

Heterogeneous Antimicrobial Resistance Patterns in Polyclonal Populations of Coagulase-Negative Staphylococci Isolated from Catheters

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Received 30 September 1999/Returned for modification 2 November 1999/Accepted 4 January 2000

Most cases of nosocomial bacteremia are catheter related, and coagulase-negative staphylococci (CoNS) are the microorganisms most frequently associated with these infections. Subtle morphological differences are frequently found among CoNS colonies cultured from infected catheters. The aim of this study was to analyze the significance of the morphological heterogeneity observed in these CoNS populations. With this purpose in mind, the clonal composition of the CoNS populations obtained from a selection of nine catheters was analyzed by two different molecular techniques, arbitrarily primed-PCR and DNA macrorestriction analysis by pulsed-field gel electrophoresis. Twenty CoNS morphotypes were included for analysis, and four single colonies representative of each morphotype were selected. Morphological differences between colonies were found to correlate in all cases with differences at the molecular level. Unique fingerprints were also obtained for some isolates which were indistinguishable from other representatives of the same morphotypes. Differences in the molecular patterns among the isolates were associated in most of the cases with differences in the antimicrobial susceptibility patterns. The frequent isolation of polyclonal CoNS populations from catheters, with heterogeneous antimicrobial susceptibility patterns, has relevant epidemiologic and therapeutic implications in the context of catheter-related infections.

Catheter-related infection is the most frequent cause of nosocomial bacteremia (4, 8). The role of catheters as the origin of bacteremia has been precisely tracked by molecular methods in several reports (5, 10).

Coagulase-negative staphylococci (CoNS) are the microorganisms most frequently isolated from catheters (16). At our institution, 64% of all isolates cultured from these devices in 1998 were CoNS.

The finding of polymorphic CoNS populations in catheter tip cultures is a frequent event. In our institution, of all the catheters received in 1998 in which CoNS were isolated exclusively, more than one morphologically distinguishable strain was found in 17.3% of the cases (82 of 474).

The aim of this study was to analyze by molecular techniques, supported by antibiotyping, the significance of the subtle morphological differences frequently observed among CoNS colonies from catheter cultures. We therefore analyzed the clonal composition of the CoNS populations involved in the infection of catheters.

The role of polyclonal populations in bacteremic episodes has been reported for microorganisms other than CoNS to constitute up to one-third of the cases described (1, 12–14). However, analyses of clonality focused on the potential sources of infection are still lacking. Catheter-related infections are a suitable context in which to study clonality in the source of an infection since the source of such infections is well defined and accessible to analysis. The fact that different clones of a microorganism could be involved in the same infectious event is both clinically and epidemiologically relevant (3, 12).

MATERIALS AND METHODS

During 1998, our laboratory received 2,547 intravascular catheter tips. After processing them by the Maki semiquantitative procedure (7), 474 showed growth of CoNS exclusively after 72 h of incubation. The same observer examined all plates with CoNS by use of a dissecting microscope and detected that 17.3% of them (82 of 474) corresponded to cultures with at least two morphologically distinguishable strains. At 24 h, all CoNS cultures for each of the catheters analyzed were apparently morphologically homogeneous; at 48 h, morphological heterogeneity among the colonies was suspected in some cases but it was only after 72 h that the cultures revealed a polymorphic composition.

For study, nine catheters were selected in which a heavy growth ($n > 50$ colonies) was observed for each of two or more different morphotypes of CoNS. Four single colonies representative of each of 20 different morphotypes were selected and stored frozen until analysis.

Identification and antimicrobial susceptibility testing were performed on a representative of each morphotype, after homogeneous cultures for each clone were obtained, by using the POS Combo Panel Type 2S (DADE Behring; MicroScan, Sacramento, Calif.). For the 20 representatives of the morphotypes, 16 corresponded to *Staphylococcus epidermidis*, two to *S. haemolyticus*, one to *S. hominis*, and one to *S. schleiferi*. All differences in the antibiotic profiles for the different morphotypes were confirmed by the disk diffusion technique of The National Committee for Clinical Laboratory Standards (NCCLS). Analysis of the antimicrobial susceptibility patterns was performed blind by someone other than the one who had selected the morphotypes. Isolates were considered different when changes from susceptible to resistant, according to the NCCLS criteria, or vice versa, were recorded for at least one antibiotic.

