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The combined use of VIGI@ct® (bioMérieux) and fluorescent amplified length fragment polymorphisms in the investigation of potential outbreaks

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Summary   Even with good surveillance programmes, hospital-acquired infections (HAIs) are not always recognized and this may lead to an outbreak. In order to reduce this risk, we propose a model for prompt detection of HAIs, based on the use of a real-time epidemiological information system called VIGI@ct® (bioMèrieux, Las Balmas, France) and on the rapid confirmation or exclusion of the genetic relationship among pathogens using fluorescent amplified length fragment polymorphism (f-AFLP) microbial fingerprinting. We present the results of one year's experience with the system, which identified a total of 306 suspicious HAIs. Of these, 281 (92%) were 'confirmed' by clinical evidence, 16 (5%) were considered to be simple colonization and the latter nine (3%) were archived as 'not answered' because of the absence of the physician's cooperation. There were seven suspected outbreaks; of these, f-AFLP analysis confirmed the clonal relationship among the isolates in four cases: outbreak 1 (four isolates of *Pseudomonas aeruginosa*), outbreak 2 (three *Escherichia coli* isolates), outbreak 6 (two *Candida parapsilosis* isolates) and outbreak 7 (30 ESβL-producing *Klebsiella pneumoniae* subsp. *pneumoniae*). Based on our results, we conclude that the combination of VIGI@ct® and f-AFLP is useful

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in the rapid assessment of an outbreak due to Gram-positive or Gram-negative bacteria and yeasts.

Introduction

Hospital outbreaks of infectious diseases often result from exposure to a common source of an aetiological agent. The latter are usually derived from a single cell whose progeny is genetically identical or closely related to the source organism.[1] Demonstrating that micro-organisms involved in a hospital outbreak are 'closely related' suggests cross-transmission of pathogens in the hospital. Another possibility is that there may be a common source in a focal cluster without patient-to-patient transmission. In the study of pathogens causing hospital outbreaks, several molecular typing methods have been used.[1,2] The main characteristics that a typing method must possess are: excellent discriminatory power, reproducibility, user-friendliness and an acceptable price.[2,3] However, the molecular typing of a micro-organism is only one aspect of an outbreak investigation; their early detection is also relevant.[3] The latter is one of the challenges for the Hospital Infection Control Service (HICS), which operates for the identification and control of hospital-acquired infections (HAIs). HAIs are identified by epidemiological surveillance, generally based on the data produced by the microbiology laboratory, as well as from an active search for HAI cases.[3,4] The aim of this study was to evaluate the usefulness of a combined system based on the use of software specifically created for the identification of HAIs and on the use of a genotyping method that could confirm the genetic relationship among isolates involved in an outbreak.

Methods

Setting

The Polyclinic of 'Tor Vergata' University in Rome is a new 273-bed hospital, planned to be extended to 600 beds in 2007.

Software operational use

Our hospital is equipped with a real-time epidemiological information system called VIGI@ct® (bioMérieux, Marcy L'Etoille, France), version 1.0.

The system is a comprehensive bacteriology program designed for exams management statistical and epidemiological analysis of the data, as well as real-time and deferred management of hospital infections and target organisms. The VIGI@ct® is designed to work with Windows NT® and is connected to the Laboratory Information System (LIS; Dasilab-Delphi; Dasit) so that the VIGI@ct® receives all the examinations requested from the clinicians in the wards and collected in the LIS. This connection is also used to acquire additional patient information such as admission and discharge dates or internal location changes, as well as demographic data supplied by the LIS. The LIS is also linked with the automated system VITEK 2 (bioMèrieux) used in our laboratory to perform microbial identification as well as the antimicrobial susceptibility testing of the isolates. Both links are represented on the desktop screen of the LIS by two dialogue windows named VIGI@ct-results and VIGI@ct-requests. These windows are always open and are helpful in verifying the status of the connection between the LIS and the VIGI@ct®. At the same time, on the desktop of the VIGI@ct® system, two icons representing these links (called GO-vitek link and GO-LIS link) are present and appear in the task ruler. Every day the microbiologists launch the data integration from the LIS and VIGI@ct® using two program functions: 'receiving the requests' and 'receiving the results' from the LIS. In this manner, the VIGI@ct® received all the bacteriological data coming from the LIS connection which are concentrated in the general bacteriology database of the VIGI@ct®, according to the user-defined criteria, where they can be viewed or used at any time for epidemiological studies. This type of connection allows the VIGI@ct® the real-time identification of all pathogens responsible for HAIs using selection criteria established by the user and monitored continuously by the program. The strategy is identical whether it is applied to HAIs or MDRs (multidrug-resistant infections): early detection triggers the automatic printing of an alarm document to be sent to clinicians as a communication tool. For hospital infections, a simple document is printed automatically every time a presumed hospital-acquired pathogen is detected (Document of Presumed Hospital Infection: DPHI).

