Food Microbiology 36 (2013) 57–62

Contents lists available at SciVerse ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Short communication

# Microbiological quality of chicken- and pork-based street-vended foods from Taichung, Taiwan, and Laguna, Philippines

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ARTICLE INFO

*Article history:*
Received 1 July 2012
Received in revised form
31 March 2013
Accepted 11 April 2013
Available online 25 April 2013

*Keywords:*
Street food
Ready-to-eat food
Food pathogens
Microbiological quality
Microbial hazard
Antimicrobial resistance

ABSTRACT

The microbiological quality of chicken- and pork-based street-food samples from Taichung, Taiwan's night markets (50) and Laguna, Philippines' public places (69) was evaluated in comparison to a microbiological guideline for ready-to-eat foods. Different bacterial contamination patterns were observed between 'hot-grilled' and 'cold cooked/fried' food types from the two sampling locations with 'hot grilled' foods generally showing better microbiological quality. Several samples were found to be unsatisfactory due to high levels of aerobic plate count, coliform, *Escherichia coli*, and *Staphylococcus aureus*. The highest counts obtained were 8.2 log cfu $g^{-1}$, 5.4 log cfu $g^{-1}$, 4.4 log cfu $g^{-1}$, and 3.9 log cfu $g^{-1}$, respectively, suggesting poor food hygiene practices and poor sanitation. *Salmonella* was found in 8% and 7% of Taichung and Laguna samples, respectively, which made the samples potentially hazardous. None of the samples was found to be positive for *Listeria monocytogenes* and *E. coli* O157, but *Bacillus cereus* was detected at the unsatisfactory level of 4 log cfu $g^{-1}$ in one Laguna sample. Antimicrobial resistance was observed for *Salmonella*, *E. coli*, and *S. aureus* isolates. Food preparation, cooking, and food handling practices were considered to be contributors to the unacceptable microbiological quality of the street foods. Hence, providing training on food hygiene for the street vendors should result in the improvement of the microbiological quality of street foods. The data obtained in this study can be used as input to microbial risk assessments and in identifying science-based interventions to control the hazards.

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## 1. Introduction

Street foods are popular because of their accessibility, low cost, variety, and nutritional value, but sometimes they are perceived as unsafe because of the unsatisfactory handling practices of food servers. Most foods are prepared and distributed in mobile and temporary shops that lack the primary facilities and infrastructure required to guarantee safe preparation of the foods (WHO, 1996). In addition, reports have shown that street vendors are generally unaware of basic food-safety issues, lack knowledge about food hygiene, and have little education (WHO–INFOSAN, 2010; WHO, 1996).

The proliferation of street-food vendors continues to increase because the business is very profitable and requires very low capitalization (Cho et al., 2011; WHO–INFOSAN, 2010), and controlling their number and the quality of food they offer is becoming a challenge. The contribution of the street-vending industry to socio-economic growth is enormous (Von Holy and Makhoane, 2006). This emphasizes the importance of placing a priority on assisting them in understanding the importance and the requirements of food safety. Doing so will protect public health and simultaneously improve the image of the street vending industry.

Various reports have identified the risks associated with consuming contaminated street-vended foods that have high levels of coliform bacteria and the presence of pathogenic bacteria, such as *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens*, and *Vibrio cholerae* (Cho et al., 2011; Hanashiro et al., 2005; Mankee et al., 2005). These reports clearly indicate that people who patronize the vendors of ready-to-eat (RTE) street foods might be putting their health at risk. In addition, the prevalence of multi-drug resistance among important microorganisms, such as *Salmonella*, *E. coli*, and *S. aureus* has been increasing and poses a real threat to public health (Guven et al., 2010; Harakeh et al., 2005) because street foods could possibly become a medium by which these antimicrobial-resistant pathogenic bacteria are transmitted to people.

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0740-0020/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fm.2013.04.005

Chicken- and pork-based street foods are commonly found in Taiwan and in the Philippines. In both countries, it is very common to use poultry (chicken/duck) and pork entrails and other parts that typically are not used for food, including intestines, blood, anal base, feet, neck, head, skin, heart, liver, gizzard, and proventriculus. Inherently, these parts are unclean and therefore require additional and careful cleaning procedures to be acceptable for human consumption. These meat-based street foods are usually available in Taiwan night markets in grilled form, while in the Philippines, they are sold in many public places, such as bus terminals, parks, and day markets.

This study was undertaken to obtain data on the microbiological quality of chicken- and pork-based street-vended foods in Taichung, Taiwan, and Laguna, Philippines. The data provide information on the microbial hazards present in the street-vended food category and can be used as inputs to microbiological risk assessments. The results of the risk assessments provide the basis for the development of science-based interventions to control the hazards and to implement and improve food-safety management systems for the street-food vending industry. In addition, in the study, we attempted to determine the antimicrobial resistance of the resulting microbial isolates, the results of which may offer valuable information on the potential of street foods to contribute to the spread of multidrug-resistant microorganisms.

## 2. Materials and methods

### 2.1. Sample collection

Street-vended food consisting of chicken- and pork-based samples, mostly grilled and fried, were obtained in two cities, i.e., Taichung, Taiwan, and Laguna, Philippines. From October 2010 through September 2011, 50 samples were taken from four night markets in Taichung, and 69 samples were taken from public places in three cities in Laguna. Each sample consisted of approximately 200–250 g, and the samples were collected from the point-of-sale in packages provided by the vendors, just as a consumer would do. The packed samples were placed in a cooler and immediately transported to the laboratory and stored at 4 °C until they were analyzed; the holding time did not exceed 16 h. The samples to be tested were grouped into hot-grilled and cold-cooked or fried categories, and the specific types of food includes hot-grilled chicken (hgc), hot-grilled pork (hgp), cold-cooked chicken (ccc), cold-cooked pork (ccp), and cold-fried chicken (cfc). The chicken-based samples included heart, skin, intestines, buttocks, bits, rice-blood cake, neck, feet, gizzard, proventriculus, and *kwek–kwek* (chicken egg). The pork-based samples included sausage, intestines, wieners, bits, liver, and head.