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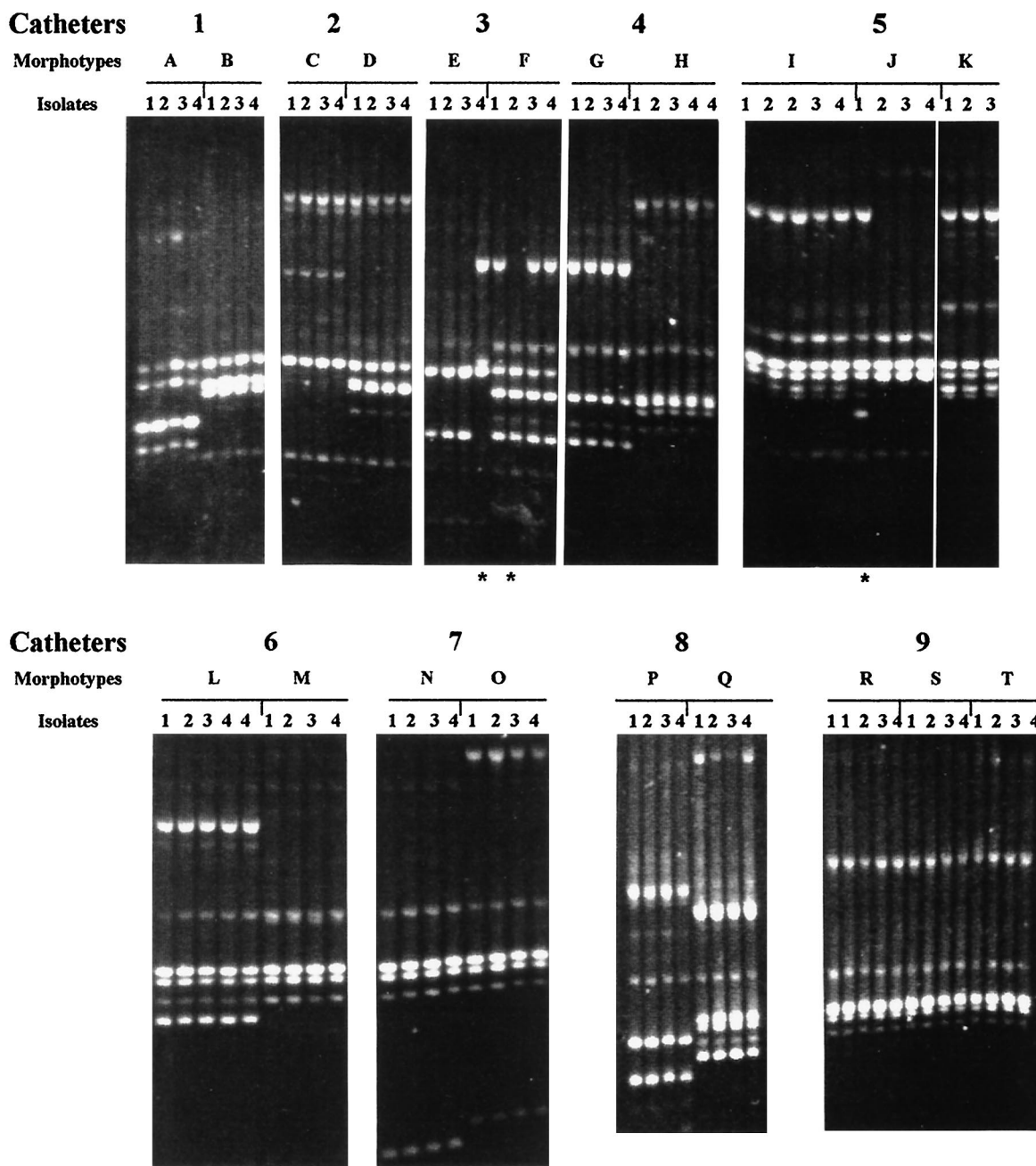


FIG. 1. AP-PCR profiles obtained with primer 2 for the CoNS isolates analyzed. Each panel corresponds to the isolates for each catheter. Nomenclature for each isolate is formed by combining the three characters on the top of the gel; from top to bottom these characters are the number indicating the catheter, the letter indicating the different morphotypes observed for each catheter, and the numbers for the four representatives of each morphotype. An asterisk at the bottom of the figure indicates isolates with unique fingerprints with respect to those within the same morphotype.

