Comparative Analysis of Hospital-Acquired and Community-Acquired *Pseudomonas aeruginosa* Strains in a Tertiary Care Medical Center

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ABSTRACT

Objective: Comparison of genotypes of nosocomially acquired and community-acquired *Pseudomonas aeruginosa* isolates in a hospital setting will shed light on the genomic relatedness between the 2 populations. To that purpose, random amplified polymorphic DNA (RAPD) analysis was performed to correlate genotypes of nosocomially identified *P aeruginosa* isolates to community-acquired isolates from clinical specimens encountered in a tertiary care medical center in Beirut, Lebanon.

Methods: RAPD was performed on 32 community-acquired and 90 nosocomially acquired isolates of *P aeruginosa*. RAPD patterns were visually observed and analyzed. Dendrograms were generated by using GelCompar II software (Applied Maths NV, St-Martens-Latem, Belgium).

Results: These data showed 23 RAPD patterns distributed among the 32 *P aeruginosa* isolates in patients with otitis externa and 31 RAPD patterns in patients with nosocomially acquired infections. Dendrogram analysis performed to compare the 2 *P aeruginosa* populations showed 2 main clusters that were genomically distant. Few strains from both populations were closely related.

Conclusion: The dendrogram analysis confirmed the significant genomic diversity of this organism and demonstrated that community-acquired *P aeruginosa* infections were genomically different from those obtained from nosocomial infections.

INTRODUCTION

Community-acquired *Pseudomonas aeruginosa* is the most frequent etiology of otitis externa.[1] Community-acquired *P aeruginosa* infections in patients reporting to the emergency room (ER) of a tertiary care medical center in

Beirut, Lebanon, were shown to be mostly associated with otitis externa.[2] The patients had either unilateral or bilateral otitis externa. A significant number experienced severe symptoms such as ear discharge, ear canal swelling, pain, periauricular cellulitis, and fever.

P aeruginosa is also one of the most common pathogens that cause nosocomial infections, particularly in patients who suffer from immunosuppression.[3] *P aeruginosa* is a ubiquitous pathogen prevalent in the hospital environment, and it can cause severe nosocomial infections.[4] The latter involve a broad spectrum of infections, including those in the respiratory, gastrointestinal, and urinary tracts as well as wound infections, sepsis, and others.[5] Various possible sources of *P aeruginosa* infection in hospitals have been identified and include tap water, medical equipment, hospital personnel, and other patients.[4,6] *P aeruginosa* accounts for 10% of all hospital-acquired infections, a site-specific prevalence that may vary from 1 unit to another and from study to study.[7]

During the last year, the average prevalence of *P aeruginosa* nosocomial infections in the medical center was 18%. Such a high rate prompted us to study the *P aeruginosa* genotypes circulating in the various units and to correlate them with the genotypes obtained from cases of community-acquired infections, mainly otitis externa, to determine whether there is a genomic relatedness from community-acquired strains and those endemic in the medical center setting.

METHODS

Isolation and Identification of *P aeruginosa*

Isolates were collected from a tertiary care medical center in Beirut, Lebanon, during a 1-year period from patients suffering from otitis externa (community acquired) and nosocomial infections. Thirty-two community-acquired isolates of *P aeruginosa* were obtained from 22 patients who presented to the ER with the diagnosis of either unilateral or bilateral otitis externa.[8] Patients had yellowish to greenish discharge, moderate to severe external auditory canal swelling, moderate to severe pain, and periauricular cellulitis. None of these patients had intrinsic predisposing factors.

Consecutive nosocomially identified *P aeruginosa* (90) isolates were also recovered from different patients' clinical specimens (1 per patient) submitted for bacteriological investigations at the Clinical Microbiology Laboratory at the American University of Beirut Medical Center.[9] Only those patients who acquired a nosocomial infection due to *P aeruginosa* as determined by clinical and laboratory testing and indicated in their medical records were considered in this study. Fever and recovery of *P aeruginosa* from the site of infection during the stay at the medical center constituted the most important criteria that defined patient's infection with this organism. All isolates were identified using standard procedures and tested for susceptibility to a panel of antimicrobial agents according to Clinical and Laboratory Standards Institute (CLSI guidelines).

