Research Note

Microbiological Evaluation of Sprouts Marketed in Mumbai, India, and Its Suburbs

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ABSTRACT

A study was undertaken to assess the microbiological quality of sprouts marketed in Mumbai and its suburbs. A total of 124 sprout samples of four different legumes—mung (Phaseolus aureus), matki (Phaseolus aconitifolius), chana (Cicer arrietinum), and vatana (Pisum sativum)—were analyzed over a period of 12 months for aerobic plate counts, coliforms, yeast and mold counts, staphylococci, Salmonella, Listeria monocytogenes, Escherichia coli, E. coli O157:H7, and coagulase-positive Staphylococcus aureus. Aerobic plate counts ranged from 7.6 to 8.9 log CFU/g, coliforms counts ranged from 5.4 to 7.9 log CFU/g, yeast and mold counts ranged from 3.6 to 7.3 log CFU/g, and staphylococci counts ranged from 3.3 to 6.6 log CFU/g. Nonpathogenic E. coli was detected in 13% of the mung, 26% of the matki, 40% of the chana, and 19% of the vatana samples. Salmonella Typhimurium was detected in 21% of the mung, 40% of the matki, and 4% of the chana samples. Salmonella Dublin was detected in 2% of the mung samples, and Salmonella Washington was detected in 4% of the matki samples. L. monocytogenes and E. coli O157:H7 were not detected in any of the samples examined. Coagulase-positive S. aureus was detected in 4% of the mung, 11% of the matki, and 4% of the chana samples. The results indicated that the marketed sprouts were of poor microbiological quality; therefore, further processing, such as radiation processing, is needed to ensure their safety.

Minimally processed vegetables have become very popular among health-conscious consumers in recent years (6). Ready-to-eat salads, sprouts, and other raw vegetables constitute a suitable meal for today’s lifestyles because they need no preparation and provide a great variety of vitamins, minerals, and other phytochemicals, which are important for human health. In metropolitan cities like Mumbai, with its growing urban population, it has become increasingly popular to eat raw salad vegetables, fruits, and sprouts (22). Various types of sprouts are popular because of their nutritional attributes; they are rich in fiber, vitamins, and minerals and are considered low-calorie foods (3). They add taste and texture to salads, sandwiches, soups, and other dishes. A typical sprout making process in India involves soaking of seeds for 12 h and sprouting for 24 h. In the United States and other countries, sprout production is a 3- to 7-day process (8). Sprouts may be contaminated during germination, processing, or consumer handling; seeds that are used for sprout making can be contaminated by the water used in the field or the fertilizers applied (8, 10). During sprouting, constant moisture, nutrients released by the sprouting seeds, and warm temperatures are conducive to the growth of microorganisms (1). A variety of bacteria, such as Salmonella, Listeria monocytogenes, Escherichia coli O157:H7, and Staphylococcus aureus, which are potential human pathogens, can gain entry and proliferate during sprouting. A number of studies have reported the presence of Salmonella, Aeromonas, and L. monocytogenes in mung bean and alfalfa and bean sprouts from countries such as the United States, Malaysia, Sweden, Thailand, and Spain (5). None of the chemical or physical treatments currently used to disinfect raw fruits and vegetables can be relied on to eliminate all types of pathogens from the surface or internal tissues of foods when applications are used that will not adversely affect sensory or nutritional qualities (5). Taormina and Beuchat (18) were able to reduce the population of E. coli O157:H7 in germinated seeds but could not eliminate the pathogens on sprouts with chemical treatments. Sprouts are generally consumed raw and thus can act as a vehicle for foodborne disease. A large number of reported outbreaks have been due to the consumption of sprouts contaminated with Salmonella and E. coli O157:H7 (8). The most common varieties of sprouts implicated in Salmonella outbreaks are alfalfa and clover sprouts (16, 19, 21). Two recent Salmonella outbreaks related to the consumption of sprouts were reported in 2001 in the United States and Canada (4, 12). Although sprouts are increasingly sold in India, very few studies have been reported about the microbiological quality of the sprouts. Viswanathan and Randhir (22) have shown the presence of Salmonella, Serratia, Enterobacter, S. aureus, fecal E. coli, and Pseudomonas aeruginosa in sprouts. To our knowl-

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edge, however, no outbreaks due to the consumption of sprouts have been reported, possibly because of the lack of surveillance in India.

The purpose of this study was to examine the microbiological quality of sprouts marketed in Mumbai and its suburbs and to check for the presence of *Salmonella*, *L. monocytogenes*, *E. coli* O157:H7, and coagulase-positive *S. aureus*.

**MATERIALS AND METHODS**

**Sampling.** Sprout samples from mung (green gram; *Phaseolus aureus*), matki (dew gram; *Phaseolus aconitifolius*), chana (chickpea; *Cicer arietinum*), and vatana (garden pea; *Pisum sativum*) were collected from different vendors in Mumbai and its suburbs. Samples were brought directly to the laboratory at ambient temperature (26 to 28°C) and analyzed immediately.

