Journal of Clinical Microbiology, June 2007, p. 1954–1962 Vol. 45, No. 6
0095-1137/07/$08.00+0 doi:10.1128/JCM.02187-06
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Loss of Bacterial Diversity during Antibiotic Treatment of Intubated Patients Colonized with *Pseudomonas aeruginosa*▽

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Received 25 October 2006/Returned for modification 8 January 2007/Accepted 26 March 2007

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Management of airway infections caused by *Pseudomonas aeruginosa* is a serious clinical challenge, but little is known about the microbial ecology of airway infections in intubated patients. We analyzed bacterial diversity in endotracheal aspirates obtained from intubated patients colonized by *P. aeruginosa* by using 16S rRNA clone libraries and microarrays (PhyloChip) to determine changes in bacterial community compositions during antibiotic treatment. Bacterial 16S rRNA genes were absent from aspirates obtained from patients briefly intubated for elective surgery but were detected by PCR in samples from all patients intubated for longer periods. Sequencing of 16S rRNA clone libraries demonstrated the presence of many orally, nasally, and gastrointestinally associated bacteria, including known pathogens, in the lungs of patients colonized with *P. aeruginosa*. PhyloChip analysis detected the same organisms and many additional bacterial groups present at low abundance that were not detected in clone libraries. For each patient, both culture-independent methods showed that bacterial diversity decreased following the administration of antibiotics, and communities became dominated by a pulmonary pathogen. *P. aeruginosa* became the dominant species in six of seven patients studied, despite treatment of five of these six with antibiotics to which it was sensitive in vitro. Our data demonstrate that the loss of bacterial diversity under antibiotic selection is highly associated with the development of pneumonia in ventilated patients colonized with *P. aeruginosa*. Interestingly, PhyloChip analysis demonstrated reciprocal changes in abundance between *P. aeruginosa* and the class *Bacilli*, suggesting that these groups may compete for a similar ecological niche and suggesting possible mechanisms through which the loss of microbial diversity may directly contribute to pathogen selection and persistence.

Mechanically ventilated patients develop bacterial colonization of the oropharynx and endotracheal tube within 12 h of intubation (14, 36). During mechanical ventilation, the endotracheal tube decreases normal airway defenses and allows microbe-laden oropharyngeal or gastric secretions to be aspirated around the endotracheal tube into the lower airways (14). The fate of such bacteria in the lungs of intubated patients is largely unknown. To date, investigations of intubated patients have used aerobic cultures of either endotracheal secretions or bronchoalveolar lavage (BAL) specimens to determine the presence of pathogenic bacteria. However, these techniques determine neither the dominant bacterial species nor the range of bacterial diversity within the community. Culture-independent methods provide a more comprehensive view of bacterial diversity and may be used to evaluate complex bacterial community dynamics, particularly during antibiotic therapy.

Culture-independent analyses are typically based on biomarker identification. The 16S rRNA gene is the most commonly used biomarker for bacterial community studies (44). Highly conserved regions of the 16S rRNA gene enable amplification of this gene from most bacteria with "universal" PCR primers, while variable regions within this gene permit discrimination between bacterial types (16). This approach has been applied successfully to the analysis of environmental (2, 20, 35, 37, 42) and human (18, 26, 28, 29, 38) bacterial communities and has revealed a much broader bacterial diversity than has traditional culture-based techniques (38). However, to our knowledge, no previous study has examined the extent of bacterial diversity within the lungs of intubated patients by culture-independent methods.

Here, we report the use of two 16S rRNA gene-based culture-independent methods, clone library sequencing (the current "gold standard" in microbial ecology) (1) and a novel high-density oligonucleotide microarray (PhyloChip) (3). Clone libraries typically involve sequencing of a few hundred 16S rRNA genes following PCR amplification; while this strategy provides great specificity, it may profile only the dominant organisms of a complex bacterial population, while less-abundant species that may contribute to disease pathogenesis remain undetected. For this reason, the PhyloChip approach was applied in parallel with 16S rRNA clone libraries to determine changes in the bacterial community composition of the lungs of intubated patients with hospital-acquired *Pseudomonas aeruginosa* during antimicrobial treatment.

MATERIALS AND METHODS

Patient selection and sampling protocol. Daily endotracheal aspirates (EAs) were collected from all intubated patients in the medical, surgical, neurovascular, and cardiac intensive care units of the University of California at San Francisco

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▽ Published ahead of print on 4 April 2007.

(UCSF). Daily EA samples were obtained, and all samples were screened by culture on Difco *Pseudomonas* isolation agar (Becton Dickinson). *Pseudomonas*-positive patients were approached, they gave informed consent, and daily quantitative cultures of *P. aeruginosa* were performed on their respiratory samples. Blind mini-bronchoalveolar lavage (mini-BAL) was performed with a Combi-Cath catheter when clinical infection was suspected. The right bronchoalveolar tree was irrigated with three 20-ml aliquots of nonbacteriostatic saline. The resulting lavage was used for *P. aeruginosa* culture and 16S rRNA analysis. Blind mini-BAL and EA samples were centrifuged and stored at −80°C. Clinical data were collected from patients' charts and recorded in an Access database so that severity of illness could be correlated with 16S rRNA data. Antibiotic drug history was also recorded. All protocols were approved by the Committee on Human Research of UCSF.

