

RESEARCH NOTE

MICROBIOLOGICAL QUALITY ASSESSMENT OF DELI MEATS SOLD IN SINGAPORE

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Abstract. Deli meats have been linked to several foodborne outbreaks worldwide. However, to the best of our knowledge, limited published data are available on microbiological quality of deli meats sold in Singapore. Deli meat samples ($n = 120$) obtained from retail establishments in Singapore were tested using standard plate counting for *Bacillus cereus*, *Escherichia coli*, *E. coli* O157, *Listeria* spp, *Salmonella* spp, *Staphylococcus aureus*, and *Vibrio* spp. *B. cereus* was found in 2% of the samples with concentrations $<3 \log \text{CFU/g}$ and non-pathogenic *L. welshimeri* in 2% of the samples with concentrations $<10 \text{CFU/g}$; overall, 90% of the samples and a standard plate count of $<6 \log \text{CFU/g}$. Counter-top ham contained a significantly higher standard plate counts than factory-packed ham. These findings provided an update to the hygiene status of deli meats sold in Singapore, which should be useful to support policy development in food safety and hygiene in the future.

Keywords: bacteria, food safety, meat product, Singapore

INTRODUCTION

Deli meats or otherwise known as lunchmeats, are ready-to-eat (RTE) meat products popularly consumed by people worldwide. Despite their popularity, deli meats have been implicated in various foodborne outbreaks (Attaran *et al*, 2008; Daniels *et al*, 2000; Gottlieb *et al*, 2006; Knabel *et al*, 2012; ProMED-mail, 2014; Sim *et al*, 2002; Williams *et al*, 2000; Wong

et al, 2005), where microbiological hazards in deli meats pose concerns in food safety and public health. In most foodborne outbreaks linked to deli meats, *Listeria monocytogenes* is frequently identified as the etiological agent, and has been detected in retail ham samples (Food Safety Authority of Ireland, 2003).

Ng and Seah (1995) reported detection of *L. monocytogenes* in deli meats sold in Singapore with prevalence of 17.6%. However, being a country that is heavily reliant on imported food including deli meats, updated microbiological data of deli meats sold in the country is necessary to assess local public health risks, support policy development and reduce information uncertainty in the

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development of food safety measures. Here, deli meats from retail establishments in Singapore were tested for presence of a variety microorganisms associated with foodborne diseases.

MATERIALS AND METHODS

Sample collection and transportation

Sliced ham and salami samples were collected as representatives of deli meats sold in Singapore. Samples ($n = 120$) [pre-packed sliced ham, counter-top sliced ham, pre-packed sliced salami, and counter-top sliced salami ($n = 30$ each)] were obtained from randomly selected supermarkets across the country. Samples were immediately stored at $<4^{\circ}\text{C}$ in insulated bags containing ice packs and then were transported to the laboratory where samples were processed and tested within 4 hours upon arrival.

Sample preparation

Each sample (25 g) was aseptically added into stomacher bag containing 225 ml aliquot of sterile Universal Pre-enrichment Broth (Accumedia, Lansing, MI) and homogenized using Stomacher[®] 400 Circulator (Seward, West Sussex, UK) at 240 rpm for 30 seconds. Serial dilutions were carried out using Butterfield's buffer (3M, St Paul, MN) and diluted samples were used for plate counting of *Bacillus cereus*, *Escherichia coli*, *Listeria* spp, *Salmonella* spp, *Staphylococcus aureus*, and *Vibrio* spp. Homogenized samples were incubated for 24 hours at 37°C for testing of *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* spp, *V. cholerae*, and *V. parahaemolyticus* as previously described (Aung *et al*, 2016; Chau *et al*, 2017).

Standard plate assay

One ml aliquot of each diluted homogenized sample was transferred

onto a 3M Petrifilm Aerobic Count Plate (3M, St Paul, MN) and incubated for 48 hours at 37°C . Colonies on petrifilm were counted according to the manufacturer's instructions.

E. coli plate assay

One ml aliquot of each diluted homogenized sample was transferred onto 3M Petrifilm *E. coli*/Coliform Count Plates (3M, St Paul, MN), and incubated for 48 hours at 37°C . Positive colonies were identified and counted according to the manufacturer's instruction. Confirmation was conducted by culture on Levine's Eosin Methylene Blue (LEMB) agar (Accumedia, Lansing, MI) and incubated for another 24 hours at 37°C . Suspected colonies were inoculated into tryptone water and incubated for 24 hours at 37°C followed by addition of 300 μl of Kovac's reagent (Remel, Lenexa, KS) (indole test). Colonies from 3M *E. coli*/Coliform Count Plates are considered valid if suspected colonies are positive.