Molecular typing methods. (i) **AP-PCR.** Bacterial cultures, DNA extraction procedures, and arbitrarily primed (AP)-PCR assays were done as described elsewhere (6). We used primers 1 (5'-GGTGC GGAA-3'), 2 (5'-GTTTCGCT CC-3'), 3 (5'-GTAGACCCGT-3'), 4 (5'-AAGAGCCCGT-3'), 5 (5'-AACGCG CAAC-3'), and 6 (5'-CCCGTCAGCA-3') from Pharmacia. Control reactions to discard false differences between fingerprints and amplification thermal profiles were as detailed elsewhere (6). Long-performance agarose gels were required to achieve enough resolution to analyze the typing patterns.

(ii) **PFGE.** Bacterial cultures, preparation of plugs, and cell lysis were performed as described previously (6). Two sequential *Sma*I digestions were performed. First, 50 U of the enzyme was added to the plugs to perform a 3-h restriction, and then another 50 U was added and the restriction reaction was left overnight. Restricted DNA fragments were separated by pulsed-field gel electrophoresis (PFGE) performed in a CHEF (contour-clamped homogeneous-

electric-field) electrophoresis system (Bio-Rad) by using 1% agarose gels and 0.5× TBE buffer. Gels were run for 20 h at 6 V/cm and 14°C with pulses ramping from 5 to 35 s.

RESULTS

It was necessary to incubate plates for up to 72 h to be able to discriminate all the morphological differences between the CoNS isolates. All isolates showing different morphotypes correlated with differences at the molecular level, and most representatives of the same morphotype shared the same fingerprint pattern.

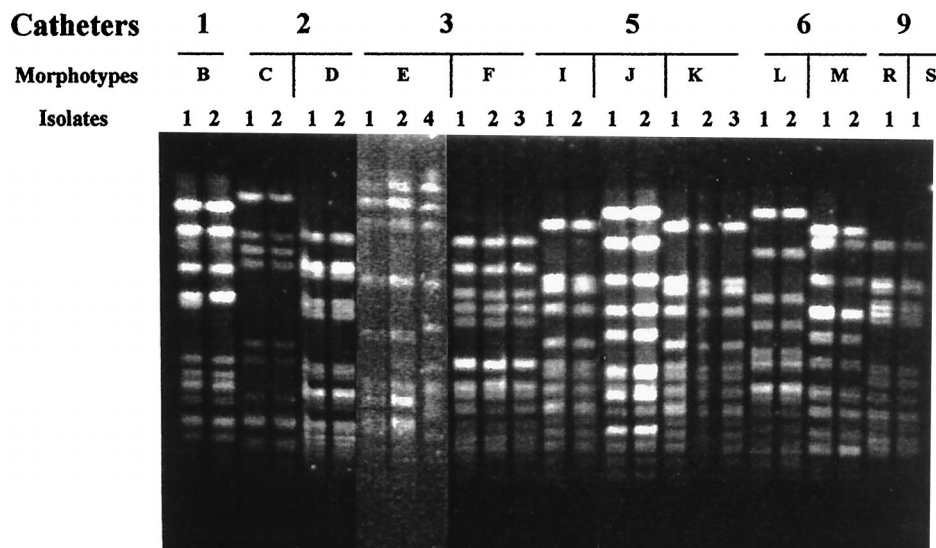


FIG. 2. DNA macrorestriction analysis after digestion with *Sma*I and PFGE with a selection of isolates. The catheter number, morphotypes, and number of each representative are indicated as for Fig. 1.

AP-PCR results (primer 2) for the CoNS isolates analyzed are shown in Fig. 1. Unique molecular fingerprints were recorded for each morphotype except for catheter 9, in which a common typing profile was obtained. This catheter was included in the analysis as a control for “false morphotypes” due to the proven pleomorphic character of their colonies. Replicates of the same isolate, included as reproducibility controls (4H4, 5I2, 6L4, and 9R1 [Fig. 1]), produced identical fingerprints. All representatives of the same morphotype shared the same fingerprint pattern, except for three isolates (3E4, 3F2, and 5J1 [Fig. 1]). When not all representatives of the same morphotype shared the same fingerprint, the morphology of the clones was reanalyzed to confirm they all were representatives of the same morphotype.

An additional AP-PCR analysis with a different primer which also offered high typing resolution with CoNS (primer 4) was performed in order to confirm the validity of the fingerprints obtained. No discrepancies were found with any of the colonies when the similarity patterns obtained with primer 4 were compared with those previously described (data not shown).

