Bacteriological Examination of Some Ready-To-Eat Foods in Faisalabad with Special Reference to *Listeria monocytogenes*

Iftikhar Hussain*, Muhammad Shahid Mahmood, Faisal Siddique and Ayesha Sarwar

Institute of Microbiology, University of Agriculture, Faisalabad-38040, Pakistan.

Corresponding Author: driftikharuaf@gmail.com

**ARTICLE HISTORY**
Received: November 10, 2014
Revised: January 06, 2015
Accepted: March 10, 2015

**Key Words:**
Daheebaray
Goal gappay
Fruit chat
Patties
Faisalabad

**ABSTRACT**
Rapid population, urbanization and changing life style are the major factors to used Ready-to-Eat (RTE) foods and numerous inhabitants meet their daily nutritional needs. This situation may be a risk for consumers due to microbial contamination in RTE foods. The microbiological quality as well as quantity of ready-to-eat food products sold in different streets and canteens in the city of Faisalabad was judged. A total of one hundred and twenty RTE food samples, including Daheebaray, Goal gappay, Fruit chat and Patties were collected from different canteens and streets in Faisalabad city between March and August, 2010. The most prevalent bacterial such as *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes* were isolated and counted. The viable bacterial counts were $7.4 \times 10^4$ cfu mL$^{-1}$, $3 \times 10^4$ cfu mL$^{-1}$, $2.5 \times 10^4$ cfu mL$^{-1}$ and $4 \times 10^4$ cfu mL$^{-1}$ for Daheebaray, goal gappay, fruit chat and patties, respectively. Precautionary measures and sanitary conditions were improved for the outbreak of food-borne diseases by the consumption of these RTE foods.

All copyright reserved to Mr.Scholar


**INTRODUCTION**

Ready-to-Eat (RTE) foods are describes as the foods being ready for immediate consumption at the point of sale. RTE foods could be raw or cooked, hot or chilled and can be consumed without further heat treatment (Tsang, 2002). Different terms have been used to describe such foods; these include convenient, ready, instant and fast foods e.g., pastries, sausages, rolls, burgers, salad, fried meat, fried chicken, milk and milk products (Caserani and Kinston, 1974). Increased consumption of RTE foods result in food-borne illness (Sivapalasingam et al., 2004). Microbiological studies carried out on RTE foods in several developing countries reported high bacterial
counts (Bryan et al., 1997, Umoh and Odoba, 1999). Food-borne bacterial pathogens commonly detected in RTE foods including E. coli O157:H7, Salmonella spp., Listeria monocytogenes, campylobacter, Clostridium perfringens, Bacillus cereus, Staphylococcus aureus (Mosupye and Von Holy, 1999). RTE foods have also been implicated in outbreaks of food-borne diseases. In 1981 a cholera epidemic in Pune city, India was attributed to contaminated sugar juice with added ice (WHO, 1996). In Senegal more than 200 cases of food poisoning were reported by RTE foods prepared by dairy products (Dawson and Canet, 1991). Between 1992-1997 in England and Wales, there were 20 reported outbreaks of food poisoning attributed to consumption of contaminated cold meat from catering premises with 573 people affected in months of June to August. Cold meat consumption resulted in salmonella outbreak (Little et al., 2000). Microbiological quality of fruit chat assessed in Patiala city of India showed food borne illness in consumers (WHO, 1998). Standard techniques have been used to access the microbiological quality of RTE foods including spread plate technique which separates duplicate APC and Gram Staining (GS) (Mosupye and Von Holy, 1999). The consumption of RTE foods has increased dramatically in recent years. It is important to know the type and number of different bacteria contaminating the RTE foods. The objective of this work was to estimate bacterial concentration levels in various RTE foods through Aerobic plate count method on nutrient agar as well as on selective media.

**MATERIAL AND METHODS**

**Collection of samples:** A total 120 sample of various types RTE foods were collected from different canteens and street in Faisalabad from March to August, 2010. Among them, there were 30 samples of Daheebaray, 30 samples of goal gappay, 30 samples of fruit chat and 30 were of patties. The 120 samples which collected from different streets and school canteens were also grouped on basis of their major component from which these were made i.e., 30 samples were mainly composed of milk and milk products, 30 samples were mainly composed of meat and meat products and 30 samples were mainly composed of salads and vegetables.

