

Survival of Fastidious and Nonfastidious Aerobic Bacteria in Three Bacterial Transport Swab Systems[∇]

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In the present study, we followed the CLSI procedure M40-A to evaluate three specimen transport systems [the new BD CultureSwab MaxV(+), the new Remel BactiSwab, and the Medical Wire & Equipment Transwab] for the survival of fastidious and nonfastidious organisms for 0, 6, 24, and 48 h at room temperature. BD CultureSwab MaxV(+) outperformed the other two swabs for the recovery of the three fastidious organisms, *Haemophilus influenzae*, *Neisseria gonorrhoeae*, and *Neisseria meningitidis* for up to 48 h. Indeed, BD CultureSwab MaxV(+) maintained a constant number of viable *H. influenzae* and *N. meningitidis* for up to 48 h, and only a 2 log reduction was noted for *N. gonorrhoeae*, fulfilling the requirements of M40-A guidelines. However, unlike Remel BactiSwab and the Medical Wire & Equipment Transwab, which fulfilled the M40-A requirements for maintaining the viability of *Streptococcus pneumoniae*, BD CultureSwab MaxV(+) could not maintain the viability of *S. pneumoniae* reference or clinical strains past 6 h. Excellent overall sensitivity (98%) (95% confidence interval, 89.5 to 99.7) was observed when the BD CultureSwab MaxV(+) rectal swabs were compared to the “gold standard” stool cultures. Thus, the BD CultureSwab MaxV(+) rectal swab can be used when investigating gastrointestinal bacterial outbreaks or when health care providers face difficulties in obtaining stool samples, particularly from children.

One of the crucial steps for accurate laboratory diagnosis of bacterial infections is adequate specimen collection and transport to the laboratory. Aspirates of fluids and exudates from suspected infected sites are superior to samples collected on swabs (2, 13). However, because of the ease of using swabs, clinical microbiology laboratories continue to receive patient samples in swab transport systems (3, 12).

Swab transport systems with semigel-stabilizing ingredients are effective methods for specimen collection and transport in the event that aspirates cannot be collected. Moreover, these swabs have been shown to protect and maintain the viability of both fastidious aerobic and anaerobic organisms (1, 5, 7). Swab transport systems are easy to use and cost-effective and can maintain the viability of a number of bacterial pathogens. The new BD CultureSwab MaxV(+) has the advantage of being flushed with nitrogen gas to stabilize low oxidation reduction (E_h) potential throughout its shelf life and to prevent medium oxidation. Moreover, vegetable protein and amino acids have been included on the swab fiber to maintain the viability of the bacteria (8).

The recent availability of CLSI procedures M40-A (quality control of microbiological transport systems) for evaluating swab systems has tremendously helped in standardizing the methods of evaluating the newly manufactured swab systems (4).

The aim of this study was to evaluate the performance of the new Copan M40 Transystem Amies without charcoal [now introduced as Becton Dickinson's product, BD CultureSwab

MaxV(+)] with the new version of Starplex StarSwab II (now introduced as Remel BactiSwab modified Amies Clear; Remel, Eobicoke, Ontario, Canada) and the Medical Wire & Equipment Transwab in maintaining the viabilities of fastidious and nonfastidious aerobic bacteria. In addition, rectal swabs using the new BD CultureSwab MaxV(+) were compared with stool cultures for the recovery of gastrointestinal bacterial pathogens. Maintaining the swabs at 4°C instead of at room temperature has been reported to be superior for recovering viable bacterial pathogens (14). However; because such conditions will most likely not be followed on a day to day basis, we decided to challenge the swab systems at room temperature.