Macrorestriction DNA analysis was performed with a selection of 25 isolates representing 12 morphotypes. The results obtained with AP-PCR were confirmed by PFGE. The restricted products after PFGE are shown in Fig. 2. Again, for all the cases, the morphological differences corresponded to different molecular fingerprints except for the control catheter (catheter 9), which harbored pleomorphic colonies. Isolates representative of the same morphotype shared the same fingerprint profile, except in one case (i.e., 3E4).

To rule out the effect of an excessively high and uninformative resolution in the molecular typing approach, antibiotic susceptibility profiles were obtained for a selection of clones. Resistance profiles for a representative of each morphotype, and several of those within the same morphotype, are shown in Table 1. Seventeen different susceptibility patterns were obtained for all of the isolates analyzed. A high correlation was found among differences at the molecular level and those in the antimicrobial susceptibility patterns. For each of the catheters, isolates with different fingerprints also showed differences in their susceptibility patterns (Fig. 1 and Table 1), with the only exception of isolates 5I1 and 5K1, which shared the

same antibiogram despite the differences found among their fingerprints (Fig. 1 and Table 1). All isolates sharing the same fingerprint (Table 1, in italics) showed identical antibiograms.

It should be noted that those cases in which unique AP-PCR fingerprints were found in one of the four representatives of the same morphotype also showed differences in their susceptibility patterns (3E4, 3F2, and 5J1 [Table 1]).

In summary, all of the catheters analyzed but one harbored CoNS clones with differences in their morphologies, molecular fingerprints, and susceptibility patterns (Table 2). Most of the

TABLE 1. Antibiotic resistance profiles obtained for a selection of isolates^a

Antibiotype	Resistance profile	Isolate(s) ^b
1	Ery, Fos	1A1, 1A2
2	Oxa	1B1, 1B2
3	Pen, Tet	2C1, 2C2
4	Oxa, Ery, Cip	2D1, 2D2, 9R1, 9S1, 9T1
5	Oxa, Ery, Cip, SXT, Gen	3E1
6	Oxa, Ery, Cip, Gen, Fos	3E4*
7	Oxa, Ery, Cip, SXT, Gen, Rif	3F1, 4G1, 4G2
8	Oxa, Ery, Cip, SXT, Gen, Fos, Rif	3F2*, 5J1*
9	Oxa, Cip, SXT, Ery	4H1, 4H2
10	Oxa, Ery, Cip, SXT	5I1, 5K1
11	Oxa, Ery, Tet	5J2
12	Oxa, Ery, Cip, SXT, Gen, Fos	6L1
13	Oxa, Gen, SXT, Tet	6M1, 6M2
14	None	7N1
15	Pen, Ery	7O2
16	Oxa, Ery, Gen, Fos	8P1, 8P2
17	Oxa, Gen, Ery, Cip	8Q1

^a One isolate for each fingerprint and several representatives of the same typing pattern as controls were tested. Antibiotics: Ery, erythromycin; Fos, fosfomicin; Oxa, oxacillin; Pen, penicillin; Tet, tetracycline; Cip, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; Gen, gentamicin; Rif, rifampin.

^b Nomenclature for isolates: the first number refers to the catheter; the capital letter refers to the morphotype, and the last number refers to the representatives of the same morphotype. Isolates included as reproducibility controls of the same morphotype are indicated in italics. An asterisk indicates an isolate with a unique fingerprint with respect to isolates within the same morphotype. The only two isolates for the same catheter with unique fingerprints and shared antibiograms are in boldface.

TABLE 2. Compilation of features of the CoNS isolates analyzed

Relevant anti-microbial ^a	Feature with the indicated catheter, morphotype, fingerprint, and antibiotic ^b																						
	1		2		3		4		5			6		7		8		9					
	A	B	C	D	E		F		G	H	I	J		K	L	M	N	O	P	Q	R	S	T
	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	u	u	u
	1	2	3	4	5	6	7	8	7	9	10	8	11	10	12	13	14	15	16	17	4	4	4
Van	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Oxa	S	R	S	R	R	R	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R	R	R
Gen	S	S	S	S	R	R	R	R	R	S	S	R	S	S	R	R	S	R	R	S	S	S	S
Rif	S	S	S	S	S	S	R	R	R	S	S	R	S	S	S	S	S	S	S	S	S	S	S
SXT	S	S	S	S	R	S	R	R	R	R	R	S	R	R	R	S	S	S	S	S	S	S	S

^a Van, vancomycin; Oxa, oxacillin; Gen, gentamicin; Rif, rifampin; SXT, trimethoprim-sulfamethoxazole.