DNA extraction and amplification of the bacterial 16S rRNA gene. Bacterial genomic DNA was isolated from 0.5 ml of EA and BAL samples with the Promega (Carlsbad, CA) Wizard genomic DNA purification kit, according to the manufacturer's instructions for purification of both gram-negative and gram-positive bacteria. The 16S rRNA gene was amplified from extracted DNA with the universal bacterial primers Bact-27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and Bact-1492R (5′-GGTTACCTTGTTACGACTT-3′) (22). The reaction mixture (50 μl, final volume) contained 5 μl of 10× PCR buffer (Amersham, NJ), 1 μl of deoxynucleoside triphosphates (10 mM), 0.7 μl of forward primer and reverse primer (100 pmol/μl each), 0.35 μl of *Taq* polymerase (5 U/μl; Amersham), and 1 μl of template DNA. PCR was performed with the DNA Engine Tetrad thermal cycler (Bio-Rad). To maximize the number of bacterial species that could be recovered by PCR, three different annealing temperatures (48°C, 52°C, and 56°C) were used for each sample to amplify the 16S rRNA genes. The following cycling parameters were used: 3 min of initial denaturation at 95°C followed by 25 cycles of denaturation (30 s at 95°C), annealing (30 s), and elongation (120 s at 72°C), with a final extension at 72°C for 7 min. Amplified products from all samples were verified by gel electrophoresis. All PCR products were gel purified with the QIAquick gel extraction kit (QIAGEN), and, for each sample, the purified products amplified at three different annealing temperatures were pooled for cloning, sequencing, and microarray analysis.

Cloning and sequencing. To generate libraries for each sample, the respective full-length 16S RNA PCR products were cloned into pCR4-TOPO vectors (Invitrogen) according to the manufacturer's instructions. One hundred ninety-two transformants from each library were picked randomly. Double-ended sequencing reactions of the entire 16S RNA sequence were carried out with PE BigDye terminator chemistry (Perkin Elmer) and resolved with an ABI PRISM 3730 (Applied Biosystems) capillary DNA sequencer. Sequencing was performed at the DOE Joint Genome Institute.

Sequence alignment and phylogenetic analysis. Paired-end sequencing reads of 16S rRNA clones were assembled with Phrap (12, 13), and only clones in which >80% of the bases had quality scores of at least Phred 20 (indicating a probability of error of ≤1%) were included in the analysis. Chimeric sequences were then detected with an online tool at Greengenes (8) (http://greengenes.lbl.gov) by using an updated version of Bellerophon (19) and were also excluded from the analysis. For the 19 samples we report here, an average of 90% of sequenced clones met these criteria and were included in subsequent analyses.

Sequences were aligned to the Greengenes 7,682-character format with the NAST (9) web server prior to being assigned to a taxonomic node with a sliding scale of similarity thresholds (34) and the Greengenes classifying tool. Distance matrices were constructed for each library with the distance matrix tool at Greengenes, with NAST-aligned sequence data as input.

Phylotype clustering and diversity estimates. Using the distance matrices generated, numbers of 16S rRNA gene phylotypes were calculated at 99% sequence identity with the furthest neighbor clustering in the program DOTUR (33), with 1,000 iterations for bootstrapping. A representative 16S rRNA gene-based phylogenetic tree was constructed with the software package ARB (32) using data from the Greengenes database. Microbial diversity was estimated with the Shannon diversity index (H′). This index takes into account both the number of species present and the proportion of the total accounted for by each species, increasing with species number and greater unevenness of species prevalence (11, 15, 23). The number of species present and the diversity index were compared with the nonparametric Wilcoxon signed-rank test for paired samples.

PhyloChip processing, scanning, probe set scoring, and normalization. The pooled PCR product was spiked with known concentrations of synthetic 16S rRNA gene fragments and non-16S rRNA gene fragments as internal standards for normalization, with quantities ranging from 5.02×10^{8} to 7.29×10^{10} molecules applied to the final hybridization mix. Target fragmentation, biotin labeling, PhyloChip hybridization, scanning, and staining were as described by Brodie et al. (3), while background subtraction, noise calculation, and detection and quantification criteria were essentially as previously reported (3), with some minor exceptions. For a probe pair to be considered positive, the difference in intensity between the perfect match (PM) and mismatch (MM) probes must be at least 130 times the squared noise value (*N*). A taxon was considered present in the sample when 90% or more of its assigned probe pairs for its corresponding probe set were positive (positive fraction, ≥0.90). Hybridization intensity (referred to as intensity) was calculated in arbitrary units for each probe set as the trimmed average (maximum and minimum values removed before averaging) of the PM minus MM intensity differences across the probe pairs in a given probe set. All intensities of <1 were shifted to 1 to avoid errors in subsequent logarithmic transformations. To account for scanning intensity variations from array to array, the intensities resulting from the internal standard probe sets were natural log transformed. Adjustment factors for each PhyloChip were calculated by fitting a linear model by the least-squares method. A PhyloChip's adjustment factor was subtracted from each probe set's $\ln_{intensity}$. Intensities for patient 1 were also normalized by total array intensity. When summarizing PhyloChip results to the subfamily, the taxon with a probe set producing the highest intensity within a subfamily was used.