MATERIALS AND METHODS

Swab transport systems. Three swab transport systems were evaluated according to the CLSI M40-A standard procedures. The bacterial transport systems evaluated were the new Copan M40 Transystem Amies without charcoal (C-M40; Copan Innovation, Inc., Corona, CA) [now introduced as Becton Dickinson's product, BD CultureSwab MaxV(+)], the new version of Starplex StarSwab II manufactured under the Remel BactiSwab modified Amies Clear (R-BS; Remel, Eobicoke, Ontario, Canada), and the Medical Wire & Equipment Transwab (MWE-TS; Medical Wire & Equipment Co. Ltd., England).

Bacterial strains. Ten ATCC bacterial strains and three bacterial clinical isolates were used in the study. The ATCC strains were *Streptococcus pneumoniae* (ATCC 49619), *Haemophilus influenzae* (ATCC 49766), *Neisseria gonorrhoeae* (ATCC 49226), *Streptococcus pyogenes* (ATCC 19615), *Pseudomonas aeruginosa* (ATCC 27853), *Moraxella catarrhalis* (ATCC 25239), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Listeria monocytogenes* (ATCC 19115), and *Neisseria meningitidis* (ATCC 13090). The clinical isolates evaluated were *Streptococcus agalactiae* and two *Streptococcus pneumoniae* isolates.

Bacterial culturing. Well-isolated colonies from each of the bacterial strains were streaked for isolation on 5% sheep blood agar (Hy laboratories Ltd. Rohovot, Israel), with the exception to *H. influenzae* and *N. gonorrhoeae*, which were streaked on chocolate agar (Hy laboratories Ltd. Rohovot, Israel). The

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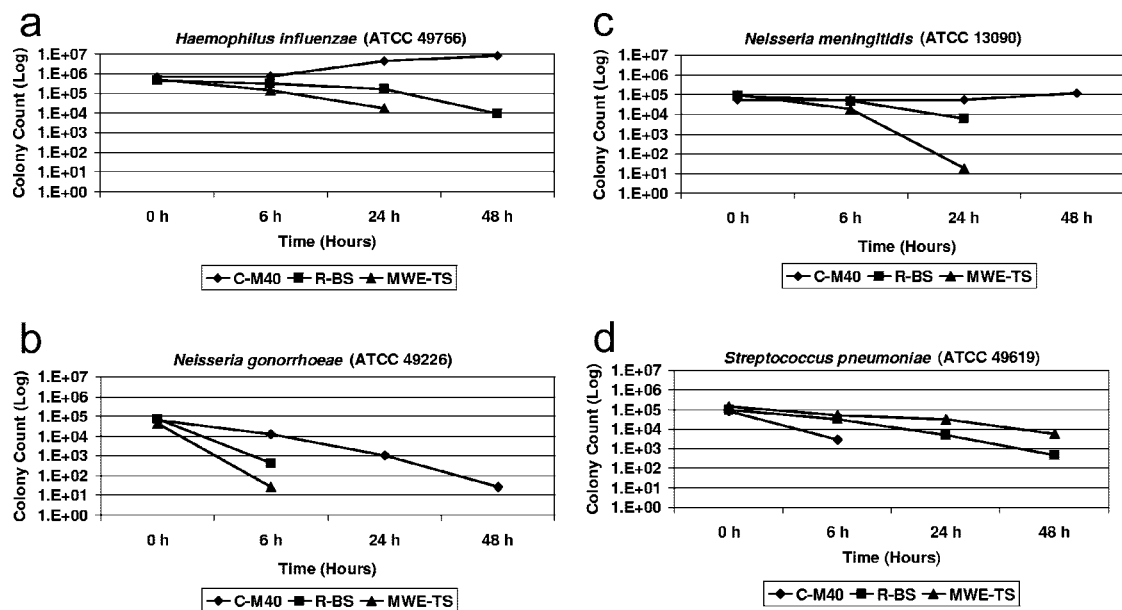


FIG. 1. Survival (log recovery) of fastidious bacteria in three different transport systems (C-M40, R-BS, and MWE-TS) for 0, 6, 24, and 48 h at room temperature.

plates were incubated for 18 to 24 h in a 5% CO₂ incubator at 37°C. Fresh, well-isolated colonies were utilized for evaluating the three swab systems.