DNA Extraction and Random Amplified Polymorphic DNA Analysis

DNA was extracted from *P aeruginosa* American Type Culture Collection (ATCC) strain and from all isolates of *P aeruginosa* by the GFX Genomic Blood DNA Purification Kit (Amersham PharmaciaBiotech, Uppsala, Sweden) according to the manufacturer's specifications. Random amplified polymorphic DNA (RAPD) analysis of the clinical and environmental isolates using 2 in-house oligonucleotide primers, Pa1 (5′ AGGGGTCTTG 3′) and Pa2

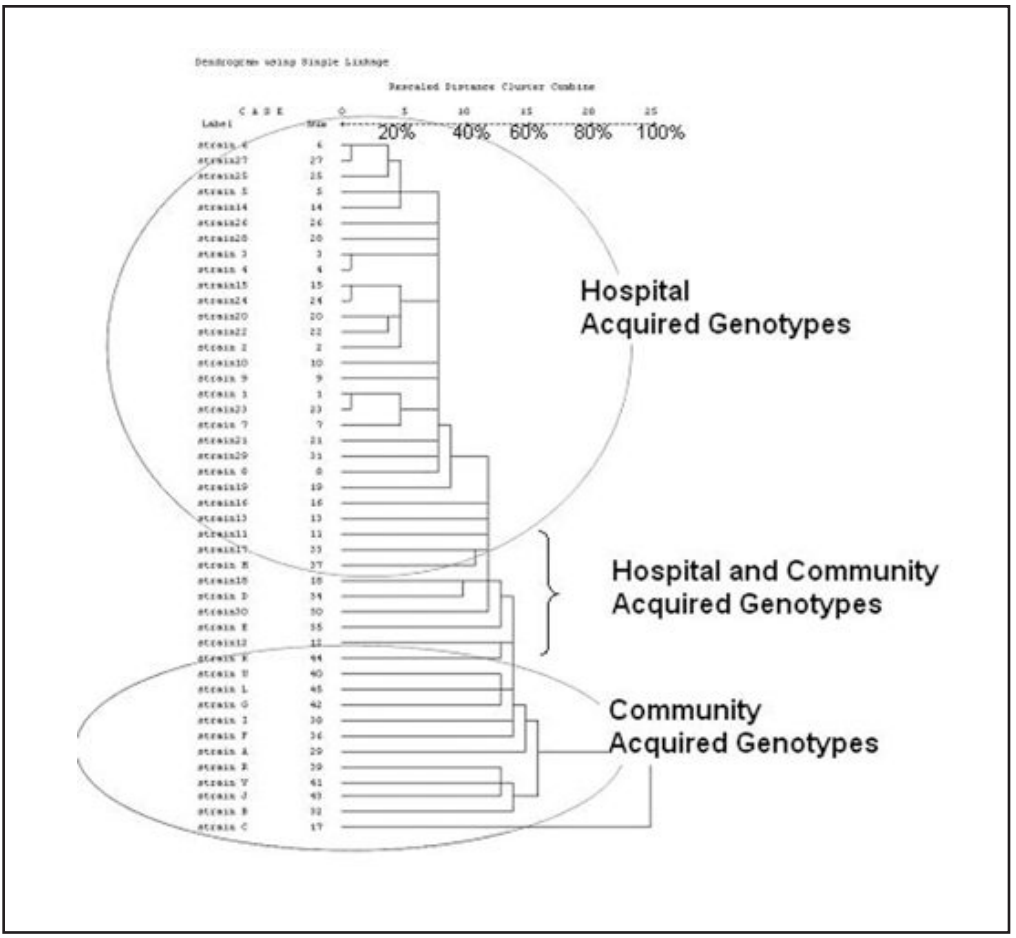

Figure 1. Dendrogram representing the genotypes found in both hospital- and community-acquired strains.

