ORIGINAL ARTICLE

Detection of cross-transmission of multiresistant Gram-negative bacilli and *Staphylococcus aureus* in adult intensive care units by routine typing of clinical isolates

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Objective: To determine the potential of laboratory services in identifying cross-transmission of multiresistant Gram-negative bacilli (MR GNB) and *Staphylococcus aureus* in adult intensive care units by routine typing of clinical isolates.

Methods: Over a 12-month period, isolates with indistinguishable PCR fingerprints were traced back to the source patients and their epidemiologic relationships were investigated. Possible episodes of cross-transmission were ascertained, and the validity of antibiograms in identifying the same cluster assessed.

Results: Of 3503 specimens received by the microbiological laboratory during 5372 patient days, 1295 cultures showed bacterial growth. Of these, 132 were primary isolates of MR GNB and 92 were primary isolates of *S. aureus*. Thirty-two MR GNB isolates (24%) shared fingerprints with one or more other isolates. Indistinguishable isolates from epidemiologically related patients suggested 17 episodes of cross-transmission. The positive and negative predictive values of antibiogram-based identification of these episodes were 19% and 72% respectively. *S aureus* displayed limited genetic diversity. The two most frequent genotypes contained 19 and 16 isolates, of which the majority appeared to be epidemiologically unrelated.

Conclusions: Endemic transmission of MR GNB occurs mainly between two patients and remains unrecognized by conventional laboratory investigation. Rapid genetic typing methods identify patients involved in cross-transmission and give an insight into the population dynamics of MR GNB on adult intensive care units.

Key words: Nosocomial infections, intensive care units, bacterial cross-transmission, multiresistant Gram-negative bacilli, *Staphylococcus aureus*, genetic diversity, PCR fingerprinting, RAPD-ALFA

INTRODUCTION

Nosocomial infections are among the most important complications that affect patients in intensive care units (ICUs). Although ICUs often make up less than 5% of all hospital beds, nosocomial infections identified in these units can account for over 20% of the entire hospital case load [1–3]. The high prevalence in ICUs is conventionally explained by the severity of underlying illness of patients treated in these units, the high frequency of invasive procedures including mechanical ventilation, and the amount of antibiotic selection pressure, which favors the transmission of multiresistant bacteria between patients. Causative organisms originate either from endogenous or exogenous sources. In contrast to endogenous infections, exogenous infections are more likely to be amenable to infection control by reducing overall rates of transmission [4]. In this respect, the microbiological laboratory plays a crucial role in the effort to minimize nosocomial spread of infections in this setting. It serves as an early-warning system by identifying similar organisms likely to be part of a chain of transmissions [5]. In order to achieve this goal during routine services, phenotypic characteristics, such as susceptibility patterns (antibiograms), are used to determine whether organisms are similar or different. Genetic typing techniques (DNA fingerprinting) are seldom utilized in non-outbreak situations, mostly

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because the typing of many different pathogens requires several typing approaches. Moreover, they are time-consuming and thus not cost-effective.

The design of PCR-based fingerprinting methods [6,7] for the analysis of microbial DNA means that a standard typing strategy can potentially be applied to any microorganism of significance [8]. Results can be made available within a single work day and may have an impact on infection control procedures in sensitive areas of hospitals. The present study was set up to describe the potential of routine typing of bacterial pathogens that are frequently involved in nosocomial infections, with the aim of early identification of possible cross-transmissions, and to compare this approach with conventionally employed discrimination techniques (antibiograms). The typing was performed by PCR fingerprinting using random amplification of polymorphic DNA (RAPD), coupled with automated laser fluorescence analysis (ALFA), which allows the generation of digital PCR fingerprint data of high accuracy [9]. With this approach, the strain frequency of multidrug-resistant Gram-negative bacteria (MR GNB) and *Staphylococcus aureus* was continuously investigated in two adult ICUs over a period of 12 months.

