Abstract

Purpose

Nosocomial infections caused by multidrug-resistant gram-negative bacilli are a global problem. Since the conventional investigation algorithm for detection of nosocomial outbreaks seems incapable of curbing the widespread of multidrug-resistant strains, the major purposes of the present project were to establish an effective real-time detection system, which was reinforced by modern computer technology and included a genetic and epidemiologic database and a rapid strain typing method.

Material and Methods

The database included gram-negative bacilli which produce extended-spectrum beta-lactamases, AmpC cephalosporinase, or metallo-beta-lactamases. These bacterial strains were collected between 1999 and 2002 and the beta-lactamases produced by these strains have been characterized recently. The multilocus sequence typing (MLST) method, a recently developed bacterial typing method, was developed in this study. The method is based on polymorphisms of structural genes among different strains of a bacterial species. Firstly, polymerase chain reaction assays were performed to amplify 14 structural genes of *Escherichia coli* and nucleotide sequencing was followed to select the most polymorphic genes. A total of 25 *E. coli* strains and 15 *Klebsiella pneumoniae* strains were tested by MLST and the results were compared with those obtained by the "gold standard" typing method, pulsed-field gel electrophoresis (PFGE). After the most polymorphic genes were selected, a total of 285 *E. coli* isolates and 439 *K. pneumoniae* isolates, which had been known to produce extended-spectrum beta-lactamases, were further typed by MLST and the data were saved in the database. Phylogenetic analysis can be done easily by comparing several polymorphic genes among different isolates, and thus the isolates can be typed. Moreover, the results can be saved easily in a digitalized manner so that they can be retrieved very quickly. A genetic and epidemiologic database was also established in this study.

Results

Among the 14 structural genes tested, six genes were found to be most polymorphic; they were *adk*、*gcl*、*gdh*、*mdh*、*metA*, and *ppk*. After phylogenetic analysis, a number was given for each variant of a structural gene for each isolate, and a MLST type was given for each isolate with six numbers together.

Among 25 *E. coli* strains and 15 *K. pneumoniae* strains tested, there were 24 and 13 PFGE types, respectively. However, only 18 and 7 MLST types were found for *E. coli* and *K. pneumoniae*, respectively. The 285 *E. coli* isolates and 439 *K. pneumoniae* isolates, which had been known to produce extended-spectrum beta-lactamases, were further typed by MLST and 37 and 29 MLST types were found among *E. coli* and *K. pneumoniae* isolates, respectively.

Conclusions

A genetic and epidemiological database was established in this study. MLST was developed but the typing method was found to be less discriminatory than PFGE. Since MLST is easier to perform and to save in a database, the method may become an auxiliary typing tool to PFGE or a useful screening tying method. More multidrug-resistant bacterial strains should be tested to intensify the functions of the system; further retrospective and prospective studies should be performed to evaluate the usefulness of the system. Furthermore, the system may be used in a nationwide surveillance program for monitoring multidrug-resistant bacterial strains.

Keyword: nosocomial outbreak; multilocus sequence typing; bioinformatics; genetic database