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Molecular Epidemiology Analysis in Nosocomial Bacterial Outbreaks

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A mi papá, mi mamá y mi hermana,
por ser mi fuerza y templanza
por su amor y apoyo
porque todo lo que soy es gracias a ellos

General Introduction

Nosocomial infections represent a serious public health problem; annual incidence in the United States is estimated in 2 million cases or about 10% of the hospitalized patients. In developing countries, the rates of nosocomial infections are 3 to 20 times higher than those in industrialized countries (1); and represent a major cause of death in infants. Reports in South America indicate that annual incidence of nosocomial infections in Colombia are around 300,000 per year and in Peru around 200,000 per year. There is no an official report in Ecuador because underreporting is common (2).

Nosocomial infections are currently a major health problem because they are major causes of morbidity and mortality, and cause high costs (3). Due to the use of large amounts of anti-microbials in the hospital environments, nosocomial bacteria tend to acquire multi antimicrobial resistance; therefore, treatments are difficult and costly. United States spends 4,500 million dollars average each year. In Ecuador a retrospective study was conducted at the hospital Vozandes (from 1999 to 2000) showing that the additional cost per patient of a ventilator associated pneumonia averaged $ 1,349 (3). Another study at the Military Hospital in Quito reported that nosocomial infections of the nervous system add an additional cost of $ 4,045 per patient, urinary tract infections $ 536 per per patient, and bacteremia $ 603 per patient (3).

The bacterial adaptation to different environments is the result of evolutionary processes in the constantly changing bacterial genomes. As consequence, nosocomial infections are the result of multiple factors that favor the microbial proliferation and colonization in healthcare centers and personnel. For these reasons, nosocomial pathogens tend to acquire virulence and antimicrobial multi resistance phenotypes (4).

Surveillance is critical in the fight against nosocomial infections, because it allows to assess the real impact of these infections and the risk factors associated to them. This information enables better decision-making for prevention and control measures (5) .Despite its benefits, many times this type of studies are a big challenge for hospital medical practice (5,6).

Molecular typing is an important epidemiological tool which utilizes analysis of molecules in order to identify the relationship of isolates and thus allows the detection of outbreaks, cross-transmission of pathogens, identification of the source of infection, recognition particularly virulent strains, and monitoring of vaccination programs (6). Molecular epidemiology studies basically the genetic lineage of pathogens in order to establish their patterns of dissemination.

There are several molecular typing techniques that analyze macro or micro genome diversity. The micro diversity techniques are focused in comparison of one or few genes to infer clonality (phylogenetic relationships) among isolates. Techniques such as restriction length fragment polymorphism (RLFP) using Southern blot of a gene or RFLP of polymerase chain reaction (PCR) products are good examples of this category. The macro diversity techniques analyze whole genome variations and

are useful to study endemic bacteria and outbreaks (6). There are a number of molecular typing procedures, and their utility depends of the characteristics of the genome. The PCR reactions of repetitive sequences are useful and sensible, but do not have a good reproducibility. Multilocus Assay Typing (MLST) is an recently developed approach which uses the sequence of housekeeping genes, and it has the advantage of lacking variation between laboratories. It allows to develop phylogenetic trees, but the selection of the genes to be analyzed is crucial (6). The other advantage of MLST is the existence of databases which locate of the clonally related strains around the world; however it is an expensive approach (6).

Pulsed-field gel electrophoresis (PFGE) is a technique that measure macro-diversity of bacterial genomes through the difference in length of DNA restriction fragments (around 4-MB) band patterns. The movement of these bands is facilitated by intermittent polarity changes. The restriction enzyme and the periodicity of electric pulses depend of bacteria species and bands size (6). PFGE is a highly reproducible method with good discriminatory power, relatively easy to perform and interpret, which can be used in many bacterial species and it is considered a standard technique for analyzing genomic DNA in epidemiological studies (6). The device type most frequently used is a Contour-clamped homogeneous electric field (CHEF), which has electrodes arranged in hexagonal form.

Finally, it is important to mention that the cost of implementing a molecular typing program in a 300 beds hospital is around $400,000 USD per year; however, these costs led to a savings of $5.00 for each dollar spent, for the high reduction of nosocomial infections (6) .

Staphylococcus aureus

Microbiological Characteristics

Staphylococcus aureus is a Gram-positive coccus that belongs to the *Micrococcaceae* family. The species of *Staphylococcus* genera have between 0,5 – 1 µm diameter, are immobile, facultative anaerobes, do not form spores and have no capsule. When is viewed through a microscope appears as grape-like clusters, and it is the origin of its name *Staphilé* that means grape clusters. When it is cultivated on blood agar plates, forms large, round, and golden colonies (this golden appearance is the reason because is named *aureus*), and sometimes it produces hemolysis (7).

S. aureus is catalase positive, this test is useful to distinguish staphylococci from enterococci and streptococci. *S. aureus* is also coagulase-positive, and this test is useful to distinguish *S. aureus* from the other *Staphylococcus* species. Another main characteristic is that it can ferment the Manitol sugar; for this reason, it grows yellow in manitol medium (7).

S. aureus is as a commensal of human skin and nasopharynx, but it can survive also in domestic animals such as dogs, chickens, cats, and horses. The importance of these bacteria is because several strains can develop virulent forms and act as opportunistic pathogens (8).

Genetic Characteristics

In the genome of this bacterium have been characterized both pathogenicity islands and multiple antibiotic resistance genes (9). In *Staphylococcus aureus* genome has been identified multiple pathogenicity islands transferred through phage, have been characterized 4 phages (ϕ80α, ϕ11, ϕ12 and ϕ147) that carry several types of different pathogenicity islands like the toxic shock syndrome exotoxin (TSST-1), the genes *saPIbov12* and *saPIbov2*, which encode a protein associated to biofilm formation.

The resistance against betalactams occurs by changes in proteins involved with peptidoglycan synthesis or by synthesis of betalactamases. (9). It has been recently determined that the stimulus to some antibiotics such as betalactams, trigger the SOS response in *Staphylococcus aureus* through the synthesis of the protein RecA and hence inhibiting LexA repressive mechanisms. This activates recombination mechanisms which activate of the lytic phases of these phages (10).

Within the multiple virulence genes of *S. aureus* it has been characterized the operon *agr* which encodes for a quorum sensing system mediated by a two components system, AgrD which is processed and exported by the conveyor AgrB to produce the signal peptide AIP (11). With an AIP increasing concentration, the histidine kinase AgrC acts as sensor, and produces the phosphorylation of response regulator AgrA that promotes the transcription of:

1. The promoter 2 that regulates RNA II transcription where the proteins encoded operon agr2. (11)

2. The promoter 3 which regulates the transcription of multiple virulence genes as alpha, beta and delta hemolysins, and exotoxins secreted as virulence factors. (11)

Additionally, *S. aureus* produce several toxins, such as pyrogenic toxin superantigens such as the toxin TSS1, which has been linked to toxic shock syndrome and other staphylococcal toxins associated to food poisoning. *Staphylococcus aureus* also can produce exfoliative toxin and which is though thought to be responsible of scalded-skin syndrome (SSS), hemolysins (alpha, beta, and delta) that act on cell membranes , and several two component toxins (like the Panton-Valentine leukocidin (PVL) associated with severe necrotizing pneumonia in children) (12).