MATERIALS AND METHODS

Description of the ICUs

The study was based on clinical specimens obtained between January and December 1994 from patients in two adult ICUs at the University Hospital Freiburg (1800 beds). One ICU was an adult surgical intensive care unit (SICU) where trauma patients or those after major abdominal surgery were treated. The unit had nine beds. All patients received mechanical ventilation and were referred to a high-dependency ward once their critical condition had improved. Patients were treated in bays of two and four beds. Two single rooms were available for patients who needed category-specific isolation or extracorporeal lung support. The other ICU was a medical intensive care unit (MICU) and provided treatment to patients with mainly cardiovascular illness. The MICU had seven beds, of which five were in a single room separated by barriers and two were in an adjacent room. Like SICU patients, these patients were mechanically ventilated and referred to a different ward if their acute condition had stabilized. Both ICUs were equipped with handwashing basins and hand disinfectant (70% isopropanol/ethanol) dispensers near all beds. Category-specific isolation and universal precautions were in practice in all the units. Handwashing procedures were routinely emphasized.

Bacterial isolates

We restricted our investigations to isolates of multiresistant Enterobacteriaceae (see below) and *Staphylococcus aureus,* and included non-fermenting Gram-negative rods, such as *Acinetobacter calcoaceticus/baumannii, Pseudomonas aeruginosa,* and *Stenotrophomonas maltophilia.* These pathogens are frequently isolated on ICUs, where they are often involved in nosocomial outbreaks. They are thus widely used for laboratory-based surveillance of nosocomial infections and regarded as indicator or 'alert' pathogens [10,11]. Patient specimens for microbiological investigations were obtained whenever infections were suspected or if requested by the care-giving practitioner. Materials were cultured and subcultured using routine procedures. Bacterial species were identified using standard protocols. When necessary MR GNB were tested by an API system (API-bioMérieux, Nürtingen, Germany). Antibiotic susceptibility was assessed by the ATB 7 semi-automatic resistance test (API-bioMérieux, Germany) at breakpoint concentrations; the assessment included 17 test substances. Susceptibility was expressed using the categories fully susceptible, intermediately susceptible, or resistant [12]. Gram-negative organisms that were resistant to broad-spectrum penicillins, and second-generation cephalosporins, or aminoglycosides, or ciprofloxacin or imipenem and all *Staphylococcus aureus* were selectively subtyped by RAPD-ALFA. Only the first isolate of any given species from each individual patient was typed.

RAPD-ALFA

PCR fingerprints for the ICU isolates were routinely obtained as described previously [9]. Briefly, two to four bacterial colonies, from overnight growth on 5% horse blood agar (BBL, Heidelberg, Germany), were suspended in 100 μL of water and heated to 95°C for 10–15 min. Crude lysates were centrifuged briefly; 2-μL portions of the supernatant provided template DNA for successive amplification reactions.

For MR GNB, a single primer, 5′-GAG GGT GGC GGT TCT- 3′, forming part of the phage M13 core region [13,14], was custom synthesized and labeled with fluoroisothiocyanate (FITC) at the 5′ end (Pharmacia, Freiburg, Germany). Each 25-μL reaction mix contained 1× PCR buffer (Pharmacia), 1 μM primer, 200 μM of each dNTP, 3 mM magnesium acetate, 0.5 IU *Taq* polymerase (Pharmacia) and target DNA. Amplification comprised 35 cycles of 94°C for 20 s, 50°C for 60 s, and 72°C for 20 s, with 2 min final extension time. *Staphylococcus aureus* isolates were subtyped using an FITC-labeled primer termed primer R1, as described by van Belkum et al [15]. Typing results for *Staphylococcus aureus* obtained by RAPD-

ALFA were confirmed by pulsed-field gel electrophoresis (PFGE) using a conventional protocol [16].

Amplification products were analyzed with a high-speed automated DNA fragment analysis device (ALF, Pharmacia), using short plates. Denaturing separating gels were prepared containing 5% Hydrolink Long Ranger (A&T Biochemicals, Malvern, USA) 7 M urea (ALF grade, Pharmacia), and 0.6× TBE. After PCR amplification, two FITC-labeled fragments, a 100-bp fragment of lambda DNA and a 1064-bp fragment of the *Escherichia coli* small-subunit rDNA, were added (0.1–1 fmol each) to each sample as internal size standards. Subsequently, 1 μL of each sample was denatured in 5 μL of loading dye (dextran blue 5 mg/mL formamide) at 95°C for 5 min and applied to the gel. The electrophoresis buffer was 0.6×TBE and the temperature was kept constant at 45°C. Fluorescence density data were stored on data files, normalized with the internal standards (100 bp, 1064 bp) and calibrated by 100-bp ladder size markers run every six lanes using Fragment Manager software (Pharmacia). In order to identify qualitative or quantitative variations between different sets of PCR amplifications, single species-specific reference strains (repro strains) were included in each PCR and assessed for reproducibility. Amplification profiles were pairwise correlated by Pearson's product–moment correlation coefficient for linear data, and the degree of correlation was illustrated by dendrograms using the unweighted pair-group method for arithmetic averages (UPGMA) of GelCompar software (Applied Maths, Kortrijk, Belgium).

