A Research Note

Thermal Resistance of Disease-Associated Salmonella typhimurium in Milk

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ABSTRACT

The thermal resistance of Salmonella typhimurium cultures that had been associated with a major milkborne outbreak of salmonellosis was determined in raw whole milk. Thirteen patient stool isolates and 24 implicated pasteurized milk isolates at concentrations of $1 \times 10^5$/ml were screened for heat resistance at 51.8°C. A representative milk strain was heated in replicate at four temperatures from 51.8 to 68.3°C. The zD value was calculated to be 5.3°C. Mean D-value estimates at 51.8°C were 24.0 and 22.8 min for patient and milk isolates, respectively. Extrapolated $D_{70.7°C}$ values were 0.24 and 0.22 s, and did not differ significantly ($\alpha = 0.05$). These isolates would not survive proper pasteurization.

Between March 22 and mid-April, 1985, more than 16,284 culture-confirmed cases of salmonellosis occurred in northern Illinois and surrounding states (10). This was the largest outbreak of salmonellosis ever documented in the United States. Epidemiologic and laboratory investigation implicated 2% low-fat pasteurized milk as the source of an outbreak-related strain of Salmonella typhimurium (5,6). The outbreak organism directly or indirectly contributed to the deaths of seven persons (7). All outbreak isolates exhibited an uncommon antimicrobial resistance pattern; almost all possessed a rare plasmid profile not seen before 1984 (12). The source of the rare organism that caused the outbreak is unknown; however, the bulk of evidence pointed to an unidentified, persistent focus within the plant (10,12).

Preliminary inspection of the dairy plant involved revealed no evidence of faulty pasteurization. It was therefore of immediate interest to know whether outbreaks strains possessed an unusual heat resistance that would enable them to survive pasteurization.

MATERIALS AND METHODS

Cultures and culture conditions

A total of 37 outbreak-related S. typhimurium strains, including 13 patient stool isolates and 24 strains recovered from milk, were studied. All patient isolates and four milk isolates were received from J. Wells, Centers for Disease Control, Atlanta, GA. The remaining 20 milk strains were isolated by U.S. Food and Drug Administration (FDA), Cincinnati, OH. All strains were verified by their reactions in triple sugar iron (TSI) and lysine iron (LI) agar (BBL Microbiology Systems, Cockeysville, MD), and in API 20E Systems (Analytab Products, Plainview, NY). They were confirmed by agglutination tests with Salmonella polyvalent and individual O-group antisera (Difco Laboratories, Detroit, MI).

Milk cultures were grown in Trypticase soy-0.6% yeast extract (TSYE) broth (BBL) at 37°C for 24 h and maintained at 4°C with monthly transfers. Test cultures were incubated in TSYE broth at 37°C for 24 h in three serial transfers. The cell density of the final culture was adjusted at 625 nm to an absorbance of 0.20, yielding approximately $10^9$ bacteria/ml.

Microbiological procedures

Total aerobic plate counts were determined in duplicate for the pooled contents of tubes at each heating interval on TSYE agar after 48-h incubation at 37°C. Surviving Salmonella were enumerated by verifying 10 randomly selected colonies/plate. Salmonella were distinguished from indigenous milk flora by reactions in LI and TSI agars and by agglutination tests with polyvalent O and O-group B antisera.
Statistical methods

The rates of thermal inactivation were determined and a
linear regression of log_{10} counts/ml vs. heating times was com­puted (13) for each outbreak strain tested at 51.8°C. The least-
square estimate of slope was calculated and is the rate of ther­
al inactivation at that temperature. An estimate of the D-value
was obtained by taking the absolute value of the inverse of
the slope. For one strain, an estimate of D-value was obtained at
four temperatures. A linear regression was computed from log_{10}
D-value vs. temperature for this strain and an estimate of the
zD value was obtained from the absolute value of the inverse
of the slope. The estimated D-values were corrected for lethality
during heating and cooling (4).

RESULTS AND DISCUSSION

All 37 outbreaks isolates were verified biochemically
and serologically as S. typhimurium. The thermal resis­
tance characteristics of a representative milk isolate, S. typhimurium
strain 4SCBS, determined by heating bac­
teria suspended in raw milk at temperatures between 51.8
and 68.3°C, are shown as D-values in Table 1. The zD-
value is 5.3°C.

The mean D-value estimates (Table 2) for patient and
milk isolates screened at 51.8°C are 24.0 and 22.8 min,
respectively. When D_{71.7°C} values were calculated from
these data using the estimated zD from the representative
strain of 5.3°C, the results (0.24 and 0.22 s) did not differ significantly (α = 0.05). When two of the most resis­
 tant strains in each group were confirmed in a heat ex­
 changer at 71.7°C, the D-values were equivalent to or
less than the extrapolated values.

The outbreak S. typhimurium strains exhibited a heat
susceptibility common to most vegetative bacteria. For example, when Read and co-workers studied the heat re­
sistance of six salmonellae isolated from dry milk, the
most resistant, S. binza, had an extrapolated D-value at
71.7°C of only 0.3 s (14).

| TABLE 1. D-value estimates for S. typhimurium strain 4 SC-
<p>| BS in raw milk. |</p>
<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>D-value (s)</th>
<th>% CV</th>
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<tbody>
<tr>
<td>51.8</td>
<td>1267.2</td>
<td>6.0</td>
</tr>
<tr>
<td>57.2</td>
<td>102.0</td>
<td>16.6</td>
</tr>
<tr>
<td>62.8</td>
<td>6.7</td>
<td>6.3</td>
</tr>
<tr>
<td>68.3</td>
<td>0.9</td>
<td>6.1</td>
</tr>
</tbody>
</table>

zD = 5.3°C.  

TABLE 2. Mean D-value estimates for outbreak strains of S.
typhimurium heated in raw milk.

<table>
<thead>
<tr>
<th>Source of strains (number)</th>
<th>D_{51.8°C} (min)</th>
<th>D_{71.7°C} (s)</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient (13)</td>
<td>24.0</td>
<td>0.24</td>
<td>33.3</td>
</tr>
<tr>
<td>Milk (24)</td>
<td>22.8</td>
<td>0.22</td>
<td>20.1</td>
</tr>
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^aFrom duplicate determinations.

^bNot significantly different at α = 0.05 level.

In contrast, species implicated in several recent disease
outbreaks associated with the consumption of dairy prod­
ucts were considerably more thermoresistant. The esti­
mated D_{71.7°C} for Listeria monocytogenes, the cause of
a milkborne listeriosis outbreak in Massachusetts in 1983
(8) and a cheeseborne outbreak in California in 1985 (7)
was 0.9 s (2). The extrapolated D_{71.7°C} for a milk isolate
of Yersinia enterocolitica, a species that has been impli­
cated in milkborne gastroenteritis, was 1.17 s (11).

Heating milk according to current pasteurization pro­
cess guidelines recommended by the FDA (9) would en­
sure the destruction of even the relatively heat-resistant
organisms. At 161°F, for example, 15-s heating time
would provide a 12-D process for Y. enterocolitica. It
seems clear that the presence of the outbreak strain of
S. typhimurium in the implicated milk was the likely re­
result of post-pasteurization contamination.

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