A Study of Microbial Quality of Vacuum Packaged, Sliced Bologna

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ABSTRACT

A study of 113 samples of vacuum packaged sliced bologna offered for sale in the retail marketplace revealed a wide range of total microbial loads. The pH was not closely related with age or microbial load, within the manufacturers' expected shelf-life of the product. Approximately 55% of old samples had pH >6.0, and pH was influenced by manufacturer. Confirmed coliform bacteria were detected in 5% of samples, but Escherichia coli was absent (<3/10g). In contrast, group D streptococci were present, sometimes in large numbers, depending on manufacturer. Potentially pathogenic bacteria, including Clostridium perfringens (>10g), coagulase positive Staphylococcus aureus (>25/g) and Salmonella (in 25 g of sample) were generally not detected. Only one sample contained >25 S. aureus/g. No relationship was observed between total microbial load and indicator organisms or pathogenic bacteria.

Bologna is an emulsion-type, non-fermented sausage which is cooked, cured and smoked during manufacture. Frequently sold as sliced, vacuum packaged luncheon meat, bologna is one of the most widely used sausage types in North America. During manufacture, bologna is cooked for 6 to 10 h to an internal temperature near 70 C. Slicing has been shown to be the most important source of contamination by both saprophytic and pathogenic bacteria in these meats (23, 24). The microflora of vacuum packaged luncheon meat changes during storage. Most bacteria on freshly packaged product die during storage (23), whereas lactic acid bacteria tend to increase and often predominate the population within 2 weeks (1, 2, 23).

Initial counts in vacuum packaged, sliced, processed meats have been reported as 10^3 bacteria/g (I, 30). These counts increase to 10^8/g during refrigerated storage (I, 20, 21, 23). Although there is usually a marked drop in pH, growth to maximum bacterial population does not necessarily result in product spoilage (I, 20, 23, 37).

Many factors, including the saprophytic microflora, pH, nitrite and salt concentrations, available water, and oxygen partial pressure influence survival and growth of pathogens (10, 21, 22, 27). Storage temperature will also have a marked effect on the microflora. Mesophiles, and therefore many pathogenic bacteria, have minimum growth temperatures of 10 to 15 C, and refrigerated storage of cured meats (below 10 C) allows lactic acid bacteria to develop (4).

With many variable factors influencing the microflora of bologna, this study was undertaken to determine the bacterial load of new and old, vacuum packaged, sliced bologna offered for sale in the retail marketplace.

MATERIALS AND METHODS

Sampling

Vacuum packaged, sliced, processed meat, labelled "Bologna", representing the product of six Canadian federally inspected manufacturers was purchased from stores of four different retail chains. Samples were selected to represent "new" bologna (≤15 days of manufacturer's shelf life expired) and "old" bologna (>21 days of manufacturer's shelf life expired). "New" samples were also purchased and stored in the laboratory at 4 C for bacterial analysis at the end of designated shelf-life.

Sample preparation

An 11-g wedge was cut aseptically through all slices in the package and homogenized with 99 ml of sterile, 0.1% peptone water in a sterile Waring Blender jar at high speed for 2 min. All bacteriological determinations, except Salmonella, were carried out on this sample homogenate. A separate 25-g wedge was cut, weighed and homogenized in 150 ml of sterile nutrient broth (Difco) for Salmonella enrichment.

Bacteriological analyses

Appropriate dilutions of the homogenized 11-g samples were inoculated in duplicate onto the following media (all media were Difco brand, unless otherwise specified):

Acidic plate counts. Plate count agar incubated at 35 C for 48 h for Standard Plate Count (SPC); incubated at 21 C for 72 h for Total Aerobic Plate Count (TPC); and incubated at 4 C for 10 days for psychrotroph count.

Group D streptococci. The presumptive group D Streptococcus count was determined on KF Streptococcus agar and incubated at 35 C for 48 and 72 h (15).

Lactic acid bacteria. Determined on Nitrite-Acidolone-Polymyxin (NAP) agar (6), incubated at 30 C for 72 h. Randomly picked colonies from NAP were screened using gram stain and catalase activity (14).

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Lactobacilli. Determined on LBS agar (26), adjusted to pH 5.60 ± 0.05 according to Costilow et al. (6), and incubated 30 C for 72 h. 