Definitions and statistical tests

During the study, it was not necessary to distinguish colonization from symptomatic infection, since the aim was to identify organisms acquired during the patients' stay in the ICUs. Any set of isolates with PCR fingerprints that correlated at a level of $r>0.7$ was reported to the infection control team and the possibility of cross-transmission explored. Average waiting times for health events were compared by two-sample Wilcoxon rank sum test using Stata release 5.0 (College Station, Texas, USA).

RESULTS

During the 12-month observation period, 1262 patients were treated in the SICU and MICU with a mean stay of 4.8 days (median 4 days, range 2–74), resulting in an entire observation period of 5,372 patient days. From these patients, 3503 specimens were sent to the clinical microbiological laboratory for bacteriologic investigation. Bacteria were grown from 1295 cultures, and 238 MR GNB and 159 *Staphylococcus aureus* isolates were identified. Since only primary isolates of a given species from each patient were subtyped, 132 MR GNB and 92 *Staphylococcus aureus* isolates were further analyzed. The anatomic sites from which the samples were obtained are given in Table 1. Of the 132 MR GNB isolates, 100 displayed unique fingerprints. Thirty-two isolates had PCR fingerprints that were identified more than once from different patients. *Pseudomonas aeruginosa*, *Enterobacter cloacae* and *A. calcoaceticus/baumannii* were the most commonly isolated MR GNB in the ICUs. They also contained the highest proportion of isolates with indistinguishable fingerprints (Table 2).

For *Pseudomonas aeruginosa*, 11 of 48 primary isolates (34%) had RAPD types that were identified more than once in different patients. Three groups of primary isolates had visually indistinguishable fingerprints (correlation level >0.9). They were isolated from six, three, and two patients respectively. Patients identified as carrying visually indistinguishable isolates were generally treated on the same ward during overlapping periods. Among the cluster of six patients who shared visually indistinguishable isolates, time–space interaction had only occurred between pairs. Isolates of these patient pairs also showed the highest degree of pattern correlation when analyzed by computer-

Table 1 Patient specimen received by the clinical microbiological laboratory from two ICUs during the investigation period of 12 months

	R	BC	U	IVC	WS	D	CSF	Total
No. of patient specimens	1282	1019	469	323	174	160	76	3503
No. of culture-positive specimens	943	17	84	27	157	64	3	1295
No. of primary cultures positive for MR GNB	101	2	5	2	14	8	-	132
No. of MR GNB isolates with individual RAPD types	73	2	5	2	12	6	-	100
No. of primary cultures positive for *Staphylococcus aureus*	72	1	10	1	7	1	-	92
No. of *Staphylococcus aureus* isolates with individual RAPD types	12	-	1	-	1	-	-	14
No. of *Staphylococcus aureus* with individual PFGE types	10	-	2	-	1	-	-	13

R, respiratory secretion (bronchoalveolar lavage, protected specimen brush, tracheal secretions); BC, blood culture, including isolator; U, urine; IVC, tips from intravascular catheters; WS, wound swaps; D, drainage fluid; CSF, cerebrospinal fluid.

Table 2 Primary isolates and RAPD types of MR GNB from patients on two ICUs during the 12-month investigation period

