**Introduction:** Chronic infection with *P. aeruginosa* is commonly encountered in patients with cystic fibrosis (CF), but the ability to delay, prevent, or better manage infection with multiply-resistant *P. aeruginosa* (MRPA) can potentially increase quality of life and extend survival. By utilizing molecular typing for multi-year epidemiological surveillance, the CF Care Center in our children’s hospital identified an endemic MRPA strain (dominant clone). The study aimed to identify risk factors for acquisition of the clone and determine differences in patient outcome associated with subsequent infection with the clone. Molecular typing was useful for ongoing surveillance of overall incidence and prevalence of infection with the dominant clone. **Methods:** Respiratory specimens were collected from nearly 300 pediatric patients with CF from 2004-2009. MRPA isolates were identified in 71 patients through conventional microbiological techniques, and bacterial DNA was extracted. Rep-PCR was performed, and amplified products were separated by size via microfluidics-based electrophoresis. Relative similarities of molecular profiles were analyzed by a web-based application. Patient demographic information and clinical parameters prior to MRPA infection were evaluated as potential risk factors. Differences in patient outcome including lung function, nutritional status, and hospitalization rate were compared. **Results:** The dominant and non-dominant clone groups included 32 patients and 39 patients, respectively. Recent hospitalization (< 90 days) was a statistically significant (p = 0.035) risk factor for acquisition of the dominant clone, and significant overlap in hospitalization of patients in the dominant clone group was present. Molecular typing results prompted evaluation of CF infection control practices including stricter contact isolation guidelines. Trends toward higher hospitalization rates and declines in lung function and nutritional status were observed in the dominant clone group as compared to the non-dominant clone group. **Conclusions:** Molecular typing of MRPA isolates in children with CF led to the discovery of an endemic strain and enabled the identification of risk factors for acquisition of the dominant clone. Multi-year molecular epidemiological surveillance provided data for clinical outcome comparisons based on pathogenic clones, at the subspecies level. Distinguishing individual bacterial strains by molecular methods provides a high-resolution strategy for monitoring the effectiveness of improved infection control practices and enhanced quality of patient care.