Nosocomial Epidemiology

The evolution of nosocomial bacteria with virulent phenotype and / or antibiotic multi-resistance increases mortality, morbidity and treatment costs. *Staphylococcus aureus* regarded as normal microbiota of nasopharynx but it can produce lower respiratory infections, bacteremia, and sepsis (13,8).

S. aureus could be constitutively resistant to some penicillins, and has been classified according this in Meticillin Resistant *Staphylococcus aureus* (MRSA) and Meticillin Sensible *Staphylococcus aureus* (MSSA). The treatment of choice for MRSA is vancomycin which is a very expensive antibiotic (3). Since 1996 have been reported strains with intermediate resistance to vancomycin (CIM 8-16ug/ml) and in 2002 there were reported strains which possessed high resistance genes *vanA or*

vanB originally found in *Enterococcus spp* (14). The acronyms VISA and GISA describe strains of *Staphylococcus aureus* with intermediate sensitivity to vancomycin or glycopeptides. These strains have been reported in Japan, USA, Europe, in Hong Kong, and Korea, but the actual prevalence of GISA is unknown (10). There have been described strains with heterogeneous resistance (hVISA) which have acquired progressive resistance to vancomycin (15,13).

Staphylococcus aureus is the second most frequent cause of nosocomial bacteremia which is three times more frequent in carriers that in no carriers. Genotype analysis indicated that 80% of carriers had clones present in the hospital workers. A large study conducted on hospital workers fund that about 27.8% of the staff of intensive care unit and approximately 31.1% of all hospital staff carried *Staphylococcus aureus* in their nasopharynx (16). The presence of *Staphylococcus aureus* carriers among hospital staff increases the risk of nosocomial infections from 2 to 3 times in the ICU, dialysis units, or surgical procedures (17).

It has been reported that the combination of conventional and molecular epidemiological analysis of staff and patients, and selective topical treatment decreased staphylococcal nosocomial infections from 0.97% to 0.56% (18). Similarly, another study found that intervention to healthy carriers caused a decrease in an OR of 0.16 (95% CI 0.04-0.59) in the development of nosocomial infections (19).

Burkholderia cepacia

Microbiological characteristics and Epidemiology

Burkholderia cepacia Complex (CCB) before named as *Pseudomonas cepacia*, is composed of at least nine different species, these species were initially referred to as 'genomovars' based on their very close phenotypic and phylogenetic relatedness. It is a β-proteobacteria group of Gram negative aerobic that produces catalase (7). The CBC is commonly found in water and soil and can survive long periods in moist environments including some common disinfectants. The dispersion could be person to person, especially in close environments like hospitals. *B. cepacia* is an important human pathogen that most often causes pneumonia in immunocompromised individuals with underlying lung disease like in cystic fibrosis patients. Accurate identification of *Burkholderia species* using routine methods continues to be difficult; misidentification as other related species also found in cystic fibrosis sputum, such as *Pandoraea spp., Ralstonia spp*., and *Achromobacter spp*. is common (20).

The CBC bacterial organisms are naturally resistant to many common antibiotics, such as aminoglycosides (gentamicin and tobramycin) and polymyxin B (21).

Burkholderia spp are being recognized with increasing frequency as nosocomial pathogens. Due to their wide distribution in the natural environment, nutritional adaptability, and ability to form biofilms, outbreaks of infection in hospitals are frequent however the source of these bacteria is seldom identified. Several recent

reports identified a variety of infection vehicles, including ultrasound gel, nebulized medications, nasal spray, hospital water, and lipid emulsion (22, 23).

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Paper 1

Staphylococcus aureus outbreak analysis in the Intensive Care Unit of the Social Security Hospital in Ecuador

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Abstract

Staphylococcus aureus is a frequent cause of nosocomial pneumonia and bacteremia. Classical and molecular epidemiology were used to study *S. aureus* outbreaks in Intensive Care Unit (ICU) of the Social Security Hospital in Quito. ***Methods:*** *S. aureus* Isolates from 17 patients and 19 posible personnel carriers were collected from March 2007 to February 2008 and analyzed by Pulsed-field gel electrophoresis (PFGE) to determine the clonal relationships. ***Results:*** In this period there were 16 cases of nosocomial acquired pneumonia and an outbreak occurred from June to September 2007 with 7 different PFGE patterns, isolates fell into 4 clonal groups, and 2 groups of clonally-related isolates. Molecular typing failed to identify any staphylococcal reservoir among staff members. ***Discussion:*** The current study demonstrated that one staphylococcal outbreak occurred

during the summer of 2007 and it was caused by different bacterial clones. An historical analysis of the staphylococcal infections in the Intensive Care Unit (ICU) showed an increased incidence during the summer months (June to September). The time of the outbreaks coincided with the programmed personnel shift. The data suggested that outbreaks were produced by the introduction of improperly trained personnel to UCI.

Resumen

Staphylococcus aureus es una causa frecuente de neumonía nosocomial y bacteriemia. Técnicas de epidemiología clásica y molecular fueron utilizadas para estudiar los brotes epidémicos de *S. aureus* en la Unidad de Cuidados Intensivos (UCI) del Hospital del Seguro Social en Quito. **Métodos:** Los aislamientos de S. aureus de 17 pacientes y 19 posibles portadores sanos en el personal, fueron obtenidos a partir de marzo de 2007 a febrero de 2008 y analizadas por electroforesis de campo pulsado (PFGE) para determinar las relaciones clonales. **Resultados:** En este período se identificaron 16 casos de neumonías nosocomiales, y un brote epidémico ocurrió entre junio y septiembre de 2007, cuyos aislados mostraron 7 diferentes patrones de PFGE; los aislamientos en su mayoría pertenecían a 4 grupos clonales, pero existieron además 2 grupos con patrones relacionados. La tipificación molecular no logró identificar a la fuente de infección entre los miembros del personal de la UCI considerados posibles portadores. **Discusión:** El presente estudio demostró que el brote epidémico que se produjo durante el verano de 2007 fue causado por bacterias originarias de diferentes clones. Un análisis histórico de las infecciones en la Unidad de Cuidados Intensivos (UCI) mostró un aumento en la incidencia de *S. aureus* durante los meses de verano (junio a septiembre). El tiempo de los brotes coincidió con la rotación programada del personal por vacaciones. Los datos sugirieron que los brotes fueron

producidos por la introducción de personal inadecuadamente capacitado en la UCI.

Introduction

Nosocomial infections represent a serious public health problem. In developing countries, the rates of nosocomial infections are 3 to 20 times higher than those in industrialized countries (1). Infections caused by nosocomial bacteria, many of which are resistant to many antimicrobials, increases mortality, morbidity, and treatment cost (2).

Staphylococcus aureus is regarded as normal microbiota of nasopharynx but it can produce lower respiratory infections, bacteremia and sepsis (3)(4); indeed, *S. aureus* is the second most frequent cause of nosocomial bacteremia. The presence of *Staphylococcus aureus* carriers among hospital staff increases the risk of nosocomial infections from 2 to 3 times in the Intensive Care Unit (ICU), dialysis units, or surgical procedures (5). The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) has increased even more the burden of staphylococcal infections in hospitals (6).

