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Comparison of Selective and Nonselective Media for Recovery of *Campylobacter pylori* from Antral Biopsies

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Antral biopsy specimens from patients with acid peptic disease were cultured in parallel on Skirrow medium (SM) and sheep blood agar (SBA) for *Campylobacter pylori*. In institution 1, 23 of 88 (26.1%) endoscopies were positive on SBA compared with 37 of 88 (42.0%) on SM ($P = 0.0001$). In institution 2, 29 of 49 (59.2%) endoscopies were positive for *C. pylori* on SBA and 27 of 49 (55.1%) were positive on SM ($P = 0.50$). The addition of SM optimizes the recovery of *C. pylori* from antral biopsy specimens.

Recently the original nomenclature of *Campylobacter pyloridis* was recognized to be a linguistic error and has now been revised to *Campylobacter pylori* (7).

After initial reports by Marshall et al. (6, 8, 9), several investigators have cultured *C. pylori* from the stomachs of patients with acid peptic disease (1, 2, 4). The pathogenic significance of this organism in gastritis remains to be established.

C. pylori has been cultured from antral biopsy specimens on selective (3, 8) and nonselective (4, 9) media, but the optimal method of recovery remains to be established. An important biochemical feature of this species is a rapid urease reaction, and this reaction has been exploited for making a rapid presumptive diagnosis of acid peptic disease by inoculating biopsy material into a urease medium (2, 9).

In this study, we compared the isolation of *C. pylori* from nonselective 5% sheep blood agar (SBA) with that from selective Skirrow medium (SM). The strains of *C. pylori* were isolated from patients attending the endoscopy suites of two hospitals in Toronto, Canada, who were referred for evaluation of upper gastrointestinal symptoms. Identical protocols for collection, transport, culture, and identification of *C. pylori* were followed at the two institutions.

Statistical analysis was performed by comparing paired proportions in the sign test (12).

In a pilot study to assess the efficacy of a nonselective 5% SBA medium in the recovery of *C. pylori*, 39 patients underwent 41 endoscopies at the two institutions, with biopsy specimens being taken from the gastric antrum. These specimens were placed in 5 ml of normal saline and brought to the microbiology laboratory within 0.5 h. The biopsy specimens were finely minced with sterile scalpel blades and inoculated onto 5% SBA. After incubation at 37°C in a microaerobic atmosphere (Campy Pak; Oxoid Ltd., Basingstoke, England) with examination after 5 days, the bacteria were identified as *C. pylori* according to published criteria (2, 9). No quantitative cultures were performed with the clinical specimens. Of the 41 biopsy cultures, 11 (26.8%) were positive for *C. pylori*, and an additional 9 were urease test positive but negative for *C. pylori* on culture. Five of these nine biopsy cultures were overgrown by *Proteus* sp., which may have accounted for the positive urease test; however, it was also possible that the overgrowth by *Proteus* sp. had masked *C. pylori*. Of great interest was the fact that four of the nine biopsy cultures (urease positive, *C. pylori* culture negative) were overgrown by bacteria that do not produce a rapid positive urease reaction. These organisms included *Pseudomonas aeruginosa*, *Streptococcus* sp., and *Citrobacter freundii*. Other authors have noted similar problems with bacterial overgrowth on cultures of gastric biopsy specimens (5, 10). This observation suggested that the *C. pylori* present were missed on culture because of overgrowth by other organisms. Indeed, the nine biopsy cultures which were urease positive and culture negative when stained by Dieterle stain revealed organisms morphologically consistent with *C. pylori*.

In the next phase of the study, we compared the efficacy of recovery of *C. pylori* with 5% SBA and SM. The ability of SM to support the growth of small inocula of *C. pylori* was tested by examining viable counts (11) of suspensions of three strains (A, B, and C) of *C. pylori* on SM and on nonselective SBA. Suspensions of each strain were made from 5-day-old SBA cultures in phosphate-buffered saline (0.01 M, pH 7.4) to a turbidity equivalent to a McFarland no. 2 standard. The viable counts on both media were similar, indicating that SM was able to support small inocula of this organism. The viable counts of each suspension on SBA and SM, respectively, were 1.2×10^7 and 1.5×10^7 CFU/ml (strain A), 2×10^7 and 1.6×10^7 CFU/ml (strain B), and 4×10^6 and 4×10^6 CFU/ml (strain C).

In addition, the efficacy of disinfection of the endoscopes after endoscopy was assessed at both institutions by random cultures of the endoscopes for bacteria including *C. pylori*; these cultures were consistently negative.

In institution 1 (Table 1), 23 of 88 endoscopies (26.1%) were positive for *C. pylori* on 5% SBA, and 37 of 88 endoscopies (42.0%) were positive on SM, a difference in recovery rates which is statistically significant ($P = 0.0001$, sign test). Therefore, SM detected all strains that were found

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TABLE 1. Culture results of nonselective 5% SBA compared with selective SM

Culture result[a] with:		Total no. of endoscopies in[b]:	
SBA	SM	Institution 1	Institution 2
+	+	23	27
+	−	0	2
−	+	14	0
−	−	51	20

[a] Growth (+) or no growth (−) of *C. pylori*.
[b] Total number of patients in institution 1 was 69. Total number of patients in institution 2 was 42.

on SBA (23 strains) and then some (14 more strains), for a total of 37 strains detected on SM.

In institution 2 (Table 1), 29 of 49 endoscopies (59.1%) were positive for *C. pylori* on 5% SBA, and 27 of 49 endoscopies (55.1%) were positive on SM, a difference in recovery rates which is not statistically significant ($P = 0.50$, sign test).

The reasons for the apparent difference in the performance of the selective SM at the two institutions are unclear; however, the fact that there was a significant improvement in the isolation rate of *C. pylori* at institution 1 suggests that the use of selective and nonselective media in parallel is superior to the use of one medium alone.

The fact that the viable counts of the three strains were similar on both media indicates that the selective SM was not inhibitory, compared with the nonselective SBA. However, the viable counts of the strains were lower on both media than expected for a suspension with a turbidity equal to a McFarland no. 2 standard. The reasons for these low counts were not explored, but the difference in counts could have been related to the loss of viable organisms in the 5-day-old cultures that were used to prepare the suspension.

SBA and SM plates were examined after a 5-day incubation; therefore, no difference in the rate of growth could be evaluated. The subjective impression was that the colony was slightly larger on SM than on SBA, but no precise measurements were made. In addition, the colonies of *C. pylori* were easier to detect on SM than on SBA because of fewer competing flora.

The choice of SM as a selective medium was based on convenience and ready availability in all clinical microbiology laboratories, thereby obviating the formulation of additional antibiotic combinations.

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