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International Pneumococcal Clones Match or Exceed the Fitness of Other Strains despite the Accumulation of Antibiotic Resistance▽

Daniel Rudolf,[1] Nikolay Michaylov,[1] Mark van der Linden,[2] Ludwig Hoy,[3] Keith P. Klugman,[4] Tobias Welte,[1] and Mathias W. Pletz[5]* on behalf of the CAPNETZ Study Group

Department of Pulmonary Medicine, Hannover Medical School, 30625 Hannover, Germany[1]; National Reference Center for Streptococci, University Hospital RWTH, 52074 Aachen, Germany[2]; Institute of Biometrics, Hannover Medical School, 30625 Hannover, Germany[3]; Rollins School of Public Health, Emory University, Atlanta, Georgia 30322[4]; and Department of Internal Medicine II, Jena University Hospital, 07470 Jena, Germany[5]

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A few international pneumococcal clones dominate the population of antibiotic-resistant pneumococci. Despite the scientific paradigm that a loss in fitness is the price for acquisition of resistance, these clones spread successfully. One hundred fifty-four isolates from adult patients with community-acquired pneumonia (CAP) were analyzed. Thirty percent showed a close relationship to international clones and had fitness equal to or exceeding that of other strains ($P = 0.015$); these factors may result in the endurance of these strains despite a reduction of antibiotic usage.

Most studies investigating the etiology of community-acquired pneumonia (CAP) have identified *Streptococcus pneumoniae* as the most frequent pathogen (23). A recent review by Welte and Kohnlein (in 2009) showed a mortality of patients hospitalized with CAP of 5 to 20% (20). Treatment failure due to antimicrobial resistance may account for at least a part of that.

A small number of global pneumococcal clones dominate the population of antibiotic-resistant pneumococci (12). In 1997, the Pneumococcal Molecular Epidemiology Network (PMEN) was established with the aim of defining the nomenclature of global antibiotic-resistant *Streptococcus pneumoniae* (14). Nationwide studies from individual countries assigned single clones to be responsible for the majority of antibiotic-resistant pneumococci (2, 10, 18).

To date, it remains unclear why PMEN clones spread so successfully despite the scientific paradigm that a loss in biological fitness is the price for acquisition of resistance. It was the aim of this study to detect PMEN clone-related clinical isolates and to compare them to unrelated clones in terms of antibiotic resistance, biological fitness, and clinical parameters.

Isolates and patient data were collected between 2002 and 2006 from patients with CAP by the German Competence Network CAPNETZ. A detailed description of the CAPNETZ methodology is given elsewhere (21).

Serotyping, multilocus sequence typing (MLST), and MIC testing of CAPNETZ strains were performed at the German National Reference Center for Streptococci. Serotyping was performed by the standard Quellung method (15), MLST was performed as previously described (5), using modified primers (9), and MIC testing was performed by the broth microdilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI) (1). Current breakpoint tables developed by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) were used to interpret MICs and define antimicrobial resistance (6). Multidrug-resistant *Streptococcus pneumoniae* (MDRSP) was defined as resistant to ≥3 classes of antibiotics (11).

We analyzed 154 pneumococcal isolates. Forty-six (30%) of these isolates could be assigned to the global PMEN clones regarding their allelic profile based on MLST. Relatedness was defined by the sharing of alleles at ≥5 of 7 loci (22).

PMEN clone-related isolates accumulated resistance to antimicrobial agents, whereas other strains mainly presented susceptibility (Table 1). The accumulation of resistance also accounts for a higher rate of MDRSP (13% versus 3%; $P = 0.023$). In total, nine PMEN clones constituted 80% of antibiotic-resistant isolates. Forty-six percent of clarithromycin-resistant isolates belonged to the England^{14}-9 clone, and 42% of clindamycin-resistant isolates belonged to the Portugal^{19F}-21 clone, as did 31% of the tetracycline-resistant isolates. Antibiotic-specific values for MIC_{50} and MIC_{90} of both groups were equal or higher for PMEN-related isolates.