Bacterial species	No. of patients with indicated isolates			
	Primary isolates	Individual RAPD type	Involvement in possible cross-transmission (by episode)	Indistinguishable RAPD type[a] (% of total)
Pseudomonas aeruginosa	48	37	(3) (2) (2) (2) (2)	11 (34)
Enterobacter cloacae	23	17	(2) (2) (2)	6 (19)
Acinetobacter calcoaceticus/baumannii	18	13	(3) (2)	5 (16)
Proteus vulgaris	9	5	(2) (2)	4 (12.5)
Stenotrophomonas maltophilia	8	8	–	–
Serratia marcescens	6	2	(2) (2)	4 (12.5)
Morganella morganii	3	3	–	–
Hafnia alvei	2	0	(2)	2 (6)
Escherichia coli	2	2		–
Klebsiella pneumoniae	2	2		–
Enterobacter aerogenes	2	2		–
Pseudomonas putida	2	2		–
Burkholderia cepacia	1	1		–
Proteus mirabilis	1	1		–
Citrobacter freundii	1	1		–
Klebsiella oxytoca	1	1		–
Enterobacter agglomerans	1	1		–
Acinetobacter xylosoxidans	1	1		–
Flavobacterium meningosepticum	1	1		–
Total	132	100		32 (24.2)

[a]Obtained from more than one patient.

assisted similarity analysis (Figure 1), indicating a closer resemblance. An overall comparison of all *Pseudomonas aeruginosa* isolates cultured during the investigation period identified a group of 14 with correlation values of >0.7. Based on epidemiologic data, nine of these 14 patients from whom the organisms were isolated were also involved in possible cross-transmissions. No temporal association between these clusters could be identified (Figure 1).

PCR fingerprinting of *Enterobacter cloacae* revealed indistinguishable patterns for six of 23 isolates (19%), and in the case of *A. calcoaceticus/baumannii* for five of 18 primary isolates (16%). Epidemiologic analysis showed concordance with the typing results, and three and two episodes of possible cross-transmissions involving six and five patients respectively could be demonstrated. Other possible cross-transmissions included two episodes for *Proteus vulgaris* (four patients), two episodes for *Serratia marcescens* (four patients) and one episode for *Hafnia alvei* (two patients).

Of the 32 MR GNB isolates with PCR fingerprints that were identified more than once among different patients, seven had identical antibiograms. Twenty-five had susceptibility profiles that differed among isolates with indistinguishable fingerprints. Moreover, 42 isolates would have been regarded as being possibly related on the grounds of identical antibiograms and species identification alone (Table 3). Thus the discrimination of different strains based on antibiograms had a sensitivity of 22% and a specificity of 65% when compared with genetic typing as the standard.

Among the 92 *Staphylococcus aureus* isolates, only 14 patterns were identified by RAPD and 13 by PFGE (Table 1), whereby both techniques showed a concordance of 94.6%. Only two isolates were methicillin resistant (MRSA), indicating a low prevalence of MRSA in the ICUs. The four most prevalent genotypes consisted of 19, 16, nine and nine isolates respectively. The average waiting time until organisms with indistinguishable fingerprints were isolated from different patients was 44.1 days (CI_{95} 32.3–55.8) as opposed to 10.1 days (CI_{95} 4.7–15.6) for MR GNB ($p=0.0004$), and patients who harbored indistinguishable MR GNB were 5.6 times less likely to have stayed in ICUs during overlapping periods than those with isogenic MR GNB (CI_{95} 1.49–23.08). Thus, the majority of *Staphylococcus aureus* isolates with indistinguishable fingerprints were regarded as epidemiologically unrelated, since they mostly originated from patients who were treated during different periods or in different ICUs with no transfer of other potential

