therapeutic decisions, because of the slow growth of the bacteria, the cost and complexity of testing methods, and a

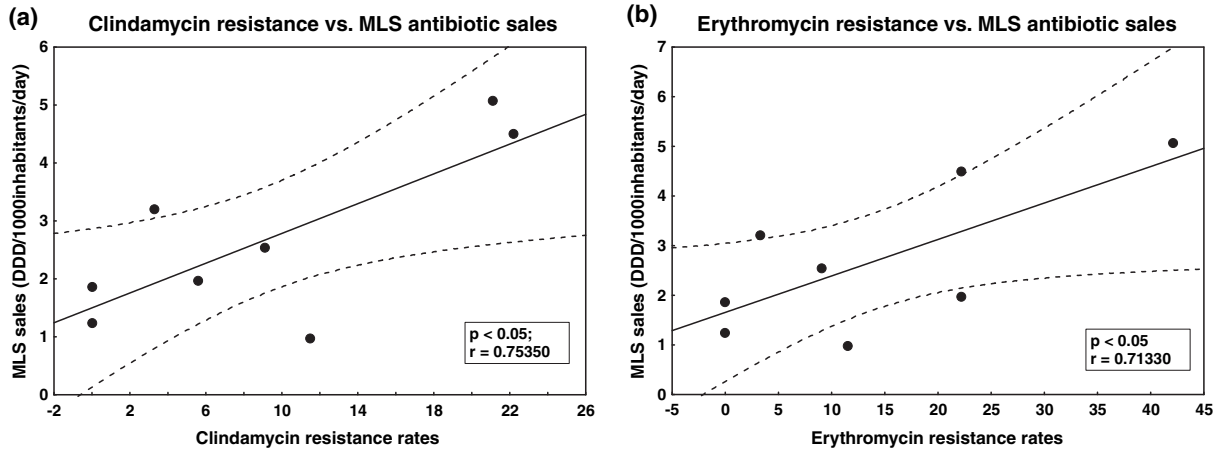

Fig. 2. Correlation between the reported sales of macrolides/lincosamides (MLS) in eight EU countries and the resistance rates to (a) clindamycin, and (b) erythromycin in these countries. By linear regression analysis, the clindamycin and erythromycin resistance rates correlate with the reported sales of MLS (1.4998 + 0.12846 × clindamycin resistance rates, $p < 0.05$, $r = 0.753$; and 1.6551 + 0.07345 × erythromycin resistance rates, $p < 0.05$, $r = 0.713$, respectively). Solid lines show mean values; dashed lines show 95% confidence intervals.

Table 7. Country of origin of 89 resistant *Propionibacterium acnes* isolates and their assignment by pulsed-field gel electrophoresis to individual clones (two reference strains, ATCC 6919 and ATCC 11828, were included in the analysis)

Clonal type (no. of resistant isolates)	Country (no. of isolates)
A (9)	ITA (6), SVN, SWE (1); ATCC 11828
B (5)	ITA (3); CZE, FIN (1)
C (1)	DEN (1)
D (2)	ITA, SVN (1)
E (1)	ITA (1)
F (32)	ITA (9), SVN (7), HUN (5), CRO (4), SWE (2); CZE, DEN, FIN, GBR, NOR (1)
G (11)	CZE, GRE, ITA (2); GER, HUN, SVN, SWE (1); ATCC 6919
H (20)	ITA (11); SVN (5); DEN (3); CRO (1)
I (2)	ITA, SVN (1)
J (1)	CZE (1)
K (1)	CZE (1)
L (1)	SVN (1)
M (1)	SVN (1)
N (1)	GBR (1)
O (1)	CZE (1)
P (1)	SWE (1)
Q (1)	SWE (1)

DNK, Denmark; FIN, Finland; NOR, Norway; SWE, Sweden; GRE, Greece; ITA, Italy; CRO, Croatia; CZE, Czech Republic; HUN, Hungary; SVN, Slovenia; GER, Germany; GBR, Great Britain; NLD, The Netherlands.

Table 8. Types of infection from which 89 resistant *Propionibacterium acnes* isolates were obtained

Type of infection (no. of resistant strains)	Clonal type (no. of strains)
Blood (45)	C, E, M (1); B, D, I, G (2); A (3); F (17); H (14)
Soft tissue (10)	O, P (1); G (2); F (6)
Skin (8)	B, J, K (1); F (2); G (3)
Abdominal abscess (7)	G (1); A, F, H (2)
Meningitis (6)	A, B, F, L (1); H (2)
Eye (2)	F (2)
Head/neck (3)	B, H, N (1)
Prosthetic device (2)	F, H (1)
Pleuresia (2)	A, G (1)
Bone (2)	A, Q (1)
Biliary infection (1)	G (1)
Faeces (1)	F (1)

general opinion that susceptibility patterns among anaerobes are predictable [23]. In the present study, different selection pressures appeared to operate for each country or region, but the highest rates of resistant isolates were found in southern European countries and the lowest rates in western European areas (Table 6). Surprisingly, despite a very restrictive policy regarding antibiotic prescription, the overall resistance rate for Swedish *P. acnes* isolates was higher than for other northern and western European countries.