Each type of infection is associated with a DPHI according to the classification reported by the Center for Disease Control and Prevention in Atlanta. The infection file is created automatically when the first organism of an infection episode is detected. All organisms detected thereafter may be included in the same or in different infection files, depending on the nature of the specimens from which they were isolated. The DPHIs are promptly sent to the department where the patient was admitted as well as to the HICS. The DPHI sent to the Department contains a questionnaire in two parts: the first requesting more detailed patient information, and the second regarding the infection itself. The latter appears to be most important in the definition of HAI cases, including the option that the clinician must flag to confirm or to exclude the HAI as well as to qualify a nosocomial infection (e.g. site of infection, date and type of surgery, presence/absence of a particular device or mechanical ventilation and so on). Following compilation, the document is then returned to the laboratory where the microbiologist archives the dossier of the patient using several options. These are by confirming the case, excluding the HAI or by archiving the case as contamination or as colonization. In the eventuality that the department fails to answer the questionnaire, the programme is designed to finalize the DPHIs which have not been returned after a user-defined interval of '*n*' days, in our case after 30 days, as 'not answered'. This option consents to document to the HICS if there is a relationship between 'lack of answers' and the spreading of a pathogen in the same area. The HAIs and MDRs are duplicated in specialized databases where they can be processed without interfering with the general bacteriology database. The data therein could be used for epidemiological studies. Continuous data analysis also allows the microbiologist to monitor the spread of pathogens within the hospital. Setting the detection parameters for HAIs or MDRs can be done at two different levels, either as trial parameters for experimental detections (test criteria) or as real-time detection parameters. General detection parameters are: (a) interval between the admission date and the specimen collection date (this interval can be defined based on the type of infection); (b) detection of all organisms or only those which were included in an antibiotic susceptibility test as well as those expressing a particular phenotype. It is important to eliminate the duplication of pathogens; this task can be easily achieved by a function of the VIGI@ct® named 'marking organism duplicates', which allows the marking of duplicates based on serotype, species or specimen.

Bacterial isolates

All strains, suspected of being HAIs, were stored at −80 °C in 0.5 ml of defibrinated bovine blood.

Outbreaks

From January 2004 to May 2005 we identified 306 suspected HAIs. By examining data concerning circulating pathogens in each department as well as in the entire hospital weekly, we observed seven possible outbreaks (numbered 1 to 7), which were promptly investigated. An outbreak is defined as the occurrence of a number of cases of a disease in a short period of time. Microorganisms responsible for these infections were: outbreak 1, *Pseudomonas aeruginosa* (four isolates); outbreak 2, *Escherichia coli* producing extended-spectrum β-lactamases (ESβLs) (three isolates); outbreak 3, *Enterococcus faecium* phenotype VanA (teicoplanin MIC ≥ 32 and vancomycin ≥ 64; three isolates); outbreak 4, *Enterobacter cloacae* ESβL-producers (three isolates); outbreak 5 *Staphylococcus epidermidis* (four isolates); outbreak 6, *Candida parapsilosis* (2 isolates); and outbreak 7, ESβL-producing *Klebsiella pneumoniae* subsp. *pneumoniae* (30 isolates). Biochemical identifications and antibiotic susceptibility tests were performed using the VITEK 2 automated system (bioMérieux) following the manufacturer's instructions. ESβL production by *E. coli* and *K. pneumoniae* was confirmed according to indications set by the NCCLS (National Committee for Clinical Laboratory Standards; now called the CLSI: Clinical and Laboratory Standards Institute). For *E. faecium*, the presence of the *van*A gene was confirmed by PCR as described by Jayaratne *et al.*[5,6] *E. coli* strains were isolated from a blood culture of a haematological patient and from two urine samples collected from catheterized patients admitted to a surgical ward. *E. cloacae* strains were isolated from blood cultures of haematological patients with intravascular catheters. *P. aeruginosa* strains were isolated from ulcerous lesions in patients admitted to the medical ward in beds which were situated next to each other. Oxacillin-resistant *S. epidermidis* strains were isolated from blood cultures of patients admitted to the ICU and *E. faecium* strains were isolated from blood cultures from patients admitted to haematology (one isolate) and the ICU (two isolates). *C. parapsilosis* isolates were cultured from the tips of intravascular catheters from two patients admitted to the ICU during the same period. Outbreak 7 was composed of 30 *K. pneumoniae* strains isolated from 14 different patients, from December