Microbacterium thermosphactum. Surface streaked on STAA agar (18), incubated 21 C for 72 h. Countable STAA plates were flooded with 2-3 ml of oxidase reagent (N,N-Dimethyl-p-phenylene-diamine monohydrochloride, Eastman Chemicals). Oxidase-positive colonies were excluded from the count.

Clostridium perfringens. Determined on Tryptose-Sulfite-Cycloserine (TSC) agar (16) and on egg-yolk free TSC agar (17). Both TSC and egg-yolk free TSC plates were incubated at 35 C, anaerobically in a H2/CO2 atmosphere (using BBL anaerobic jars and “gas-pak” cartridges) for 24 h. 

Staphylococci. Determined on Mannitol Salt agar (MSA, presumptive staphylococci) and Baird-Parker (BP) medium and incubated at 35 C for 48 h. Colonies on BP medium were counted according to the Canadian Health Protection Branch acceptable method (18), differentiating between type I (shiny, black, smooth colonies causing clearing of the opaque egg-yolk medium, with or without a zone of precipitation around the colony) and type II colonies (Similar colonies without egg-yolk reaction). A selection of type I and II colonies was inoculated into Brain Heart Infusion (BHI) broth, streaked on MSA slants and incubated at 35 C for 24 h. MSA positive cultures were inoculated from BHI broth into EDTA coagulase plasma to test for coagulase positive Staphylococcus aureus (18). 

Coliforms and fecal coliforms. The 3-tube method of the Most Probable Number (MPN) technique was adapted from the Canadian Health Protection Branch acceptable method (19) and the International Committee on Microbiological Specifications for Foods (ICMSF) procedures (20). Appropriate dilutions were inoculated into Lauryl Tryptose (LST) broth. Gas-positive LST tubes at 24 and 48 h were incubated into Brilliant Green Lactose 2% Bile (BGB) and EC broths. Similarly, gas-positive BGB tubes at 24 and 48 h were streaked onto Levine EM agar for the completed coliform test. All media, except EC broth, were incubated at 35 C. EC medium was incubated at 44.5 C for 24 h. Gas-positive EC tubes were used to determine the MPN of fecal Escherichia coli.

Salmonella. The 25-g sample was homogenized in non-selective nutrient broth enrichment, incubated at 35 C for 20 h, and an aliquot transferred to Selenite Cystine (SC) broth, selective enrichment, and incubated at 35 C for 24 and 48 h. The SC selective enrichment was streaked onto Brilliant Green (BGA) agar and Bismuth Sulfite (BSA) agar and incubated at 35 C for 24 and 48 h. Salmonella-type colonies were screened on MacConkey agar and TSI slants.

Positive controls were done during the study using known cultures of coagulase-positive S. aureus, S. typhimurium, S. cholerae-suis, E. coli and C. perfringens.

In addition to bacteriological tests, pH was measured on 111 of the bologna samples using a 1:10 blended homogenate of bologna in de-ionized, distilled water (23, 40) and for ca. 70% of the samples using a combination (single probe) electrode (Fisher Scientific Co., Cat. No. 13-639-90) directly between the bologna slices (29, 31, 32, 36).

**Analyses**

Data were analyzed statistically using Pearson's correlation coefficients (r) as outlined by Nie et al. (25). Chi-square analysis for independence (25) was carried out to determine the influence of manufacturer and age upon bacteria counts and pH. In addition, using only the results from “new” bologna samples (all brands) and “old” bologna samples (those samples which were purchased “new” and stored at 4 C in the laboratory to pull date) an analysis of variance (25) to determine effects of manufacturer and age was carried out.

**RESULTS AND DISCUSSION**

A total of 113 samples of bologna were analyzed, representing “new” and “old” samples from six manufacturers. The pH profile of 111 of the bologna samples is shown in Table 1. The pH ranged from 4.9 to 6.7, and almost 50% of samples fell in the range of 6.1 to 6.5. The pH of bologna has been reported as 6.2 to 6.4 (37), 5.2 to 5.3 (26) and 5.0 to 6.5 (23). Results of this study supported the wider range of pH levels reported by Kempton and Bobier (23). They noted that the pH drop did not occur until after 4 weeks of refrigerated storage. In this study, however, pH dropped before 4 weeks of storage had expired, possibly as a result of poor storage conditions at retail level. Age had a significant effect on pH (p<0.002). New bologna generally fell in the pH range of 6.1 to 6.5, while old product often had pH values below 5.5. However approximately 55% of old samples failed to develop low pH. This poor correlation (r = -0.2) was due to manufacturer effect (p = 0.0001). The old product of one manufacturer generally had low pH, while that of another manufacturer was generally high pH. This might explain reports by Steinke and Foster (37) and Riemann et al. (26) that samples had a narrow pH range.