Nucleotide sequence accession numbers. The nucleotide sequences generated in this study have been submitted to the GenBank database under accession numbers EF508731 to EF512008.

RESULTS

We began our study by comparing sampling techniques. To do this, the bacterial species compositions of samples obtained with blind mini-BAL were compared to those obtained with EAs from two patients with hospital-acquired *P. aeruginosa* by using 16S rRNA clone library sequencing. While there were distinct differences in community compositions between these two patients, the community compositions of the EA and BAL samples from each individual were highly similar (Fig. 1A and B). Therefore, in the remainder of this study, EAs were used due to the simplicity and cost-effectiveness of this less-invasive sample collection method.

Next, as a control for this study, EA samples were collected from three healthy individuals who had been briefly intubated for elective surgery. No 16S rRNA PCR product was detected from these patients (data not shown) under the conditions that readily yielded 16S rRNA amplicons in study patients, confirming that the normal lung is sterile and that our techniques identify organisms present only after colonization of the endotracheal tube and airway has occurred.

We next collected EAs from seven patients colonized by *Pseudomonas aeruginosa*. Patient age, sex, timing of EA sampling, periods of antibiotic administration, and antibiotic sensitivity details are presented in Table 1. All patient samples yielded a 16S rRNA PCR product, and a total of 3,278 nonchimeric 16S rRNA sequences from patient-derived 16S libraries were subjected to phylogenetic analysis. Almost all organisms detected by cloning and sequencing were from five bacterial phyla, the *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, and *Fusobacteria* (Fig. 1C). Over half (55%) of the sequences obtained were from *Pseudomonas aeruginosa*, followed by *Stenotrophomonas maltophilia* (9.7%), *Prevotella* spp. (5.8%), *Acinetobacter* spp. (5.7%), *Serratia marcescens* (5.0%), *Haemophilus* spp. (3.8%), *Neisseria* spp. (3.3%), *Mycoplasma* spp. (2.4%), and *Streptococcus* spp. (2.3%). An additional18 genera were detected but together represented less than 7% of all clones sequenced. Of these less-abundant species, many are known oral, nasal, and gastrointestinal tract inhabitants, e.g., *Porphyromonas*, *Campylobacter*, *Fusobacterium*, *Lactobacillus*, *Enterococcus*, *Rothia*, *Actinomyces*, *Abiotrophia*, *Alcaligenes*, *Corynebacterium*, *Staphylococcus*, and *Veillonella*

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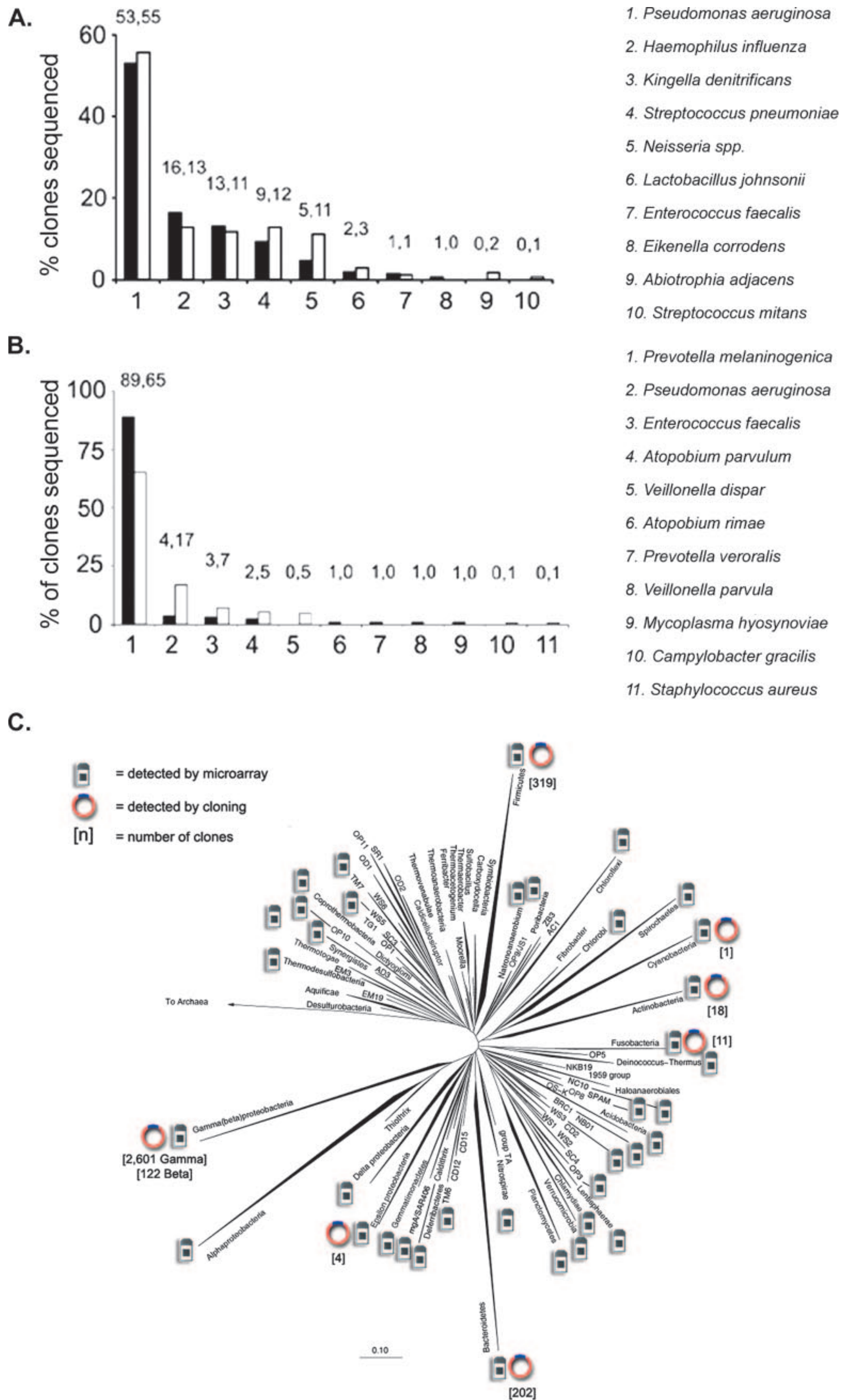

