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Correlation between the genetic diversity of nosocomial pathogens and their survival time in intensive care units

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Summary Bacteria differ in their ability to survive in the hospital environment outside the human host. Species remaining viable and infectious have a higher chance of being transmitted, giving them a fitness advantage in hospitals. This differential fitness could be expected to alter the genetic population structure of bacterial populations in hospitals, and should be reflected by the relative abundance of several successful clones. The objective of this study was to test for a potential correlation between tenacity, i.e. environmental survival, and clonal abundance determined by the genetic diversity in different bacterial species from prospectively collected isolates of intensive care patients. A literature review was performed to identify mean environmental survival times for the most important pathogens in intensive care units (ICUs): *Staphylococcus aureus*, enterococci, *Acetinobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp., *Escherichia coli*, *Klebsiella pneumoniae* and *Stenotrophomonas maltophilia*. To determine the genetic diversity of the natural population of these species in ICUs, a prospective 18-month study was conducted in five units with median nosocomial infection rates. All clinical isolates were collected, and highly discriminatory DNA fingerprinting techniques were used to identify specific clones. A diversity index for each species was calculated as the number of distinguishable genotypes in the population divided by size. The correlation between survival times and the diversity indices for the individual pathogens was investigated using non-parametric

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methods. Although 21 studies were identified in the literature, only two were relevant. They showed median survival times between 1.5 days (*P. aeruginosa*) and 60.0 days (*Enterococcus faecium*). During the prospective ICU study, 1264 pathogens were investigated and simple diversity indices between 49.1 (*Enterococcus faecalis*) and 89.8 (*E. coli*) were found. A correlation between survival times and the diversity indices for the individual pathogens was found (correlation coefficient 0.821, $P=0.024$). Environmental survival may be an important factor contributing to the ecological fitness of some nosocomial pathogens in ICUs. Infection control measures should consider this finding.

Introduction

Healthcare-associated infections are a particular threat to patients who are immunocompromised and need intensive care. As treatment becomes limited due to the relentless increase in antibiotic resistance, preventive measures are gaining importance. Most healthcare-associated infections are endogenous, but exogenous infections might be prevented by appropriate infection control procedures.

Pathogens originating from patients or environmental reservoirs are mainly transmitted by hands of personnel or other vectors (such as fomites or devices). Where transmission is vector-borne, bacteria have to survive outside the host milieu that normally supports their survival and replication.

However, the role of the inanimate hospital environment in the transmission of nosocomial infections is controversial.[1–5] Limited data are available on the survival of nosocomial pathogens outside the human host, and the influence of their tenacity on their success in healthcare institutions has never been studied. Therefore, the objective of this study was to see whether the frequency with which certain strains were isolated from intensive care patients is associated with environmental survival time, using the most important nosocomial micro-organisms in intensive care untis (ICUs) described in the literature.

Methods

The investigation focused on 10 of the most frequent nosocomial pathogens in ICUs: *Acetinobacter baumannii* complex, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Escherichia coli*, *Enterococcus faecium*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Stenotrophomonas maltophilia*. A MEDLINE search was performed for the terms 'survival' and 'tenacity' using the Boolean operator 'AND' for each species for the period from 1966 until 2004. Only original articles were considered. The articles were scanned for information on survival time, and the methods used were evaluated critically in order to identify studies sufficiently comparable for the different pathogens under investigation. Furthermore, the inocula and test surfaces used had to have been relevant to the ICU environment in order for the study to be included.

Diversity of pathogens

During an 18-month period (February 2000–July 2001), patients who stayed $\geq$48 h on five ICUs at two university hospitals were enrolled. The ICUs were encouraged to maintain routine microbiological investigations, and all collected microbiological specimens on clinical grounds at high frequency. All clinical and screening isolates of the 10 'indicator organisms' were collected and stored at $-80\,^{\circ}C$ (Microbank, Viva). Only primary isolates from each patient were submitted for molecular typing [*S. aureus* and enterococci by pulsed-field gel electrophoresis (PFGE); Gram-negative pathogens by AP-polymerase chain reaction technique and a double primer amplified fragment-length polymorphism (AFLP) method]. After typing, genetically indistinguishable organisms isolated from different sites of the same patient were excluded. Full details of the methods used have been published elsewhere.[6]

To describe the genetic population structure for each bacterial species, a simple diversity index was used, as follows. In a population of N isolates of a species in a defined environment, a given isolate