Many isolates were resistant to clindamycin and/or erythromycin (Table 3), and these were isolated principally from severe infections. In some countries (Italy and Greece) there was a correlation with sales of antibiotics, but this was not observed for Sweden and Denmark (Table 6). It is possible that topical clindamycin, used commonly for the treatment of acne, could account for the correlation, but it is unlikely that all patients carrying resistant isolates were acne patients or had contact with acne patients. Great Britain has a high consumption rate of macrolides/lincosamides, but the resistance rates were still lower than in Italy or Greece. Overall, a higher prevalence of erythromycin resistance was observed in southern Europe, and a higher

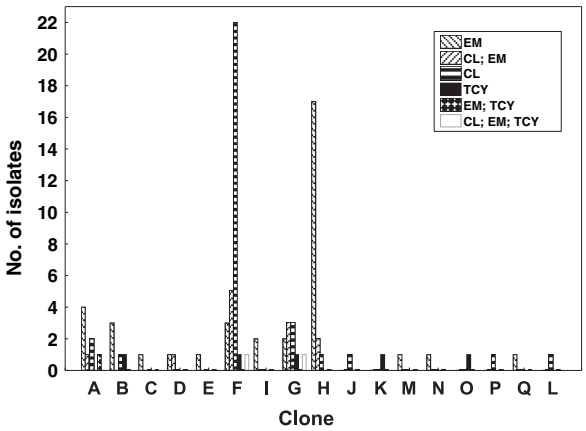

Fig. 3. Distribution of six resistant phenotypes among 17 clones identified by pulsed-field gel electrophoresis. CL, clindamycin; EM, erythromycin; TCY, tetracycline.

prevalence of clindamycin resistance in southern and eastern Europe (Table 6).

Tetracycline resistance was encountered less frequently than clindamycin and erythromycin resistance, and most of the tetracycline-resistant isolates were from northern European countries, whereas no tetracycline-resistant isolates were identified in western European countries. Although tetracycline is still used for a variety of diseases, fewer resistant isolates were found than for other antimicrobial agents, as has also been shown for skin samples from acne patients [17,24]. In comparison to other countries from the same region, lower resistance rates were found for isolates from Hungary and the Czech Republic (Table 6). These results are in agreement with those of other recent studies investigating skin samples [24].

Linezolid belongs to a new class of antimicrobial agents, the oxazolidinones, and is considered to be active against Gram-positive aerobic and anaerobic bacteria [11,25]. However, Auckland *et al.* [26] described the isolation of linezolid-resistant enterococci from patients who had received this antibiotic. The present study did not detect any resistance to linezolid among the *P. acnes* isolates tested, but clinicians should be aware of the risk that *P. acnes* isolates may become resistant to this antibiotic in the future.

As demonstrated in the present study, there is a potential risk of life-threatening infections, e.g., septicaemia or meningitis, with antibiotic-resistant *P. acnes.* Fortunately, this bacterium is usually susceptible to β-lactams, such as penicillin or vancomycin, that are used to treat serious infections, and shows resistance mainly to erythromycin, clindamycin and tetracycline [17,27,28]. Acne patients are usually treated with many oral and topical antimicrobial agents, and their normal flora is exposed to a great selective pressure for resistance development. Although resistance in *P. acnes* seems to result mainly from a mutational event in the 23S rRNA ribosomal binding site for MLS antimicrobial agents or the 16S rRNA ribosomal binding site for tetracycline [29,30], it has been reported that a corynebacterial transposon, Tn*5432*, which carries the rRNA methylase *erm* (X) (erythromycin ribosome methylase) gene, can determine resistance to MLS agents [31]. It is not impossible that other mobile resistance genes coming from different species may be transferable to skin propionibacteria [31], and this could lead subsequently to acute infections caused by resistant *P. acnes* isolates.

