The bacterial quality of red meat and offal in Casablanca (Morocco)

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The present study aimed to evaluate the bacteriological quality of beef (n = 52), lamb (n = 52) and beef offal (n = 52) marketed in Casablanca, Morocco. Meat and offal samples (n = 156), were collected randomly from butcheries, supermarkets, and slaughterhouses. Two sampling periods were considered, one during the hot season and the second one during the cold season. The samples were analyzed for the presence of the following bacteria: Escherichia coli, coagulase-positive Staphylococcus, Clostridium perfringens, Salmonella, and Listeria monocytogenes. Results indicated that counts of the aerobic plate count, and fecal coliforms were particularly high in all the samples analyzed. E. coli, coagulase-positive Staphylococcus and C. perfringens were detected in 37.8, 16, and 4.5% of the meat samples, respectively. Neither Salmonella nor L. monocytogenes were isolated from meat samples. Approximately 26.9% of beef, 34.6% of lamb and 28.8% of beef offal samples contained bacteria above the maximum limits established by the Moroccan regulatory standards for meat and meat products. Seasonality and the distribution location significantly (p < 0.05) affected bacterial populations: the hot season and butcheries appeared to be cases where the highest populations of bacteria in meat were observed. These high levels of microbiological contamination attest the poor hygienic quality of meat and offal, possibly due to uncontrolled processing, storage, and handling of these products.

Keywords: Bacterial quality / Offal / Red meat / Sampling location / Season

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1 Introduction

Recent data from either developing or developed countries indicate that, at least 10% of the population may experience a food borne disease. The situation is equally serious in developing countries [1], with obvious economic consequences [2]. In 2002, the Centers for Disease Control in the United States reported 76000 cases of food borne diseases, the majority being of bacterial origin [3]. In Europe, monitoring the impact of food borne disease mortality is not a major priority; however, 50000 cases of acute gastro-enteritis per million inhabitants annually are reported. In France, 559 cases of food borne diseases were reported in 2001, of which 64% were due to salmonellosis [4].

In Morocco, although several efforts have been made to improve food safety and quality, food borne diseases still represent one of the main causes of morbidity [5]. During the last 5 years (2000 to 2004), 7118 cases of food borne diseases have been reported among which 86% were of bacterial origin (Morocco foodborne disease outbreaks, searchable data 2000–2005. Yearly Reports 2000–2004). According to the same report, 21.3% of the bacterial food borne diseases was caused by red meat and meat products and 14.7% occurred in the city of Casablanca. Many factors have contributed to increased food borne diseases. Mass production, environmental factors, and inadequate knowledge on food handling have contributed to increased contamination of foodstuffs [1]. Other factors were also reported including increased consumption of fresh-chilled, minimally processed foods rather than highly processed foods, and dine-out and take-away meals as well as alternative cooking methods (Red meat safety and clean livestock. Food stand. agency, 2002). With highly perishable foodstuffs such as fresh red meat, the threat of food poisoning is particularly important [2]. Because of the nature of meat and the means by which it has to be obtained from the animal, some level of microbial contamination is inevitable, and may include pathogenic bacteria. With regard to micro-

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Abbreviations: SAS, Statistical Analysis Systems; APC, aerobic plate counts; cfu, colony forming units; GLM, general linear model
bacterial hazards, meat is generally a safe product when cooked and handled appropriately prior to consumption, but problems may arise when hygienic practices are not observed and any present food borne pathogens are allowed to survive and multiply. For this reason, any kind of raw meat is regarded as a high-risk category food and should be handled accordingly [6].

Red meat is an important part of the human diet. Moroccan official data reported that between 2000 and 2004, an average of 152,000 tons of beef, 116,000 tons of lamb, 51,000 tons of beef offal, and 47,000 tons of other red meats (goat, camel, and horse) are presumably produced yearly in Morocco, with more than 12% of red meat production in Casablanca (National Statistics Rapport for department of animal producing. Ministry of Farming, Morocco 2005).

The main studies performed on red meat and beef offal, focused on the evaluation of the hygienic quality [7–10]. None of these studies assessed the hygienic quality of raw red meat produced during various seasons and at different distribution locations. In order to provide such information, objectives of the present work were (i) to determine incidence and/or levels of pathogenic and nonpathogenic microorganisms present in red meat and beef offal produced in Casablanca, and (ii) to analyze the effect of seasonality and distribution location on the incidence of such microorganisms.