**Chemicals and media.** Microbiological media used in the study were from Hi Media Laboratories, Mumbai, India. Blood used for blood agar was obtained from human volunteers. Rabbit plasma, *E. coli* O antiserum O157, and *E. coli* H antiserum H7 were obtained from Becton Dickinson (Sparks, Md.).

**Microbiological analysis.** Microbiological analysis was performed per standard methods adopted from the online *Bacteriological Analytical Manual*, U.S. Food and Drug Administration, for the detection, enumeration, and identification of individual organisms (20). *Salmonella* Typhimurium MTCC 98 (obtained from the Microbial Type Culture Collection, Chandigarh, India), *L. monocytogenes* NCAIM-B-01442 (an avirulent strain, kindly supplied by Dr. Cs. Mohacsi-Farkas, Szent Istvan University, Budapest, Hungary), *E. coli* ATCC 35218, and *S. aureus* FM002 (a coagulase-positive strain isolated from poultry meat) were used as standards for biochemical tests.

**Aerobic plate count (APC).** Twenty-five grams of the sample was homogenized in 225 ml of sterile physiological saline. After appropriate serial dilutions, the samples were poured on plate count agar. The colonies were counted after 24 h of incubation at 35°C.

**Coliform count.** Appropriate dilutions were poured plated on violet red bile agar and the medium was solidified, it was overlaid with violet red bile agar. Typical dark red colonies were counted after 24 h of incubation at 35°C.

**Yeast and mold count.** The yeast and mold counts were determined on potato dextrose agar by the spread plate technique. The colonies were counted after 48 h of incubation at room temperature (~26°C).

**Staphylococci count.** The spread plate technique was used to determine the staphylococci counts on Baird-Parker agar. The colonies were counted after 48 h of incubation at 35°C.

**Salmonella.** Twenty-five grams of each sample was homogenized in lactose broth and incubated overnight at 35°C. After the initial preenrichment step, the samples were further enriched in tetraionate broth and Rappaport-Vassiliadis medium at 42°C for 24 h. A loopful of culture from each of these media was streaked onto bismuth sulfite agar, xylose lysine deoxycholate agar, and Hektoen enteric agar. Both typical and atypical colonies from each of these plates were picked, purified, and identified by subsequent biochemical tests. Isolates confirmed by biochemical tests were further serotyped at the Salmonella and Escherichia Centre, Central Research Institute, Kasauli, India.

**RESULTS AND DISCUSSION**

A total of 124 sprout samples (46 mung, 27 matki, 25 chana, and 26 vatana) were analyzed. The results of the APCs, coliform counts, yeast and mold counts, and staphylococci counts of sprouts are shown in Figure 1. Counts from the four varieties of sprout samples were not significantly different (*P < 0.05*). APCs were in the range of 7.6 to 8.9 log CFU/g. Similar values for APCs (7 to 9 log CFU/g) were observed in mixed sprouts by Prokopowich and Blank (11). Rajkowski et al. (13) have reported APCs to be between 6 and 7 log CFU/g in broccoli sprouts. Analysis of seeds showed that APCs were <10^2 CFU/g (data not shown). This naturally occurring population can rapidly increase during germination and sprouting because of the favorable conditions for bacterial growth. Coliforms constituted almost 90% of the bacterial flora, ranging from 5.4 to 7.9 log CFU/g, and may have come from a variety of sources, such as the soil in which the seeds were grown,
The water used for washing the sprouts, or the people involved in making and distributing the sprouts.

The yeast and mold counts ranged from 3.6 to 7.3 log CFU/g; the main sources of yeast and mold contamination could be air and dust. Staphylococci counts ranged from 3.3 to 6.6 log CFU/g, indicating unhygienic handling.