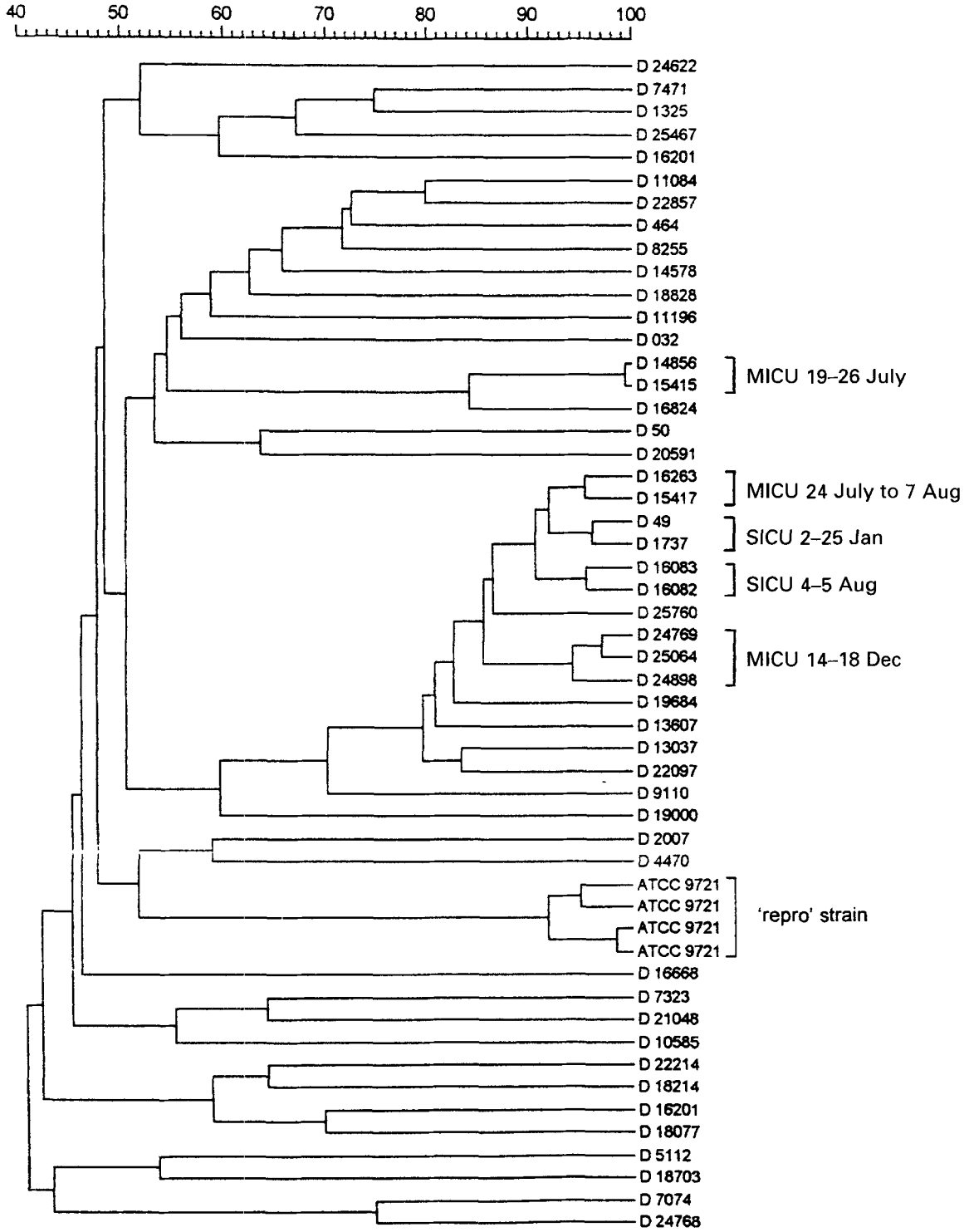

Figure 1 Dendrogram for 48 isolates of *Pseudomonas aeruginosa* obtained from patients in two ICUs over a 12-month investigation period, including replicate analysis of a 'repro' strain. Illustrated are the degree of correlation between isolates from different patients ($r \times 100$), the sample number, type of ICU, and date of the first and last isolation of possible cross-transmitted pathogens.

Table 3 Contingency table for calculating sensitivity[a] and specificity[b] of antibiogram typing compared to genetic typing by PCR fingerprinting

	No. of isolates indistinguishable by antibiogram	No. of isolates with different antibiogram	Total
No. of isolates with indistinguishable PCR fingerprints	7	25	32
No. of isolates with different PCR fingerprints	35	65	100
Total	42	90	132

[a]Sensitivity=22%. [b]Specificity=65%. Positive predictive value=19%, negative predictive value=72%.

carrier patients or staff between the wards. Phage typing and PFGE carried out independently at the National Reference Center for Staphylococcal Infections showed that the two most frequent genotypes belonged to lysogroups II and III and clustered with reference strains known to have a wide overall distribution in Germany.

DISCUSSION

It is commonly assumed that transmission from exogenous sources is the most frequent route of nosocomial infection [17,18]. However, this opinion has been increasingly challenged, as a result of attention being paid to infections caused by organisms derived from the patient's endogenous flora [19–21]. Assessing the relative contributions of these dichotomous pathways has implications for preventive strategies, since exogenous transmission is more amenable to intervention and control [22]. This study was designed to determine the strain frequencies of MR GNB and *Staphylococcus aureus* in routine microbiological specimens in order to determine the potential of routinely employed molecular typing methods in identifying nosocomial transmissions between patients on ICUs that otherwise would remain unrecognized.