### 2.2. Microbiological analysis

Samples were analyzed for Aerobic Plate Count (APC), coliform bacteria, *E. coli*, *B. cereus*, *S. aureus*, *Salmonella* spp., *E. coli* O157, and *Listeria monocytogenes*. With some modifications, the test procedures basically were conducted in accordance with the requirements of the Bacteriological Analytical Manual (BAM Online). Twenty-five grams of each sample were mixed with 225 ml of sterile, buffered, phosphate water in a sterile filter bag (Whirl-Pak®, Nasco, Wisconsin, USA) and macerated for 2 min (Stomacher, Seward, Florida, USA). Serial dilutions up to $10^{-6}$ were prepared using 0.1% peptone water, and samples were placed on pour plates in duplicate using Plate Count Agar (Difco BD, New Jersey, USA) for aerobic plate count (APC). The plates were incubated at 37 °C for 24–48 h. Simultaneous enumeration of coliform bacteria and *E. coli* was done by pour plate in duplicate using Chromucult® Coliform Agar (Merck, Darmstadt, Germany). The plates were incubated at 37 °C for 24–48 h. *E. coli* BCRC 11634 and ATCC 25922 were used as reference strains. Dark-blue to violet colonies were counted as *E. coli*, and salmon-to-red colored colonies were counted as coliforms. The total of all of the dark-blue-to-violet colonies were considered to be the total coliform count. The presence of *E. coli* was confirmed by the Indole test using Kovacs reagent. For the enumeration and isolation of *B. cereus*, 0.1 ml of the sample suspension ($10^{-1}$ dilution) was inoculated onto the surfaces of *B. cereus* selective agar plates (Oxoid, Hampshire, England) by the spread-plate method. The plates were incubated at 37 °C for 24–48 h. Presumptive identification of *B. cereus* was confirmed by the Rapid Confirmatory Staining Procedure (Oxoid). *B. cereus* ATTC 10876 was used as the reference strain.

For *S. aureus*, 225 ml of nutrient-enriched broth with 7% NaCl were added to 25 g of each sample in a sterile filter bag (Whirl-Pak®, Nasco), and the resulting mixture was homogenized/macerated for 2 min (Stomacher, Seward, USA). One milliliter of diluted ($10^{-1}$) sample suspension was separated into quantities of 0.4, 0.3, and 0.3 ml and distributed on three Baird Parker Agar plates (Merck, Darmstadt, Germany) plates by spread plating. The plates were incubated at 37 °C for 48 h. The presence of typical *S. aureus* colonies was confirmed by the coagulase test using Bactident® coagulase (Merck) or Staphylase test kit (Oxoid) and by polymerase chain reaction (PCR) using an *S. aureus* DAS™ detection kit (Los Baños, Philippines). The catalase test and gram-staining also were conducted. *S. aureus* ATCC 25923 and BCRC 12653 were used as reference strains.

For *Salmonella* detection, 25 g of each sample were placed in a sterile filter bag (Whirl-Pak®, Nasco), mixed with 225 ml of buffered peptone water, and incubated at 37 °C for 20–24 h. From the enriched sample, 0.1 ml was added to Rappaport Vassiliadis broth (Merck) and 1 ml was added to Tetrathionate broth (Merck), and the two were incubated for 24 h at 42 °C and 37 °C, respectively. Then, the enriched solutions were streaked on Xylose Lysine Desoxycholate agar (Difco BD, Madison, USA), Bismuth Sulfite Agar (Merck), and Hektoen Enteric Agar (Merck), after which they were incubated at 37 °C for 24 h. Presumptive positive results were confirmed biochemically according to the Biochemical Identification of *Salmonella* and *Shigella* Using Abbreviated Panel Tests (WHO, 2010) and by PCR using a Salmonella DAS™ detection kit. Microscopic examinations of Gram-stained cells also were conducted. *Salmonella enterica* ATCC 10749 and ATCC 9842 were used as reference strains. For serotyping, the *Salmonella* isolates were submitted to the Antimicrobial Resistance Surveillance Reference Laboratory of the Research Institute for Tropical Medicine in the Department of Health in Manila, Philippines.

The presumptive presence of *E. coli* O157:H7 was confirmed using modified Tryptic Soy Broth with novobiocin (mTSB, Merck) as selective pre-enrichment (37 °C, 24 h) and streaked on Sorbitol MacConkey Agar plates (Pronadisa, Madrid, Spain), Fluorocult *E. coli* O157:H7 agar plates (Merck), and CHROMagar™ O157 plates (Paris, France) as selective/differential media (37 °C, 24 h). Biochemical tests were conducted using Triple Sugar Iron agar (TSI), Indole, Urea, lysine iron agar (LIA), and PCR using an *E. coli* O157 DAS™ detection kit. *E. coli* O157:H7 BCRC 15377 was used as the reference strain.

CHROMagar™ (Paris, France) *Listeria* plate was used for the detection of *L. monocytogenes* in the food samples. For enrichment, 25 g of sample were placed in 225 ml of Demi Frazer Broth (Acumedia, Michigan, USA) with ferric ammonium citrate and incubated at 37 °C for 24 h. The enriched solution was streaked to CHROMagar™ *Listeria* agar plate and incubated at 37 °C for 24 h. *L. monocytogenes* BCRC 14848 was used as the reference strain.

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