QUANTIFICATION AND MOLECULAR CHARACTERIZATION OF SALMONELLA ISOLATED FROM FOOD SAMPLES INVOLVED IN SALMONELLOSIS OUTBREAKS IN RIO GRANDE DO SUL, BRAZIL

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ABSTRACT

Data concerning the prevalence and populations of Salmonella in foods implicated in outbreaks may be important to the development of quantitative microbial risk assessments of individual food products. In this sense, the objective of the present study was to assess the amount of Salmonella sp. in different foods implicated in foodborne outbreaks in Rio Grande do Sul occurred in 2005 and to characterize the isolated strains using phenotypic and genotypic methods. Nineteen food samples involved in ten foodborne outbreaks occurred in 2005, and positive on Salmonella isolation at the Central Laboratory of the Health Department of the State of Rio Grande do Sul, were included in this study. Food samples were submitted to estimation of Salmonella using the Most Probable Number (MPN) technique. Moreover, one confirmed Salmonella colony of each food sample was serotyped, characterized by its XbaI-macrorestriction profile, and submitted to antimicrobial resistance testing. Foods containing eggs, mayonnaise or chicken were contaminated with Salmonella in eight outbreaks. Higher counts (>10^7 MPN.g^-1) of Salmonella were detected mostly in foods containing mayonnaise. The isolation of Salmonella from multiple food items in five outbreaks probably resulted from the cross-contamination, and the high Salmonella counts detected in almost all analyzed samples probably resulted from storing in inadequate temperature. All strains were identified as S. Enteritidis, and presented a unique macrorestriction profile, demonstrating the predominance of one clonal group in foods involved in the salmonellosis outbreaks. A low frequency of antimicrobial resistant S. Enteritidis strains was observed and nalidixic acid was the only resistance marker detected.

Key-words: Salmonella, foodborne outbreak, quantification, PFGE.

INTRODUCTION

In Southern Brazil, a high prevalence of Salmonella isolation has been found in pigs (2), pork (6) and pork products (24). In opposite to that, pork is rarely involved in salmonellosis outbreaks reported in this region (8,25). Data collected in Rio Grande do Sul, during the period of 1997 to 1999, pointed salad prepared with homemade mayonnaise as the most often implicated food in salmonellosis outbreaks, accounting for 42.45% of all identified food vehicles (8). Factors responsible for the discrepancy between Salmonella prevalence in pork and the frequency of foodborne outbreaks attributed to pork consumption need to be better investigated.

It is well documented that exposure to larger quantities of foodborne pathogens usually results in a greater risk to human health (14,18,34). Consequently, the final concentration of Salmonella in food is an important parameter contributing to overall disease risk. In this sense, data on the prevalence and
populations of *Salmonella* in foods implicated in outbreaks may be important to the development of quantitative microbial risk assessments of individual products.

Another aspect contributing to the understanding of *Salmonella* epidemiology is the characterization of the strains involved in outbreaks. Serotyping is traditionally conducted as a first approach in the characterization and usually forms the background for all other typing methods (28). Over the past two decades genotyping has been associated to the traditional typing methods to achieve a better discrimination of strains and to identify bacterial clones (28). Among the molecular methods, pulsed-field gel electrophoresis (PFGE) is considered the standard method for DNA fingerprinting in *Salmonella*, and has been performed to investigate salmonellosis outbreaks (11,16,17,21,40).

In this sense, the objective of the present study was to assess the amount of *Salmonella* sp. in different foods implicated in foodborne outbreaks in Rio Grande do Sul occurred in 2005 and to characterize the isolated strains using phenotypic and genotypic methods.

**MATERIAL AND METHODS**

Food samples

Nineteen food samples involved in ten foodborne outbreaks occurred in Rio Grande do Sul in 2005, and positive for *Salmonella* isolation at Laboratório Central da Secretaria Estadual da Saúde (Central Laboratory of State Health Department, LACEN/RS, Porto Alegre, Rio Grande do Sul) were included in this study. Food samples were stored during the analysis period in sterile flasks at 4ºC. Data available for each confirmed salmonellosis outbreak were obtained from the epidemiological investigation report received with the food samples by LACEN.

*Salmonella* quantification and serotyping

Food samples were submitted to estimation of *Salmonella* using the Most Probable Number (MPN) technique as previously described (4) with modifications. From each positive sample, 25 g were added to 225 mL of Buffered Peptone Water (BPW). The samples were homogenized for 1 min (Stomacher, Interscience, St. Nom, France) and decimal dilutions up to 10⁸ were prepared in BPW. Triplicate tubes of all dilutions were incubated at 35ºC for 18 h. From each dilution tube, aliquots of 0.1 mL were transferred to 9.9 mL of Rappaport-Vassiliadis broth (Merck, Darmstadt, Germany). Following incubation on the selective enrichment media at 42ºC for 24 h, samples were streaked onto XLT4 (Difco, Sparks, USA) agar. After 24 h incubation at 37ºC, suspected colonies from each plate were confirmed as *Salmonella* by biochemical tests and agglutination using poly O-antisemur (Probac, São Paulo, Brazil). The number of tubes in each dilution, from which colonies were confirmed as *Salmonella*, was used to estimate *Salmonella* counts using the MPN table (1). One confirmed *Salmonella* colony of each food sample was serotyped at Fundação Oswaldo Cruz (Brazilian Salmonella Reference Institute, Rio de Janeiro, Brazil).