Surveillance is critical in the fight against nosocomial infections, because it allows to assess the real impact of these infections and the risk factors associated to them. This information enables better decision-making for prevention and control measures (7). Molecular typing is an important epidemiological tool which utilizes analysis of genetic lineage of pathogens in order to identify the relationship of isolates and thus allows the detection of outbreaks, cross-transmission of pathogens, source of infection, recognition particularly virulent strains and monitoring of vaccination programs (8).

This study is the result of the implementation of an epidemiological surveillance system for staphylococcal infections in the Intensive Care Unit (ICU) of the largest public hospital in Quito, from March 2007 to February 2008. The goal of the study was to determine the origin and the factors involved in the occurrence of staphylococcal outbreaks in this hospital.

Materials and Methods

Subjects

The study was performed in Carlos Andrade Marín Hospital in Quito, a 300-bed tertiary care center. The ICU has 18 beds admitting critically ill patients. This study was previously reviewed and accepted by the Bioethics Committee of San Francisco de Quito University and by Bioethics Committee of Carlos Andrade Marín Hospital.

We analyzed 17 isolates from patients collected over one year period (March 2007 to February 2008). All patients with confirmed nosocomial acquired *S. aureus* were asked to fill out an outbreak analysis form (Annex 1) containing cause of admission, co-morbidities, time in hospital, bed location, and type of nosocomial infection.

Also, there were screened 43 health workers of ICU, and we obtained 19 *S. aureus* isolates from their nasopharynx as potential nosocomial bacteria carriers. Statistical analysis was conducted by STAT VIEW version 5.0 and Microsoft Excel 2007. We used odds ratio test to determine the likelihood of patients acquiring MRSA infections when undergoing antibacterial prophylaxis.

Bacterial isolates

A total of 17 *S. aureus* isolates from inpatients with pneumonia were collected from March 2007 to February 2008 from Carlos Andrade Marín Hospital in Quito. Nineteen

strains were obtained from nasal samples of 43 health workers from the Intensive care unit. Samples were cultivated in Manitol medium and identified as *S. aureus* with coagulase test. Then, isolated colonies were cultured in BHI medium and stored at -80℃.

Isolates from patients were named as P1 to P17 according the chronological date of isolation. Isolates from carriers were named according the occupation of each carrier: assistant nurse (A), assistant nurse 2 (C), nurse (E), respiratory physioterapist (F), cleaning staff person (L), medical doctor (M), personnel in charge of equipment maintenance (Q), and secretary (S).

Antimicrobial sensitivity profiles

Antibiotic susceptibility tests were carried out by disk diffusion method using Mueller-Hinton agar. The antimicrobial agents tested included cephoxitin (FOX), erythromycin (E), clindamycin (DA), nofloxacin (NOR), vancomycin (VA) and penicillin (P).

Pulsed Field Gel Electrophoresis

Isolates were analyzed by pulsed-field gel electrophoresis (PFGE). A sample of each isolate of *S. aureus* was plated into brain-heart infusion agar and incubated overnight at 37℃. Agarose plugs were prepared by mixing 200µl of a bacterial suspension in 75 mM NaCl–25 mM EDTA (pH 7.5) buffer (absorbance of 0.63 OD (± 0.02) a 610 nm) with 200 µl molten solution of 1% BioRad PFGE agarose in 0.5TBE 0,5X , 1% SDS solution containing 4 µg of lysostaphin (Sigma-Aldrich, Oakville, Ontario, Canada). Cells were lysed in situ at 37℃ for 4 h in lysis buffer (6 mM Tris-HCl [pH 8.0], 1 M NaCl, 100 mM EDTA, 0.5% Brij-58, 8 µg of lysostaphin, 0.5%

sodium lauroylsarcosine). Cells in plugs were lysed overnight at 50℃ in 1.5 ml of lysis buffer (50 mM Tris, 50mM EDTA, 1% Sarcosil) containing 0,8 mg of Proteinase K (Bio-Rad Laboratories). The plug slices were washed with sterile water and TE and each plug was digested overnight at 30°C in 100 µL of React4 Buffer, BSA 1 µl and 10 U *SmaI* restriction enzyme. The slices were loaded into a 1% agarose gel, and DNA was separated by PFGE using a CHEF DRII system (Bio-Rad) in 0.5X TBE Buffer with pulse times of 5 to 40 s at 6 V/cm for 21h at 14℃. Concatameric bacteriophage lambda DNA molecules were used as molecular weight standard (New England Biolabs) (9).

The PFGE patterns of the clinical isolates were compared and clonal relatedness was established based on the recommendations of Tenover et al (10,11).

Results

Isolates from patients

A total of 17 strains were isolated during one year study. The characteristics of the isolates are summarized in Table 1. The most common nosocomial infection was ventilator-associated pneumonia (12 patients), the most common cause of admission was severe cranium-encephalic trauma (9 patients), and there were isolated 9 meticillin sensible *Staphylococcus aureus* (MSSA) and 8 meticillin resistant *Staphylococcus aureus* (MRSA). An evident outbreak occurred from July and September 2007 with 11 cases of nosocomial acquired pneumonia (Fig 4). Statistical analysis for relation between *S. aureus* type and antibiotic prophylaxis did not prevent the acquisition of MRSA infections (Odds Ratio=2,28).

Clonal Analysis of Isolates

Analysis of isolates showed 12 different PFGE patterns. There were 4 groups of isolates with identical PFGE patterns, and 2 groups that had related profiles. The groups of isolates with identical PFGE pattern were: P4 and P10 (isolated in July and August 2007), P5 and P6 (isolated in July 2007), P15 and P16 (isolated in November and December 2007), and P13, P14, and P17 isolated in September, October 2007 and February 2008) (Fig 2 and 3) (Table 3).

The first partial relation was between P4 and P10 with P8 (Pattern IVa and IVb). The second partial relation was between P13, P14 and P17 with P12 (Pattern IXb and IXa) (Fig 2 and 3) (Table 3). Isolates P13, P14 and P17 have an identical

PFGE pattern (IXb) but a different antimicrobial sensitivity profile (g, h and c) (Table 3).

An isolate from community acquired pneumonia (isolate P7) was used as unrelated isolate (Fig 2 and 3). All the relations were supported in phylogenetic analysis by UPGMA with DICE coefficient (Fig 4). The distribution of PFGE patterns in time shows the circulation of a variety of clones during the outbreak between July 2007 and February 2008 (Fig 5).

Potential Carriers

Although there were staff members carrying *Staphyloccoccus aureus*, none of their isolates was involved in the pneumonia outbreaks (Fig 6 and 7).