The distribution of saprophytic bacterial counts, including potential indicator organisms, is shown in Table 2. Total aerobic and psychrotroph counts varied over a wide range, which agrees with other literature reports for bologna and frankfurters (1, 20, 21, 23, 38, 40). Results for total counts at different temperatures can be used to infer storage history of samples (38). Samples stored continuously at 4 C or below should have psychrotroph counts equal to or greater than total counts at 21 or 35 C. For these samples, 44% of counts at 21 C and 29% of counts at 35 C were at least five times higher than at 4 C. In comparison, 40% of counts at both 4 and 21 C were at least five times lower than at 35 C. This suggests widely varying temperature histories of samples, resulting in growth of mesophilic organisms (i.e. storage temperature of 10 C and above). New samples had significantly lower total aerobic counts (p<0.001). Manufacturer also had a significant effect on total aerobic counts at 35 and 21 C (p<0.01) but not at 4 C, suggesting that differences in handling of product at manufacturing level influenced the mesophile count, but not the psychrotroph count. The psychrotroph count appeared to depend on post-manufacturing storage conditions.

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>4.6-5.0</th>
<th>5.1-5.5</th>
<th>5.6-6.0</th>
<th>6.1-6.5</th>
<th>6.6-7.0</th>
<th>(Number of samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>11</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>9</td>
<td>1</td>
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<tr>
<td>C</td>
<td>3</td>
<td>17</td>
<td>3</td>
<td>8</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>9</td>
<td>0</td>
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</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>7</td>
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<td>New samples</td>
<td>0</td>
<td>2</td>
<td>6</td>
<td>27</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Old samples</td>
<td>3</td>
<td>19</td>
<td>12</td>
<td>27</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>All samples</td>
<td>3</td>
<td>21</td>
<td>18</td>
<td>54</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

*Results for 111 samples out of 113 analyzed.*
TABLE 2. Profiles of saprophytic and indicator organism counts for 113 samples of vacuum-packaged, sliced bologna.

<table>
<thead>
<tr>
<th>Count</th>
<th>35 C</th>
<th>21 C</th>
<th>4 C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent of samples with counts/g in the ranges</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10⁴</td>
<td>1.8</td>
<td>21.2</td>
<td>19.4</td>
</tr>
<tr>
<td>&lt;10³</td>
<td>24.8</td>
<td>18.5</td>
<td>23.0</td>
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<tr>
<td>&lt;10²</td>
<td>23.0</td>
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</tr>
<tr>
<td>&lt;10¹</td>
<td>30.0</td>
<td>8.1</td>
<td>27.0</td>
</tr>
<tr>
<td>&lt;10⁰</td>
<td>20.4</td>
<td>7.1</td>
<td>4.9</td>
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</table>

Numbers of samples

<table>
<thead>
<tr>
<th>Count</th>
<th>35 C</th>
<th>21 C</th>
<th>4 C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total count (35 C)</td>
<td>1.8</td>
<td>21.2</td>
<td>19.4</td>
</tr>
<tr>
<td>Total count (21 C)</td>
<td>11.5</td>
<td>18.5</td>
<td>23.0</td>
</tr>
<tr>
<td>Psychrotroph count</td>
<td>22.7</td>
<td>13.6</td>
<td>13.6</td>
</tr>
<tr>
<td>Lactic acid bacteria (NAP)</td>
<td>0.89</td>
<td>0.61</td>
<td>0.73</td>
</tr>
<tr>
<td>Lactobacilli (LBS)</td>
<td>32.8</td>
<td>16.8</td>
<td>13.3</td>
</tr>
<tr>
<td>M. thermosphactum</td>
<td>38.9</td>
<td>13.3</td>
<td>15.9</td>
</tr>
</tbody>
</table>

LBS = presumptive lactic acid bacteria count.
NAP = presumptive lactobacilli count.

Values of r:
r = 0.66 and r = 0.63, respectively. These counts were influenced by manufacturer and age of the product. However, this was not true of M. thermosphactum. Contrary to expectation (I4), M. thermosphactum did not appear to be associated with growth of bacteria in this vacuum packaged bologna, and correlations with other saprophytic organisms were extremely low (see Table 3). Furthermore, M. thermosphactum was not detected in 71 of the samples, indicating counts <100/g in 63% of samples tested.

