Incidence of microbial flora in lettuce, meat and Spanish potato omelette from restaurants

J. M. Soriano1*, H. Rico2, J. C. Moltó1 and J. Mañes1

A total of 370 samples including lettuce, meats (beef, pork and chicken) and Spanish potato omelette from restaurants were studied to evaluate the incidence of Escherichia coli, E. coli O157:H7, Staphylococcus aureus, Salmonella spp., Yersinia enterocolitica, enterococci and some micro-organisms that can cause spoilage or can be used as indicators for food safety. Escherichia coli and enterococci were harboured with the highest incidence in lettuce, whereas incidence of Staphylococcus aureus was higher in meat than in the other foods studied. Enterobacter cloacae and Klebsiella pneumoniae were isolated from the three food groups. Chryseomonas luteola, Enterobacter sakazakii, Klebsiella ozaenae, Moraxella spp. and Serratia odorifera were isolated from lettuce, whilst Providencia spp. were detected only in beef. Salmonella spp., E. coli O157:H7 or Yersinia enterocolitica were not isolated from any of the raw or 'ready-to-eat' samples analysed.

Introduction

Spain has more than sixty thousand restaurants with a production estimate of €1·32 X 1010 (Martín Cerdeño 1999). Some meals served in these establishments can be contaminated with foodborne micro-organisms due to incorrect food processing operations or to management practices common in agriculture (ICMSF 1980, Kaneko et al. 1999).

Several micro-organisms capable of causing human illness such as Escherichia coli, Salmonella spp. and Staphylococcus aureus have been isolated from vegetables (García-Villanova et al. 1987, Rodriguez de Lecea and Soto Estéras 1981, Soriano et al. 2000a), from meats, (Ferrer et al. 1992, Ubach et al. 1988, Soriano et al. 2000b) and from omelettes (Arnedo et al. 1998, Eisenberg et al. 1975, Soriano et al. 2000b). In these foods, other micro-organisms such as members of the genus Chryseomonas, Citrobacter, Enterobacter, Klebsiella, Moraxella and Serratia have also been found (García Arribas et al. 1982, Kaneko et al. 1999, King et al. 1991, Lund 1992, Membre and Kubaczka 1998, Soriano et al. 2000a, b, Stiles and Ng 1981, Stutz et al. 1991, Tapia de Daza and Díaz 1994, Zhao et al. 1997). Some members of the genuses Citrobacter, Enterobacter, Erwinia and Klebsiella are of vegetal and environmental origin (Mossel and Struijk, 1995), whilst Chryseomonas luteola and Moraxella spp. can cause spoilage by synthesising catabolic enzymes such as pectinolytic enzymes (Membre and Kubaczka, 1998).

The objective of this study was to determine the incidence of E. coli, S. aureus, enterococci and several micro-organisms in three food
groups: lettuce, meats and Spanish potato omelette (this product is a popular meal due to its high market demand and its formulation is characterized by the presence of potatoes, eggs, olive oil, onion and salt). The presence of certain micro-organisms can be used as an indicator of food safety and/or detection of incorrect processing.

Materials and Methods

Sampling

A total of 370 samples from 20 University restaurants in Valencia (Spain) were procured from September 1999 to March 2000 for bacteriological examination. Raw and ‘ready-to-eat’ lettuces were analysed in this study. The terms ‘raw’ and ‘ready-to-eat’ refer to lettuce obtained before and after sanitation with sodium hypochlorite, respectively. Meats such as pork, beef and chicken were obtained in both raw and ‘ready-to-eat’ forms, whilst Spanish potato omelette was regarded as a ‘ready-to-eat’ food. ‘Ready-to-eat’ foods were taken at the end stages of elaboration. These foods were selected in this study as the most consumed. The temperature of the studied foods was measured after the sampling with a Crison 638 Pt digital thermometer (Crison Instruments, Barcelona, Spain). The samples were collected and placed in sterilized plastic bags, transported in ice chests to the laboratory and cultured immediately upon arrival.

Microbiological methods

The samples studied (25 g) were weighed aseptically into sterile Stomacher bags, diluted with 225 ml buffered peptone water (BPW) (Oxoid, Unipath, Hampshire, UK) and homogenized in a Stomacher (Classic IUL, Barcelona, Spain). The homogenate from the sample preparation was used for the following plating and incubation procedures: to detect *E. coli*, BPW was subcultured in Brilliant Green Bile (2%) broth (BGB) (Oxoid) at 35°C for 24 h, the positive tubes were inoculated onto Rapid *E. coli* 2 agar (REC) (Sanofi Diagnostics Pasteur, Steenvoorde, France) and incubated at 37°C for 24 h. Non-violet and violet colonies were tested by oxidase test strips (Microkit Ibérica, Madrid, Spain), Gram reaction and identified using the API 20E test strips (Bio-Mérieux, Marcy l’Etoile, France). Violet colonies were presumptive *E. coli*. These isolated violet colonies on REC agar were inoculated onto sorbitol-MacConkey agar (SMAC) (Oxoid) at 37°C for 24 h. Colourless colonies on the SMAC agar were tested for agglutination using an *E. coli* O157:H7 latex agglutination test (Oxoid) (Blood and Curtis 1995).