FIG. 1. (A and B) Comparisons of 16S rRNA clone libraries of EA (white bars) and BAL (black bars) patient samples. (A) A total of 173 clones were sequenced from the EA samples, and 153 clones were sequenced from the BAL samples. (B) A total of 154 clones were sequenced from the EA samples, and 141 clones were sequenced from the BAL samples. Species abundances (as percentages) of the sequenced clones are indicated above the bars. (C) Phylogenetic tree showing all recognized bacterial phyla/divisions. Phyla detected in EAs by cloning and PhyloChip microarray analyses are shown. The five main phyla detected by clone library are indicated. Many additional phyla, undetected by cloning, were detected by array analysis.

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TABLE 1. Patient information and antimicrobial treatment

Patient	No. of days after enrollment (sample no.)	Sex	Patient age	Antimicrobial treatment	
				Within 24 h before study enrollment	Following sampling (sensitivity[a])
1	1 (1)	Female	57 yr	Cefazolin, piperacillin-tazobactam	Piperacillin-tazobactam (S), fluconazole, cefazolin
	7 (2)				Cefazolin (S), fluconazole (S), levofloxacin (S)
2	1 (1)	Male	79 yr	Cefazolin, ceftazidime	Antifungal, ceftazidime (S), vancomycin
	11 (2)				Ceftazidime (R), vancomycin, piperacillin-tazobactam (S), ciprofloxacin (S)
	15 (3)				Vancomycin, piperacillin-tazobactam, ciprofloxacin
3	1 (1)	Female	54 yr	None	Ciprofloxacin (S)
	5 (2)				Ciprofloxacin
4	1 (1)	Male	55 yr	None	Piperacillin-tazobactam, vancomycin
	7 (2)				Piperacillin-tazobactam
5	1 (1)	Female	85 yr	Clindamycin	Clindamycin, piperacillin-tazobactam (S)
	4 (2)				Piperacillin-tazobactam (S), vancomycin, ciprofloxacin (S)
	15 (3)				None
	18 (4)				None
	23 (5)				None
6	1 (1)	Female	45 yr	None	Meropenem (I), fluconazole, linezolid
	23 (2)				Tobramycin(S), imipenem (I), cefpirome, cefazolin, cefepime (I)
	102 (3)				Timentin, trimethoprim-sulfamethoxazole, imipenem, vancomycin, fluconazole, cefepime, cefpirome, amphotericin B, tobramycin
7	1 (1)	Female	2 mo	Ampicillin, gentamicin, trimethoprim-sulfamethoxazole	Ampicillin (R), gentamicin
	82 (2)				Gentamicin (S)

[a] S, sensitive; R, resistant; I, indeterminate.

(6, 10, 27, 39). These results support the hypothesis that oral, nasal, and gastrointestinal tract microbiota are the major reservoirs for bacteria that colonize the lower airway in intubated patients (17, 40, 41).