Discussion

The present report describes an outbreak of *Staphylococcus aureus* which took place from July to September 2007 in the ICU of the largest public hospital in Quito. Molecular characterization of the infecting isolates in the outbreak showed 7 different bacterial clones and there was no relationship with *Staphylococcus aureus* isolated from personnel. The outbreak occurred from June to September, which coincided with rotation of personnel due to vacation. We were able to detect another outbreak of *Staphylococcus aureus,* during the same period of time in the year 2006. The data strongly suggests that the outbreak was caused by the introduction of improperly trained personnel to ICU. This report is in contrast to previous studies in which they were healthcare workers, the ones who carried the bacteria and infected patients (12,13,14) and outbreaks usually are caused by a limited number of clones (15,16,17,18).

One clone (PFGE pattern IXb) was isolated from cases occurring from September 2007 to February 2008. These finding may indicate that some strains could have been carried by personnel or other patients during this time, however we failed to identify them (80% of the staff was screened once).

Epidemiological analysis shows that the most common risk factor of acquiring a nosocomial infection by *S. aureus* was the use of mechanic ventilation. Indeed, the most prevalent nosocomial infection was ventilator associated pneumonia. All the

patients with Ventilator Associated Pneumonia had others risk factors such as catheter enteric nutrition and previous surgery. Interestingly, there was a higher risk of acquire a MRSA strain when using antibiotic prophylaxis.

In conclusion, there were 7 main clusters of *S. aureus* circulating in the ICU during June and September 2007, and caused a multiclonal outbreak. This study, suggested that changes in personnel of ICU may have been the principal cause of high rates transmission of *S. aureus*. The study suggests that improper sanitation of the hands and equipment during manipulation of the breathing tubes and gastric catheters may have contributed to the spread of the staphylococcal infection among patients.

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Paper 2

Infect Control Hosp Epidemiol, 2008; 29(4):364-6.

Investigation of an Outbreak of Central Venous Catheter–Associated Bloodstream Infection Due to Contaminated Water

Richard W. Douce, MD; Jeannete Zurita, MD; Olga Sanchez, RN; Paul Cardenas Aldaz, MD

Abstract

We report an outbreak of infections transmitted by the central venous line catheters in a small hospital of Ecuador. We found ampoules of reportedly sterile water for injection to be contaminated with *Burkholderia cepacia* and *Myroides odoratus*. Retrospective review of charts revealed that the problem had been present in Quito for 18 months. Pulse field gel electrophoresis (PFGE) suggested clonality between the clinical isolates of *B. cepacia* with the isolates from the ampoules of sterile water. Some of the patients with bacteremia due to *Burkholderia cepacia* had severe symptoms. In some cases we also isolated *Myroides odoratus* from the blood of infected patients, but in this case it did not present symptoms of infection. . Isolation of *Burkholderia cepacia* from blood obtained from reportedly "sterile ampoules" indicates the importance of monitoring of solutions for injection used in health care institutions.

Resumen

Este estudio presenta el análisis de un brote epidémico de infecciones transmitidas por catéteres de vía central que ocurrió en un pequeño hospital de Ecuador. Al parecer se han encontrado ampollas de agua estéril para inyección contaminados con *Burkholderia cepacia* y *Myroides odoratus*. La revisión retrospectiva de historias clínicas determinó que el problema había estado presente durante los últimos 18 meses en este hospital. El análisis mediante electroforesis de campo pulsado (PFGE) sugiere clonalidad entre los aislados clínicos de *B. cepacia* con los aislamientos de las ampollas de agua estéril. Algunos de los pacientes con bacteriemia debido a *Burkholderia cepacia* desarrollaron síntomas graves. En algunos casos también *Myroides odoratus* fue aislado de hemocultivos de pacientes infectados, pero en este caso los pacientes no presentaron síntomas de infección. El aislamiento de *Burkholderia cepacia* de sangre obtenida de supuestas "ampollas estériles" indica la importancia de la vigilancia de las soluciones utilizadas para la inyección en las instituciones de salud.

Introduction

An outbreak of central venous catheter–associated bloodstream infections was reported in a hospital in Ecuador. Commercially produced ampoules of water for injection were found to be contaminated with *Burkholderia cepacia* and *Myroides odoratus.* Removal of these ampoules yielded a 10-fold reduction in the incidence of catheter-associated infection.

Burkholderia cepacia is a rare cause of bloodstream infection (BSI). It has been isolated from solutions for disinfection[1-3] and from water,[2] and has been implicated in several nosocomial outbreaks. We report an investigation of an outbreak after 4 patients with central venous catheters (CVCs) were identified as having bacteremia due to *B. cepacia* in September 2006. The objective of the investigation was to identify and eradicate the source of *B. cepacia* contamination.

Methods

Setting and participants. The setting was an acute care teaching hospital in Ecuador with a total of 75 beds, of which 8 were in the intensive care unit. The study was performed by the infection control committee, which includes a part-time nurse epidemiologist (O.S.), a part-time infection control physician (R.D.), a microbiologist (J.Z.), and representatives of the nursing staff.

Case definition and laboratory methods. A case patient was defined as a patient with a CVC who had a culture of peripheral blood sample, of blood drawn from the catheter, or of the catheter tip that was positive for *B. cepacia.* All results of routine cultures for current patients and all medical records from patients whose blood cultures were positive for *B. cepacia* were reviewed, and all isolates from culture were saved for further analysis. Results of cultures performed at the hospital during the preceding 2 years and procedures for obtaining blood samples for culture and for placing CVCs were also reviewed. Samples were obtained for culture from all the materials used for catheter maintenance, including povidone iodine, alcohol swabs, and heparin for flushing lines. Finally, the medical records of the index patients were reviewed to see what medicines they received in common between the date of admission and the date of the first positive blood culture result. Samples of those medicines and their diluents were cultured.

Blood cultures were performed using an automated system (Bactec 9120; Becton Dickinson). The organisms isolated were identified by conventional methods, by the

ERIC Electronic Rapid Compendium (Remel), and by the MicroScan system NC32 (Siemens Medical Solutions Diagnostics). Catheter tips were cultured using the technique described by Maki et al.[4] Liquids were cultured by direct inoculation into blood culture bottles.

Isolates were submitted for analysis by pulsed-field gel electrophoresis (PFGE) using the methods described by Heath et al.[5] The PFGE patterns of the clinical isolates were compared, and clonality was identified on the basis of the recommendations of Tenover et al.[6]

Results

After the identification of 4 patients with CVC-associated BSI, there was concern about a possible outbreak, and we subsequently identified 8 patients retrospectively and 4 patients prospectively who fit the case definition. The Figure 1 gives the temporal distribution of these case patients. All 16 patients had CVCs made of polyurethane and had been in the intensive care unit sometime during their hospitalization. Two of the 16 patients had no fever and were initially considered to have pseudobacteremia. They were identified on the basis of cultures of blood obtained at the time CVC use was discontinued after a surgical procedure. Four patients had recurrent low-grade fever and had blood cultures that were positive for *B. cepacia* within 4 days after catheter removal. One of these patients was found to have sepsis with multiorgan failure and persistent bacteremia after removal of the catheter; this patient finally improved 11 days later after beginning use of 3 intravenous antibiotics. After *B. cepacia* was identified in the other 3 patients, they were treated with oral trimethoprim and sulfamethoxazole and did well. Five of the 16 patients died of the disease process for which they were hospitalized.

