

Routine Culture of Stool Specimens for *Yersinia enterocolitica* Is Not a Cost-Effective Procedure

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Cefsulodin-Irgasin-novobiocin (CIN) agar was used to isolate *Yersinia enterocolitica* from 3,622 stool specimens received in our laboratory during a 1-year period. Seven specimens (0.2%) yielded *Y. enterocolitica* strains from a total of five patients. The low frequency of *Y. enterocolitica* isolation observed, coupled with the isolation of this pathogen from three of the five patients by our standard stool examination protocol, leads us to conclude that routine culture of stool specimens on CIN agar is not a cost-effective procedure.

Although *Yersinia enterocolitica* is well known as an agent of gastroenteritis, the magnitude of its importance as a cause of diarrhea is open to debate. Although some authors (7, 12) have found the incidence of *Y. enterocolitica* to be equal or nearly equal to that of other common stool pathogens, others (8) have concluded that the extremely low incidence of *Y. enterocolitica* noted by them makes routine examination of stool specimens for this pathogen unjustifiable. Weissfeld (A. S. Weissfeld, Clin. Microbiol. Newsl. 3:91-93, 1981) summarized reports of the incidence of *Y. enterocolitica* and commented that the apparent geographical variation of *Y. enterocolitica* isolation rates, along with the use of inadequate detection methods, could explain the reported discrepancies in *Y. enterocolitica* incidence.

Selective enrichment techniques for *Y. enterocolitica* include cold enrichment and alkali treatment of specimens. Although cold enrichment is effective (9, 12), it is a time-consuming technique and may not allow for completion of the culture within a clinically relevant time frame. Assessments of the usefulness of alkali treatment have produced conflicting results (10, 13). Various selective and differential media for *Y. enterocolitica* have been described (1, 4, 5, 11) and compared (6), and as a result of these reports we chose cefsulodin-Irgasin-novobiocin (CIN) agar as a selective and differential medium for screening all stool specimens received in our laboratory over 1 year. Our aim was to determine the incidence of *Y. enterocolitica* in samples received at our institution and hence to assess the usefulness of routine culture for this pathogen.

During the study period, 3,622 stool specimens were examined. In our routine stool screening protocol, specimens are planted directly on blood (BAP), MacConkey (MAC), salmonella-shigella (S-S), and Campylobacter agar plates and a portion of the specimen is incubated in selenite broth before being subcultured on MAC and S-S agar plates. Plates selective for *Campylobacter* spp. are incubated at 42°C in an atmosphere of 5% O₂ and 10% CO₂ and examined daily for 3 days. The BAP and S-S primary plating media and the S-S subculture plate from selenite broth are incubated at 35°C in 4 to 6% CO₂ for 2 days and examined daily. Finally, both the primary and selenite broth subculture MAC plates are incubated at 35°C in 4 to 6% CO₂ for 24 h and then at

room temperature in an ambient atmosphere for an additional 24 h. The MAC media are examined for the appearance of newly formed, small, lactose-positive or negative colonies as a screening procedure for the presence of possible *Yersinia* isolates.

During the study period, CIN agar plates were inoculated with stool samples, incubated at room temperature for 3 days, and examined daily. During the first part of the study all organisms growing on CIN agar were identified by using the Gram Negative Identification Card in the AutoMicrobic system (Vitek Systems, Inc., Hazelwood, Mo.). Later in the study all isolates growing on CIN agar were screened by subculture to nutrient agar plates incubated at 35°C. Since *Yersinia* isolates are distinguished from other physiologically similar genera by their inability to form colonies with diameters greater than 1.0 mm on nutrient agar at 37°C (3), only pinpoint colonies appearing on the nutrient agar plates were further identified. All media used in the study were purchased from either GIBCO Diagnostics, Madison, Wisc., or BBL Microbiology Systems, Cockeysville, Md.

Of the 3,622 stool specimens examined, only 7 (0.2%) yielded *Y. enterocolitica*. *Salmonella* spp. were isolated from 130 (3.6%), *Campylobacter* spp. were isolated from 89 (2.5%), and *Shigella* spp. were isolated from 36 (1.0%) of the specimens. The seven *Yersinia* isolates were found in single specimens from four patients and in three specimens from a single patient. *Y. enterocolitica* was recovered from specimens of all five patients by using CIN agar, whereas our standard protocol revealed three of the five cases.

Close to half (1,764 of 3,622) of the CIN plates examined in the study yielded growth of organisms other than *Yersinia* spp. Isolates from the first 500 plates examined were distributed as follows: *Citrobacter* spp., 267; *Morganella* spp., 103; *Kluyvera* spp., 30; *Pseudomonas* spp., 22; *Serratia* spp., 17; *Klebsiella* spp., 14; *Providencia* spp., 13; *Escherichia* spp., 10; *Aeromonas* spp., 8; *Enterobacter* spp., 7; *Proteus* spp., 3; *Hafnia* sp., 1; and CDC group VE-2, 1. Altorfer et al. (2) noted that CIN medium may be useful for isolation of *Aeromonas* spp. as well as *Yersinia* spp. They reported an *Aeromonas* isolation frequency of 2.4% by using a medium and incubation conditions comparable to ours, but we observed only 1.6% isolation.

Technologists who examined the clinical specimens in our study did not observe the distinctive bull's eye (a red center

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with a transparent margin) colony morphology of *Yersinia* isolates on CIN agar within the first 48 h of incubation. Thus, we believe that virtually all organisms growing on this medium should be screened to rule out their possible identity as *Yersinia* spp. Our experience suggests that this practice would result in a large expenditure of time and money for an extremely small yield of *Yersinia* isolates over the number revealed by our standard protocol.

We assessed the clinical relevance of culturing *Yersinia* spp. by asking the clinician what changes, if any, were made on the basis of this report. Data were available for only three of the five patients. Two of these three patients were still symptomatic and were treated; for one of the two patients potential surgery was averted. The third patient was not treated, since his diarrhea had largely resolved by the time his culture results were available.

In conclusion, we believe that it is not cost effective to routinely culture all stool specimens on CIN agar. Additional media for *Yersinia* culture should be available, however, when clinical or epidemiological circumstances warrant their use.

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