We next evaluated the bacterial diversity in five patients (Table 1, patients 1 to 5) for whom an initial sample was obtained within 24 h of parenteral antibiotic administration and a second sample was obtained 4 to 10 days later. Analysis of microbial diversity in the 16S clone libraries demonstrated a substantial reduction in bacterial diversity during antibiotic administration (Fig. 2). The mean number of bacterial species identified fell from 16.2 to 5.6 ($P < 0.05$), and Shannon's diversity index, a commonly used measure of microbial diversity, fell from 1.48 to 0.59 ($P < 0.05$). In each case, antibiotic therapy led to selection of a pathogenic species that dominated the community. In four of five patients, *P. aeruginosa* came to dominate the microbial community at the second time point, despite the administration of antibiotics to which it was susceptible in vitro (Table 1). In the fifth patient, the community became dominated by another pathogen, *Klebsiella pneumoniae.* Even when *P. aeruginosa* constituted the majority of the population at the initial time point, as in patient 3, who was chronically ventilated, there was initial diversity in the remainder of the community that was lost with antibiotic therapy.

To investigate how the bacterial community changed over a longer period of antibiotic treatment, we obtained additional samples at later time points for two patients (patients 2 and 5), and during prolonged antibiotic therapy in two additional patients (patients 6 and 7). All of these samples showed reduced diversity compared with the initial time point, and *P. aeruginosa* was the predominant species in six of seven samples, suggesting that once this organism is established as the dominant species, microbial diversity is slow to recover. Patient 2 did demonstrate a reduction in the relative abundance of *P. aeruginosa* following adjustment of antipseudomonal therapy 11 days after colonization, and, importantly, this was accompanied by substantial recovery of microbial diversity. This patient improved clinically, and extubation was attempted. Collectively, these data demonstrate that the loss of bacterial diversity and *Pseudomonas* dominance are highly correlated during antipseudomonal therapy.

Dominance of bacterial communities by one or a few species may result either from overgrowth of the dominant species or from loss of the nondominant species. Due to the limited number of clones that can feasibly be sampled from clone libraries, highly abundant species may mask the presence of less-abundant but clinically significant species. To determine whether the decline in diversity observed in clone libraries was

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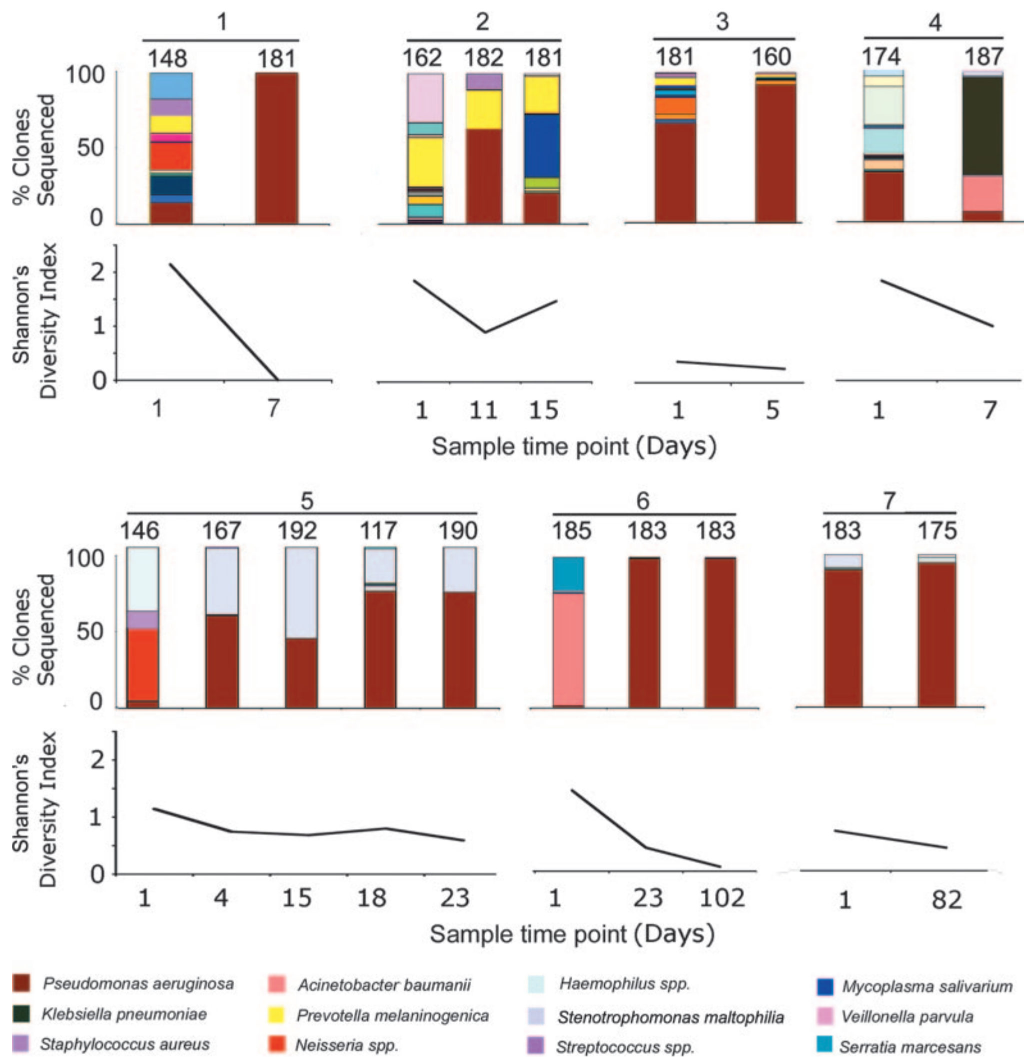