The 5 remaining patients had no apparent complications. After performing 47 cultures of disinfectants and solutions used for catheter maintenance, we finally isolated *B. cepacia* from 5-mL glass ampoules of reportedly sterile water used to dilute antibiotics for infusion. Quantitative culture of this commercial product revealed 3,000 cfu/mL of *B. cepacia* and 10 cfu/mL of *Myroides odoratus.* Four different lots of ampoules of

water for injection from the same manufacturer were found in the hospital pharmacy. Samples of the ampoules of water from each lot were uniformly culture-positive for the same organisms. The pharmacy had been purchasing water ampoules from this source for 18 months before the outbreak was identified. Five isolates of *B. cepacia* recovered from patients in this investigation and 3 isolates recovered.

After *M. odoratus* was identified in the commercial water, we performed a retrospective review of the medical records and found that 21 blood cultures had been positive for *M. odoratus.* All of them had been considered to represent pseudobacteremia before this investigation. No patient had both *M. odoratus* and *B. cepacia* isolated from blood culture. All but 1 of the isolates of *M. odoratus* and *B. cepacia* were recovered during the period when the implicated water was being used by the hospital pharmacy. As soon as the commercial water was confirmed to be contaminated with *B. cepacia* and *M. odoratus,* the product was removed from the hospital, and a formal report was submitted to the Ecuador Ministry of Health. Intrinsic contamination of the water was confirmed by the National Microbiology Laboratory.

Information on the number of catheter-days was available for the 2 months that included the case identification and prospective study. During that time, there were 8 CVC-associated infections during 263 catheter-days (30 infections per 1,000 catheter-days). During the 10 months after removal of the implicated water, no further isolates of either *B. cepacia* or *M. odoratus* were recovered from blood samples, and a total of 3 CVC-associated infections occurred in our hospital during 877 catheter-days (3.4 infections per 1,000 catheter-days).

Discussion

B. cepacia is not an organism that typically infects CVCs.[7] At the beginning of the outbreak, few *B. cepacia* isolates were recovered, and the organism was not consistently isolated each month. A full investigation was not initiated until after sepsis due to *B. cepacia* was documented in a patient and after several patients had the same organism recovered from blood in the same month.

M. odoratus is common in soil and water but is rarely isolated from patients.[8] It was not considered pathogenic in any of our patients, and, as a contaminant, it was inappropriately attributed to a faulty procedure in obtaining blood samples for culture—until after it was isolated from the contaminated commercial product. In the literature, it has been implicated in cellulitis and bacteremia associated with catheters and is considered to be resistant to carbapenems, βlactams,and aminoglycosides.[8]

This outbreak was caused by commercial ampoules of sterile water intrinsically contaminated by 2 different bacteria. Sterile water for injection has been implicated in outbreaks of bacteremia due to *B. cepacia* in other countries.[9,10] Isolation of *B. cepacia* or *M. odoratus* from the blood of patients should stimulate investigation that includes the culture of any commercial product that contains water that has been involved in the treatment of those patients.

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FIGURES AND TABLES

Isolate ID	Date of isolation	Mechanic Ventilation	Sample	PFGE Group
P-001	may-07	+	Trachea	I
P-002	jun-07	+	Trachea	II
P-003	jun-07	+	Trachea	III
P-004	jul-07	+	Trachea	IVa
P-005	jul-07	+	Trachea	V
P-006	jul-07	+	Trachea	V
P-007*	ago-07	-	Trachea	VI
P-008	ago-07	+	Trachea	IVb
P-009	ago-07	+	Blood	VII
P-010	ago-07	-	Trachea	IVa
P-011	ago-07	-	Trachea	VIII
P-012	ago-07	+	Trachea	IXa
P-013	sep-07	+	Trachea	IXb
P-014	oct-07	+	Trachea	IXb
P-015	nov-07	+	Trachea	X
P-016	dic-07	+	Blood	X
P-017	feb-08	+	Trachea	IXb

Table 1. Epidemiological analysis of all patient samples. It shows the date of isolation, age, gender, type of infection.

***Community acquired Pneumonia**

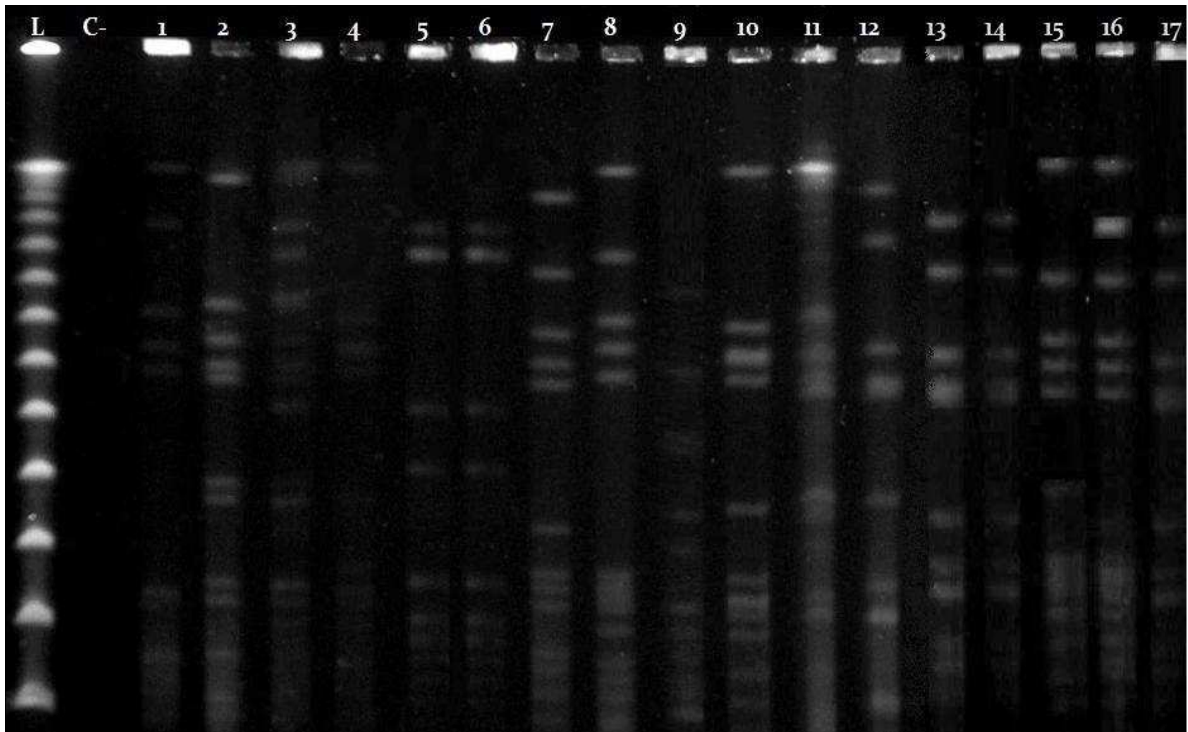

Figure 1 . **PFGE analysis of Patients´ isolates from 1 to 17 (P1 to P17), Lambda Ladder (L), Negative control (C-).**