2 Materials and methods

2.1 Sample collection

Samples were collected between April 2002 and March 2004. A total of 156 raw meat and beef offal samples were randomly collected from butcheries (n = 18), supermarkets (n = 18), and slaughterhouses (n = 6) in Casablanca, Morocco. Samples of beef (n = 52), lamb (n = 52), and beef offal (n = 52) were collected. Two sampling periods were considered: a hot season (April to September) with temperatures varying between 25 and 39°C, and relative-moisture between 32 and 72%, and a cold season (November to March) with temperatures varying between 5 and 20°C and relative-moisture between 56 and 84%. At 2–week intervals, approximately 200 g of raw meat and beef offal were aseptically collected. All samples were sent to the laboratory in sterile bags at 4°C for ≤ 2 h. A portion (25 g) of each sample was placed into a separate sterile stomacher bag with 225 mL 0.1% sterile peptone water, and then pummeled with a MIX I mixer (AES Laboratory, Combourg, France). Samples were subsequently diluted serially in 0.1% sterile peptone water for bacterial analyses.

2.2 Bacteriological analysis

Viable cell counts were performed by the spread-plate method after tenfold serial dilutions in 0.1% w/v peptone solution as follows: (i) Aerobic total count (ATC) was carried out on plate count agar (PCA; Bio-Rad, Marnes la Coquette, France) and incubated at 30°C for 72 h. (ii) Fecal coliforms (FC) counts were carried out on Violet Red Bile Lactose Agar (Bio-Rad) incubated at 44°C for 24 h, typical colonies were considered as round, red-to-pink, 0.5–2 mm in diameter, surrounded with a red-to-pink halo. (iii) E. coli counts, on RAPID’E. coli Agar (Bio-Rad) incubated at 37°C for 18 to 24 h, typical E. coli colonies were considered as violet-to-pink. (iv) S. aureus on Baird-Parker agar with egg yolk-potassium tellurite emulsion plates (Bio-Rad) incubated at 35 ± 1°C for 24 to 48 h. Typical colonies (black surrounded by clear zones) were tested for coagulase activity using rabbit plasma after activation by overnight incubation in brain heart broth (Bio-Rad) at 35°C. (v) C. perfringens and other sulfite-reducing clostridia on tryptone-sulfite agar with Cyclocerine (Bio-Rad) incubated anaerobically at 44 ± 1°C for 24 to 48 h, followed by a confirmation on lactose-sulfite broth and thioglycolate with resazurin broth (Bio-Rad).

In addition to the above-mentioned enumerations, 25-g samples were analyzed for the presence or the absence of Salmonella spp, and L. monocytogenes using enrichment procedures. For confirmation of Salmonella, 25 g of each samples was homogenized with 225 mL of 0.1% peptone water and was incubated at 37°C for 24 h. Portions of 1 and 0.1 mL of this pre-enrichment culture were transferred, for enrichment, to 9 mL of Muller Kauffman with added Tetra-thionate broth, and to Rappaport Vassiliadis Soy enrichment broth (Bio-Rad), respectively. Inoculated enrichment media were incubated at 37 and 42°C, respectively. After 24 h of incubation, a loopful of each enrichment medium was streaked onto Xylose-Lysine-Desoxycholate agar (Bio-Rad), Edel and Kampelmarcher agar and Hektoen agar (Bio-Rad) and incubated at 35°C for 24 to 48 h. Viable cell counts were performed by the spread-plate method after tenfold serial dilutions in 0.1% w/v peptone solution as follows: (i) Aerobic total count (ATC) was carried out on plate count agar (PCA; Bio-Rad, Marnes la Coquette, France) and incubated at 30°C for 72 h. (ii) Fecal coliforms (FC) counts were carried out on Violet Red Bile Lactose Agar (Bio-Rad) incubated at 44°C for 24 h, typical colonies were considered as round, red-to-pink, 0.5–2 mm in diameter, surrounded with a red-to-pink halo. (iii) E. coli counts, on RAPID’E. coli Agar (Bio-Rad) incubated at 37°C for 18 to 24 h, typical E. coli colonies were considered as violet-to-pink. (iv) S. aureus on Baird-Parker agar with egg yolk-potassium tellurite emulsion plates (Bio-Rad) incubated at 35 ± 1°C for 24 to 48 h, followed by a confirmation on lactose-sulfite broth and thioglycolate with resazurin broth (Bio-Rad).