^b From top to bottom: the number of the catheter, the capital letter indicating the different morphotypes, the lowercase letter indicating the different molecular fingerprints, and the numbers indicating the antibiotic types. Susceptibility (S) and resistance (R) patterns for the most relevant antimicrobials, from a therapeutic point of view, are shown for the different morphotypes. The differences in the susceptibility profiles when found for clones within the same catheter are in boldface.

isolates with differences at the morphological and molecular levels were indistinguishable at the species level and were identified as *S. epidermidis*. The only catheter with isolates sharing fingerprints and antibiotic types corresponded to a control, which harbored pleomorphic colonies. When considering therapeutically relevant antimicrobials, differences in the susceptibility patterns were found between clones within the same catheter for most of the cases analyzed (Table 2, in boldface).

DISCUSSION

In this study, we performed a molecular analysis using two different molecular techniques on a selection of CoNS single colonies isolated from catheters. Incubation times of less than 72 h were not long enough to reveal the polymorphic character of cultures, and therefore overnight incubation would have incorrectly indicated that the catheter cultures were homogeneous.

It seems unlikely that the polymorphic character of these populations could be attributed to contamination during sample culture. In this case, lower colony counts would be expected.

This study has shown that the subtle differences at a morphological level in CoNS colonies are informative since they are highly correlated with differences at the molecular level. The only case in which morphologically different isolates shared the same fingerprint was a control catheter with proven pleomorphic colonies. The proportion of morphologically distinguishable isolates linked to unique fingerprints was much higher than previously reported for polyclonal gram-negative populations responsible for bacteremic episodes (14).

It is worth noting that molecular differences were found not only among representatives of different morphotypes but also for some representatives within the same morphotype. This observation indicates that the variability in CoNS populations infecting catheters is higher than that expected from morphological differences only and means that several single colonies should be selected to assure correlation with the CoNS clones infecting blood.

It is possible that, in some cases, the polyclonal character of infections could not have been detected by standard typing approaches (2, 11). In this study, the application of AP-PCR has enabled us to obtain a high resolution for CoNS typing. Our AP-PCR experimental conditions allowed detection of molecular differences among some representatives within the same morphotype. It is remarkable that not all these differ-

ences could be detected by PFGE, the typing method considered as a reference for CoNS typing.

The polyclonal composition of the catheters analyzed could have clinical implications, as shown by the antimicrobial susceptibility tests. Most of the cases in which differences at the molecular level were found corresponded to colonies with differences in the antibiotic susceptibility profile. On the other hand, common antibiotic types were found among colonies sharing fingerprints. The high correlation between molecular variability and differences in antibiotic types obtained in our study rule out the risk of selecting an excessively high and uninformative degree of molecular resolution.

Therefore, most of the catheters analyzed were infected by at least two clones, each with a different antibiotic resistance profile. When we considered only the most relevant antimicrobials, from a therapeutic point of view, differences in the susceptibility patterns could be found for certain clones within the same catheter. This occurred in most of the catheters analyzed and points out the limitations of performing susceptibility tests from single colonies if polyclonal populations are involved (1, 12).

Relapse and reinfection concepts should be applied with caution in cases of catheter-related infections. Reinfections are considered when different clones of the same microorganisms are sequentially isolated (9, 15). If we take into account the frequent polyclonal composition of CoNS populations, different clones may be randomly selected from successive cultures if only single colonies are picked. In that case, relapses due to polyclonally infected catheters could be misinterpreted as two independent episodes (reinfection) caused by different CoNS isolates.

The finding of polymorphic CoNS populations in catheter tip cultures is a frequent event. In our institution, of all the catheters received in 1998 in which CoNS were isolated, more than one different morphologically distinguishable strain was found at least in 17.3% of the cases. We should also allow for increased clonal variety given that, in our study, molecular differences among isolates were found to be not exclusively linked to morphological differences.

To conclude, polyclonality in CoNS populations infecting catheters should be considered. Several single colonies of the same episode should be studied either to allow the precise tracking of the source of bacteremia or to accurately define the susceptibility pattern of the bacterial population responsible for a catheter-related infection.

ACKNOWLEDGMENTS

We thank Carmen Aldea for her help in the molecular typing assays. We are also indebted to Thomas O'Boyle for his help in the preparation of the manuscript.

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