Failure To Detect Salmonella enterica Serovar Dublin on Aes Laboratoire Salmonella Agar Plate

Isolation and identification of Salmonella enterica from clinical specimens are an important component of the workload of clinical laboratories. Detection of S. enterica in specimens of feces is dependent on plating on selective and differential media both directly and following enrichment in selenite broth. Recently new differential media have become available (1, 4) including the Aes Laboratoire Salmonella Agar Plate (ASAP) medium (Aes Laboratoire). S. enterica produces distinctive pink to purple colonies on ASAP medium based on the enzymatic action of S. enterica C₉ esterase on a chromogenic substrate, magenta-cap (5-bromo-6-chloro-3-indolylcaprylate). The medium also contains a second chromogen, X-β-d-glucopyranoside, which is hydrolyzed by β-d-glucosidase produced by Klebsiella spp. and Enterobacter spp. (blue to blue-green colonies). Serratia spp. produce both C₉ esterase and β-glucosidase, resulting in violet blue colonies. Most other species of bacteria result in white colonies. Following resuscitation from storage at ~70°C on unselective medium, we subcultured a single colony of each of 320 isolates of S. enterica comprising S. enterica serovar Typhi (n = 6), S. enterica serovar Enteritidis (n = 99), S. enterica serovar Typhimurium (n = 83), and 34 other serovars (n = 132) on ASAP medium. All isolates produced colonies of the expected color, except for two isolates of S. enterica serovar Dublin, which produced white colonies. Thirty additional nonduplicate isolates of Salmonella serovar Dublin were subcultured onto ASAP medium. All 30 yielded white colonies. Isolates of Salmonella serovar Dublin were from diverse sources including humans and animals from Ireland and two isolates received as part of quality assurance panels from outside Ireland. Pulsed-field gel electrophoresis (PFGE) analysis (restriction enzyme XbaI, Pulse-Net protocol) on the 32 Salmonella serovar Dublin isolates showed six patterns differing by one or two bands. Twenty-six isolates were indistinguishable, and the remaining six isolates gave five distinguishable patterns. One isolate from outside Ireland was indistinguishable from most local isolates, but the other differed from the predominant pattern by one band.

The failure of Salmonella serovar Dublin isolates to produce the expected pink to purple colonies suggests that these isolates do not produce C₉ esterase sufficiently quickly or in sufficient quantity to result in color change after overnight incubation. After reincubation for a total of 72 h a faint pink color was noted for some isolates.

Limited diversity of Salmonella serovar Dublin on PFGE analysis with XbaI has been reported previously (3). Likewise for S. enterica serovar Enteritidis (also a group D1 salmonella) isolates of different phase types and from different countries are often indistinguishable on PFGE with XbaI and additional enzymes (2). This collection included two isolates from outside Ireland that were very closely related to isolates from Ireland on PFGE. This collection of isolates is probably representative of the spectrum of the diversity that exists within Salmonella serovar Dublin. Failure to produce the expected color on ASAP medium is likely to be a frequent, though perhaps not universal, property of isolates of this serovar.

These results indicate that Salmonella serovar Dublin may go undetected if laboratories use only ASAP medium as a differential medium for detection of S. enterica. Given the virulence of Salmonella serovar Dublin (5), its detection is particularly important.

REFERENCES