JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1988, p. 175–177
0095-1137/88/020175-03$02.00/0
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Vol. 26, No. 2

Simple Method for Rapid Diagnosis of Catheter-Associated Infection by Direct Acridine Orange Staining of Catheter Tips

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Received 10 August 1987/Accepted 9 October 1987

Direct acridine orange (AO) staining was used to detect bacteria adherent to intravascular catheters (IVC). Samples from 710 IVC tips were first cultured on blood agar plates by a semiquantitative technique and then independently colored with AO and screened dry at a magnification of ×100 for 3 min. In the absence of fluorescence, they were considered negative. When fluorescence was present, they were further examined for the presence of microorganisms at ×1,000 with immersion oil. Of 710 IVC tips, 37 (5.2%) were positive upon culture (≥15 colonies) and 673 were negative (640 were sterile and 33 [4.6%] had 1 to 14 colonies). The AO sensitivity was 84%, and the AO specificity was 99%. When restricted to the 212 long IVC, AO sensitivity rose to 94%. AO staining was positive in all cases of catheter-associated bacteremia. The negative predictive value of the preliminary screening at ×100 was 99.5%. The direct examination of IVC tips stained by AO appears to be a simple and rapid method for diagnosing IVC-associated infections. In addition, AO staining is easier to perform than Gram staining.

When an intravascular-catheter-associated infection is suspected, it is generally recommended that the device be removed and the tip be cultured. Maki et al. (5, 6) have standardized a semiquantitative method for culturing vascular cannulas on solid media. However, the confirmation of an infection by culture requires an additional day. An accelerated microbiological detection of catheter-associated infection was proposed by Cooper et al. (1), who developed a Gram-staining technique of the catheter tips. This technique depends on the optical properties of the different catheters and is time-consuming, since it requires the microscopical examination of at least 200 oil immersion fields.

Acridine orange (AO), a fluorochrome dye buffered at low pH, was described as enhancing differential staining of bacteria in clinical specimens (2, 3). This fluorescent staining technique is more rapid to perform than Gram staining, since a single staining step, rather than four, is required. Furthermore, the AO-stained smear can subsequently be colored by Gram stain, if a confirmation is desired (7).

The purpose of the present study was to evaluate AO staining as a possible method for the detection of bacteria adherent to intravascular catheters.

MATERIALS AND METHODS

Collection of catheters. Over a 6-month period, intravascular catheters submitted from nonburned adult medical and surgical patients were systematically sent to the laboratory, independently of any clinical suspicion of infection, and prospectively examined. Any type of intravascular catheter was accepted for the study. For short catheters (<6 cm), the entire length of the cannula was cut 1 cm below the skin surface/catheter junction. For long catheters, two 5-cm segments were cultured: the tip and the intracutaneous segment. The catheter segments were transported to the laboratory in sterile, dry containers.

Catheter culture. Catheters were cultured by using the semiquantitative method described by Maki et al. (6). Flamed forceps were used to transfer the entire catheter segment onto the surface of a 100-mm 5% sheep blood agar plate (Columbia base; Oxoid Ltd., London, England), and the catheter was rolled back and forth four times across the agar surface. The catheter was then placed back in a dry, sterile tube and held for AO staining. Plates were incubated at 37°C in an atmosphere of air containing 8% CO_2 for 4 days and inspected daily for microbial growth. Colony counts were performed by visual inspection; confluent growth was defined as more than 1,000 colonies per plate. All organisms were identified by standard microbiological methods.

Preparation of AO staining solution. AO can be obtained ready for use (Difco Laboratories, Detroit, Mich.) or prepared as follows: 20 mg of AO powder (Fluka AG, Buchs, Switzerland) is added to 190 ml of sodium acetate buffer (stock solution of 100 ml of 1 M sodium acetate and 90 ml of 1 M HCl); 1 M HCl is added if necessary to yield a final pH of 3.5 and a final AO concentration of about 100 mg/liter (4). At pH 3.5 to 4.0, AO binds to nucleic acids of bacteria and stains them orange. The staining solution was stored at room temperature and was protected from light.

Catheter staining with AO. Catheters were examined without previous knowledge of culture results. The entire lengths of the catheter segments were first fixed at 56°C for 2 min in a dry incubator. Each catheter was then immersed in AO staining solution for 3.5 min, rinsed with water, drained, and air dried.