2004 to May 2005 with increased transmission in January. *K. pneumoniae* strains were cultured from different samples for each patient (including blood, urine and venous devices as well as respiratory samples). In all cases micro-organism biotypes and antibiotypes were extremely similar (at most a minor error was observed in the antibiograms of the isolates for each case). The median age of patients involved in outbreak 7 was 65.5 years and their therapy often included a third generation cephalosporin (for the index patient: cefotaxime).

f-Amplified fragment length polymorphism

The genetic relationship among the isolates was determined by fingerprinting using the commercial kit f-AFLP Microbial fingerprinting (Applera, Foster City, CA, USA) according to the manufacturer's instructions. The f-AFLP reactions were loaded and run on the ABI 310 DNA genetic analyzer (Applera). Each f-AFLP reaction was analysed using Genescan software and Genographer program version 1.6.0 (kindly provided by James J. Benham, free of charge online at http://hordeum.oscs.montana. edu/genographer; ©1999 Montana State University). Cluster analysis was performed using the unweighted pair group method with the averages algorithm (UPGMA).[7] The percentage similarity between patterns was calculated using the Dice correlation coefficient.[4] The cluster analysis of a single outbreak was completed in a time ranging from 72 to 96 h.

Results

During the observation of pathogens circulating in our hospital, the VIGI@ct® system identified a total of 306 suspected HAIs. Of these, 281 (92%) were 'confirmed', 16 (5%) were 'colonized', and the latter nine (3%) were recorded as 'not answered'. Less than 30% of the questionnaires were returned by the clinician within 48 h and the remaining were obtained after being requested by the laboratory. f-AFLP analysis confirms the clonal relationship of the isolates in four cases: in outbreak 1 (*P. aeruginosa* strains), in outbreak 2 (*E. coli* ESβL producer), in outbreak 6 (*C. parapsilosis* isolates), and in outbreak 7 (*K. pneumoniae* strains). Dice's index was 0.97 for *P. aeruginosa* isolates, 1 for *E. coli* strains, and 0.99 for *C. parapsilosis*, indicating a genetic relationship between the isolates, ranging from 0.80 to 1 for *K. pneumoniae* isolates. In outbreak 7 fingerprinting analysis of the 30 strains, isolated

from several samples from 14 different patients, confirmed that the strains isolated from each patient were clonally related. However, the isolates of three patients proved to be the same clone, having a Dice index of 1. Therefore, we reduced the study to 12 clones: 11 composed of a single isolate representative of each patient (one clone—one patient), and clone 5, representative of isolates from three different patients (clone 5: 5, 1 and 2). By analysing the f-AFLP profiles we were able to identify four different clusters: cluster C, composed of two clones (nos. 3 and 9); cluster AB articled in three branches, the first (A-1) composed of seven isolates (nos. 14, 4, 6, 10, 11, 12 and 13), the second (A-2) composed of a single clone (no. 5, representing three patients) and the third (B) composed of two clones (nos. 7 and 8). Dice's index of the strains of cluster A-1 was 1, and these branched with the clones of A-2 with a Dice index of 0.92. A-1/A-2 also branched with cluster B (whose strains present a Dice index of 0.92) with a Dice index of 0.85. Cluster AB joined to clones of cluster C (expressing a Dice index of 1) with a Dice index of 0.80. Over a six-month period the first clone (that of the index patient) to appear was included in branch A-1, followed by that of branch A-2 (after 20 days from the appearance of the last clone of A-1). Clones of branch B followed A-2 by 15 days, while a period of more than a month separates the clones of branch AB from those of branch C (Figure 1). In many patients the infection began from the colonization of a device such as an arterial, venous or urinary catheter, as well as from endotracheal tubes for mechanical ventilation. This confirms the central role of the ICU staff in the transmission of nosocomial pathogens, since microbiological investigation of the environment did not produce isolation of this pathogen (data not shown). All isolates showed similar decreased susceptibility to ampicillin (MIC > 32) cefotaxime and ceftazidime (MIC > 64). β-Lactamase inhibitors such as clavulanic acid had little influence on the MIC values while tazobactam reduced the MIC values of piperacillin. None of the isolates showed resistance to carbapenems. All of the isolates were resistant to ciprofloxacin and 50% of the strains tested were resistant to gentamicin.[8,9]