The correlation coefficients between pH and different saprophytic bacteria parameters are shown in Table 3. A correlation of 0.7 or more is necessary to achieve 50% predictability between counts, such relationships were only observed in many of the 28 correlations, but many of the correlations were significant. These data indicate that lactic acid bacteria, lactobacilli and group D streptococci grew in the samples during storage, and their growth was reflected in the total aerobic counts at 35 and 21 C, but not to the same extent at 4 C.

Group D streptococci were detected in 78% of bologna samples analyzed, ranging from 10 to 2.1 × 10⁴ organisms/g. In 60% of samples, group D Streptococcus counts were above 100/g, indicating that many samples had been exposed to undesirable storage temperature conditions (5, 39). The group D Streptococcus count was significantly affected by age (p = 0.0004) and manufacturer (p = 0.0001). The group D Streptococcus count was significantly correlated with total aerobic count at 35 C (r = 0.685), again suggesting high storage temperatures. In a separate study (Stil, unpublished data), it was shown that 49% of isolates were Streptococcus faecium var. durans, 38% were S. faecium and 5% were Streptococcus faecalis. S. faecium var. durans has often been associated with heat processed meats, and in cheese it is not considered to indicate fecal contamination (12). However, both S. faecium and S. faecalis are considered by some to indicate fecal contamination (5). In these samples, numbers did not indicate degree of contamination because of the apparent growth opportunities for these organisms. A poor correlation was observed between coliform bacteria and group D streptococci in these samples, confirming the reports of others (5, 39). However, the total absence of fecal-type E. coli in the samples raised doubts about the fecal implications of S. faecium and S. faecalis.

For coliform bacteria and fecal E. coli, the lowest concentration that could be detected using the MPN technique was 3 organisms/10g. Only 5% of samples had detectable confirmed coliform counts. None of the samples were gas-positive in EC medium at 44.5 C and all samples were salmonella-negative in 25-g enrichments. This contrasted markedly with the group D streptococci, confirming that the group D streptococci were most likely from equipment, implicating sanitation of equipment and storage temperature, rather than poor hygiene as their source.

Potentially pathogenic bacteria were only present at low concentrations in the bologna samples tested. Only 10 (9%) samples had detectable levels of anaerobic spore...
forming bacteria. One sample had coagulate-positive *S. aureus* at 10^3/g, the remaining samples were <25/g. Only on Mannitol Salt agar (MDA) were appreciable counts, (up to 10^5/g) obtained. However, 54% of samples had counts <1,000/g, and in 44% of samples there was no detectable count. Counts on MSA represent salt tolerant bacteria that ferment mannitol. Although *S. aureus* would be included in this count, it appeared from the Baird-Parker medium results that potentially pathogenic, coagulase-positive *S. aureus* counts were low. Since the samples had been stored and handled such that many bacteria could grow, it was apparent that for these samples the potentially pathogenic bacteria did not compete well with the saprophytic flora. High saprophytic counts would not necessarily indicate a potentially hazardous product.

**SUMMARY AND CONCLUSIONS**

The samples of bologna in the retail marketplace differ widely in bacterial load, both as a function of age and manufacturer. The latter is somewhat difficult to explain, but probably reflects different manufacturing techniques and different bacterial flora predominating in processing plants. Among the bacteria that grow in the bologna samples, the group D streptococci are the only group that might represent some concern. While group D streptococci are generally not accepted as indicators of fecal contamination of foods, they are suspected of having a food poisoning potential (39). Results of this study suggest that group D streptococci are not indicating fecal contamination. However, the source of *S. faecium*, in particular, and also *S. faecalis* in these samples should be known before this conclusion can be confirmed.

Low pH is an important protective factor against growth of potentially pathogenic bacteria in vacuum packaged luncheon meats. This study indicated that low pH may not develop in the samples, right up to the end of shelf-life (30 to 35 days from manufacture). Low pH alone, therefore, cannot be relied on for protection against growth of food poisoning bacteria in bologna. The low levels of potential pathogens in the bologna samples analyzed, even in samples with high total bacterial loads, indicate either a low incidence of contamination or failure of the pathogens to survive in the product. Bacterial standards, at least for total bacterial loads, would not appear to be meaningful for vacuum packaged bologna. Inoculation studies using enteropathogenic bacteria would help to clarify the food poisoning potential of bologna.

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