(5′CTTCTTCAGCTCGACGCGACG3′), was performed. RAPD was carried out according to Matar et al[8] using the PTC-100 Programmable Thermal Controller (MJ Research, Inc., Watertown, MA). Briefly, RAPD analysis was performed on all isolates in 100-µL reaction mixtures, each containing 10 µL of template DNA, 16 µL of dNTPs (0.2 mM), 10 µL of 10X PCR buffer (100 mM TrisHCl [pH 8.3], 500 mM KCl, 4mM MgCl2), 1 µL of primer 1 (0.5 µM), 1 µL of primer 2 (0.3 µg/µL), 2.5 U of *Taq* DNA polymerase, and 61.5 µL of nanopure sterile water. The amplification program included the following steps: denaturation at 94°C for 3 seconds, annealing at 53°C for 1 minute, and extension at 72°C for 1 minute for 44 cycles. The cycles were followed by a final extension step at 72°C for 10 minutes. Amplicons were subjected to electrophoresis on 2% agarose gels at 107 volts for 2 hours.

The gels were then visualized using an ultraviolet UV-transilluminator (UVP, Uppland, CA) and photographed with an Olympus 3.0 camera (Japan) and the Digidoc-it program (UVP). Digital TIFF image files were imported into the GelCompar II software (Applied Maths NV, St-Martens-Latem, Belgium). Gels were normalized using the DNA ladder markers as reference. Band calling of the RAPD patterns was

performed by visual inspection of the normalized gel images. A dendrogram was generated with GelCompar II statistical software using the Dice coefficient and unweighted paired-group methods for arithmetic averages algorithm.

RESULTS

The genotypic analysis of the 32 otitis externa isolates revealed 23 RAPD patterns. Bilateral otitis externa was associated with 20 of the 32 isolates. Four of these 20 isolates, obtained from 2 patients, had RAPD 1 pattern, while 8 isolates obtained from both ears of 4 patients had 4 RAPD patterns. The remaining 8 isolates from 4 patients with bilateral otitis each showed a different RAPD pattern. Twelve of 32 isolates associated with unilateral otitis exhibited all 12 RAPD patterns.[8] RAPD analysis of the nosocomial infections have shown 31 genotypes present among the clinical isolates.[9] Thirty-eight of ninety (42%) of the clinical isolates showed genotype 1 to be distributed among the medical center units. Each of genotypes 2-30 represented from 1% to 8% of the strains. Dendrogram analysis of nosocomially and community-acquired strains was performed and showed 2 main clusters; the first cluster represented the genotypes of the nosocomially acquired *P aeruginosa*, while the second showed the community-acquired *P aeruginosa* (Figure 1). Most of the strains within the 2 clusters illustrated closer genomic relatedness among each other. Most of the genotypes from nosocomial infections were highly interrelated whereas community-acquired genotypes were less interrelated. Although similarities between the 2 clusters were seen, both populations were not related to each other because the genotypes did not overlap.

DISCUSSION

P aeruginosa is known to be a versatile and opportunistic pathogen in terms of its genetics, metabolic potential, and mechanisms of virulence. The pathogenesis of otitis externa is multifactorial. Both host-related factors as well as pathogen factors play major roles in colonization and infection.[10] The *P aeruginosa* that caused the infection may have been part of the patient's endogenous flora, originated from other patients, or spread to humans from various environmental sources.[11-13] Frequent recombination of chromosomal genes between different isolates of *P aeruginosa* may have lead to the genetic diversity of the organism.[14] The mechanisms of genetic exchange, including transformation, transduction, and conjugation, affect *P aeruginosa* to adapt to changing conditions by acquiring new genetic information.[15] This versatility is reflected in the results of this study, whereby 23 different RAPD patterns were generated from 32 isolates of patients having otitis externa and 31 genotypes from 90 isolates obtained from patients with nosocomial infections with predominance of genotype 1.

The predominance of a genotype in the medical center indicates that cross contamination among patients led to the spread of this genotype among the various units, possibly through transient hand carriage by health care personnel due to contact with contaminated surfaces or by patient contact with contaminated surfaces or medical equipment.[6] These findings suggest that cross colonization may be an important means of *P aeruginosa* spread and infection especially after identification of a potentially virulent clone (genotype 1) of this organism that had been propagated in various units over a period of 1 year.

The genotypes found in both populations were different from one another. The dendrogram analysis confirmed the great genomic diversity of this organism and demonstrated that *P aeruginosa*

strains obtained from community-acquired infections were genomically different from those obtained from nosocomial infections. Nevertheless, a small number of isolates from the 2 sources were closely related as seen in the middle portion of the dendrogram.

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