Isolation and identification of *Salmonella*, *S. aureus* and enterococci (Lancefield group-D streptococci) were performed using the homogenate in BPW at 37°C for 24 h. *Salmonella* were analysed using selenite cystine broth (Oxoid) and Rappaport–Vassiliadis enrichment broth (Oxoid) for 24 h at 37 and 42°C, respectively, and the positive cultures were finally streaked onto Hektoen enteric agar (HEA) (Oxoid) and Brilliant Green agar (BGA) (Oxoid) and incubated 24–48 h at 37°C. The purified suspect colonies were identified using the API 20E system (Busse 1995). To detect *S. aureus*, BPW was subcultured in duplicate in Giolotti-Cantoni (GC) (Oxoid) supplemented with potassium tellurite solution 3.5% (Oxoid) and incubated at 37°C for 24–48 h, the positive tubes were spread onto Baird–Parker (BP) agar (Oxoid) supplemented with egg-yolk emulsion (Oxoid) at 37°C for 24 h. After incubation, the plates were examined for the typical black, convex colonies, with or without a light halo, and these were subcultured in tryptone soy agar (TSA) (Oxoid) at 37°C for 24 h. Colonies obtained on the last agar were examined microscopically, Gram stained, tested for catalase reaction and confirmed with an agglutination Staphytect Plus test (Oxoid) (Baird and Lee 1995). For enterococci, the procedure was continued by enrichment in azide dextrose broth (ADB) (37°C, 24 h) (Oxoid), and the positive tubes were kept on KF Streptococcus agar (Oxoid) at 37°C for 24 h. Suspect colonies were picked onto slants of bile esculin agar (Oxoid) at 37°C for 24 h. Colonies obtained on the last agar were examined microscopically, Gram stained, tested for catalase reaction and confirmed with an agglutination Staphytect Plus test (Oxoid) (Baird and Lee 1995). For *Yersinia enterocolitica*, the samples studied (25 g) were also weighed into sterile Stomacher bags, but were diluted instead with

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225 ml Yersinia-selective enrichment broth according to Osmer (Merck, Darmstadt, Germany). The homogenate was plated onto a Yersinia-selective enrichment agar base according to Shiemann (Oxoid). Broth and agar were supplemented with Yersinia selective solution (Oxoid) and incubated at 30 °C for 24 h. Colonies with typical Yersinia morphology (i.e., dark red centre and a transparent periphery halo on agar) were tested microscopically, Gram stained, tested for catalase reaction and identified using the API 20E system (de Boer 1992).

Results

The microbial incidence in raw and ‘ready-to-eat’ restaurant foods is presented in Table 1. The highest percentage of S. aureus (9.2%) was found in raw meat. Escherichia coli and enterococci were detected with the highest incidence (10% and 20%, respectively) in raw lettuce. Also, the detection of other members of the Enterobacteriaceae family was performed in the raw and ‘ready-to-eat’ samples studied. The highest percentage of Citrobacter freundii, Chryseomonas luteola, Enterobacter cloacae, Enterobacter sakazakii, Klebsiella pneumoniae, Klebsiella oxytoca, Klebsiella ozaenae, Moraxella spp. and Serratia odorifera was found in raw lettuce, whereas Providencia spp. were isolated only from raw meat (1.7%) (Table 1). No Salmonella spp., E. coli O157:H7 or Y. enterocolitica were detected in any of the samples studied.

The restaurants evaluated reported the use of sodium hypochlorite to sanitize lettuces and the use of this disinfectant reduced the incidence of all the micro-organisms studied. Similarly, in meat, a reduction in the percentages of the micro-organisms studied was observed when an adequate heating treatment was applied (Table 1).

The average of temperatures was 10.1–15.1 and 7.6–16.2 °C from raw and ‘ready-to-eat’ lettuce, respectively. For raw and ‘ready-to-eat’ chicken, beef and pork was 5.1–7.2 and 27.4–37.5°C, respectively and Spanish potato omelette was 22.4–31.8 °C.

Discussion

For lettuce, our study is in agreement with that of King et al. (1991), who showed a lower contamination of the end product than in the raw vegetables. Our results show a lower incidence than previous studies (Ercolani 1976, Fang et al. 1999, García-Villanova Ruiz et al. 1987, Rodríguez de Lecea and Soto Esteras 1981). Mossel and Struik (1995) suggested that Citrobacter spp., Enterobacter cloacae, Enterobacter sakazakii, Erwinia spp., Klebsiella pneumoniae and

Table 1. Incidence of microbial flora in raw and ‘ready-to-eat’ foods from restaurants