FIG. 2. Temporal changes in bacterial diversity. Each bar represents the color-coded relative abundance of bacteria in a single EA. Numbers above the horizontal bars represent individual patients, and the numbers of clones analyzed for each sample are indicated directly above each bar. For each sample, Shannon's diversity statistic, which reflects both species numbers and evenness of species distribution, is plotted below the histogram.

a true reflection of the bacterial community in these patients, bacterial diversity was also analyzed with high-density microarrays (PhyloChips), which have enhanced sensitivity for low-abundance species compared with cloning (7). For four patients, the same 16S rRNA gene amplicon pools from which clone libraries were prepared were subsequently hybridized to PhyloChips, and the bacterial communities were compared (Fig. 3). While the microarray approach detected >30 times more bacterial types than did clone library sequencing ($P < 0.0001$), the PhyloChip data clearly mirrored the loss of diversity found in the clone library data, with the mean number of observed phylotypes falling from 517 to 280 during antibiotic treatment ($P < 0.05$). These data clearly show that the loss of diversity demonstrated in clone libraries is not an artifact of sampling and confirm that antibiotic treatment leads to lower diversity.

Due to the great sensitivity of the PhyloChip, entire bacterial community responses can be monitored by this technique. Figure 4A illustrates temporal changes in the fluorescence

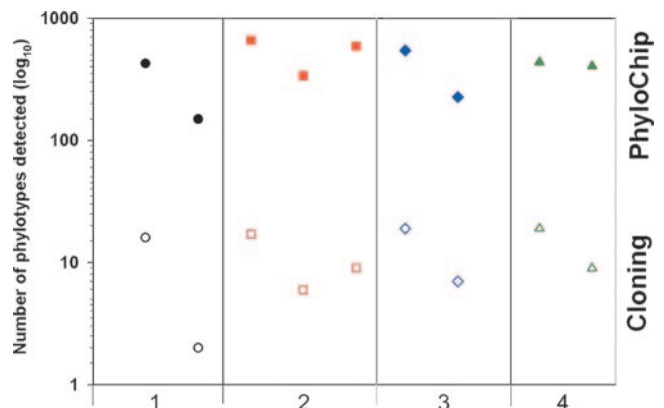

FIG. 3. Comparison of PhyloChip and clone library monitoring of bacterial diversity (phylotype numbers) over time for four patients. Closed symbols show numbers of bacterial phylotypes detected by PhyloChip analysis; open symbols show numbers of bacterial phylotypes detected by clone library on a logarithmic scale. The PhyloChip reveals many more bacterial groups, but the data exactly mirror the loss of diversity revealed by clone library sequencing.