For confirmation of L. monocytogenes, a 25-g sample was homogenized in a sterile bag with 225 mL of Half Fraser broth (Bio-Rad) and incubated at 30°C for 24 h. A 1-mL portion from this pre-enrichment culture was transferred to 9 mL of enrichment broth (Complete Fraser broth) (Bio-Rad) and incubated at 35°C for 24 h. A loopful of the...
enrichment culture was streaked onto Palcam (Bio-Rad) and Oxford agars (AES Laboratory), and incubated at 35°C for 24 to 48 h. Typical colonies were characterized biochemically by the Listeria API commercial test-kit (Bio-Merieux) [12].

2.3 Data analysis

For each organism, duplicate plates were enumerated and the means calculated. All bacterial counts were expressed as log10 colony forming units (cfu) per g (log 10 cfu g –1 ). The mean log10 (X) value and SD were calculated on the assumption of a log normal distribution. Data for counts from beef, lamb, and beef offal were analyzed separately. Preliminary analysis of fixed effects for data from beef, lamb, and beef offal using the general linear model (GLM) procedure of Statistical Analysis Systems (SAS®) v.8.2 (SAS, 2002) indicated that populations were dependent on season and distribution location. Data for beef and lamb were separated by organism and evaluated using a 2 × 2 (distribution location × season, respectively) factorial design. Data for beef offal were separated by organism and evaluated using a 2 × 2 × 2 (distribution location × season × type, respectively) factorial design. For beef and lamb, individual fixed effects and up to two-way interactions were evaluated with ANOVA using the model y = a + x1 + x2 + x1x2 in the GLM procedures of SAS®, where x1 represents distribution locations, x2 represents season. For beef offal, individual fixed effects and up to three-way interactions were evaluated with ANOVA using the model y = a + x1 + x3 + x1x2 in the GLM procedures of SAS®, where x1 represents distribution locations, x2 represents season, and x3 represents type of beef offal. Least-squares means were separated using a protected pair wise t-test of SAS®. All differences were reported at a significance level of alpha = 0.05.

3 Results and discussion

3.1 General remarks

Microbiological profiles of beef, lamb and beef offal are reported in Tables 1, 2, and 3, respectively. Morocco has legislative microbiological safety criteria for foods, including raw meats [13]. For fresh red meat, acceptable upper limits are 2.5 log10 cfu g −1 for fecal coliforms, 2 log10 cfu g −1 for S. aureus, and 1.3 log10 cfu g −1 for C. perfringens. In addition, according to regulations, Salmonella and L. monocytogenes should be undetectable in a 25-g

Table 1. Microbiological profile of beef (mean log10 cfu g −1 ± SD)

<table>
<thead>
<tr>
<th>Distribution location</th>
<th>Slaughterhouse Season</th>
<th>Butchery Season</th>
<th>Supermarket Season</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot (n = 26)</td>
<td>Cold (n = 26)</td>
<td>Hot (n = 26)</td>
<td>Cold (n = 26)</td>
</tr>
<tr>
<td>Aerobic plate counts</td>
<td>5.2 ± 0.8a</td>
<td>5.1 ± 1.5a</td>
<td>7.3 ± 1.1b</td>
</tr>
<tr>
<td>Fecal coliforms</td>
<td>1.6 ± 0.6a</td>
<td>1.6 ± 0.9a</td>
<td>3.7 ± 1.0a</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>1.0 ± 0.0a</td>
<td>1.2 ± 0.5a</td>
<td>3.2 ± 1.3a</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>2.1 ± 0.3a</td>
<td>2.1 ± 0.3a</td>
<td>2.3 ± 0.4a</td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>1.0 ± 0.0a</td>
<td>1.0 ± 0.0a</td>
<td>1.3 ± 0.5a</td>
</tr>
</tbody>
</table>

a, b) Means in the same row with different superscript letters are different (p < 0.05).

