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Survival of Aerobic and Anaerobic Bacteria in Purulent Clinical Specimens Maintained in the Copan Venturi Transystem and Becton Dickinson Port-a-Cul Transport Systems

DIANE M. CITRON,[1]* YUMI A. WARREN,[1] MARIE K. HUDSPETH,[1] AND ELLIE J. C. GOLDSTEIN[1,2]

R. M. Alden Research Laboratory, Santa Monica-UCLA Medical Center, Santa Monica,[1] and UCLA School of Medicine, Los Angeles,[2] California

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Recovery of aerobic and anaerobic bacteria from clinical specimens maintained in the Copan Venturi Transystem and the Becton Dickinson Port-a-Cul transport was assessed. Of 54 anaerobes, 53 were recovered after 4 h, and 52 were recovered after 24 h, from both systems. After 48 h, 45 and 50 were recovered from the two systems, respectively.

Protection of anaerobic bacteria from exposure to oxygen during the transport of clinical specimens to the laboratory is crucial for the survival of these organisms. Because the use of swabs may encourage collection of superficial specimens that represent colonizing bacteria instead of the etiologic agents found deeper in the infected tissues, aspirates have always been preferable to swab systems for anaerobic cultures. However, swabs are frequently used in many hospitals to collect many types of specimens, including surgical specimens, simply because of their convenience. We previously evaluated the Venturi Transystem (Copan Diagnostics, Corona, Calif.) for its ability to maintain the viability of five American Type Culture Collection and five clinical strains of anaerobic bacteria and found it to be comparable to the Port-a-Cul swab transport system (Becton Dickinson Microbiology Systems [BD], Cockeysville, Md.) (1). In the present study, we obtained excess pus collected at surgery from patients with suspected anaerobic infections and used it to compare the abilities of these two swab systems to maintain the viability of the organisms after 4, 24, and 48 h at room temperature. The transported samples were plated onto both selective and nonselective media for isolation of aerobic and anaerobic bacteria. After incubation, the quantity of growth of the different species was enumerated, the strains were identified, and their recovery was compared to their presence at the starting time.

Surplus specimens consisting of at least 2 ml of pus were collected from patients undergoing surgical procedures and injected by syringe into a Port-a-Cul vial (BD) or Anaerobic Transport System (Anaerobe Systems, Morgan Hill, Calif.) for transport to the research laboratory for the study protocol, usually within 10 h of collection. The specimens were screened by Gram stain for the presence of multiple types of organisms suggestive of anaerobes before entry into the study. The specimen sources were as follows, with numbers of samples in parentheses: peritoneal fluid (six), rectal abscess (two), pelvic abscess (one), and chest drainage (one).

The Copan Venturi Transystem (CVT) consists of a plastic transport tube containing Amies medium without charcoal and without an indicator. The plastic sheath is pinched above the semisolid medium to prevent spillage and to reduce the surface area for oxygen diffusion. The swab containing the specimen is placed into the agar. The BD Port-a-Cul system consists of a screw-cap glass tube with a deep column of reduced Cary-Blair semisolid agar medium with a resazurin indicator. The swab containing the specimen is placed deep into the agar.

Upon receipt in the laboratory, the transporter containing the pus was brought into the anaerobic chamber, and 0.1 ml was inoculated onto a swab. The swab was resuspended into 0.9 ml of brucella broth, and 80 μl was immediately inoculated onto supplemented brucella blood agar and onto supplemented phenylethyl alcohol blood agar. Forty microliters was inoculated onto each half of a biplate; half of the plate contained laked blood agar with kanamycin and vancomycin, and the other half contained *Bacteroides*-bile-esculin agar (Anaerobe Systems). The plates were incubated in the anaerobic chamber at 36 to 37°C for 5 days prior to inspection. The original pus specimen and the swab suspension were removed from the anaerobic chamber, and 80 μl of the suspension was inoculated onto plates of Trypticase soy agar-blood and Rose agar incubated in 5% CO_2 and MacConkey agar incubated in ambient air (Hardy Diagnostics, Santa Maria, Calif.) at 36 to 37°C for recovery of aerobic and facultative organisms. This represented the 0-h culture.

Working outside of the anaerobic chamber, we inoculated 0.1 ml of the pus onto three sets of swabs of the CVT and Port-a-Cul systems for subsequent culture after 4, 24, and 48 h of storage at room temperature. At the indicated times, the swabs were brought into the anaerobic chamber and suspended in 0.9 ml of brucella broth. Anaerobic and aerobic media were inoculated as described above.

After incubation, growth of the different colony types was scored as 1+ to 4+ according to the plate quadrant on which they grew, and the colonies were identified according to standard methods (4, 5). If the same organism grew on more than one type of medium, the medium with the greatest quantity of growth was used for calculating the growth score. The growth scores of all isolates within each species or group were averaged.