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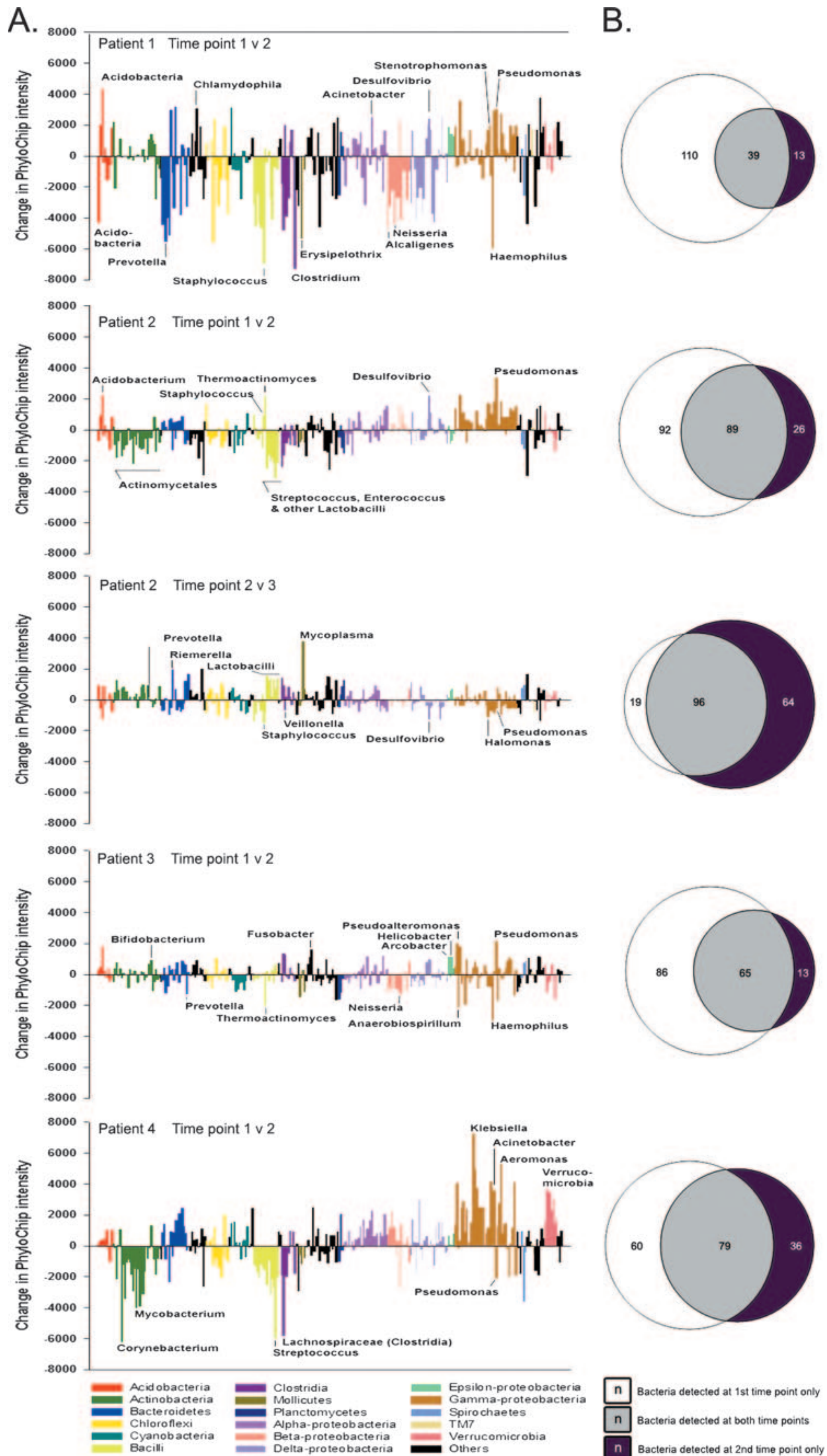

FIG. 4. PhyloChip analysis of complete bacterial communities over time in EAs. (A) Bacteria are ordered alphabetically from left to right according to taxonomic affiliation. Bars above the zero line represent bacteria that increased in abundance relative to the first EA sample being compared; bars below represent those bacteria that declined in abundance. (B) Venn diagrams demonstrate the number of bacterial subfamilies detected at each time point. Note the significant overlap in composition between successive time points.

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intensity of bacteria detected by PhyloChip. Bacteria demonstrating large changes in intensity between time points are labeled, and these correspond well to the dominant bacterial species detected by clone library analysis. The dominance of a few species within a community analyzed by 16S rRNA clone library clearly reflects the limited number of clones sequenced. The PhyloChip readily demonstrated that many bacteria present at the initial sampling point were indeed still present in the subsequent sampling period (Fig. 4B). Conversely, bacteria such as *Klebsiella* that became dominant in later clone library samples were detected in the initial sample by PhyloChip but were not detected in the corresponding clone library. This underscores the potential for low-abundance species to eventually dominate bacterial communities during the course of antimicrobial administration.

The microarray analysis also allowed us to correlate changes in phylogenetic groups over time when these groups of bacteria respond in similar manners to antibiotic administration. For example the γ-*Proteobacteria* (which include *Pseudomonas aeruginosa*) generally exhibit an inverse relationship in abundance with bacteria in the phylum *Actinobacteria* and in the class *Bacilli* (which includes *Lactobacillus*, *Streptococcus*, *Staphylococcus*, and *Enterococcus*) in all patients examined (Fig. 4A). Similarly *Haemophilus* and *Pseudomonas* also demonstrated an inverse relationship. The reciprocal changes in these subgroups within the bacterial community suggest they may be competing for similar niches in the endotracheal environment or otherwise influencing each other's growth.

DISCUSSION

Management of infections caused by *P. aeruginosa* is increasingly difficult due to this bacterium's metabolic versatility, intrinsic antimicrobial resistance, and remarkable armory of virulence factors. Clinically, treatment options are becoming limited due to the rapid emergence of multidrug-resistant strains, which are now estimated to account for up to 30% of strains isolated from patients in nursing homes, hospitals, and intensive care units (30). In this study, we used 16S rRNA-based culture-independent methods to determine the effect of antibiotic therapy on bacterial community dynamics in patients colonized with *P. aeruginosa*. Compared to culture-based methods, both clone library and microarray techniques have often provided a richer picture of microbial diversity (3, 10, 44), and we found that to be true in this study. Prior to, or early in, antibiotic therapy, the airways were colonized with a remarkably wide array of oral, nasal, and gut flora that are presumably aspirated into the lung around the endotracheal tube.