PFGE is a robust typing method that can establish strain relatedness among bacteria with the same multiple antibiotic resistance pattern, and can determine whether a particular clone is more prone to become resistant. Criteria established by Tenover *et al.* [32] are applicable for small sets of epidemiologically related isolates (≤ 30) and for investigating outbreaks. The present study investigated a large number of isolates, collected over a 6-year period from a wide area, and these inclusion criteria were not considered appropriate for determining the genomic diversity among the isolates. PFGE has been used previously for epidemiological studies involving *P. acnes* [17]. Thus, relative homogeneity among 69 resistant isolates from the skin of acne patients in a single geographical area has been reported [17], but until the present study, there were no data regarding the genetic diversity of resistant *P. acnes* clinical isolates from Europe. Perry *et al.* [33] used random amplification of polymorphic DNA for typing clinical isolates of *P. acnes*, and reported that two isolates of different serotypes belonged to two different clones. In the present study, *P. acnes* ATCC 6919 (serotype I) and *P. acnes* ATCC 11828 (serotype II) were also found to belong to different clones, with less than 60% similarity. In the future, PFGE could be used to distinguish between different *P. acnes* serotypes. The present study showed a high level of genomic diversity, reflected by the large number of different PFGE patterns and the presence of isolates that did not cluster with other clones.

It is important to note that some clones appear to have spread not only in related areas, but also in more distant countries (Table 7). Isolates belonging to the same clone from neighbouring countries may have similar or different patterns of antibiotic resistance. The first example may represent the spread of the same clone in geographically closed regions, while in the second case the clone may be formed of isolates that are more prone to become resistant to different antimicrobial agents. *P. acnes* isolates from different types of infection were distributed among different clones, but a correlation was observed between isolates with low-level resistance to erythromycin or clindamycin and certain clonal types. It was therefore concluded that most clindamycin-resistant or erythromycin-resistant *P. acnes* isolates are members of a single clone that has spread in different areas in Europe. Similar findings regarding the clonal distribution of clindamycin-resistant isolates were obtained in a recent study in which skin samples from acne patients were characterised [17].

The international variations in resistance rates, even within Europe, highlight the importance of the selective pressure exerted by inappropriately used antimicrobial agents. However, sales data cannot be equated with antibiotic exposure without consideration of the problem of patient compliance, which is very difficult to measure. Low compliance and self-medication may contribute to resistance [34]. It is known that the level of antimicrobial resistance can differ from one city to another, and even within the same city, differences can exist between hospitals. The data presented here are representative for each country and geographical region, which could be a limitation of the study. However, epidemiological studies can provide useful information, which can be used to stimulate discussion concerning regional differences in prevalence rates and help in the development of strategies to combat the rising problem of antibiotic resistance in *P. acnes*.

REFERENCES

1. Moncla BJ, Hillier SL. *Peptostreptococcus*, *Propionibacterium*, *Lactobacillus*, *Actinomyces*, and other non-spore forming anaerobic gram-positive bacteria. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH, eds. *Manual of clinical microbiology*, 8th edn. Washington, DC: ASM Press, 2003; 857–879.
2. McGinley KJ, Webster GF, Leyden JJ. Regional variations of cutaneous propionibacteria. *Appl Environ Microbiol* 1978; **35**: 62–66.
3. Sutter VL. Anaerobes as normal oral flora. *Rev Infect Dis* 1984; **6**(suppl): 62–66.
4. Eady EA, Cove JH, Holland KT, Cunliffe WJ. Erythromycin resistant propionibacteria in antibiotic treated acne patients: association with therapeutic failure. *Br J Dermatol* 1989; **121**: 51–57.
5. Eady EA. Bacterial resistance in acne. *Dermatology* 1998; **196**: 59–66.
6. Jakab E, Zbinden R, Gubler J, Ruef C, von Graevenitz A, Krause M. Severe infections caused by *Propionibacterium acnes*: an underestimated pathogen in late postoperative infections. *Yale J Biol Med* 1996; **69**: 477–482.
7. Brook I. Bacteremia due to anaerobic bacteria in newborns. *J Perinatol* 1990; **10**: 351–356.
8. Praderio L, Dagna L, Beretta G, Rubin G, Ossi C. *Propionibacterium acnes* sepsis in a previously healthy man. *Clin Infect Dis* 1998; **27**: 1330–1331.
9. Brook I, Frazier EH. Infections caused by *Propionibacterium* species. *Rev Infect Dis* 1991; **13**: 819–822.
10. Bolognia JL, Edelson RL. Spread of antibiotic-resistant bacteria from acne patients to personal contacts—a problem beyond the skin? *Lancet* 1997; **350**: 972–973.
11. Hedberg M, Nord CE. Anaerobic bacteria. In: Yu VL, Weber R, Raoult D, eds. *Antimicrobial therapy and vaccines*, 2nd edn. New York: Apple Tree Productions, 2002; 55–62.
12. Eady EA, Ingham E. *Propionibacterium acnes*—friend or foe? *Rev Med Microbiol* 1994; **5**: 163–173.
13. Brook I. *Propionibacterium acnes*. In: Yu VL, Weber R, Raoult D, eds. *Antimicrobial therapy and vaccines*, 2nd edn. New York: Apple Tree Productions, 2002; 533–535.
14. Sillerstrom E, Wahlund E, Nord CE. In vitro activity of LY 333328 against anaerobic gram-positive bacteria. *J Chemother* 1999; **11**: 90–92.
15. Celig DMSP. Clinical evaluation of the RapID-ANA II panel for identification of anaerobic bacteria. *J Clin Microbiol* 1991; **29**: 457–462.
16. National Committee for Clinical Laboratory Standards. *Methods for antimicrobial susceptibility testing of anaerobic bacteria*, 6th edn. Approved standard M11-A6. Wayne, PA: NCCLS, 2004.
17. Oprica C, Emtestam L, Lapins J *et al*. Antibiotic-resistant *Propionibacterium acnes* on the skin of patients with moderate to severe acne in Stockholm. *Anaerobe* 2004; **10**: 155–164.
18. Maslow JN, Slutsky AM, Arbeit RD. Application of pulsed-field gel electrophoresis to molecular epidemiology. In: Persing DH, Smith TF, Tenover FC, White TJ, eds. *Diagnostic molecular microbiology: principles and applications*. Washington, DC: American Society for Microbiology, 1993; 563–572.
19. Dice LR. Measures of the amount of ecological association between species. *J Ecol* 1945; **26**: 297–302.
20. Kahlmeter G, Brown D, MacGowan A *et al*. EUCAST—the European Committee on Antimicrobial Susceptibility Testing. *Clin Microbiol Infect* 2003; **9**(suppl 1): 422.
21. Cars O, Molstad S, Melander A. Variation in antibiotic use in the European Union. *Lancet* 2001; **357**: 1851–1853.
22. Leyden JJ, McGinley KJ, Mills OH, Kligman AM. *Propionibacterium* levels in patients with and without acne vulgaris. *J Invest Dermatol* 1975; **65**: 382–384.