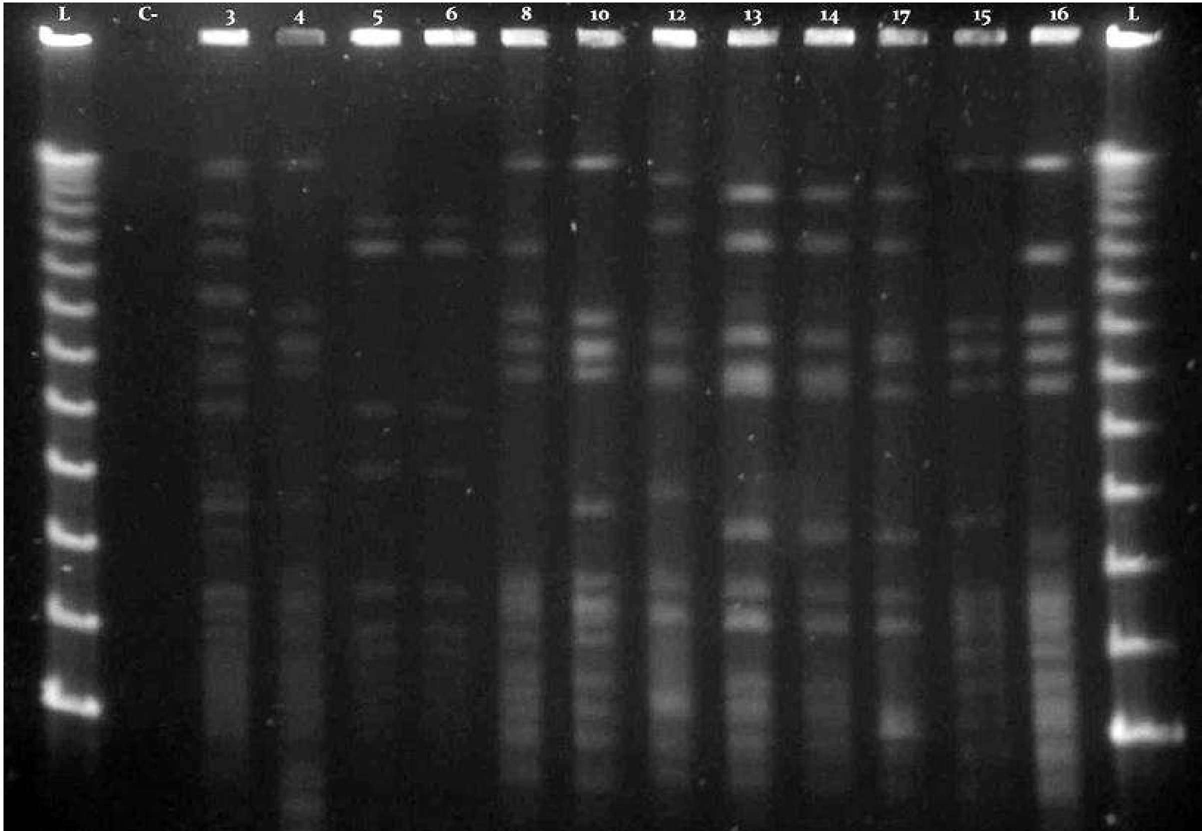

Figure 2 . PFGE analysis of Patients´ isolates grouped by similar PFGE patterns from 3 to 17 (P3 to P17), Lambda Ladder (L), Negative control (C-).

ISOLATE	DATE	TYPE	ANTIBIOTIC PATTERN	PFGE PATTERN
P-001	may-07	MRSA	a	I
P-002	jun-07	MSSA	b	II
P-003	jun-07	MSSA	c	III
P-004	jul-07	MRSA	d	IVa
P-005	jul-07	MRSA	d	V
P-006	jul-07	MRSA	d	V
P-007	ago-07	MSSA	e	VI
P-008	ago-07	MRSA	d	IVb
P-009	ago-07	MSSA	e	VII
P-010	ago-07	MRSA	d	IVa
P-011	ago-07	MSSA	f	VIII
P-012	ago-07	MSSA	e	IXa
P-013	sep-07	MSSA	g	IXb
P-014	oct-07	MSSA	h	IXb
P-015	nov-08	MRSA	d	X
P-016	dic-08	MRSA	d	X
P-017	feb-08	MSSA	c	IXb

Table 2. Antimicrobial and Molecular typing of all patients´ isolates. It is showed antibiotic patterns and PFGE patterns.

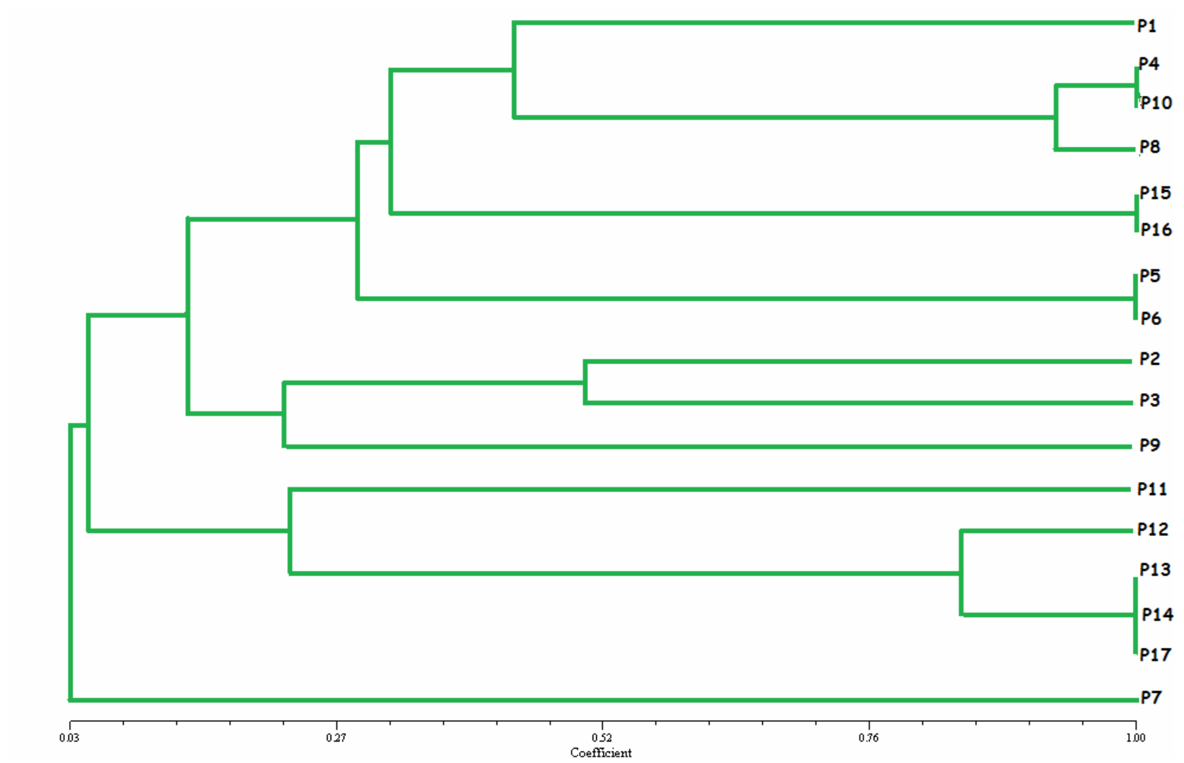

Figure 3. UPGMA tree constructed with the PFGE profiles obtained from staphylococcal isolates. DICE coefficient was used.