Gram-negative bacteria and *Staphylococcus aureus* cause the majority of nosocomial infections in ICUs [23]. If strains are multiresistant they are also considered to be highly transmissible in ICU settings, as they enjoy a selective advantage where antibiotics are heavily used [24]. Indistinguishable PCR fingerprints (RAPD types) of pathogens cultured from different patients should indicate cross-transmission if the underlying species is sufficiently diverse that the chance of finding organisms with exactly the same RAPD type is low.

Pattern analysis of the *Psdudomonas aeruginosa* isolates exhibited a group of related strains indicated by an overall correlation level of >0.7. Previous studies have shown that clonally related strains may correlate with coefficients up to 0.7, whereas cross-transmitted strains frequently achieve values of 0.9 and higher with the RAPD-ALFA technique [9,25]. Interestingly, nine of 11 isolates involved in episodes of possible cross-transmission belonged to this group. In the absence of any knowledge of the expected diversity in the species as a whole, i.e. the frequency of particular RAPD types in wild-type organisms, it is impossible to know if this distribution reflects the natural occurrence of related strains. Alternatively, it may also indicate the persistence of a clonal lineage that exhibits a particularly high transmissibility when introduced into the hospital environment. Samples from moist environments that were obtained during the investigation period did not, however, reveal a reservoir of this clone, as has been shown previously in the neonatal ICU of the same hospital [26].

In contrast to most of the epidemiologically unrelated MR GNB, *Staphylococcus aureus* exhibited only a relatively low genetic diversity in the present study. Among the 92 isolates investigated, 90 were methicillin sensitive (MSSA) and only 14 or 13 individual fingerprints could be demonstrated, depending on the typing technique (RAPD or PFGE) used. Moreover, both techniques identified two types that contained 19 and 16 isolates each (comprising 38.1% of the entire population), most of which did not have any epidemiologic relation but represented common clones prevalent in Germany. This observation suggests that correspondence of the same genetic type of *Staphylococcus aureus* in a closed facility does not necessarily indicate cross-transmission between patients and supports the conclusion of other studies that some MSSA strains are widely distributed and may independently colonize different patients [27–29]. We therefore decided not to make assumptions concerning transmission episodes based on the strain frequencies of *Staphylococcus aureus* in the present investigation.

In spite of a high average density of microbiological investigation (2.8 investigations per patient), no outbreak or significant cluster of infections was detected during the study period, and it can therefore be assumed that the results reflect the incidence of cross-transmission at endemic background levels [30]. Seventeen episodes of MR GNB transmission could be ascertained in 5372 patient-days, i.e. 3.16 episodes per 1000 ICU patient-days. Although these figures appear to be low, not less than 24.2% of MR GNB strains isolated from ICU patients in our study seemed to be involved in cross-transmissions. In the absence of firm