<table>
<thead>
<tr>
<th></th>
<th>Lettuce</th>
<th>Pork</th>
<th>Beef</th>
<th>Chicken</th>
<th>Spanish potato omelette</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw (n = 40)</td>
<td>RTE (n = 40)</td>
<td>Raw (n = 40)</td>
<td>RTE (n = 40)</td>
<td>Raw (n = 40)</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1(2.5%)</td>
<td>1(2.5%)</td>
<td>7(17.5%)</td>
<td>2(5%)</td>
<td>3(7.5%)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>4(10%)</td>
<td>2(5%)</td>
<td>4(10%)</td>
<td>2(5%)</td>
<td>1(2.5%)</td>
</tr>
<tr>
<td>Enterococci</td>
<td>8(20%)</td>
<td>6(15%)</td>
<td>5(12.5%)</td>
<td>2(5%)</td>
<td>5(12.5%)</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>8(20%)</td>
<td>4(10%)</td>
<td>2(5%)</td>
<td>2(5%)</td>
<td>4(10%)</td>
</tr>
<tr>
<td>Chryseomonas luteola</td>
<td>6(15%)</td>
<td>1(2.5%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>18(45%)</td>
<td>1(2.5%)</td>
<td>1(2.5%)</td>
<td>0(0%)</td>
<td>5(12.5%)</td>
</tr>
<tr>
<td>Enterobacter sakazakii</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>4(10%)</td>
<td>3(7.5%)</td>
<td>1(2.5%)</td>
<td>0(0%)</td>
<td>2(5%)</td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>2(5%)</td>
<td>1(2.5%)</td>
<td>3(7.5%)</td>
<td>1(2.5%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Klebsiella ozaenae</td>
<td>2(5%)</td>
<td>1(2.5%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Moraxella spp.</td>
<td>1(2.5%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Providencia spp.</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>2(5%)</td>
</tr>
<tr>
<td>Serratia odorifera</td>
<td>2(5%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
</tbody>
</table>

RTE: ‘ready-to-eat.’
Klebsiella oxytoca are of vegetal and environmental origin, whilst E. coli is of faecal origin. Consequently, E. coli can be an ideal indicator in the analysis of raw products, but the presence of other members of the Enterobacteriaceae family may not necessarily be associated with faecal contamination (Zhao et al. 1997, Mossel and Struijk 1995). In the same sense, Kaneko et al. (1999) assumed that intact vegetables are contaminated with non-faecal coliforms of soil origin and suggested that E. coli detected in the fresh vegetables might be derived from the environment of food factories from interior surfaces of equipment that are difficult to clean (Splitthoesser and Corlet 1980). Zhao et al. (1997) suggested that the faecal coliform test should be abandoned for food testing due to the frequent occurrence of faecal coliforms in leaves and other foods of plant origin.

In meat, our Staphylococcus aureus findings (Table 1) are in agreement with those percentages found in 'ready-to-eat' samples (2%) (Ubach et al. 1988), although higher incidences of E. coli (Arranz Santamaria et al. 1995, Ferrer et al. 1992) and enterococci (Di Noto et al. 1990, García Arribas et al. 1982) in meat have been reported in the literature. García Arribas et al. (1982) isolated micro-organisms of the genus Enterobacter, Klebsiella and Citrobacter from fried, cooked and raw meat in school restaurants. Stiles and Ng (1981) have pointed out that Klebsiella pneumoniae is a better indicator for sanitization and hygiene of meats at the retail level than E. coli, due to the fact that K. pneumoniae has a short survival time in meats and its detection could indicate recent contamination. Leclerc et al. (1996) have shown that the presence of enterococci cannot be considered a faecal indicator in these foods whilst Reichert et al. (1988) suggested that for preserved meat products, Enterococcus faecalis is the indicator micro-organism in heat treatment, since this is the most heat resistant among potentially harmful vegetative bacteria.

For Spanish potato omelette, a slight reduction was observed for S. aureus, E. coli, enterococci, Klebsiella pneumoniae, K. oxytoca, Citrobacter freundii and Enterobacter cloacae when compared with a previous study in this sample (Soriano et al. 2000b). Also, the prevalence of E. coli in our study was lower than that obtained by other authors (Arranz Santamaria et al. 1995, Ferrer et al. 1992).

At the moment, an improvement in the hygienic practices of the food handlers, together with an implementation of the hazard analysis and critical control point (HACCP) system (Mañes and Soriano 1999) is being carried out in the restaurants involved in this study. These measures have achieved a considerable reduction in the incidence of different micro-organisms in food with respect to previous works (Soriano et al. 2000a, b). However, the presence of some micro-organisms indicate either post-cooking contamination of the foods studied or that the length of time and temperature control in storage facilities was inadequate to prevent bacterial growth or, alternatively, improper management practices in agriculture, as part of the growing, harvesting, washing, sorting, packing and transporting procedures. Perhaps the use of indicators must be applied to improve food analysis and as a guarantee of food safety: the presence of E. coli indicates potential faecal contamination, the predominance of S. aureus indicate possible cross-contamination between food preparation, surfaces and the food itself and Klebsiella pneumoniae can be used as indicator of recent contamination. It is to be noted here that the HACCP system is well organized and applied in most of the restaurants studied, but a few of them are still not fully aware of the importance of food quality control. Hence, our objective remains to fully convince workers and management to apply the HACCP system as a procedure to ensure food safety.

Acknowledgements

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References

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Ercolani, G. L. (1976) Bacteriological quality assess-