Swab evaluation protocol (i) Inoculum preparation. The CLSI M40-A (quantitative swab elution method) was followed to evaluate the three transport swabs. Briefly, the swab elution method, which allows for quantitative measurement of the ability of a transport system to maintain viable organism, was utilized. Inocula of the isolates were prepared in 0.85% sterile physiological saline (pH 6.8 to 7.2) to a concentration of approximately 1.5×10^8 CFU/ml (equivalent to a 0.5 McFarland standard) from an 18- to 24-h plate culture of each organism. Turbidities were checked with a nephelometer (Biomeurieux, France). Each of the organism's 0.5 McFarland standard was diluted 10-fold in sterile 0.85% physiological saline solution to provide a concentration of approximately 1.5×10^7 CFU/ml. The inoculum was prepared just prior to transfer of the organism suspension to the swab system to be evaluated. The whole procedure did not exceed 20 min, to prevent the loss of the organism's viability in the inoculum prior to incubation of inoculated swabs.

(ii) Inoculation procedure. In triplicate, 100 μ l (10^6 organisms) was used to inoculate each of the swabs evaluated. The survival of the organisms on each of the swabs at room temperature was determined at 0, 6, 24, and 48 h.

(iii) Recovery of viable organisms. At each of these time points, viable organisms on the swabs were recovered in 1 ml of sterile physiological saline after being vortexed for 30 s. This was followed by serially diluting the viable organisms 1:10, 1:100, and 1:1,000 in sterile saline. Depending on the organism evaluated, the recovery at time point 0 was between 10^5 to 10^6 organisms.

(iv) Quantitation of viable organisms. In triplicate, 100- μ l samples were used to quantify the organisms for each of the dilutions on 5% sheep blood agar or chocolate agar when required. The organisms were spread over the agar surface with a plate spreader, and the plates were incubated at 37°C in the appropriate incubator. Bacterial recovery was determined by counting the colonies recovered in each of the dilutions. The number of organisms recovered was expressed as an average for triplicate samples evaluated.

Stool and rectal culture bacterial cultures. Paired rectal swabs and stool samples were obtained from 198 consecutive children 1 week to 10 years of age presenting with gastroenteritis at Caritas Baby Hospital between August 2004 and October 2004. The samples were collected by well-trained health care providers and transported to the laboratory within 1 h of sample collection. Rectal swabs were obtained within 10 min after obtaining the stool specimen by inserting the swab just beyond the rectal sphincter and rotating the swab, and feces should be present on the swab upon removal. Both specimen types were inoculated on MacConkey agar, XLD agar, Preston Campylobacter selective agar plate, and 5% blood agar (all prepared in-house by Oxoid Ltd., England) and in selenite broth (BioLife, Milano, Italy). Inoculated plates were incubated at 35°C for 24 h, while the Preston agar was incubated at 42°C in 5% O₂, 10% CO₂, and

85% N₂ conditions for 48 h. A subculture at day 2 from the selenite broth was performed on all samples. Biochemical analyses were performed on all suspected colonies. Conformation and typing of *Salmonella* isolates were performed with *Salmonella* O group antisera (A, B, C, D, and E) using the slide method according to the manufacturer's guidelines (Israel National *Salmonella* Center). *Shigella* conformation and typing were performed with antisera against *Shigella sonnei*, *Shigella flexneri*, *Shigella boydii*, and *Shigella dysenteriae* using the slide method according to the manufacturer's guidelines (Denka Seiken Co. Ltd., Japan).