Macrorestriction analysis

Genomic DNA of one *Salmonella* isolate obtained from each positive food sample was extracted as previously described (22,36). Slices of DNA-containing agarose plugs were digested with 20 units of *XbaI* (Promega, Madison, USA) at 37ºC for 18 h. The respective fragments were separated by pulsed field gel electrophoresis (PFGE) in 1% PFGE-certified agarose gel (BioRad, Hercules, USA) in a CHEF DR II system (BioRad, California, USA) at 5.6 V.cm⁻¹ with 0.5 × TBE as the running buffer. In order to avoid the DNA degradation of *Salmonella* isolates, 50 μmol of Thiouire (Acros Organics, Geel, Belgium) was added to the running buffer. The pulse times were increased from 10 to 30 s during the first 11 h and subsequently from 30 to 50 s during the next 13 h. The gel was stained with ethidium bromide (2 mg/mL, Sigma, St. Louis, USA) and photographed under UV-illumination. Patterns produced by PFGE were compared using the GelCompar II software package (Applied Maths, Kortrijk, Belgium).

Antimicrobial susceptibility testing

Antimicrobial resistance was determined by agar disk diffusion tests using disks with the following antimicrobials (Cefar Diagnóstica, São Paulo, Brazil): amikacin (30 μg), ampicillin (10 μg), cefaclor (30 μg), ciprofloxacin (5 μg), chloramphenicol (30 μg), gentamicin (10 μg), nalidixic acid (30 μg), tetracycline (30 μg), tobramycin (10 μg), streptomycin (10 μg), sulfamethoxazole-trimethoprim (23.75/1.25 μg), and sulfonamide (300 μg). The testing was conducted and evaluated according to the document M100-S15 of the Clinical and Laboratory Standards Institute (7). *Escherichia coli* ATCC 25922 was used for quality control testing.

**RESULTS**

In eight salmonellosis outbreaks analyzed in the present study, a total of 212 people were exposed and 15 patients needed hospitalization. In two outbreaks, epidemiological data regarding the city of origin and/or the number of exposed people were not available (Table 1). The major symptoms observed were fever, diarrhea and abdominal pain, while nausea and vomiting were reported in six outbreaks. The median incubation period varied from 9 to 24 hours, and in most part of the outbreaks, symptoms appeared between 13 and 17 hours after the food ingestion (data not shown).

Foods containing eggs, mayonnaise or chicken were contaminated with *Salmonella* in eight outbreaks (Table 1).
Characterization of *Salmonella*

Higher counts (>10⁷ MPN.g⁻¹) of *Salmonella* were detected mostly in foods containing mayonnaise. Other food items with high counts were the cake in outbreak #8, and the pork sausage in outbreak #4. In five outbreaks more than one food item were positive on *Salmonella* isolation. In these cases, food items with low as well as high *Salmonella* counts were detected in a same outbreak.

All fourteen *Salmonella* strains submitted to serotyping were classified as *S. Enteritidis* and resulted in a single PFGE profile (Fig. 1). Six *S. Enteritidis* strains were resistant only to nalidixic acid, while the remaining eight strains were sensible to all tested antimicrobials (data not shown).

### DISCUSSION

In the present study we analyzed foods collected in ten confirmed salmonellosis outbreaks investigated in Rio Grande do Sul in the year 2005. These outbreaks probably represent a small fraction of all salmonellosis cases that occurred in this region, since the lack of reporting to the sanitary authority and failure on identification of the responsible food is not a rare event in foodborne disease surveillance (35).

Salmonellosis has been the most important foodborne disease, in Rio Grande do Sul, since 1993 (8,25). In most salmonellosis outbreaks investigated in Brazil (8,25,30) and in

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Table 1. *Salmonella* quantification in foods involved in outbreaks in Rio Grande do Sul, Brazil, in 2005.