Concerning outbreak 4, Dice's coefficient between the *E. cloacae* isolates was 0.80, showing a correlation but excluding clonality. However, in outbreaks 3 and 5, *E. faecium* and *S. epidermidis* respectively, Dice's coefficient was <0.70, indicating the absence of a genetic relationship that could confirm patient-to-patient transmission of the pathogens.
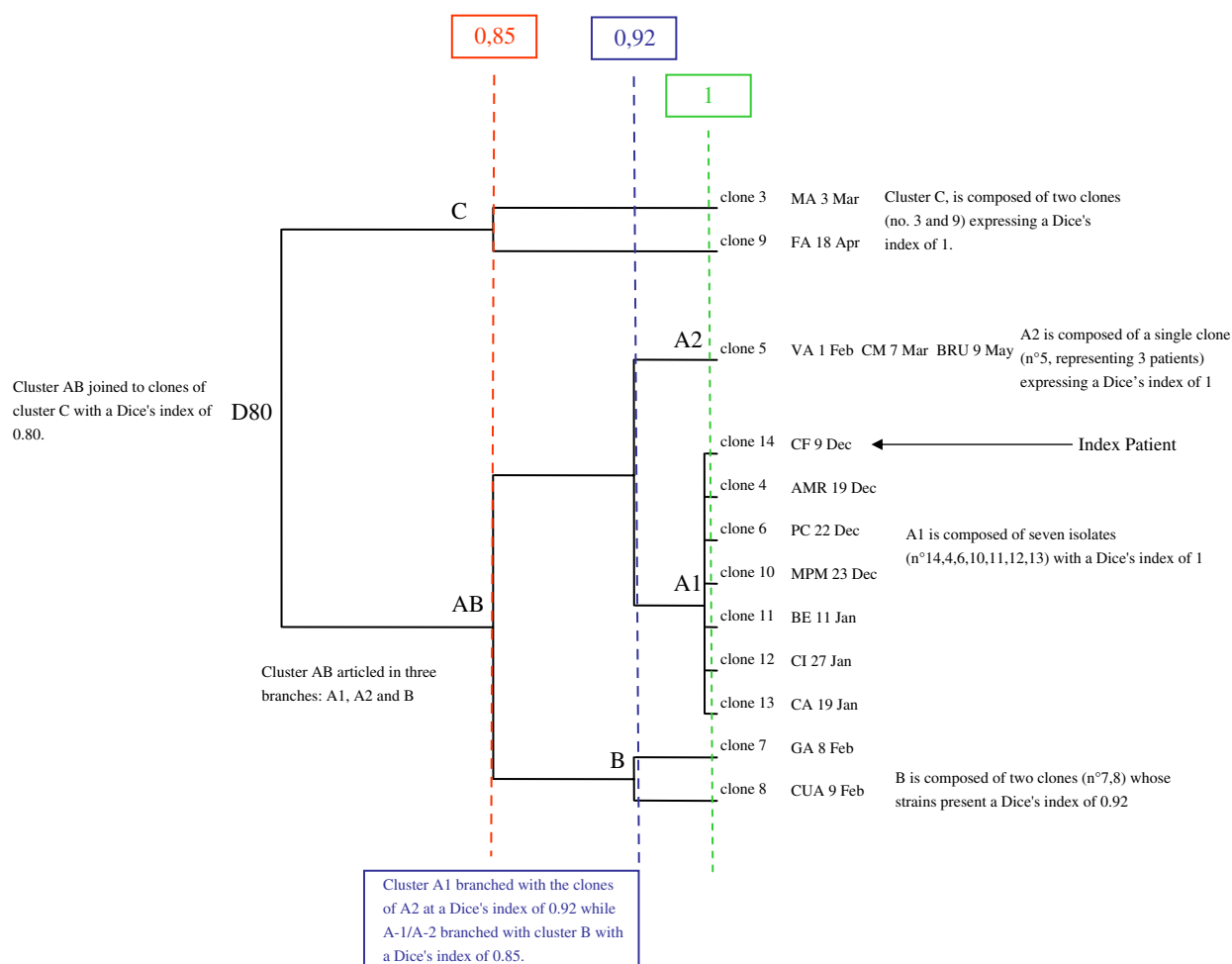
Figure 1 Dendrogram of *K. pneumoniae* isolates.