The percentage of the isolates recovered at the various times and their average quantities are presented in Table 1.

A total of 54 (range, 2 to 9) anaerobic and 34 (range, 1 to 5) aerobic bacteria were isolated from the 10 clinical specimens for an average of 8.8 isolates per specimen at 0 h. After 4 h, one isolate of *Clostridium subterminale* that was initially present at 1+ growth was not recovered from either system; however, the isolate was present in both systems again after

* Corresponding author. Mailing address: R. M. Alden Research Laboratory, 1250 16th St., Santa Monica, CA 90404. Phone: (310) 319-4325. Fax: (310) 319-0040. E-mail: dcitron-rmarl@juno.com.

TABLE 1. Survival of aerobic and anaerobic bacteria after 4, 24, and 48 h in two transport systems

Organism	0 h		4 h				24 h				48 h			
			CVT		Port-a-Cul		CVT		Port-a-Cul		CVT		Port-a-Cul	
	Total no. of strains	Avg quantity (range)[a]	% Survival	Avg quantity (range)	% Survival	Avg quantity (range)	% Survival	Avg quantity (range)	% Survival	Avg quantity (range)	% Survival	Avg quantity (range)	% Survival	Avg quantity (range)
Bacteroides fragilis	2	3.5 (3–4)	100	3 (3–3)	100	3 (3–3)	100	3 (3–3)	100	3 (3–3)	100	3.5 (3–4)	100	3.5 (3–4)
Bacteroides thetaiotaomicron	4	2.75 (1–4)	100	3.25 (3–4)	100	3.75 (3–4)	100	3 (2–4)	100	3.75 (3–4)	100	3.5 (3–4)	100	3.5 (2–4)
Other *B. fragilis* group organisms[b]	13	3.24 (1–4)	100	3.07 (2–4)	100	2.77 (2–4)	100	2.93 (2–4)	100	3.08 (2–4)	100	3.32 (2–4)	100	3.23 (2–4)
Prevotella spp.[c]	5	2.6 (1–3)	100	2.2 (1–4)	100	2.4 (2–3)	80	2.2 (0–4)	100	2.4 (1–3)	40	1 (0–4)	80	2.2 (0–4)
P. asaccharolytica	2	2 (1–3)	100	1 (1–1)	100	1 (1–1)	100	1.5 (1–2)	50	1.5 (0–3)	0	0 (0–0)	50	0.5 (0–1)
F. nucleatum	1	3	100	1	100	2	100	2	100	2	0	0	0	0
Bilophila wadsworthia	3	2.33 (2–3)	100	2.33 (2–3)	100	2.67 (2–3)	100	2.67 (2–3)	100	2.67 (2–3)	100	3 (2–4)	100	3 (3–3)
Peptostreptococcus spp.[d]	5	3 (2–4)	100	2.2 (2–4)	100	2.4 (2–3)	100	2.2 (1–3)	100	2.8 (1–4)	80	1.6 (0–3)	80	1.8 (0–3)
C. innocuum	7	2.43 (1–4)	100	2.71 (1–4)	100	2.57 (1–4)	85.7	2.43 (0–4)	100	3.14 (2–4)	85.7	2.43 (0–4)	100	2.86 (1–4)
Other *Clostridium* spp.[e]	7	2.14 (1–3)	85.7	1.57 (0–2)	85.7	2 (0–3)	100	2.14 (1–3)	100	2.42 (1–4)	85.7	2.14 (0–4)	100	2.42 (1–4)
Eubacterium spp.[f]	4	2.75 (2–4)	100	2.5 (2–4)	100	2.25 (2–3)	100	2 (1–3)	100	2.75 (1–4)	100	2.75 (1–4)	100	2.25 (1–3)
Lactobacillus catenaformis	1	3	100	3	100	3	100	3	100	3	100	3	100	3
E. coli	8	2.38 (1–4)	100	2.13 (1–4)	100	2.29 (1–4)	100	3.5 (2–4)	100	3.5 (3–4)	100	4 (4–4)	100	4 (4–4)
Other members of the *Enterobacteriaceae*[g]	6	2.83 (1–4)	83.3	2.5 (0–4)	83.3	2.33 (0–4)	66.7	2.67 (0–4)	83.3	2.67 (0–4)	50	2.67 (0–4)	83.3	2.83 (0–4)
P. aeruginosa	3	1.67 (1–3)	100	1.33 (1–2)	66.7	1 (0–2)	66.7	2.33 (0–4)	66.7	2.33 (0–4)	66.7	2.67 (0–4)	66.7	2.67 (0–4)
Comamonas acidovorans	1	1	100	1	100	1	100	3	100	3	100	4	100	3
Enterococcus spp.[h]	4	2.25 (2–3)	100	1.5 (1–2)	100	1.25 (1–2)	100	2.25 (1–4)	100	2.25 (1–4)	100	2.75 (1–4)	100	2.75 (1–4)
Streptococcus spp.[i]	8	2.25 (1–4)	87.5	2 (0–3)	75	2 (0–3)	87.5	2.63 (0–4)	75	2.63 (0–4)	75	2.38 (0–4)	75	2.13 (0–4)
Staphylococcus epidermidis	2	3 (2–4)	100	1.5 (1–2)	100	2 (2–2)	100	1.5 (1–2)	100	1.5 (1–2)	100	1.5 (1–2)	50	1.5 (0–3)
Corynebacterium spp.[j]	2	1 (1–1)	100	1 (1–1)	100	1.5 (1–2)	100	1 (1–1)	100	1 (1–1)	50	0.5 (0–1)	50	0.5 (0–1)

