Spurious Sulfamethoxazole-Trimethoprim Resistance of Salmonella typhi

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Several studies have identified thymidine excess in susceptibility test media as the cause of spurious resistance of various bacteria to sulfamethoxazole-trimethoprim. We document the phenomenon in Salmonella typhi and Salmonella paratyphi-A and demonstrate its occurrence in 3 of 17 (18%) lots of Mueller-Hinton agars now in use in major medical laboratories in Lima, Peru. The findings are particularly significant because sulfamethoxazole-trimethoprim is an important alternative to chloramphenicol or ampicillin for the treatment of typhoid and paratyphoid fevers.

Mueller-Hinton (MH) agar is the standard medium in the United States for testing the susceptibility of aerobic and facultative anaerobic, rapidly growing bacteria (10). Several reports have documented that some lots of the medium are unsuitable for sulfamethoxazole-trimethoprim (SxT) susceptibility testing due to an excess of thymidine (3, 6, 8).

The quality of a large batch of MH medium recently purchased by a hospital laboratory in Lima, Peru, was initially suspect when discordant SxT test results were noted for a Salmonella typhi isolate tested with two lots of agar. The lot indicating resistance of the organism was later found unacceptable due to an excess of thymidine. This observation prompted a survey of the MH agars in use in other major medical centers of the city to determine the suitability of the media for testing the susceptibility of S. typhi to chloramphenicol, ampicillin, and SxT, the three drugs most often recommended for the treatment of typhoid fever (7).

A sample (30 to 50 g) of each lot of dehydrated medium in actual use for susceptibility testing was collected at each of 11 hospitals and 3 tropical medicine institutes in March and April 1984. A total of 17 samples were collected. These represented six companies (BBL Microbiology Systems, Britania, Difco Laboratories, Laphsa, Merck & Co., Inc., and the Pasteur Institute) and 16 different lot numbers. Sixteen samples were MH agar, and one was MH broth to which Bacto-Agar (Difco) was added to 1.5% before autoclave sterilization.

The media were reconstituted and prepared for use in accordance with each manufacturer's instructions. Susceptibility tests were done by the Kirby-Bauer technique (1), with modifications as follows.

A set of four plates of each medium was evaluated. Two were swabbed with a solution containing 30 IU of thymidine phosphorylase (TPP; Burroughs Wellcome Co.) per ml, and the other two were swabbed with sterile distilled water. The plates were allowed to dry for 30 min, and a standardized suspension of a clinical isolate of S. typhi was inoculated onto a TPP- and nonswabbed plate; the other two plates were similarly inoculated with a suspension of Escherichia coli ATCC 25922. Standard disks containing SxT, ampicillin, and chloramphenicol (Difco) were then applied, and all plates were incubated at 36°C for 18 h.

The ampicillin and chloramphenicol disks yielded zones of inhibition of similar size on the E. coli and S. typhi on all the media. The zones measured 15 to 19 mm around the former disks and 21 to 26 mm around the latter disks. With these zones in the acceptable range for the reference organism (1), all media in the survey were shown to be adequate for chloramphenicol and ampicillin susceptibility testing, and the S. typhi strain was judged susceptible to both antimicrobial agents.

SxT susceptibility tests in 14 of the 17 MH media revealed well-demarcated zones of inhibition measuring 24 to 30 mm. There was no difference in the zone sizes of the TPP- and nonswabbed plates (Table 1); these media met standard criteria for SxT testing. Results of the remaining three MH agars, all from Labpsa, are also shown in Table 1. The diameters of inhibition zones on the non-TPP-swabbed plates were difficult to measure because of the gradual tapering and feathery edges of bacterial growth projecting towards the disks. The TPP-swabbed plates of these media yielded large, clear zones of inhibition that were easy to measure. These three medium lots caused spurious resistance of the organisms unless TPP was added.

Stock cultures of S. typhi and Salmonella paratyphi-A, isolated up to 4 months before the survey by the laboratory in which defective MH agar had been initially detected, were retested with acceptable media to evaluate the accuracy of the original susceptibility reports. Five of 15 (33%) S. typhi and 7 of 13 (54%) S. paratyphi-A isolates had been reported resistant to SxT by the laboratory. All these isolates were susceptible in vitro when retested on media of acceptable standards; 43% of the enteric-fever salmonella isolates had been erroneously reported as resistant. Perhaps all had not been reported resistant because of variability in interpretation of the test results by the technicians or due to intermittent use of acceptable medium lots.

The false SxT resistance in S. typhi and S. paratyphi-A reported here is similar to that in Haemophilus influenzae and Streptococcus pyogenes, which Leers (8) reported was probably in error as a result of the use of unacceptable media. The presence of thymidine in the medium allows certain organisms to bypass the growth inhibition caused by trimethoprim and the sulfonamides (5). TPP converts thymidine to thymine (2, 6), making thymidine-abundant media acceptable for SxT testing of most organisms. The enterococci, however, can bypass this inhibition with either thymidine or thymine, and Crider and Colby (3) recently em-
TABLE 1. Zones of inhibition of reference cultures with \textit{SxT} disks* on MH agars

<table>
<thead>
<tr>
<th>Bacterial test strain and additive</th>
<th>Zone of inhibition (mm [mean range]) on the following media:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acceptable (14 samples) Unacceptable (3 samples)</td>
</tr>
<tr>
<td>\textit{E. coli}</td>
<td></td>
</tr>
<tr>
<td>TPP added</td>
<td>28.1 (24–30) 23 (22–24)</td>
</tr>
<tr>
<td>TPP not added</td>
<td>25.6 (24–30) 11 (8–15)</td>
</tr>
<tr>
<td>\textit{S. typhi}</td>
<td></td>
</tr>
<tr>
<td>TPP added</td>
<td>28.0 (24–32) 24 (22–26)</td>
</tr>
<tr>
<td>TPP not added</td>
<td>26.1 (24–30) 10 (9–12)</td>
</tr>
</tbody>
</table>

* Sulfamethoxazole, 23.75 µg; trimethoprim, 1.25 µg.

phosphized that even very low concentrations of either metabolite can profoundly affect the \textit{SxT} susceptibility of the organism. Moreover, they recommended that special strains of \textit{Streptococcus faecalis} be used to detect tiny traces of inhibitor. The spurious resistance reported here was readily detected by use of a more common reference culture, \textit{E. coli} ATCC 25922.

This study showed that an excess of thymidine in susceptibility test media causes spurious \textit{SxT} resistance in enteric-fever \textit{Salmonella} spp. \textit{SxT} resistance in enteric-fever salmonellae is rare (4, 9, 11, 12); in the absence of quality assurance tests, unacceptable media or technician error should be suspected in all reports of resistance.

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LITERATURE CITED