RESULTS

Survival of fastidious bacteria. Survival of *H. influenzae*, *N. gonorrhoeae*, *N. meningitidis*, and *S. pneumoniae* is shown in Fig. 1. C-M40 outperformed the other two swabs evaluated, R-BS and MEW-TS, for the recovery of *H. influenzae*, *N. gonorrhoeae*, and *N. meningitidis*. *H. influenzae* was recovered for up to 48 h of incubation at room temperature in C-M40. The number of viable organisms remained stable for 6 h, followed by a gradual increase at 24 h (0.5 log) and 48 h (1 log) (Fig. 1a). Even though R-BS maintained the viability of *H. influenzae* for up to 48 h, the number of viable organisms started to decrease after 6 h, and by 48 h, a 1.5 log reduction in the number of viable organisms was noted (Fig. 1a). MWE-TS could not maintain the viability of *H. influenzae*, since rapid reduction (1 log) in the number of viable organisms was noted after 6 h (Fig. 1a). By 24 h, a 1.5 log reduction in the number of viable organisms was detected and no viable organisms were recovered at 48 h from MWE-TS.

N. gonorrhoeae viability was maintained in C-M40 for 48 h, a longer evaluation period than the 24 h recommended by M40-A. A 1 log reduction in the number of viable organisms was noted at each of the time points evaluated (Fig. 1b). By 48 h, a 3.5 log reduction in the number of viable organisms was noted. On the other hand, the R-BS and the MWE-TS could not maintain the viability of *N. gonorrhoeae* for more than 6 h (Fig. 1b). Indeed, there were 2 and 3 log reductions in the

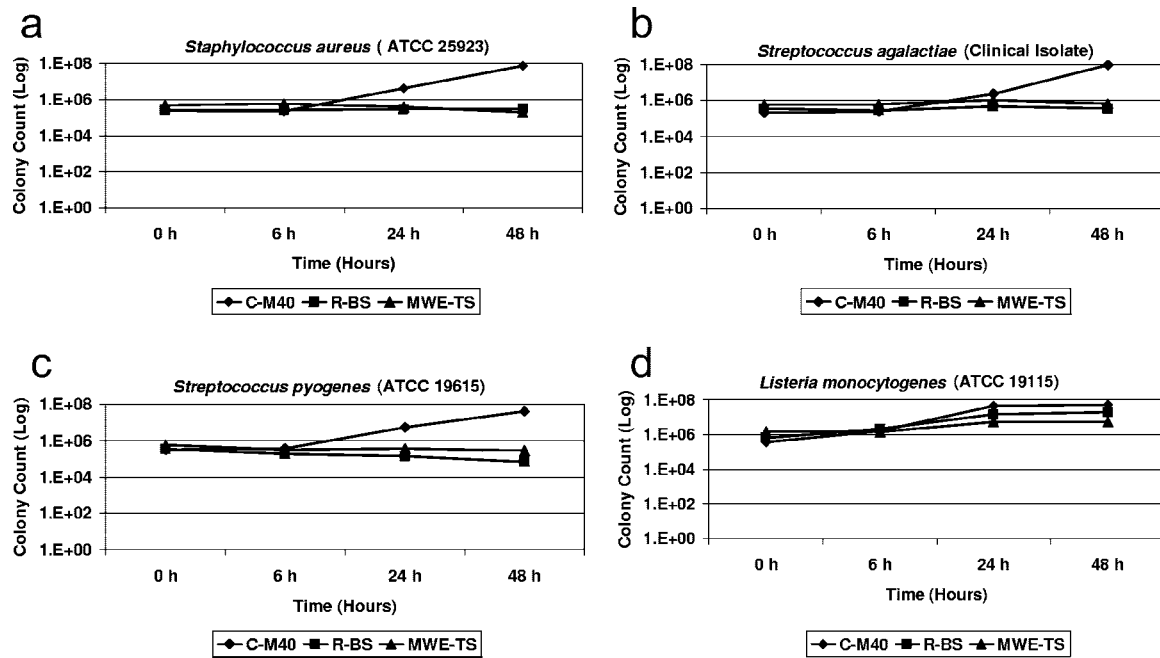


FIG. 2. Survival (log recovery) of nonfastidious gram-positive bacteria in three different transport systems (C-M40, R-BS, and MWE-TS) for 0, 6, 24, and 48 h at room temperature.

number of viable organisms at 6 h in R-BS and MWE-TS, respectively.