Table 2. Microbiological profile of lamb (mean log10 cfu g −1 ± SD)

<table>
<thead>
<tr>
<th>Distribution location</th>
<th>Slaughterhouse Season</th>
<th>Butchery Season</th>
<th>Supermarket Season</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot (n = 26)</td>
<td>Cold (n = 26)</td>
<td>Hot (n = 26)</td>
<td>Cold (n = 26)</td>
</tr>
<tr>
<td>Aerobic plate counts</td>
<td>5.7 ± 1.0a</td>
<td>5.2 ± 1.3a</td>
<td>6.7 ± 0.9a</td>
</tr>
<tr>
<td>Fecal coliforms</td>
<td>2.0 ± 0.9a</td>
<td>2.1 ± 0.8a</td>
<td>3.7 ± 1.2a</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>1.2 ± 0.6a</td>
<td>1.3 ± 0.6a</td>
<td>3.2 ± 1.4a</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>2.1 ± 0.2a</td>
<td>2.0 ± 0.0a</td>
<td>2.8 ± 1.2a</td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>1.0 ± 0.0a</td>
<td>1.3 ± 0.5a</td>
<td>1.2 ± 0.6a</td>
</tr>
</tbody>
</table>

a, b) Means in the same row with different superscript letters are different (p < 0.05).
meat sample. There are no safety criteria concerning beef offal in the Moroccan standard regulations, however, for purposes of comparison reference is made to fresh red meat upper limits.

### 3.2 Aerobic plate counts

Microbiological quality may be considered a factor in determining meat quality. Aerobic plate counts (APC) are a widely accepted measure of the general degree of microbial contamination [14]. Meat is generally considered of poor quality if APC values exceed 2.5 log10 cfu g–1 in beef, 5.0 log10 cfu g–1 in lamb and 2.3 log10 cfu g–1 in beef, lamb and beef offal, respectively. In this study, mean APC values of 6.50 log10 cfu g–1 in beef, lamb and beef offal, respectively. In this study, mean of fecal coliform counts in beef collected in slaughterhouses (i.e. 1.6 ± 0.7 log10 cfu g–1) was in accordance with that reported by Karib [8] for beef collected in the slaughterhouse of Rabat. On the other hand, the results obtained for fecal coliforms in beef samples collected from butcheries (3.2 ± 0.6 log10 cfu g–1) were consistent with those reported by Oumokhtar et al. [9] for samples beef collected from butcheries in Rabat. The statistical analysis of the data obtained revealed that the effect of the sampling location on the numbers of fecal coliforms was highly significant (p < 0.05) whereas there appeared to be no seasonal effect. The average numbers of fecal coliforms in meat samples taken from butcheries or supermarkets were significantly (p < 0.05) higher than those recorded for meat samples taken from the slaughterhouses suggesting that the hygienic conditions and temperature in which the product was handled and stored after the slaughtering process may have been inadequate.

### 3.3 Fecal coliforms

The average numbers of fecal coliforms were 2.5 ± 1.2 log10 cfu g–1, 2.8 ± 1.0 log10 cfu g–1 and 2.3 ± 1.1 log10 cfu g–1 in beef, lamb and beef offal, respectively.
3 log unit) during the various stages of carcass handling and deboning process. Such increases in contamination become further aggravated with poor hygienic conditions during handling and processing. High counts of *E. coli* in foods are not always alarming because most strains are harmless and opportunistic in nature [6]. Nevertheless, the bacterium is well recognized as a fecal indicator that provides a fair estimate of the level of fecal contamination and the hygienic conditions during handling and processing [18]. Furthermore, some *E. coli* strains are pathogenic and have been associated with severe gastroenteritis through internalization, toxin production and interference with physiological functions [19]. Benkerroum *et al.* [20] have isolated shiga-toxin producing *E. coli* O157 at a frequency of 11.1% from meat products marketed in the city of Rabat.

### 3.5 Staphylococcus aureus

*S. aureus* has long been recognized as a food poisoning bacterium of concern to human health worldwide [21]. In the present study, the pathogen was isolated from 25 samples (16%) of the 156 meat and beef offal samples with a prevalence of 11.5, and 20.5% in the cold and the hot season, respectively. The average counts of *S. aureus* recorded in beef, lamb and beef offal were $2.1 \pm 0.4, 2.3 \pm 0.7$, and $2.1 \pm 0.5 \log_{10} \text{cfu g}^{-1}$, respectively. The counts of the pathogen in beef samples collected from slaughterhouses ($2.1 \pm 0.3 \log_{10} \text{cfu g}^{-1}$) were in agreement with those reported by Karib *et al.* [8] (*i.e.* $2.4 \pm 0.4 \log_{10} \text{cfu g}^{-1}$). Enumeration of *S. aureus* revealed that the count of the pathogen exceeded $5 \log_{10} \text{cfu g}^{-1}$ in 3 out of the 156 analyzed samples (0.02%). Such high level contamination with *S. aureus* has been associated with increased risk for staphylococcal food poisoning [22]. High contamination of food with *S. aureus* has been related to improper personal hygiene of employees [23] during handling and processing.