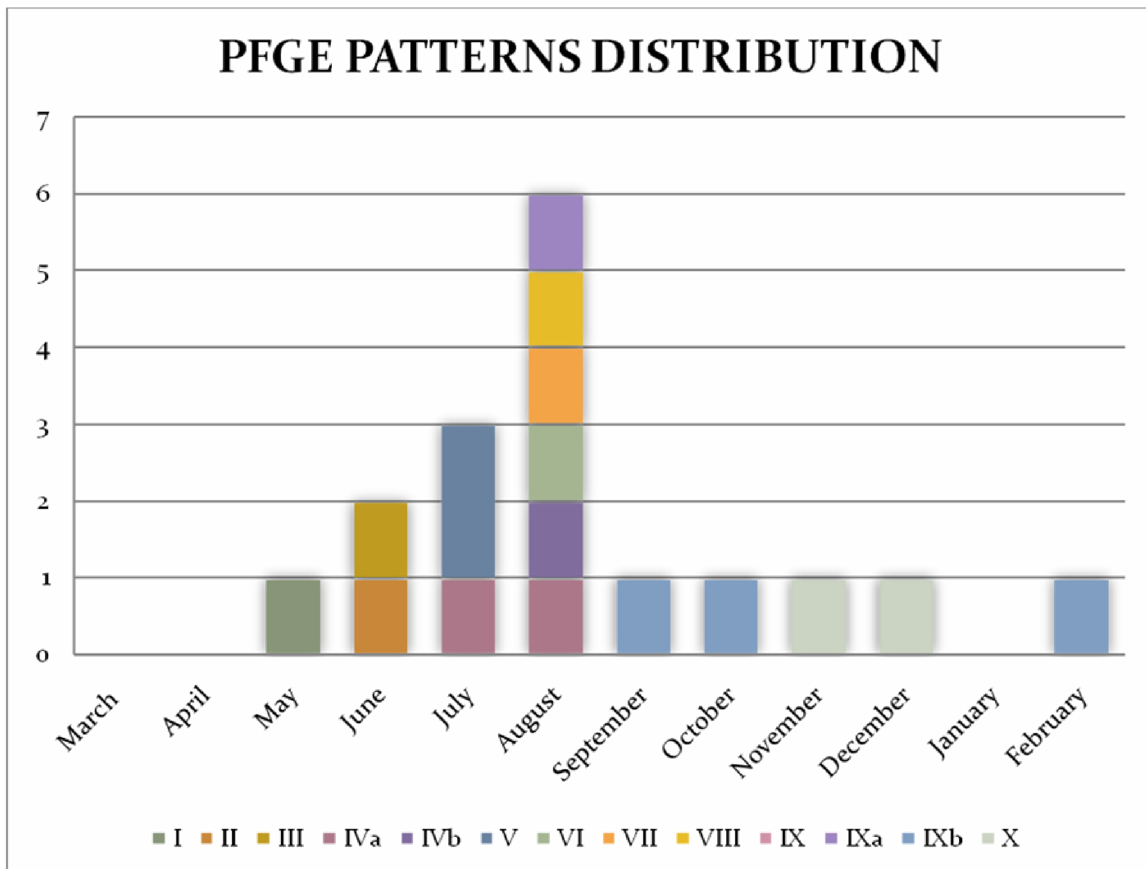

Figure 4. Time distribution of *Staphyloccocus aureus* PFGE patterns . The vertical axes indicate the number of staphyloccal infections.

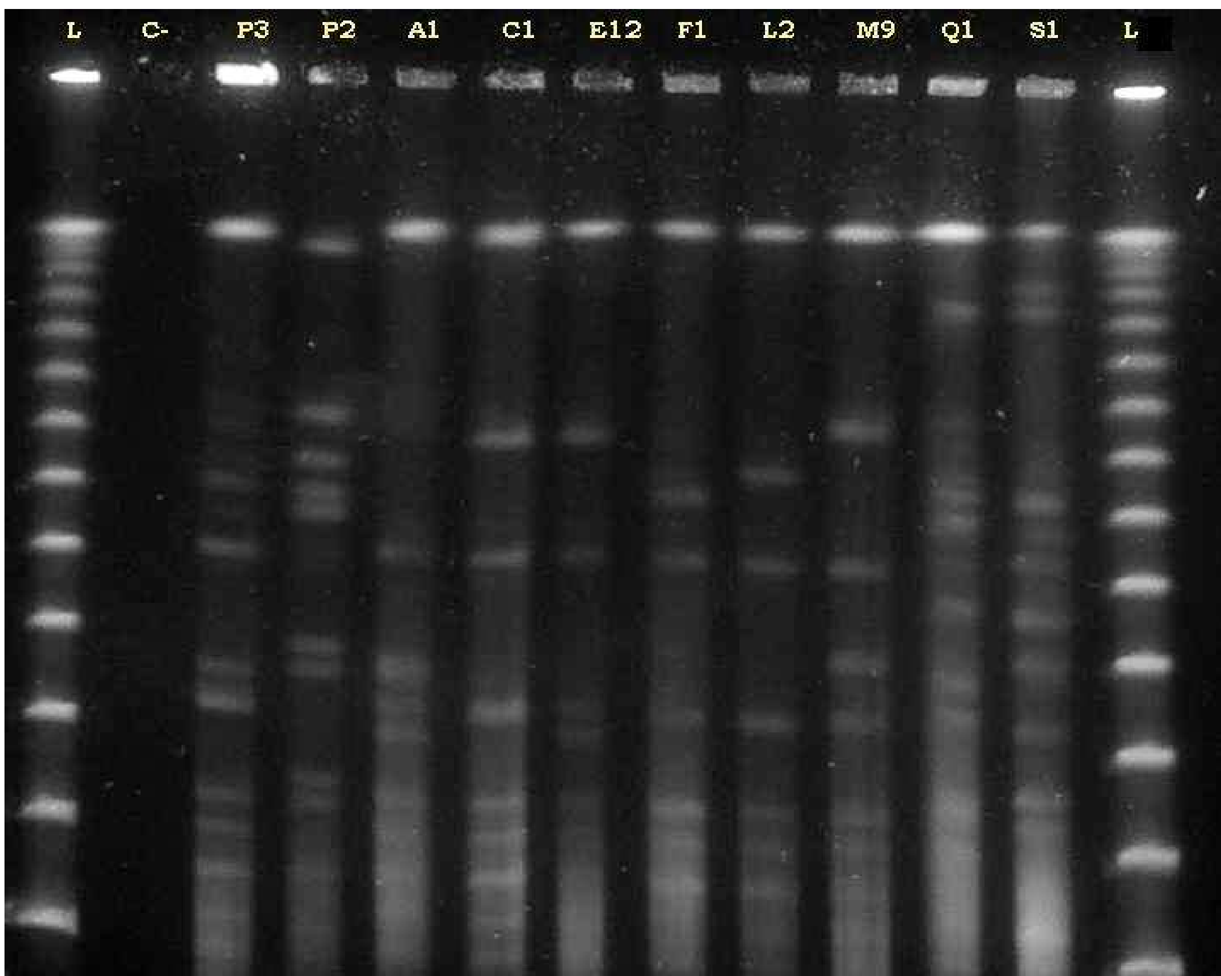

Figure 5 . **PFGE analysis of Patient 2 isolate (P2) compared with assistant nursing 2 (A2), stretcher bearer 1 (C1), nurse 12 (E12), respiratory physiotherapist 1 (F1), cleaning staff person 2 (L2), medical doctor 9 (M9), medical equipment repair staff person 1 (Q1), secretary 1 (S1), Lambda Ladder (L), Negative control (C-) and unrelated isolate from patient 3 (P3).**