Contamination of fresh produce could occur from any combination of the following: contaminated seeds, contaminated equipment, contaminated water sources, or poor hygienic handling (8). Salmonella, L. monocytogenes, nonpathogenic E. coli, and coagulase-positive S. aureus were detected in the samples analyzed (Table 1). These pathogens and nonpathogenic E. coli might be introduced into sprouts from seeds, water used during production, and improper sanitation during production and marketing (15). All of the samples analyzed were presumptive positive for Salmonella, nonpathogenic E. coli, and S. aureus; however, only 31% of Salmonella, 16% of nonpathogenic E. coli, and 2% of S. aureus isolates were confirmed by biochemical analysis (Table 1). Pathogenic E. coli O157:H7 was not detected in any of the samples analyzed. Of 62 Salmonella isolates showing typical biochemical characteristics, 24 were further confirmed by serology. Twenty-two isolates were confirmed as Salmonella Typhimurium, 1 isolate was confirmed as Salmonella Dublin, and 1 isolate was confirmed as Salmonella Washington. Nonpathogenic E. coli is common in the normal microflora of the intestinal tracts of humans and other warm-blooded animals; sprouts can get contaminated by this bacterium in the field or during handling. A total of 13% of the mung, 26% of the matki, 40% of the chana, and 19% of the vatana samples were positive for nonpathogenic E. coli (Table 2). The majority of reported outbreaks due to the consumption of sprouts and other minimally processed foods were related to Salmonella (17). There are more than 2,500 serovars of Salmonella, and all of them are considered pathogenic. Salmonella can be introduced from the seeds used for sprouting or during processing. It can survive and grow at storage temperatures greater than 8°C. Also, this pathogen has been shown to grow on the surface of alfalfa sprouts (7, 10). Among the four different sprout samples tested, Salmonella Typhimurium was detected in 21% of the mung, 40% of the matki, and 4% of the chana samples. Salmonella Dublin was detected in 2% of the mung samples, and Salmonella Washington was detected in 4% of the matki samples (Table 2). L. monocytogenes is a saprophyte that lives in a plant-soil environment and could therefore be contracted by humans and animals through many possible routes from many sources (2). L. monocytogenes, a psychrotroph, can grow at temperatures as low as 2°C (14). Risk of listeriosis is increased when products are stored for longer periods before consumption. L. monocytogenes was not detected in any of the samples analyzed. Other species of Listeria were detected: L. grayi was found in 4% of the mung and 3% of the matki samples, L. innocua was detected in 7% of the matki and 4% of the chana samples, and L. welshimeri was detected in 2% of the mung samples (Table 1). S. aureus is known to be carried in the nasal passages of healthy food handlers; contamination by this bacterium can occur as a result of unhygienic conditions. Coagulase-positive S. aureus was detected in 4% of the mung, 11% of the matki, and 4% of the chana samples (Table 2). The conditions under which seeds are sprouted are ideal for bacterial proliferation, an increase of more than 1,000-fold can occur, and bacterial numbers may exceed 10⁷ CFU in sprouted seeds per g without affecting the appearance of the product (11, 18). Sprouts have been identified as a special problem because of the potential for pathogen growth during the sprouting process (8). Good agricultural practices, such as seed cleaning, treatment with one or more methods, storage, and handling, along with hazard analysis and critical control point systems, are recommended for sprouts (8). None of the chemical treatments currently used for the disinfection of fruits, vegetables, and sprouts are sufficient to ensure complete elimination of pathogens, and this has resulted in a number of outbreaks (9, 23). Our study indicates that market sprouts are of poor microbiological quality and that they also harbor pathogens. Consumption of raw sprouts sold in Mumbai and its suburbs may lead to foodborne disease. Therefore, some sort of processing, such as

### TABLE 1. Total number of pathogens identified up to biochemical levels

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Presumptive positive</th>
<th>Confirmed positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella</td>
<td>198</td>
<td>62&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>E. coli&lt;sup&gt;c&lt;/sup&gt;</td>
<td>216</td>
<td>34</td>
</tr>
<tr>
<td>S. aureus (coagulase positive)</td>
<td>284</td>
<td>6</td>
</tr>
<tr>
<td>E. coli O157:H7</td>
<td>ND&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ND</td>
</tr>
</tbody>
</table>

<sup>a</sup> Of 62 isolates showing typical biochemical characteristics of Salmonella, 22 were identified as Salmonella Typhimurium, 1 as Salmonella Dublin, and 1 as Salmonella Washington by serology.

<sup>b</sup> L. grayi in 4% of the mung and 3% of the matki samples, L. innocua in 7% of the matki and 4% of the chana samples, and L. welshimeri in 2% of the mung samples.

<sup>c</sup> Nonpathogenic E. coli.

<sup>d</sup> ND, not detected.

### TABLE 2. Percentage of samples positive for pathogens analyzed

<table>
<thead>
<tr>
<th>Samples (no.)</th>
<th>Salmonella&lt;sup&gt;a&lt;/sup&gt;</th>
<th>L. monocytogenes</th>
<th>E. coli&lt;sup&gt;b&lt;/sup&gt;</th>
<th>S. aureus (coagulase positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mung (46)</td>
<td>23</td>
<td>0</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>Matki (27)</td>
<td>44</td>
<td>0</td>
<td>26</td>
<td>11</td>
</tr>
<tr>
<td>Chana (25)</td>
<td>4</td>
<td>0</td>
<td>40</td>
<td>4</td>
</tr>
<tr>
<td>Vatana (26)</td>
<td>0</td>
<td>0</td>
<td>19</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Salmonella Typhimurium in 21% of the mung, 40% of the matki, and 4% of the chana samples; Salmonella Dublin in 2% of the mung samples, and Salmonella Washington in 4% of the matki samples.

<sup>b</sup> Nonpathogenic E. coli.
radiation processing, which is effective in killing nonsporulating bacterial pathogens and has a minimum effect on freshness, texture, and nutrients, needs to be used for the hygienization of sprouts to ensure their safety.

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REFERENCES