Neither season nor sampling location significantly ($p > 0.05$) affected the levels of contamination of red meat and beef offal samples with *S. aureus*.

### 3.6 Clostridium perfringens

The overall mean count of *C. perfringens* in meat and beef offal was $1.1 \pm 0.3 \log_{10} \text{cfu g}^{-1}$. However, individually, the count of *C. perfringens* exceeded the tolerable limit set by the Moroccan food safety regulations (*i.e.* $1.3 \log_{10} \text{cfu g}^{-1}$) in 7 (4.5%) of the 156 samples analyzed, all of which were taken at point-of-sale. Such counts were significantly ($p < 0.05$) affected by the season of sampling.

*C. perfringens* is one of the most widespread of all pathogenic bacteria in the environment, and is commonly found (although in low numbers) in the gastrointestinal tract of healthy animals, where from it generally contaminates animal carcasses during slaughtering [6]. Nonetheless, the most frequently involved anaerobic microorganisms involved in food spoilage and/or poisoning are sulfite-reducing clostridia. These microorganisms can grow inside the carcasses, thereby producing off-flavors and/or producing toxins [24].

Correlation analysis between the five group of microorganisms, namely APC, fecal coliforms, *E. coli*, *S. aureus* and *C. perfringens* indicated a high correlation between fecal coliforms and *E. coli* ($r = 0.92, p < 0.05$), and between APC and fecal coliforms ($r = 0.77, p < 0.05$).

### 3.7 Salmonella

*Salmonella* was not detected in any of the 25-g meat and beef offal samples. These results are in agreement with those reported previously [8, 9, 25]. In studies done by Madden *et al.* [26], and Bacon *et al.* [27], the prevalence of presumptive *Salmonella* spp. was reported to be 1.3 to 1.5%, respectively.

### 3.8 Listeria monocytogenes

*L. monocytogenes* was not detected in any of the 25-g meat or beef offal samples. Results from this study contrast with those of, Kriem *et al.* [28] who reported a 14.4% overall incidence of *L. monocytogenes* in red meat and meat products marketed in Rabat. Although, it is well established that refrigeration reduces or eliminates the risk of food borne diseases and is therefore required for the storage of many foods including meats [29, 30], *L. monocytogenes* is a typical cold-tolerant pathogen in meat [2]. It may result in serious and sometimes fatal diseases in humans, especially in immuno-compromised individuals. The incidence of human listeriosis in Morocco is low and there is no evidence implicating red meat as a vehicle of the pathogen in the reported cases (Morocco food borne disease outbreaks, searchable data 2000–2005. Yearly Reports 2000–2004). Despite the ubiquity of *L. monocytogenes*, its occurrence at high levels in fresh red meat is uncommon.

### 4 Concluding remarks

Results of this study indicated that bacterial counts in 30% of all tested samples were beyond the Moroccan safety limits. These cases were observed in samples taken from butcheries and supermarkets while all of those collected from slaughterhouses were below the regulatory standards. These results suggest that there may be a lack of adequate control
strategies during post-slaughter operations. Such inadequate controls may include: lack of good hygienic practices, inadequate sanitation during manufacturing, transport, storage, and post-production handling, as well as inadequate maintenance of adequate cold chain management during distribution. Consistent with other countries, Morocco is experiencing newer consumer trends in the culinary traditions with an obvious tendency to adopt international and further processed foods, some of which are uncooked or undercooked. Such a tendency combined with poor hygienic practices may contribute to increase the prevalence of pathogens in foods, thereby increasing the risk of food borne disease for consumers. Results obtained in this study provide evidence, which may be used by the Moroccan government to adopt regulations enforcing the application of the hazard analysis critical control points (HACCP) system as a means to identify and control the hazards in foods and especially in meat products. Furthermore, these results may promote the acceptance of programs such as HACCP by the meat industry in an attempt to provide safer and more wholesome products.

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5 References