<table>
<thead>
<tr>
<th>Outbreak#</th>
<th>City</th>
<th>Date (month/day)</th>
<th>Number of exposed</th>
<th>Number of hospitalizations</th>
<th>Involved food</th>
<th><em>Salmonella</em> serovar</th>
<th><em>Salmonella</em> quantification MPN.g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alecrim</td>
<td>01/24</td>
<td>5</td>
<td>0</td>
<td>Potatoes with mayonnaise</td>
<td><em>Enteritidis</em></td>
<td>4.6x10⁹</td>
</tr>
<tr>
<td>2</td>
<td>Camaquã</td>
<td>02/21</td>
<td>5</td>
<td>0</td>
<td>Chicken</td>
<td><em>Enteritidis</em></td>
<td>1.1x10⁵</td>
</tr>
<tr>
<td>3</td>
<td>Frederico Westphalen</td>
<td>04/19</td>
<td>10</td>
<td>0</td>
<td>Cake</td>
<td><em>Enteritidis</em></td>
<td>2.4x10⁷</td>
</tr>
<tr>
<td>4</td>
<td>Teutônia</td>
<td>04/23</td>
<td>9</td>
<td>1</td>
<td>Potatoes with mayonnaise</td>
<td><em>Enteritidis</em></td>
<td>2.4x10⁷</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sausage</td>
<td><em>Enteritidis</em></td>
<td>3.6x10⁷</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chicken</td>
<td><em>Enteritidis</em></td>
<td>4.6x10⁶</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pork</td>
<td>-</td>
<td>&lt;3</td>
</tr>
<tr>
<td>5</td>
<td>Ipê</td>
<td>04/25</td>
<td>6</td>
<td>3</td>
<td>Cassava with mayonnaise</td>
<td><em>Enteritidis</em></td>
<td>2.8x10⁸</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Roasted beef</td>
<td><em>Enteritidis</em></td>
<td>4.3x10⁷</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Turkey</td>
<td>-</td>
<td>&lt;3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chicken</td>
<td>-</td>
<td>&lt;3</td>
</tr>
<tr>
<td>6</td>
<td>ND</td>
<td>05/15</td>
<td>ND</td>
<td>ND</td>
<td>Roasted beef</td>
<td><em>Enteritidis</em></td>
<td>4.6x10⁵</td>
</tr>
<tr>
<td>7</td>
<td>São Pedro da Serra</td>
<td>10/16</td>
<td>160</td>
<td>3</td>
<td>Potatoes with mayonnaise</td>
<td><em>Enteritidis</em></td>
<td>1.1x10⁸</td>
</tr>
<tr>
<td>8</td>
<td>Santa Rosa</td>
<td>11/27</td>
<td>ND</td>
<td>ND</td>
<td>Cake</td>
<td><em>Enteritidis</em></td>
<td>1.5x10⁶</td>
</tr>
<tr>
<td>9</td>
<td>Palmeira das Missões</td>
<td>11/27</td>
<td>15</td>
<td>8</td>
<td>Cooked pea</td>
<td><em>Enteritidis</em></td>
<td>1.1x10⁵</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Minced meat</td>
<td>-</td>
<td>3x10⁶</td>
</tr>
<tr>
<td>10</td>
<td>Porto Alegre</td>
<td>12/28</td>
<td>2</td>
<td>0</td>
<td>Salami</td>
<td>-</td>
<td>&lt;3</td>
</tr>
</tbody>
</table>

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**Dice similarity Coefficient (%)**

<table>
<thead>
<tr>
<th>XbaI patterns</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
</tr>
<tr>
<td>E2</td>
</tr>
<tr>
<td>E3</td>
</tr>
</tbody>
</table>

*Figure 1.* XbaI-macrorestriction profiles identified in *Salmonella Enteritidis* strains isolated from foods involved in outbreaks in Rio Grande do Sul, Brazil in 2005.
In conclusion, high counts (>10³ MPN g⁻¹) of Salmonella were detected in most foods implicated in the reported salmonellosis outbreaks occurred in 2005 in Rio Grande do Sul. Our results suggest that one PFGE clonal group of S. Enteritidis was involved in all those salmonellosis outbreaks.

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RESUMO

Quantificação e perfil molecular de <i>Salmonella</i> isolada de alimentos envolvidos em surtos de salmonelose no Rio Grande do Sul

Dados sobre a prevalência e a população de <i>Salmonella</i> em alimentos implicados em surtos podem contribuir na condução de análises de risco. Dessa forma, o objetivo desse estudo foi determinar a quantidade de <i>Salmonella</i> esp. presente em alimentos implicados em surtos ocorridos no Rio Grande do Sul em 2005 e caracterizar os isolados por meio de técnicas fenotípicas e genotípicas. Dezenove amostras de alimentos obtidas em dez surtos ocorridos em 2005 e identificadas como positivas para <i>Salmonella</i>, dezenove amostras de alimentos obtidas em dez surtos ocorridos em 2005 e identificadas como positivas para <i>Salmonella</i>, dezenove amostras de alimentos obtidas em dez surtos ocorridos em 2005 e identificadas como positivas para <i>Salmonella</i>, foram submetidas à análise de risco. Dessa forma, o objetivo desse estudo foi determinar a quantidade de 

Palavras-chave: <i>Salmonella</i>, surtos, quantificação, PFGE

REFERENCES


