Letters to the Editor
Identification of Pathogenic Serotypes of Yersinia enterocolitica

We were interested to read the report by Farmer et al. (3) on the combined use of pyrazinamidase activity (PYZ), growth on Congo red-magnesium oxide agar (CR-MOX), D-xylene fermentation (XYL), and salicin-esculin hydrolysis (SAL-ESC) for identifying pathogenic serogroups of Yersinia enterocolitica. We wish to present data from a similar, but prospective, study carried out in Italy from 1987 to 1992.

During the study period, a total of 1,619 strains of Y. enterocolitica of human and nonhuman origin in Italy were tested for PYZ, XYL, and SAL-ESC (all at 25°C for 48 h). No strains had been epidemiologically incriminated in outbreaks. Not included in this study are strains formerly referred to as biogroup 3A and 3B of Y. enterocolitica and now classified as Y. mollaretii and Y. bercovieri, respectively.

Of the 1,619 strains, 603 represented unquestionably pathogenic phenotypes as they fit into biogroup 4, serogroup O3, phase type VIII or IX, biogroup 2, serogroup O9, phase type X2, biogroup 1B, serogroup O8, phase type X2; biogroup 2, serogroup O5,27; or biogroup 3, serogroup O1,2,3. These isolates were uniformly PYZ- and SAL-ESC-. All 423 strains belonging to biogroup 4, serogroup O3, were XYL-, in contrast to other pathogenic and nonpathogenic (see below) phenotypes of Y. enterocolitica, which were uniformly positive. Thus, we agree with the authors that D-xylene fermentation is very useful for identifying this bio-serogroup.

The remaining 1,016 strains were positive for XYL, PYZ, and SAL-ESC, with the exception of 19 isolates which were XYL- and PYZ- but SAL-ESC- after 48 h of incubation at 25°C. All of these strains were biochemically more like biogroup 1A (lipase*, indole*, xylose*, trehalose*, nitrate*, pyrazinamidase*, beta-glucosidase*, Voges-Proskauer*, proline peptidase negative or positive) (6). From the standpoint of clinical and public health, we do not consider strains of biogroup 1A to be enteric pathogens. This conclusion was based on (i) their widespread presence in the environment, (ii) their high isolation rate from healthy individuals and patients whose histories were not compatible with yersiniosis, (iii) lack of their recovery upon primary plating, regardless of source of isolation, and (iv) lack of antibody response in serum samples from 34 examined patients from whose stools a biogroup 1A strain had been isolated. Surprisingly, while O serotyping isolates of biogroup 1A, we found that single O factors representative of the main pathogenic strains of Y. enterocolitica often occurred in these environmental strains. We noted this in 65 of 997 PYZ- and SAL-ESC- strains which included O4,32 (28 strains), O3 (19 strains), O21 (9 strains), O8 (5 strains), O20 (3 strains), and O18 (1 strain) serogroups. It is also noteworthy that three nonhuman O8 isolates were PYZ- and SAL-ESC- but nevertheless contained plasmid DNA of a size similar to that of plasmid DNA from the well-known human virulent O8 strains WA and 8081. Further characterization of these plasmids by Southern hybridization after BamHI restriction showed the lack of plasmid homology between our study strains and the virulent O8 strains.

Farmer et al. (3) state that PYZ, XYL, SAL-ESC, and CR-MOX can be used to screen for pathogenic serotypes of Y. enterocolitica since O antisera are not generally available. Our results reaffirm that the use of O antisera without prior complete biochemical characterization of the Y. enterocolitica isolates could lead to considerable overestimation of pathogenic serogroups (1, 2, 4, 5). We agree that simple tests such as PYZ and SAL-ESC are a primary tool to identify Y. enterocolitica isolates as a pathogenic or nonpathogenic phenotype, while O typing is important in understanding the epidemiology and ecology of this species.

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REFERENCES

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Authors’ Reply
We were encouraged by the results of the extensive survey of human and environmental Y. enterocolitica strains from the Italian National Center for Yersiniosis. The fact that only 19 of 1,619 strains had discordant results for the pyrazinamidase and salcin-esculin tests is much more encouraging than the 9% we observed in our smaller series of strains from human patients in the United States. The authors point out that strains with these discordant results can be biotyped to determine whether they belong to biogroup 1A, which contains only nonpathogenic strains, but we are not optimistic that many clinical microbiology labo-
Wellcolex Colour Salmonella Test and Selenite-F Broth

I read with interest a recent publication entitled “Evaluation of the Wellcolex Colour Salmonella Test for Detection of Salmonella spp. in Enrichment Broths” (6). However, we would like to make a number of comments on the results of the Wellcolex Colour Salmonella Test in the above publication, as they are significantly different from our own experience with the test (5).

In the study by Rohner et al., the sensitivity of the Wellcolex test is lower, even with the Selenite-F broth, than those in other studies (1, 3, 5). A number of key factors were omitted in this study which could explain the lower performance of the Wellcolex Salmonella Test.

(i) **Amount of inoculum.** The manufacturer gives precise instructions regarding the amount of inoculum to be used for a specific volume of Selenite broth, as it is added at a critical stage for optimum recovery of Salmonellae. Seeding Selenite broth with too little or too much would lead to poor growth of salmonellae.

(ii) **Emulsification.** Emulsification of fecal specimens prior to inoculation is also recommended by the manufacturer, as this should liberate Salmonella spp. and allow maximum growth in this selective environment.

(iii) **GN broth.** The recommended incubation time for GN broth is 6 to 8 h (2). In the study by Rohner et al. (6), the incubation time was 18 to 24 h, which would indicate that the laboratory procedure did not use culture conditions for optimal recovery of Salmonella spp. This appears to be confirmed by the fact that the subcultures from the GN broth missed five Salmonella spp. which were isolated with the primary plates. There was therefore no point in evaluating the performance of the Wellcolex Colour Salmonella Test on this GN broth. Furthermore, the manufacturer recommends testing only on Selenite-F Broth.

(iv) **Quality of the Selenite-F broth.** Lastly, the quality of the Selenite-F broth is obviously critical in the recovery of Salmonella spp. The above study (6) only compared GN and Selenite-F broths, without comparing different Selenite broths. Our own evaluation (5) has shown quality differences between Selenite-F broths from two manufacturers.

By changing the methodology as described above, we found that the sensitivity of the Wellcolex Colour Salmonella Test was 99% on Selenite-F broth (5). It should also be noted that the salmonella incidence in our study was over 20%, compared with 4% in the study by Rohner et al.

We wish to publish this letter in reply to Dr. Rohner’s publication, as we feel that the results of their study are incomplete and do not represent the true performance of the Wellcolex Colour Salmonella Test.

REFERENCES