N. meningitidis viability was maintained for up to 48 h in C-M40 with no loss of viable organisms, a longer evaluation period than the 24 h recommended by M40-A. (Fig. 1c). Unlike C-M40, the two other swab systems, R-BS and MWE-TS, could not maintain the viability of *N. meningitidis* beyond 24 h, the period recommended by M40-A. By 24 h, 1 and 3.5 log reductions in the number of viable *N. meningitidis* were noted in R-BS and MWE-TS, respectively.

While C-M40 outperformed the other two swabs in maintaining the viability of *H. influenzae*, *N. gonorrhoeae*, and *N. meningitidis*, C-M40 could not maintain the viability of *S. pneumoniae* past 6 h. Similar results were obtained after evaluating two *S. pneumoniae* clinical isolates in C-M40 (data not shown). R-BS and MWE-TS maintained the viability of *S. pneumoniae* for 48 h with 1 and 2 log reductions in the number of viable organisms, respectively (Fig. 1d).

Overall, C-M40 satisfied the requirements of the M40-A recommendation of no more than a 3 log drop in 24 or 48 h for the three fastidious organisms, *H. influenzae*, *N. gonorrhoeae*, and *N. meningitidis*, while it failed to pass the recommendations for maintaining the viability of *S. pneumoniae*. On the other hand, R-BS satisfied the requirements of the M40-A recommendation for the three organisms *H. influenzae*, *N. meningitidis* and *S. pneumoniae*. MWE-TS satisfied the requirements of M40-A recommendation for *S. pneumoniae* only.

Survival of nonfastidious gram-positive bacteria. The survival of *S. aureus*, *S. agalactiae*, *S. pyogenes*, and *L. monocytogenes* in the three transport systems is shown in Fig. 2. A minimal increase in the number of viable *S. aureus*, *S. agalactiae*, and *S. pyogenes* was observed in the R-BS and MWE-TS transport systems (Fig. 2a, b, c). On the other hand, there was a 2 log increase

in the number of viable organisms in the C-M40 system after 48 h (Fig. 2a, b, c). All three swabs maintained the viability of *L. monocytogenes* at all time points evaluated.

Survival of nonfastidious gram-negative bacteria. The survival of *P. aeruginosa*, *E. coli*, and *M. catarrhalis* in the three transport systems is shown in Fig. 3. All three swabs maintained a stable number of viable organisms after 6 h of incubation at room temperature (Fig. 3a, b, c). A rapid increase in the number of viable organisms (2 logs) was noted for *P. aeruginosa* and *E. coli* after 24 h in all three swabs, and the number was sustained at 48 h (Fig. 3a, b). All three swabs maintained the number of viable *M. catarrhalis* isolates for 24 h (Fig. 3c). This was followed by one- and twofold increases in the number of viable organisms at 48 h in C-M40 and R-BS, respectively. In contrast, a small reduction in the number of viable *M. catarrhalis* isolates was noted in MWE-TS (Fig. 3c).

Recovery of gastrointestinal stool pathogens from C-M40 rectal swabs. Of the 198 paired stools samples and rectal swabs evaluated for gastrointestinal bacterial pathogens, 51 samples (26%) were positive for one of the common bacterial stool pathogens, *Salmonella*, *Shigella*, or *Campylobacter* species. Indeed, of the 51 positive samples, 49 were positive in both the stool culture and the rectal swabs, while 2 samples were positive in either the stool specimen or the rectal swab. Thus, the overall sensitivity of the rectal swabs was 98% (95% confidence interval [CI], 89.5 to 99.7%) (Table 1). Stratifying the bacterial pathogens by genus, all 29 *Campylobacter* species isolated from stool cultures were also isolated from rectal swabs. Thus, the sensitivity of the rectal swabs for detecting *Campylobacter* species was 100% (95% CI, 88.3 to 100%) (Table 1). Similarly, all 15 *Shigella* species isolated from stool cultures were also isolated from rectal swabs, giving a sensitivity of 100% (95% CI, 79.6 to 100%) (Table 1). Six patient samples contained *Salmo-*