Microscopical examination of catheters. The examination under epifluorescence did not require prior longitudinal cutting of the catheter or the removal of clotted intraluminal blood, as opposed to the Gram stain technique (1), since the optical properties were satisfactory under these conditions for each kind of catheter. Catheters were attached to a cleaned glass slide (76 by 26 mm; Menzel-Gläser, Hannover, Federal Republic of Germany) with adhesive tape and immediately examined under a fluorescence microscope (Laborlux 11 [Wild and Leitz, Zürich, Switzerland] equipped with a mercury vapor arc lamp at 50 W, filter block I 2/3 for excited light in the wavelength band of 450 to 490 nm, bright-field condenser completely open). Each smear was scanned at a magnification of ×100 dry (eyepiece, 10/18;

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objective, 10/0.25). Unless fluorescent material was detected after 3 min of examination at low magnification, the result was considered negative. When fluorescent material was detected, confirmation of its morphology was obtained by using a magnification of ×1,000 with oil immersion (objective, 100/1.25 oil). Because of the cylindrical shape of the cannulas, frequent fine focusing was necessary at high magnification. Each segment scanned at low and high magnification was examined for no more than 10 min.

RESULTS

A total of 710 intravascular catheters from surgical and medical patients were analyzed: 498 (70%) short catheters (412 Venflon [Viggo AB, Helsingborg, Sweden], 52 Vasculon [Viggo], and 34 Abbocath-T [Abbott, Sligo, Republic of Ireland] catheters) and 212 (30%) long catheters (87 Intracath [Deseret Medical, Sandy, Utah] and 125 Drumcath [Abbott] catheters).

The semiquantitative cultures of 37 catheter tips (5.2%) were positive (≥15 colonies per agar plate). Only one was positive for more than one organism. The cultures of 33 (4.6%) catheter tips showed between 1 and 14 colonies per agar plate (so-called contaminated catheters), and 640 (90.1%) catheters were sterile. Table 1 lists the organisms recovered from positive catheters. Coagulase-negative staphylococci were the most common isolates. The two catheters colonized by *Candida* species (*Candida albicans* and *Candida tropicalis*) were removed from patients receiving hyperalimentation.

The microscopical examination of AO-stained catheters gave the following results. AO screening of the catheter surface under low power (×100) revealed fluorescent material on 141 catheters (20%) and no fluorescence on 569 catheters (80%). At this magnification, the fluorescent areas consisted mainly of plaques of amorphous material and clumps of polymorphonuclear leukocytes, but microorganisms could not be readily distinguished. Of these 569 catheters, 567 were culture negative and 2 were culture positive (17 and 130 colonies, respectively).

The 141 catheters showing fluorescent material at low magnification were further examined under high-power oil immersion. Bacteria or yeasts were visible in 36 (25%) of those specimens. The semiquantitative culture was positive for 31 and negative for 5 of these 36 catheters. For the 31 catheters positive by both methods, the number of colonies on the primary plate was ≥500 for 12 tips, between 100 and 500 for 17 tips, and 50 for 2 tips. All four catheter-related bacteremias recorded in this series were positive on AO stain. Of the 105 catheters showing fluorescent material but with no bacteria or yeasts visible under high power, 4 were positive upon culture (100, 23, 21, and 15 colonies).

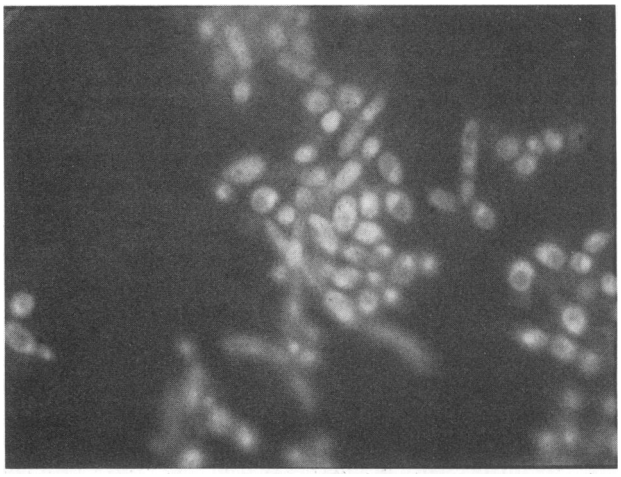

FIG. 1. Catheter colonized with *C. tropicalis* (magnification, ×1,000). Yeasts appear as big, oval blastospores with various degree of AO binding.