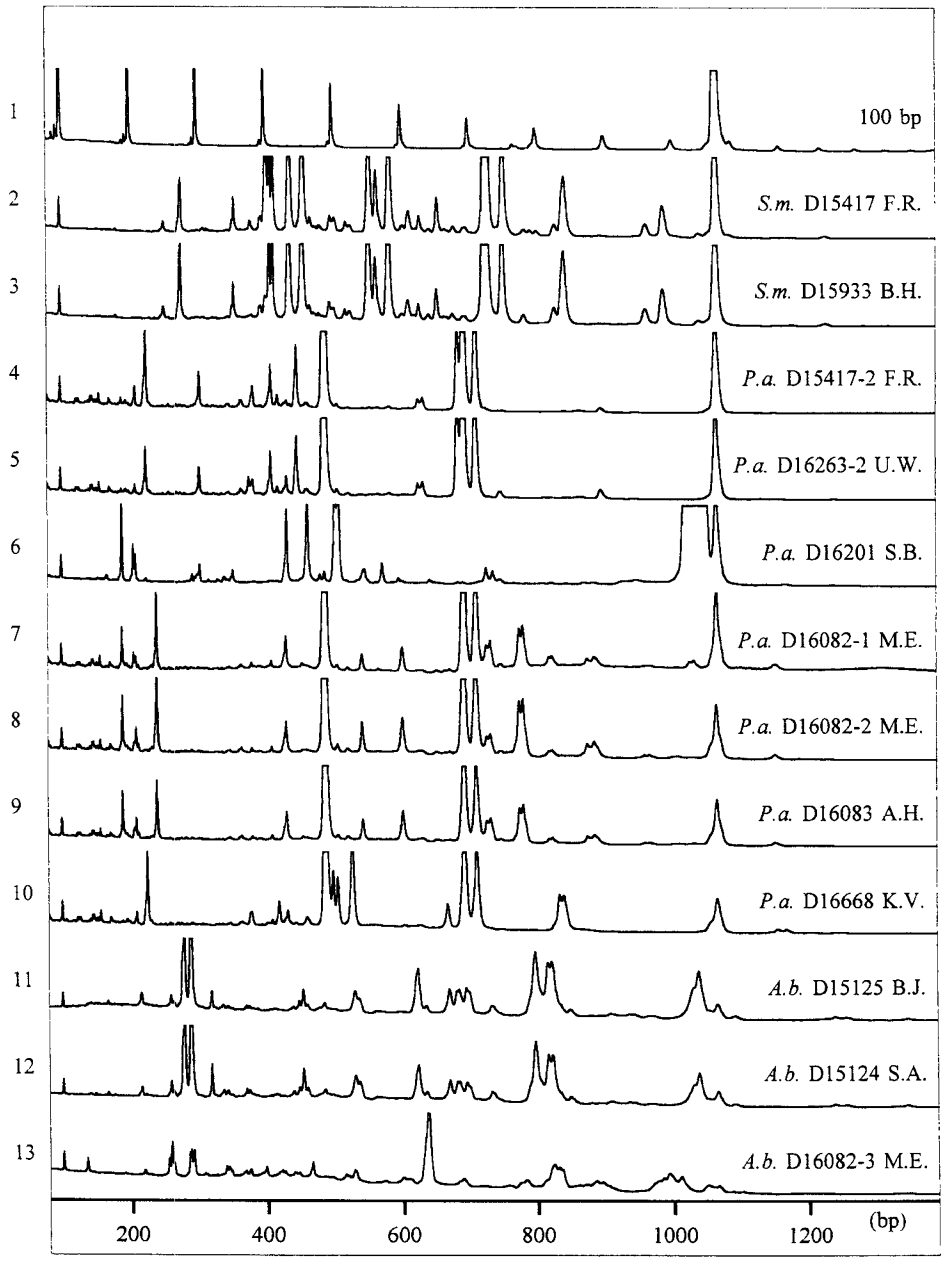

Figure 2 Representative fluorescence densitogram of MR GNB from clinical specimens isolated from patients during a 3-week interval (21 July to 12 August 1994) in two ICUs. Lanes 2–6, isolates from MICU; Lanes 7–13, isolates from SICU. Lane 1: 100-bp-ladder size marker. Lane 2: *Serratia marcescens* from tracheal secretion of patient F.R., isolated on 24 July. Lane 3: *Serratia marcescens* from tracheal secretion of patient B.H., isolated on 1 August, same clone as lane 2. Lane 4: *Pseudomonas aeruginosa* cultured from the same tracheal secretion as *Serratia marcescens* (patient F.R.) in lane 2. Lane 5: *Pseudomonas aeruginosa* from tracheal secretion of patient U.W., isolated on 7 August, same clone as in lane 4. Lane 6: *Pseudomonas aeruginosa* from tracheal secretion of patient S.B., isolated on 6 August, unrelated to the transmission clone. Lanes 7 and 8: two isolates *of Pseudomonas aeruginosa* which differed by colony morphology, from the same tracheal secretion of patient M.E., isolated on 5 August. Lane 9: *Pseudomonas aeruginosa* from wound swab of patient A.H., isolated on 4 August, same clone as lanes 7 and 8. Lane 10: *Pseudomonas aeruginosa* from tracheal secretion of patient K.V., isolated on 12 August, unrelated to transmission clone. Lane 11: *A. calcoaceticus/baumannii* from tracheal secretion of patient B.J., isolated on 21 July. Lane 12: *A. calcoaceticus/baumannii* from tracheal secretion of patient S.A., isolated on 27 July, same clone as lane 11. Lane 13: *A. calcoaceticus/baumannii* cultured from the same tracheal secretion as *Pseudomonas aeruginosa* (patient M.E.) in lanes 7 and 8 but bearing no relation to the transmission clone in lanes 11 and 12. All isolates had been produced in parallel and PCR products were run in the same gel. Lanes with repro strains and other size marker lanes have been omitted.