[a] Growth was scored as 1+ to 4+ according to the plate quadrant on which the isolate grew; the quantity of growth for all strains was averaged by genus, group, or species.
[b] *Bacteroides ovatus* (two strains), *Bacteroides uniformis* (five strains), *Bacteroides vulgatus* (two strains), *Bacteroides distasonis* (two strains), *Bacteroides splanchnicus* (one strain), and an unidentified species (one strain).
[c] *P. melaninogenica* (one strain), *Prevotella loescheii* (one strain), *Prevotella bivia* (two strains), and *P. intermedia* (one strain).
[d] *P. magnus* (three strains), *Peptostreptococcus anaerobius* (one strain), and *Peptostreptococcus micros* (one strain).
[e] *C. subterminale* (one strain), *Clostridium symbiosum* (one strain), *Clostridium clostridioforme* (two strains), *Clostridium difficile* (one strain), and unidentified species (two strains).
[f] *Eubacterium lentum* (two strains), *Eubacterium limosum* (one strain), and an unidentified species (one strain).
[g] *K. pneumoniae* (two strains), *Enterobacter cloacae* (one strain), *Proteus vulgaris* (one strain), *Proteus mirabilis* (one strain), and *C. koseri* (one strain).
[h] *Enterococcus faecium* (three strains) and *Enterococcus faecalis* (one strain).
[i] *Streptococcus constellatus* (four strains), *Streptococcus salivarius* (one strain), *Streptococcus* group C/G (one strain), *Gemella morbillorum* (one strain), and an *Aerococcus* sp. (one strain).
[j] *Corynebacterium amycolatum* (one strain) and *Corynebacterium xerosis* (one strain).

24 h. After 24 h, one strain each of *Clostridium innocuum* and *Prevotella melaninogenica* was not recovered from the CVT system, and one strain of *Porphyromonas asaccharolytica* was not recovered from the Port-a-Cul system. After 48 h, the CVT did not support the viability of both strains of *P. asaccharolytica* and one strain each of *Prevotella intermedia*, *Prevotella bivia*, *Fusobacterium nucleatum*, and *Peptostreptococcus magnus*. The *C. subterminale* isolate that was present on the 24-h culture was again not recovered. The Port-a-Cul after 48 h did not support the viability of *P. intermedia*, *F. nucleatum*, or *P. magnus*.

Some of the aerobic organisms, especially *Escherichia coli*, appeared to grow in both systems. One strain of *Citrobacter koseri* was overgrown by *E. coli* and *Klebsiella pneumoniae* in both systems and was not recovered after 24 and 48 h. Growth of the enteric bacteria may have been enhanced by the presence of small amounts of oxygen introduced into the transport systems during the setup process. One strain of *Pseudomonas aeruginosa* that was initially present at 1+ growth was lost in both systems. One strain of a viridans group streptococcus also did not survive after 4 h in the Port-a-Cul system, and another strain did not survive after 24 h in either system.

Overall, at 24 h, 52 of 54 anaerobes and 30 of 34 aerobes were recovered from the CVT transporter, while the Port-a-Cul grew 53 of 54 anaerobes and 30 of 34 aerobes. After 48 h, 45 of 54 anaerobes and 27 of 34 aerobes were recovered from the CVT transporter, while 50 of 54 anaerobes and 27 of 34 aerobes were recovered from the Port-a-Cul. The decreased recovery of anaerobes may have been due to oxygen seepage into the plastic CVT transporters.

After 24 h, the two systems were comparable in their abilities to maintain the viability of most of the organisms. After 48 h, some of the more fastidious strains were not recovered from either system, especially those strains that were initially present in small numbers. Some of the facultative organisms with large colonies increased in number after 24 and 48 h and overgrew some of the strains with smaller, slower-growing colonies. This occurred mostly on the blood agar plates, and the selective agars were necessary to isolate some of the strains.

Both systems are adequate for transport of clinical specimens if the time to reach the clinical laboratory is within 24 h.

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