When the microorganisms could be visualized, their morphology correlated well with the results of the culture. Notably, yeasts could easily be distinguished from bacteria, and for the three catheters which were colonized by rods, the bacillary morphology was readily distinguished by the AO stain. Figure 1 illustrates the appearance of a positive AO-stained catheter.

Table 2 summarizes the correlation between the AO stain and the semiquantitative culture of the catheter tip for 710 intravascular catheters. The largest group of catheters (n = 668) was negative by both methods (<15 colonies on primary plate and negative AO stain). In particular, none of the 33 contaminated catheters (1 to 14 colonies on the agar plate) was positive on direct AO examination. The five culture-negative catheters with a false-positive AO stain were all long catheters, and two of them were from patients on antibiotics when the catheter was removed. Five of the six catheters with a false-negative AO stain were short catheters, and four of them had between 15 and 23 colonies on culture.

Overall, the AO stain was 84% sensitive. When restricted only to long catheters (n = 212), the AO stain sensitivity rose to 94% (16 of the 17 culture-positive catheters were positive on AO examination). The specificity of the AO stain was 99% (97% and 100% for long and short catheters, respectively). The predictive value of a positive AO stain for catheter tip colonization was 86%, and the predictive value of a negative AO stain was 99%. The general agreement with the semiquantitative culture was 98%.

TABLE 1. Organisms recovered on semiquantitative culture of 37 positive intravascular catheter tips[a]

Organism	No. of positive catheters
Coagulase-negative staphylococci	25
Staphylococcus aureus	5
Candida spp.	2
Micrococcus spp.	1
Viridans group streptococci	1
Corynebacterium spp.	1
Klebsiella pneumoniae	1
Klebsiella oxytoca + coagulase-negative staphylococci	1

[a] A positive result was defined as the presence of ≥15 colonies per agar plate.

TABLE 2. Correlation[a] between AO staining of catheter tips and semiquantitative cultures of 710 intravascular catheters

AO stain result	No. of culture results[b]	
	Positive (≥15 colonies)	Negative (<15 colonies)
Positive	31 (16 + 15)	5 (5 + 0)
Negative	6 (1 + 5)	668 (190 + 478)
Total	37 (17 + 20)	673 (190 + 483)

[a] Sensitivity, 84%; specificity, 99%.
[b] Numbers in parentheses denote the numbers of long and short catheters.

DISCUSSION

As a complement to the semiquantitative culture, Cooper and Hopkins (1) proposed a direct Gram stain of the intravascular catheter, having shown that a colonized catheter which had been rolled on an agar plate still had sufficient adherent bacteria to be visible by Gram stain. Their study was based on use of 330 catheters, of which only 2.4% were peripherally inserted short venous catheters. There were 41 culture-positive catheters (12.4%). Their results showed that the Gram stain was 100% sensitive and 99.6% specific for the detection of catheter tip colonization.

As an attempt to speed up and simplify the direct examination of intravascular catheters, an AO direct staining procedure of catheter tips was developed and evaluated by using 710 consecutive catheter tips with a distribution representative of the various catheter types used in a general hospital. The majority were peripherally inserted short catheters. At removal, all catheters were screened and cultured independently from clinical suspicion of infection. For long catheters, the sensitivity of the AO staining method rose to 94%. It is quite conceivable that short catheters were more difficult to examine owing to their smaller size and diameter. The specificity of the direct examination was excellent (99%). The general agreement with the semiquantitative culture was 98%.

Although direct examination of catheter tips cannot replace the culture, which is essential for the identification and the susceptibility test of the isolated bacteria, it will offer early recognition of intravascular catheter-associated infection. This should allow investigators to select a prompt and appropriate therapy, especially in view of the good correlation between the morphology of the microorganisms seen during AO screening and their final identification upon culture. In conclusion, AO direct examination of intravascular catheter tips appears to be a simple and promising method for the preliminary diagnosis of catheter-associated infections.

ACKNOWLEDGMENTS

We thank V. Bonifas for his assistance in preparing the photographs. We are also grateful to H. H. Siegrist for helpful comments.

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