Not unexpectedly, antimicrobial treatment had a pronounced effect on bacterial community composition, with bacterial diversity falling in every case. In most cases, *P. aeruginosa* began as a relatively small fraction of the observed species and came to dominate the community despite the administration of antibiotics to which it was sensitive in vitro. Alarmingly, pathogenic species became dominant in every patient during antibiotic therapy, and both the loss of diversity and *P. aeruginosa* dominance often persisted during prolonged antibiotic therapy. When *P. aeruginosa* declined with antibiotic therapy after becoming dominant (patient 2), there was a substantial recovery in microbial diversity. In contrast, when *P. aeruginosa* was replaced by a dominant pathogen (patient 4), diversity declined. Together, our data demonstrate that loss of diversity is highly correlated with pathogen selection in *P. aeruginosa*-colonized patients who are treated with antibiotics. We hypothesize that reduced microbial diversity under antibiotic selection in the airways may contribute directly to pathogen selection through the loss of microbial competition. This concept is supported by the observation that the abundance of specific organisms (notably *Actinobacteria* and *Bacilli*) always appeared to change in a manner opposite that of *P. aeruginosa.*

It has long been hypothesized that the evolution of virulence is related to the number and variety of bacterial species infecting the host (4, 31). Previously, it was predicted that increased diversity of pathogenic species would promote virulence of individual species (31). However, more recently it has been demonstrated that in polymicrobial infections, less-virulent strains are often favored, suggesting that increased diversity may in fact reduce virulence (21). This hypothesis is supported by our observation that administration of antimicrobials eliminates competition by decreasing the diversity of nontarget organisms and allowing an increase in pathogen abundance. One mechanism through which a decrease in bacterial diversity might alter virulence is quorum sensing. Quorum sensing is a means of bacterial cross-talk between individual cells incorporated into a biofilm, and it may radically affect the gene expression and virulence of pathogens, including *P. aeruginosa* (24, 25, 43). Further studies investigating the contribution of bacterial dynamics to pathogenicity will be required to fully evaluate this hypothesis.

This study highlights the importance of recognizing and understanding the complex bacterial community dynamic that exists in the airways of intubated patients and that is routinely missed by traditional culture-based analyses. Treatment strategies that rely primarily on the identification of *P. aeruginosa* in the airways may often result in suboptimal clinical outcomes. Specifically, in our study, administration of antibiotics to patients colonized with *P. aeruginosa* within a complex bacterial community led to loss of microbial diversity and selection of *P. aeruginosa* or another pathogen. In previous studies, strategies to minimize antibiotic use led to improved outcomes and the emergence of fewer multidrug-resistant strains (5). While further study is clearly needed, we believe that culture-independent surveillance of microbial diversity could be a useful adjunct in the management of intubated patients colonized by *P. aeruginosa* by helping to determine both the effectiveness and the appropriate duration of antibiotic therapy.

We used two culture-independent methods in this study, both of which showed qualitatively similar reductions in bacterial diversity during antibiotic treatment. The PhyloChip is clearly superior to clone library sequencing for assessing the complete spectrum of bacteria present in the community, including low-abundance species, while the 16S rRNA library approach provides important information on relative abundance at the species level. Both culture-independent molecular methods documented a multitude of bacteria undetected by standard identification techniques. Importantly, this was true not only for fastidious, slow-growing, and/or unculturable organisms but also for routinely cul-

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tured pathogens (3, 10, 44). Unlike sequencing, the PhyloChip method does not require cloning and is therefore rapid enough that it could be used to provide information about bacterial abundance and diversity in the clinical setting.

In summary, we have documented the evolution of the complex bacterial community that exists in intubated patients, who are often subjected to a variety of antibiotics. Our data indicate that *P. aeruginosa* frequently arises to become the dominant organism in colonized patients, as competing flora are eliminated by antimicrobial therapy. This may occur even when the patient is on appropriate antipseudomonal therapy as indicated by the in vitro susceptibility profile. If bacterial community changes could be monitored in a timely and cost-effective manner, the effectiveness of antimicrobial therapy might be more readily judged. Furthermore, we hypothesize that this methodology could be utilized to gauge the effectiveness of antibiotic treatment and individualize the duration of antibiotic therapy, perhaps by treating only until microbial diversity rebounds. Ultimately, culture-independent methods such as the PhyloChip will need to be tested for the ability to help guide antibiotic therapy and improve outcomes for intubated patients.

ACKNOWLEDGMENTS

This work was supported by SCCOR grants PH50HL74005 and HL69809 to J.P.W.-K., by a UCSF Academic Senate Independent Investigator Award to S.V.L., and by NIH grant HL024075 to J.B. Part of this work was performed under the auspices of the U.S. Department of Energy's Office of Science, Biological and Environmental Research Program, and by the University of California, Lawrence Livermore National Laboratory under contract no. W-7405-Eng-48, Lawrence Berkeley National Laboratory under contract no. DE-AC02-05CH11231 and Los Alamos National Laboratory under contract no. DE-AC02-06NA25396. We declare no conflicts of interest.

We thank Susannah Tringe for helpful comments on the manuscript and Sam Pitluck and Victor Kunin for help with GenBank submissions.

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