data on other readily transmissible pathogens such as *Staphylococcus aureus*, enterococci, coagulase-negative staphylococci and fully susceptible Gram-negative bacilli, the overall frequency of transmission cannot be estimated with confidence.

The present data are in accordance with figures obtained from a study carried out by Chetchotiskad et al [28], who investigated the strain frequency of a partly different panel of Gram-negative organisms on five adult and pediatric ICUs. They reported an incidence density of 1.71/1000 ICU patient-days and found that as many as 17.9% (26/145) of the Gram-negative strains examined were involved in endemic cross-transmission. The smaller values reported in that study may be explained by differences in the analysis, such as a lower investigation frequency (one strain typed every 46.2 ICU patient-days versus 40.6 ICU patient-days in the present study) and the employment of less sensitive plasmid profile analysis for the typing of the majority of isolates. However, the figures may also simply reflect an overall lower transmission rate in a facility where patients were hospitalized in private rooms in contrast to ICUs with a bay or large-room design. Both studies, however, agree that the number of patients involved in single episodes is relatively small, rarely being more than two at a time, and thus demonstrate that cross-transmission in ICUs usually occurs at levels undetectable by methods commonly recommended for routine surveillance [31].

Clearly, there are two limitations to a study that confines the assessment of cross-transmission solely to microbiological examinations requested by the intensive care practitioner. A bias is introduced, since severely ill patients will be more frequently investigated compared to those who quickly recover or do not show signs of infection. Consequently, some transmission events may be missed. Since colonization and infection with nosocomially acquired organisms is, on the other hand, more frequent in the severely ill individuals, our results may not underestimate the true density of transmission of this selected group of pathogens. Another limitation is the fact that any transmission that would have become manifest only after discharge from the ward would certainly not have been noticed. However, post-ICU discharge would inevitably increase uncertainties regarding the time of bacterial acquisition, i.e. before or after ICU discharge.

Given the densities of endemic transmission reported in this paper, what recommendations for surveillance of nosocomial infections in ICU patients should be given? The use of antibiograms as an initial first-line screen for cross-transmission suffers considerable limitations in low-endemic situations. Obviously, the probability of correctly identifying transmission events based on identical susceptibility profiles, i.e. the positive predictive value, becomes very small, and was only 0.19 in the present study (Table 3). The negative predictive value may be slightly better. Based on antibiograms, the probability of having ruled out an episode of transmission correctly was 0.72, but that still means that over a quarter of all transmission episodes would have been missed. Routine DNA typing would be a desirable component of infection control services in an ICU setting, but is not widely available and is impractical for most diagnostic laboratories. Conventional DNA-based typing techniques are expensive and time-consuming and frequently require different approaches for different bacterial species. The identification of cross-transmission is therefore often delayed, resulting in only marginal impact on control procedures. RAPD generates results within a single working day [8] and allows an analysis of multiple species in parallel using a single technique (Figure 2). Simple agarose gel electrophoresis allows detection of genetically indistinguishable pathogens. When limiting this relatively inexpensive technique to situations when bacteria of a single species are isolated from patients in conspicuous clusters, sensible data can be made available to clinical staff in a timely fashion. In our experience, typing data have a high educational value if transmissions can be demonstrated at times when patients are still being treated in the unit. More importantly, typing data are reassuring if discordant strains are identified, and serve as an important tool in auditing the quality of hygienic precautions in ICUs.

As infections in critically ill patients cannot be efficiently controlled without a better understanding of the dynamics of bacterial populations, monitoring of the strain frequencies of microorganisms in ICUs will become an important scientific issue. Future investigations will have a bearing on decisions on the usefulness of strategies that aim at the interruption of endogenous versus exogenous acquisition of nosocomial infections. RAPD-ALFA is a convenient approach if DNA fingerprint data of high quality need to be stored and made available for comparison over extended periods of time.

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