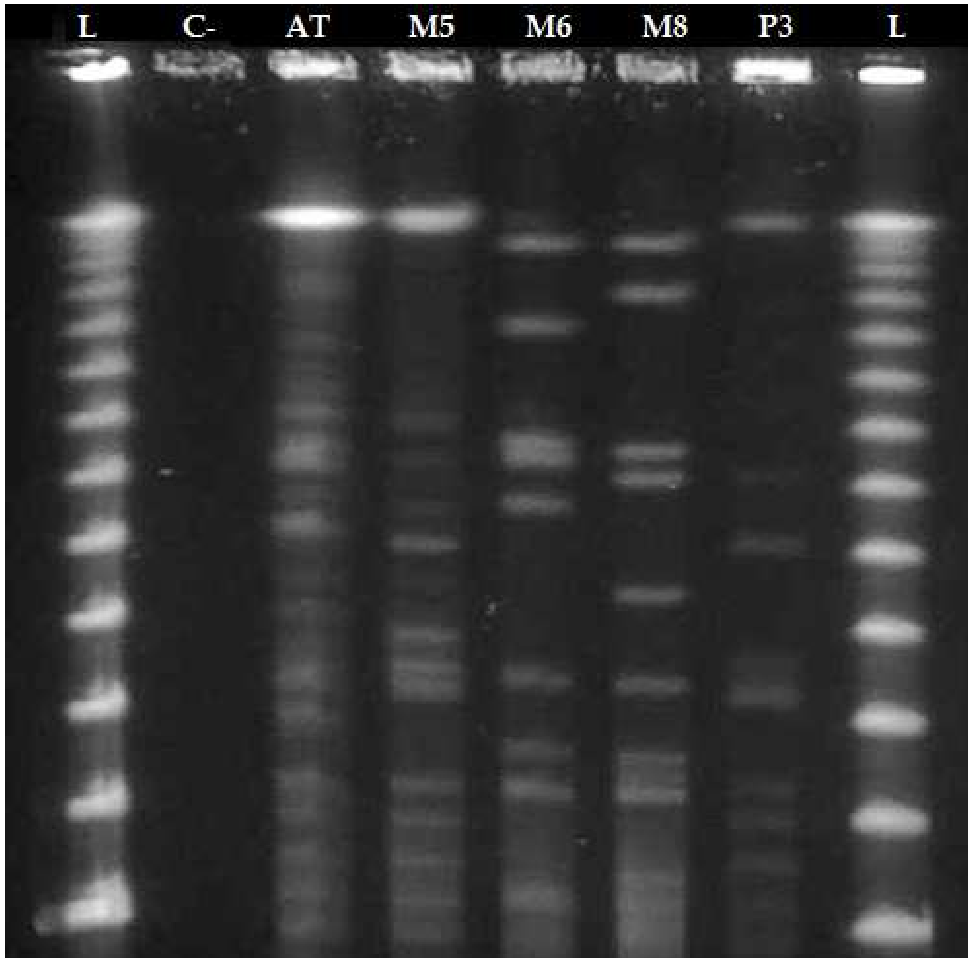

Figure 6 . PFGE analysis of Patient 3 isolate (P3) compared with isolates M5, M6, M8. Lambda Ladder (L), Negative control (C-) and unrelated isolate from S. aureus ATCC (AT).

Use of commercial water began

Use of commercial water ended

Beginning of investigation

																																O			
																																O			
																										O					O			
																										O					O			
																										O					X			
																										O					X	O		
																										O				O	X	X		
	O																X								O		O	O			O	X	X		
	X															X	X	X		X		X		O	O		O	O			X	X	X		
Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2004												2005												2006											

Figure 7. Temporal distribution of case patients investigated, by month. O, *Myroides odoratus* isolated from the blood of a patient; X, *Burkholderia cepacia* isolated from blood or catheter tip of a patient with a central venous catheter. from samples of these commercial ampoules of water were analyzed by PFGE and were shown to have identical banding patterns.

Annex 1. Nosocomial Infection Formulary: outbreak analysis form containing cause of admission, co-morbidities, time in hospital, bed location, and type of nosocomial infection

SISTEMA DE REGISTRO DE ENFERMEDADES NOSOCOMIALES

ID PACIENTE #:	**Diagnostico :**	**TIPO:**
	NOMBRE DEL PACIENTE:	**Sexo:** M F
Fecha Admisión:	**Edad:**	**Fecha Registro Datos:**

Servicio de Origen:		
C. Cardiológica	Nefrología	Oft - Otorrino
C.General	Neonatología	Onc - Hem
Emergencias	Neumología	Pediatría
Endocrinología	Neurocirugía	Traumatología
Ginecología	Neurología	U. Quemados
Med Interna	Obstetricia	Urología

ESTANCIA EN UCI	
FECHA INGRESO A UCI:	
DIAGNÓSTICO DE INGRESO:	
PRESENTA DIAG. ENF. NOSOCOMIAL AL INGRESO:	SI
	NO

Solo en Caso de Cirugía	
Día de la Operación:	**Duración:** hrs. min.
Tipo de Herida:	
Tipo de Anestesia:	
Emergencia	SI NO
Endoscopia:	SI NO
ASA clasificación:	1 2 3 4 5
Trauma:	SI NO
Cirujano:	
Primer Ayudante:	
Segundo Ayudante:	
Anestesista:	
Instrumentista:	
Circulante:	
Procedimientos Multiples:	

Infecciónes y Factores de Riesgo		
Fecha de Diagnóstico de la Infección1:		
Fecha de Diagnóstico de la Infección 2:		
Infección de Vías Urinarias		**Técnicas invasivas:**
		Cateterismo Urinario
		Intrumentasión vesical
Infección Herida Quirúrgica	SITIO	TIPO HERIDA
Neumonia	Comunitaria	Tiempo uso ventilador
	Nosocomial	
	Ventilatoria	
		Profilaxis Ab
BSI Bloodstream Infection	LCBI	Vía Periférica
	CSEP	Vía Central
Otra infección:		

FECHA ALTA:	
MUERTE POR CAUSA DE INFECCIÓN NOSOCOMIAL	
MUERTE POR OTRA CAUSA	