Discussion

Monitoring the transmission of infections in the hospital is a precise aim of the team involved in infection control.[10] Close co-operation between clinicians and microbiologists is very important. The microbiologist plays a primary role in the study of isolates circulating in the hospital, for which he must have adequate systems (hardware and software) for the real-time control of pathogen transmission. At the same time, he must also be able to rapidly confirm or exclude the possibility of an epidemic cluster by genotyping isolates. The electronic laboratory-based surveillance method has already been demonstrated to be more useful than the hospital-wide nosocomial infection detection by medical records, as the latter is insensitive and inconsistently applied.[11,12] In addition, our experience confirms the effectiveness of the use of surveillance software, such as VIGI@ct®, in the rapid identification of HAIs. Our findings also show that the combined use of this surveillance system with a fingerprinting method (such as the f-AFLP) allows for the rapid definition of the outbreaks. The VIGI@ct® is a new system introduced for the control of HAIs, which is already used in several hospitals in Italy but our experience is the first to be described. VIGI@ct® has proved to be very helpful, since it allows for the identification in real time of any suspected HAI and also monitors the circulation of MDRs that pose a serious problem for prescribing physicians. The continuous data analysis from databases allows the monitoring, department by department, of epidemic clusters. As the VIGI@ct® is a complex program it is advisable to take into account the initial accurate configuration of the system, as well as the linking with the central LIS, for its correct use. It is also important that clinicians return information, i.e. they must carefully compile the questionnaire concerning the infection and the patient. Otherwise, HAIs may remain unidentified.

In the absence of a surveillance system it is difficult to promptly identify more insidious

outbreaks, particularly those caused by the spread of a pathogen among different wards in the hospital or those critical areas such as the ICU. The ICU itself represents an epicentre of emerging infection in a hospital and therefore requires a surveillance system able to identify all possible correlations among pathogens. An example of this would be the ESβL-producing Enterobacteriaceae and *Acinetobacter*. These micro-organisms colonize patients as well as survive in the environment, thus associating them with sporadic events in addition to more obvious cross-transmission. In the absence of an electronic surveillance system, it may be extremely difficult to identify an outbreak.[13]

Equally important is the use of molecular techniques to confirm a clonal relationship among isolates involved in an outbreak. In our experience, this task has been achieved by using f-AFLP.

This molecular method, first described by Vos *et al.*, has already been demonstrated to be as discriminative as the pulsed-field gel electrophoresis, but is, on the contrary, much less labour-intensive and allows for the testing of a large number of isolates in a short time with an acceptable workload.[14] In all seven possible outbreaks we have rapidly confirmed or excluded the genetic relatedness among the isolates. The rapidity with which we achieved a result has had an important effect on the management of patients as well as in the control of the spreading of pathogens. In this view, data concerning the outbreak caused by the spread of ESβL-producing *K. pneumoniae* in the ICU are particularly interesting.

This pathogen circulated in the ICU for a period of six months, during which time it caused serious infections, though never fatal, in several patients. Various risk factors have been implicated in the selection and spreading of these strains; excessive antibiotic exposure (especially third generation cephalosporins), extended hospital stay, recent surgery, admission to an ICU, the use of arterial, venous and urinary catheters, patients' age and severity of illness.[8,15] Our *K. pneumoniae* strains were in fact isolated from patients possessing several risk factors. It is generally agreed that outbreaks of this pathogen are due to insufficient hygienic precautions. In particular, Hollander *et al.* observed that the spread via hands of patients or staff members seemed to be very likely because it is difficult to detect other reservoirs.[8] Observation of our *K. pneumoniae* outbreak strongly suggests that there has been a persistent source within the department, either human or in the environment. Unfortunately, in our case, the source of the outbreak was not identified. The ecological niches of this micro-organism are at present unknown, and it is not known if carriage is persistent or transient. Antibiotic exposure could have played a role in the selection of *K. pneumoniae*; in fact, the first isolates were found in the index patient whose therapy included cefotaxime. Preventive measures should be applied immediately to stop the spread of ESβL strains among patients and/or in the hospital environment.[9] In our case these measures comprised identification but not the isolation of patients from whom the ESβL-producing *K. pneumoniae* strains were isolated, reinforcement of barrier measures and educational programmes for the ICU staff including a handwashing procedure. These measures have only limited the spread of this clone in other departments of our hospital, but *K. pneumoniae* still causes sporadic infection in the ICU.

As hospitals are expected to improve patient safety and to contain costs, there is a widely increasing interest concerning the surveillance methods used to control HAIs. We can affirm that our experience in the use of a system such as the VIGI@ct®, in combination with f-AFLP as a pathogen typing method, is useful in the identification of all possible HAIs and consequently in the early detection of the outbreaks. In particular, the VIGI@ct® system is helpful for microbiologists because it supports them in the validation of microbiological data and in the creation of an epidemiological information system. The system is also helpful for clinicians because it helps them prescribe the most suitable antibiotic therapy, monitor patients' infection, and for epidemiologists, because it allows them to identify outbreaks and study infection dynamics.

Acknowledgements

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