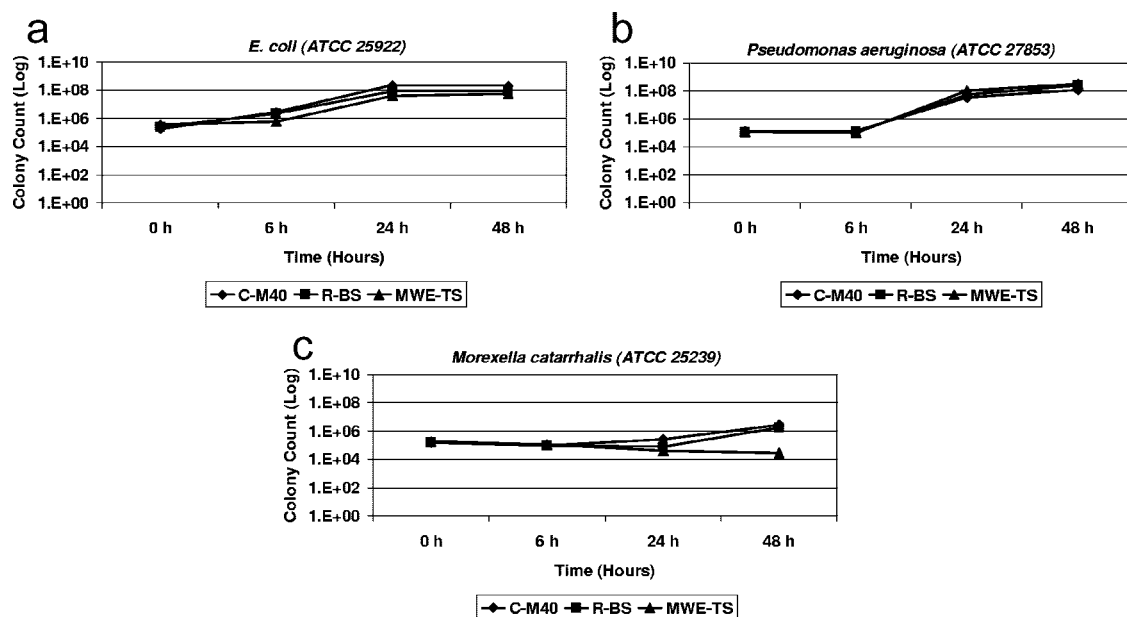


FIG. 3. Survival (log recovery) of nonfastidious gram-negative bacteria in three different transport systems (C-M40, R-BS, and MWE-TS) for 0, 6, 24, and 48 h at room temperature.

nella species. Four were detected in the paired stool samples and rectal swab cultures. For the two discordant pairs, *Salmonella* was detected in the stool culture from one and from the rectal swab culture from the other. For both of these latter samples, the *Salmonella* species in both samples were detected only after subculturing the selenite broth on XLD agar, thus suggesting low numbers of *Salmonella* organisms in the stool sample.