In vitro fitness was determined by the measurement of growth curves. Isolates were inoculated into Todd-Hewitt medium, and bacterial growth was monitored by a microplate reader (Molecular Devices) at an optical density of 600 nm at intervals of 5 min over a time period of 16 h (7, 22). To compare bacterial growth as one component of biological fitness, we analyzed the maximum slope of each curve (maximum increase of optical density/45 min). The experiment was repeated nine times, and arithmetic means were used.

The fitness of PMEN clone-related pneumococcal isolates was equal to or exceeded that of isolates without a relationship to the global clones. Growth curves of PMEN-related isolates reached a significantly higher maximum slope (mean values, 1.48 ± 0.73 versus 1.18 ± 0.54; $P = 0.015$). There was no difference in maximum optical density at stationary phase (0.22 ± 0.09 versus 0.21 ± 0.07). In total, the isolates presented a

* Corresponding author. Mailing address: Division of Gastroenterology, Hepatology and Infectious Diseases, Department of Internal Medicine II, Jena University Hospital, Erlanger Allee 101, 07470 Jena, Germany. Phone: 49 (0) 3641 932 4224. Fax: 49 (0) 3641 932 4652. E-mail: mathias.pletz@med.uni-jena.de.

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TABLE 1. MICs of pneumococcal isolates (n = 154)

Antibiotic	Non-PMEN clone-related isolates (n = 108)				PMEN clone-related isolates (n = 46)				*P* value for testing[a]	
	MIC (mg/liter)			% R[b]	MIC (mg/liter)			% R[b]	MIC	Resistance
	Range	50%	90%		Range	50%	90%			
Penicillin G	0.015–1.0	0.015	0.015		0.015–4.0	0.015	0.078	2	0.001**	0.116
Amoxicillin	0.015–0.50	0.015	0.030		0.015–4.0	0.015	0.039	2	0.233	0.117
Cefotaxime	0.015–0.50	0.015	0.030		0.015–1.0	0.015	0.057		0.007**	
Clarithromycin	0.060–32.0	0.120	0.120	4	0.060–32.0	0.310	16.0	49	0.000**	0.000**
Clindamycin	0.060–32.0	0.120	0.120	4	0.060–32.0	0.120	16.0	17	0.856	0.004**
Tetracycline	0.120–32.0	0.250	0.50	5	0.120–16.0	0.50	16.0	24	0.001**	0.000**
Levofloxacin	0.250–2.0	1.0	1.0		0.50–2.0	1.0	1.0		0.419	
Vancomycin	0.060–2.0	0.50	2.0		0.120–2.0	0.50	2.0		0.350	

[a] *, $P \leq 0.05$; **, $P \leq 0.01$.
[b] R, resistant.

high variability regarding lag phase, exponential phase, and autolysis patterns but no considerable differences between both groups. Antimicrobial resistance and biological fitness are parameters usually influencing evolution and dissemination of resistance contrary to each other. According to our study, we suggest that one reason for the success of PMEN clones is a combination of resistance and fitness.

Antibiotic selective pressure influences dissemination of resistance. Previous studies described a tendency for resistant strains to decrease selectively after reduction of antibiotic usage (8, 19). PMEN clones have a high genomic plasticity, leading to fast adaption to clinical interventions like capsule switching and evolution of resistance (3). We suggest that PMEN-related resistant strains will not completely disappear after reduction of antibiotic usage, because there is no evolutionary disadvantage to being resistant and biologically fit.

The survey of clinical and demographic characteristics, laboratory findings, and outcome parameters, e.g., CRB-65, CURB, and mortality, revealed no significant difference between the two groups. Also, the proportions of invasive pneumococcal disease were not different. Therefore, we concluded that PMEN-related clones do not infect immunocompromised or elderly patients primarily and that patient factors do not account for the observed differences in resistance between the two groups. Patients with severe immunosuppression, e.g., HIV or active tuberculosis, are not enrolled in the CAPNETZ study.