**Samples preparation:** All the samples were collected and shifted to the diagnostic laboratory in the Department of Microbiology, University of Agriculture Faisalabad and kept at room temperature. Ten grams of each sample was homogenized with 5 mL normal saline (NS). Serial dilutions were performed as required. Standardize the dilution by selecting that plate which gave dilution within range of 30-300 colonies. That was dilution fourth (4th dilution.)

**Aerobic plate count:** After homogenization, the sample for aerobic plate count was serially diluted in normal saline to \(10^{-2}\), the \(10^{-1}\) to \(10^{-4}\) dilutions were plated on plate count agar (Oxide, Basingstoke UK). The plates were incubated at 37°C for 24-48 h. Counts were made using manufacture instructions and reported on colony forming units (cfu g\(^{-1}\)).

**Isolation and identification of E.coli:** After homogenization, the sample was serially diluted in normal saline to \(10^{-4}\). Using a sterile pipette, 0.5 mL of \(10^{-4}\) dilution was spread on Nutrient agar (Oxide, Basingstoke, UK). The plates were then allowed to dry before being incubated at 37°C for 18-24 h. the off white colored colonies were identify as presumptive E. coli and confirmed using MacConkey’s agar (Oxide, Basingstoke, UK).

**Isolation and identification of Salmonella species:** The homogenate in normal saline was incubated for 18-24 h at 37°C followed by selective enrichment of 1 ml in 9 mL of Selenite tetrathionate broth. The tetrathionate broth was incubated at 37°C for 18-24 h. The broths were then sub cultured onto Salmonells-Sheigella Agar (SSA) and incubated at 37°C for 24-48 h (Oxide, Basingstoke, UK). Presumptive positive colonies (non-lactose fermenting with suitable colony morphology) were then confirmed by using IMVIC and Triple Sugar Iron (TSI) test (API, Biomerieux, Marcy L’Etoile, and France).
Isolation and identification of *Staphylococcus aureus*: Using a sterile pipette 0.5 mL of the 10⁻⁴ dilution was spread onto a plate of Staph 110 Agar (Oxoid, Basingstoke, UK). The plates were then allowed to dry before being incubated at 37°C for 48 h. The plates were then examined for typical *Staph aureus* colonies (golden yellow, shiny, convex colonies with a narrow zone of opacity surrounded by a zone of clearing). The presence of *Spathaureus* was confirmed by the catalase and coagulase tests (Pro-Lab Prolex Staph kit, Neston, Wirral, UK).

Isolation and Identification of *Listeria monocytogenese*: Ten grams samples were homogenized within 20 mL of Listeria pre enrichment broth (Oxoid) and incubated for 36-48 h at 30°C and then 0.5 mL of the 10⁻⁴ dilution was spread onto a plate of Tryptic Soy Agar-Yeast Extract (Difco) medium and incubated for 48 h at 30°C. motility test, Gram staining, catalase test, nitrate reduction test, rhamnose, xylose and mannitol fermentation, beta hemolysis activity and CAMP test was used for identification of *Listeria monocytogenese*. *L. monocytogenes* isolates were typified by using *Listeria O* antiserum type 1 and type 4 (Difco) (Gebretsadik et al., 2011).

### RESULTS AND DISCUSSIONS

The total of 120 samples were randomly selected from different streets and schools canteens around district Faisalabad. The samples were subjected to bacteriological examination (cultural, morphological and biochemical test presented in Table 1) to check the presence of different food-borne pathogens including *E. coli*, Salmonella spp., *Staphylococcus aureus* and *Listeria monocytogenese*. Distribution of these microorganisms were 50%, 40%, 40% and 46% (*E. coli*), 17%, 20%, 21% and 18% (Salmonella spp.), 17%, 22%, 16% and 20% (*Staphylococcus aureus*) and 16%, 18%, 25% and 16% (*Listeria monocytogenese*) in Daheebaray, Goal gappay Fruit chat and Patties respectively as denoted in Fig. 1 and Fig. 2. Some other researchers reported that 96%, 81%, 71% and 65% *E. coli*, Salmonella spp. And *Staph aureus* respectively in different ready to eat food (Badrie et al., 2003; Gebretsadik et al., 2011). Among these microorganisms, *Listeria monocytogenes* is most important because it cause severe type of zoonotic disease. Mainly, the transmission of this disease due to contaminated food and food products. It has been reported in many countries including 13% Japan (Inou et al.,