DISCUSSION

Swab systems are not the best way to collect patient samples for either aerobic or anaerobic specimens (7, 13). However, when swabs are the only choice, C-M40 appears to be an acceptable choice for the maintenance of viable fastidious and nonfastidious organisms for up to 48 h. C-M40 outperformed the other swabs for maintaining the viability of the majority of the fastidious and all of the nonfastidious bacteria. Indeed, all the organisms tested in our study were detected for up to 48 h postincubation at room temperature, satisfying or exceeding the requirements established by the M40-A guidelines, with the exception of the *S. pneumoniae* ATCC 49619 strain and the

two *S. pneumoniae* clinical isolates. *S. pneumoniae* survived for only 6 h in C-M40 compared to 48 h in both R-BS and MWE-TS. Unlike the observations made in our study, Morosini et al. noted survival of *S. pneumoniae* ATCC 6305 for up to 48 h (12). Indeed, Morosini et al. noted an increase in the number of viable *S. pneumoniae* at 6 h, followed by gradual decline in the number of viable organisms. We cannot explain why there was such dramatic difference between these two studies. We ruled out mishandling *S. pneumoniae*-inoculated swabs as a cause for the loss of viable organisms by monitoring the room temperature every 6 h during the evaluation period.

The survival of the fastidious organisms *H. influenzae*, *N. meningitidis*, and *N. gonorrhoeae* in C-M40 was similar to what has been reported by other investigators (8, 12). Melhus and Tano noted similar observations with regard to the survival of the two fastidious organisms *H. influenzae* and *N. gonorrhoeae* in C-M40 systems; however, the authors did not evaluate the survival of *S. pneumoniae* in their study (11). Gandhi and Mazzulli reported similar survival rates of fastidious organisms (*H. influenzae* and *N. gonorrhoeae*) for up to 24 h (6). However, unlike what we have repeatedly noted, Gandhi and Mazzulli reported a sudden reduction in the number or viable organ-

TABLE 1. Comparison between the “gold standard” stool culture and C-M40 rectal swabs for recovery of bacterial stool pathogens

Rectal swab result	No. of samples with stool culture result for:							
	Overall ^a		<i>Shigella</i> ^b		<i>Campylobacter</i> ^c		<i>Salmonella</i> ^d	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Positive	49	1	15	0	29	0	4	1
Negative	1	147	0	183	0	169	1	192

^a Sensitivity, 98.0%; 95% CI, 89.5 to 99.7%.
^b Sensitivity, 100%; 95% CI, 79.6 to 100%.
^c Sensitivity, 100%; 95% CI, 88.3 to 100%.
^d Sensitivity, 80%; 95% CI, 37.6 to 96.4%.

isms at 48 h of incubation (6). Similar results regarding the survival of nonfastidious organisms, *S. aureus*, *S. pyogenes*, and *P. aeruginosa* have been reported by other investigators (12).

Depending upon which specimen types are cultured, bacterial overgrowth in swab systems is of concern, since misinterpretation of bacterial culture results can occur. The CLSI procedure M40-A recommends the performance of overgrowth studies at 4°C (4). We chose to simulate the actual practice in our environment, which is specimen transport at ambient temperatures. Under those conditions, we noted continuous growth of *H. influenzae* in C-M40 up to 48 h. This could be beneficial, since such a phenomenon will guarantee the recovery of such fastidious organisms from some patient specimen types (e.g., eye, throat, or ear).

Continuous growth of gram-positive organisms in all swab systems was alarming, since they can overgrow some of the fastidious organisms, such as *S. pneumoniae* and *N. gonorrhoeae*, such as may occur when the infected site is contaminated with normal flora or when two pathogens are the causative agents of the infection. In C-M40, continuous growth of all gram-positive pathogens evaluated was noted at 24 and 48 h (Fig. 2), while the majority of the pathogens maintained stable numbers of organisms in R-BS and MWE-TS. The only pathogen that continued to grow in all three swabs was *L. monocytogenes*. This is acceptable in all cases, since it is not normal flora and its presence should be reported to the physicians. Other investigators noted similar continuous growth of *S. aureus* and *S. pyogenes* in C-M40, while constant numbers of viable organisms were maintained in Starplex StarSwab II and other swab types (8, 12). The stability and continuous growth of *S. agalactiae* in C-M40 could be instrumental in allowing the detection of this pathogen in vaginal swabs from pregnant women. Similar to the gram-positive organisms evaluated, the nonfastidious gram-negative organisms evaluated continued to grow in all three swabs, with the exception of *M. catarrhalis*, which maintained a constant number of viable organisms in MWE-TS. Similar observations were noted for the survival of other *K. pneumoniae* and *P. aeruginosa* isolates (12).