23. Hecht DW. Prevalence of antibiotic resistance in anaerobic bacteria: worrisome developments. *Clin Infect Dis* 2004; **39**: 92–97.
24. Ross JI, Snelling AM, Carnegie E *et al*. Antibiotic-resistant acne: lessons from Europe. *Br J Dermatol* 2003; **148**: 467–478.
25. Edlund C, Oh H, Nord CE. In vitro activity of linezolid and eperezolid against anaerobic bacteria. *Clin Microbiol Infect* 1999; **5**: 51–53.
26. Auckland C, Teare L, Cooke F *et al*. Linezolid-resistant enterococci: report of the first isolates in the United Kingdom. *J Antimicrob Chemother* 2002; **50**: 743–746.
27. Eady EA, Jones CE, Tipper JL, Cove JH, Cunliffe WJ, Layton AM. Antibiotic resistant propionibacteria in acne: need for policies to modify antibiotic usage. *BMJ* 1993; **306**: 555–556.
28. Leyden JJ, McGinley KJ, Cavalieri S, Webster GF, Mills OH, Kligman AM. *Propionibacterium acnes* resistance to antibiotics in acne patients. *J Am Acad Dermatol* 1983; **8**: 41–45.
29. Ross JI, Eady EA, Cove JH *et al*. Clinical resistance to erythromycin and clindamycin in cutaneous propionibacteria isolated from acne patients is associated with mutations in 23S rRNA. *Antimicrob Agents Chemother* 1997; **41**: 1162–1165.
30. Ross JI, Eady EA, Cove JH, Cunliffe WJ. 16S rRNA mutation associated with tetracycline resistance in a gram-positive bacterium. *Antimicrob Agents Chemother* 1998; **42**: 1702–1705.
31. Ross JI, Eady EA, Carnegie E, Cove JH. Detection of transposon Tn5432-mediated macrolide–lincosamide–streptogramin B (MLSB) resistance in cutaneous propionibacteria from six European cities. *J Antimicrob Chemother* 2002; **49**: 165–168.
32. Tenover FC, Arbeit RD, Goering RV *et al*. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995; **33**: 2233–2239.
33. Perry AL, Worthington T, Hilton AC, Lambert PA, Stirling AJ, Elliott TS. Analysis of clinical isolates of *Propionibacterium acnes* by optimised RAPD. *FEMS Microbiol Lett* 2003; **228**: 51–55.
34. Albrich WC, Monnet DL, Harbarth S. Antibiotic selection pressure and resistance in *Streptococcus pneumoniae* and *Streptococcus pyogenes*. *Emerg Infect Dis* 2004; **10**: 514–517.