### Table 1: Isolation identification and characterization of different microbes from ready to eat food

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Culture media</th>
<th>Incubation time (°C)</th>
<th>Cultural Colonies</th>
<th>Morphological Name of Tests</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>Mackonky agar</td>
<td>37</td>
<td>Pink color</td>
<td>Gram Negative Lactose fermentation</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Catalase</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Simmon’s Citrate Indole Production</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Urease</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lysine decarboxylation Voges-Prokauer</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TSI glucose</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TSI sucrose</td>
<td>Negative</td>
</tr>
<tr>
<td><em>Salmonella spp.</em></td>
<td>SSA, SCA</td>
<td>37</td>
<td>Black color</td>
<td>Gram Negative rods Rambose fermentation</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A methyl d mannoside Oxidase Indole Production</td>
<td>Negative</td>
</tr>
<tr>
<td><em>L. monocytogenese</em></td>
<td>PA</td>
<td>37</td>
<td>Greenish color</td>
<td>Gram Positive rods</td>
<td>Positive</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>Staph 110 agar</td>
<td>37</td>
<td>Whitish creamy color clusters shape</td>
<td>Coagulase</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hemolysis</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Oxidase</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Indole Production</td>
<td>Negative</td>
</tr>
</tbody>
</table>

SSA: Salmonella Shigella Agar, SCB: Selenite Cysteine Agar, PA: Palcam Agar
Fig. 1: Percentage (%) of bacterial isolates per gram in TRE foods.

Fig. 2: Microbial evaluation and distribution of RTE food products

Table 2: Aerobic plate count of different bacteria isolated from ready to eat food

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Microorganisms</th>
<th>Dahee Baray</th>
<th>Goal Gappay</th>
<th>Fruit Chat</th>
<th>Patties</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E. coli</td>
<td>7.4x10^7</td>
<td>2.5x10^7</td>
<td>2.5x10^7</td>
<td>2.5x10^7</td>
</tr>
<tr>
<td>2</td>
<td>Salmonella spp.</td>
<td>4x10^7</td>
<td>3x10^7</td>
<td>5x10^7</td>
<td>3x10^7</td>
</tr>
<tr>
<td>3</td>
<td>L. monocytogenes</td>
<td>2x10^4</td>
<td>4x10^4</td>
<td>4x10^4</td>
<td>5x10^4</td>
</tr>
<tr>
<td>4</td>
<td>S. aureus</td>
<td>3.5x10^8</td>
<td>4.8x10^4</td>
<td>2.5x10^4</td>
<td>3x10^4</td>
</tr>
</tbody>
</table>

2000), 19% Jordan (Awaisheh, 2010), 32% Spain (Capita et al., 2001), 34% Canada (Bohaychuk et al., 2006), 2.6% Ethiopia (Gebretsadik et al., 2011), 30.2% Korea (Baek et al., 2000), 17.8% Turkey (Kalender, 2012). We were further confirmed as type 1 and type 4 by using specific antisera. Bacterial count is associated with hygienic measures and type of food and utensils used for processing of these RTE foods. In developing countries such as Pakistan, Bangladesh etc., where food borne illness are common problem due to eating ready to eat foods. The bacterial load due to unhygienic measures, personal hygiene was observed 78% due to E. coli, 68% due to Salmonella spp. and 17.5% due to Staphylococcus aureus depicted in Table 2. This study correlates with past studies in which more microbial count was observed which 96.3% was for E. coli, 81.5% for Salmonella spp. 71.4% for Staphylococcus aureus (Badrie et al., 2003). Sudari et al. (1996) observed an outbreak due to consumption of iceberg lettuce which comes under category of salads and vegetables and in this study samples chosen was goal gappay and fruit chat. The major bacteria responsible for causig food-borne illness was Salmonella 81% which correlate with present study in which Salmonella isolated from goal gappay was 15.6% which was less than that salmonella isolated from leaf lettuce. In case of milk and milk products sample selected was Ice cream. (Hennessey et al., 1996) observed gastroenteritis due to consumption of Ice cream and pathogen detected was Salmonella (3%). This study correlates with present study in which Daheebaray was selected from category of milk and milk products in which Salmonella count was (6.41%)/g which was more than that isolated from Ice cream.

CONCLUSION

It is concluded that that RTE foods are cheap, economical but not healthy due to lack of hygienic measures, dirty utensils, vendor's hygiene etc. these factors contributing many species of bacteria but major pathogen are E. coli, Salmonella spp. and Staphylococcus aureus. Basic and main source of bacterial infection is poor hygienic measures and this problem may be solved by improving by supervision in food handling procedure, greater consumer education on transmission of enteric food borne diseases and food safety risks.

REFERENCE