Overall, all three swabs evaluated appear to have similar bacterial release at time point zero (Fig. 1, 2, 3). However, C-M40 was noted to have a better physical design that kept the semigel in the bottom of the swab container compared to that of R-BS and MWE-TS. The addition of specific vegetable protein and amino acids in the C-M40 formula could have played a role in the continuous growth of the bacteria evaluated, allowing it to outperform the other swab systems evaluated for the recovery of fastidious and nonfastidious bacterial strains. However, extensive evaluation of the effects of these ingredients on the survival of *S. pneumoniae* should be performed to determine if it played a role in reducing the number of viable organisms (log reduction accounts for loss of 90% of viable organisms) as we noted in our study. Inhibitory substances in R-BS and MWE-TS swab materials and the refinishing chemicals used for bleaching the rayon fibers could have also played a role in allowing C-M40 to outperform these two swabs (15). Our results from this study were consistent with what the C-M40 manufacturer claims, with the exception of *S. pneumoniae*, which the manufacturer claims to survive for at least 24 h.

Because of the problems that health care providers can face when obtaining stool specimens, particularly from children

with gastroenteritis, we decided to compare the recovery of bacterial pathogens from rectal swabs and stool samples. Since C-M40 outperformed the other two swabs in the recovery of the majority of the bacterial strains evaluated, we decided to use it for obtaining rectal swabs. Rectal swabs should only be used when health care providers have a difficult time collecting patient specimens and, in particular, before administration of antibiotics (9). Stool cultures are generally considered the “gold standard” for isolation and identification of gastrointestinal pathogens, since rectal swabs are generally considered less sensitive (10). Ours is the first study to evaluate the effectiveness of the newly manufactured C-M40 for collecting stool samples. The sensitivity of the rectal swabs in comparison to the gold standard was 98% (95% CI, 89.5 to 99.7%). C-M40 rectal swabs had 100% sensitivity for the recovery of *Shigella* and *Campylobacter* species compared to stool cultures. Kaplan et al. reported similar results with Transwab (Medical Wire and Equipment Company, Cleveland, OH) when using rectal swabs for the recovery of *Campylobacter jejuni* (9). On the other hand, due to the low *Salmonella* shedding in some of the patients evaluated, the sensitivity of C-M40 for the detection of *Salmonella* was 80% (95% CI, 37.6 to 96.4%) (Table 1). Kotton et al. recently reported 64% sensitivity and 80% specificity for the recovery of *Salmonella* from rectal swabs (10). Overall, rectal swab cultures using C-M40 appear to be as sensitive as stool culture for recovery of these three pathogens. From these results, C-M40 swab systems can be used as rectal swabs when regular stool samples cannot be collected, in particular when looking for *Campylobacter* or *Shigella* species or for investigations of large outbreaks of bacterial gastroenteritis.

The data discussed in this report emphasize the importance of processing specimens collected in swab systems as soon as possible or with a maximum delay period of up to 24 h to reduce fastidious organism death (i.e., *S. pneumoniae*, *H. influenzae*, *N. gonorrhoeae*, and *N. meningitidis*) or nonfastidious organism overgrowth (i.e., *E. coli* and *P. aeruginosa*). Of the swabs evaluated, the C-M40 outperformed the other swab systems in maintaining the viability of three of the four fastidious organisms evaluated for up to 48 h. Moreover, C-M40 rectal swabs gave excellent sensitivity compared to stool cultures for recovery of *Salmonella*, *Shigella*, and *Campylobacter* from children with gastroenteritis.

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