Conjugated pneumococcal vaccines have been demonstrated to reduce antibiotic resistance due to eradication of multidrug-resistant clones with vaccine serotypes (4, 13). In our study, the 7-valent pneumococcal conjugate vaccine covered 87% of patients infected by PMEN-related isolates, whereas conjugate vaccine coverage of isolates without a relationship to the PMEN clones was significant lower (7-valent, 87% versus 17%, $P < 0.001$; 10-valent, 89% versus 35%, $P < 0.001$; 13-valent, 89% versus 71%, $P = 0.017$). Current studies have shown replacement with novel antibiotic-resistant pneumococcal clones that are not encompassed in vaccines (see the review by Pletz et al. from 2008 [16]). However, serotypes of most clones accounting for replacement are contained in the extended 13-valent vaccine (17).

The success of international PMEN clones is based on the combination of antimicrobial resistance and biological fitness and may result in the endurance of these strains despite a possible reduction of antibiotic usage. Currently, the most effective way to eradicate these clones seems to be the widespread use of a conjugate vaccine. New vaccines can interrupt the transmission of antibiotic-resistant strains, but continued attention to the emergence of novel nonvaccine serotypes and development of vaccines with a broader coverage will be necessary for the future prevention of antibiotic-resistant pneumococcal infections.

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Members of the CAPNETZ Study Group (excluding the authors) include: S. Krüger (Medical Clinic I, University Clinic RWTH, Aachen, Germany), D. Stolz (Clinic for Pneumology, Uni-Spital, Basel, Switzerland), N. Suttorp, H. Schütte, and A. Tessmer (Department of Infectious Disease and Respiratory Medicine, Charité-University Medicine Berlin), P. Martus (Institute for Biostatistics and Clinical Epidemiology, Charité-University Medicine Berlin), T. Bauer and J. Hecht (HELIOS Klinikum Emil von Behring, Berlin, Germany), W. Pankow, A. Lies, and D. Thiemig (Hospital for Internal Medicine, Pneumology and Infectious Disease, Vivantes Clinical Center, Berlin-Neukölln, Germany), B. Hauptmeier, D. Wede, and M. Suermann (University Hospital Bergmannsheil, Department of Pneumology, Allergology and Sleep Medicine, Bochum, Germany), S. Ewig (Department of Respiratory Medicine and Infectious Diseases, Augusta Hospital, Bochum, Germany), M. Prediger and K. Zobel (Department of Internal Medicine III, Carl-Thiem-Klinikum Cottbus), J. Rademacher (Department of Respiratory Medicine, Hannover Medical School, Hannover, Germany), G. Barten, L. Gosman, and W. Kröner (CAPNETZ Main Office, Hannover, Germany), R. Bals (University Clinic Saarlandes, Internal Medicine V, Pneumology, Homburg/Saar, Germany), K. Dalhoff and P. Heyer (Medical Clinic III, Pulmonology, University Hospital Schleswig-Holstein, Lübeck, Germany), G. G. Rohde (Department of Respiratory Medicine, Maastricht University Medical Center, MUMC+, Maastricht, The Netherlands), W. Petermann, H. Buschmann, R. Kröning, and Y. Aydin (Brotherhospital St. Josef, Medical Clinic, Pneumology, Paderborn, Germany), T. Schaberg and I. Hering (Center of Pneumology, Diakonie-Hospital Rotenburg), R. Marre (University of Ulm), C. Schumann (Department of Internal Medicine II, University of Ulm), H. von Baum (Ulm University Hospital, Medical Microbiology and Hygiene), T. Illmann and M. Wallner (2mt Software, Ulm, Germany), O. Burghuber and G. Rainer (Internal Lung Department, Otto Wagner Spital Wien), and all study nurses.

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