S. aureus plate assay

One ml aliquot of each diluted homogenized sample was spread onto a Baird-Parker agar (BPA)(Oxoid, Hampshire, UK) plate and incubated for 48 hours at 37°C . Positive colonies were counted according to manufacturer's instruction. Positive isolates then were picked and added to hydrogen peroxide to check for catalase activity. Positive isolates were also added to 0.5ml of reconstituted rabbit coagulase plasma with EDTA (Remel, Lenexa, KS) and incubated for 24 hours at 37°C . Colony counts from BPA are considered valid if positive colonies are positive in both catalase and coagulase tests.

B. cereus plate assay

One hundred μl aliquot of each diluted homogenized sample was transferred onto mannitol-yolk-polymyxin (MYP)

agar (Oxoid, Hampshire, UK) and incubated for 18-24 hours at 30°C and eosin pink colonies surrounded by a zone of precipitation were counted as presumptive *B. cereus*. Positive colonies were picked and streaked on Brilliance agar (Oxoid, Hampshire, UK) and *B. cereus* colonies from MYP agar are considered confirmed if green colonies are found on Brilliance *B. cereus* agar.

Detection of *Listeria* spp

A 10 µl loopful of enriched sample was streaked onto PALCAM agar (Accumedia, Lansing, MI) and incubated for 48 hours at 37°C. Positive colonies then were streaked on tryptone-soy agar (Oxoid, Hampshire, UK), incubated for 24 hours at 37°C and subsequently tested using *Listeria* test kit (Oxoid, Hampshire, UK). Positive colonies were then inoculated onto ChromID™ Ottaviani Agosti agar (bioMérieux, Lyon, France) and incubated for 24 hours at 37°C. Colonies on ChromID™ Ottaviani Agosti agar suspected as *L. monocytogenes* were confirmed using a RAPiDEC® Lmono test kit (bioMérieux, Lyon, France).

Detection of *Salmonella* spp

A 10 µl loopful of enriched sample was streaked onto xylose-lysine-deoxycholate (XLD) agar (Accumedia, Lansing, MI) and incubated for 24 hours at 37°C. Positive colonies were inoculated into triple-sugar-iron (TSI) (Accumedia, Lansing, MI) as well as lysine-iron agar (LIA) (Accumedia, Lansing, MI) slants and incubated for 24 hours at 37°C. Colonies producing red slant with yellow butt on TSI and purple butt on LIA slants were confirmed using a *Salmonella* test kit (Oxoid, Hampshire, UK) and API20E test kit (bioMérieux, Lyon, France).

Detection of *Vibrio* spp

A 10 µl loopful of enriched homogenized sample was streaked onto

thiosulfate-citrate-bile salts-sucrose (TCBS) agar (Oxoid, Hampshire, UK) and incubated for 24 hours at 37°C. Colonies with green/yellow color on TCBS agar were confirmed using an oxidase test kit (bioMérieux, Lyon, France) and API 20E test kit (bioMérieux, Lyon, France).

Detection of *E. coli* O157:H7

A 10 µl loopful of enriched homogenized sample was streaked onto HiCrome *E. coli* O157:H7 Selective agar (Sigma-Aldrich, St Gallen, Switzerland) and incubated for 24 hours at 37°C. Positive colonies were picked and streaked on sorbitol-MacConkey (SMAC) agar (Oxoid, Hampshire, UK) and on Levine's Eosin Methylene Blue agar (LEMB) agar (Oxoid, Hampshire, UK). Colorless colonies on SMAC agar that appeared blue black with or without metallic sheen on LEMB agar were confirmed using a RIM *E. coli* O157:H7 latex test kit (Remel, Lenexa, KS).

Data analysis

Results were analyzed using SPSS version 25 (IBM, Armonk, NY). Data between groups were analyzed using Kruskal-Wallis test and if significantly different (p -value <0.05), pairwise comparisons were conducted between subgroups using Mann-Whitney U test, p -values adjusted using Bonferroni correction and tested at 5% significance level.

RESULTS

E. coli, *E. coli* O157, *L. monocytogenes*, *Salmonella* spp, and *Vibrio* spp were not detected in any of the samples tested. *B. cereus* was detected in 2/120 (2%) of the samples at concentrations <3 log CFU/g, *Listeria* spp detected in 2/120 (2%) of the samples at concentrations <10 CFU/g; all of which were later identified as non-pathogenic *L. welshimeri*.

Overall, 91% of deli meat samples were within regulatory limit of the standard plate counts (SPC), *ie* within 10^6 CFU/g (Singapore Food Agency, 2005), comprising 93% of factory-packed ham samples, 90% of counter-top ham, 97% of factory-packed salami and 83% of counter-top salami samples. Median SPC among factory-packed ham, counter-top ham, factory-packed salami and counter-top salami was statistically different

(2.77, 4.05, 5.29 and 5.32 log CFU/g, respectively; p -value <0.0001) (Fig 1). SPC in counter-top ham is significantly higher compared to factory-packed ham (p -value <0.01),

DISCUSSION

The survey shows only a small minority of deli meat products in Singapore were contaminated with

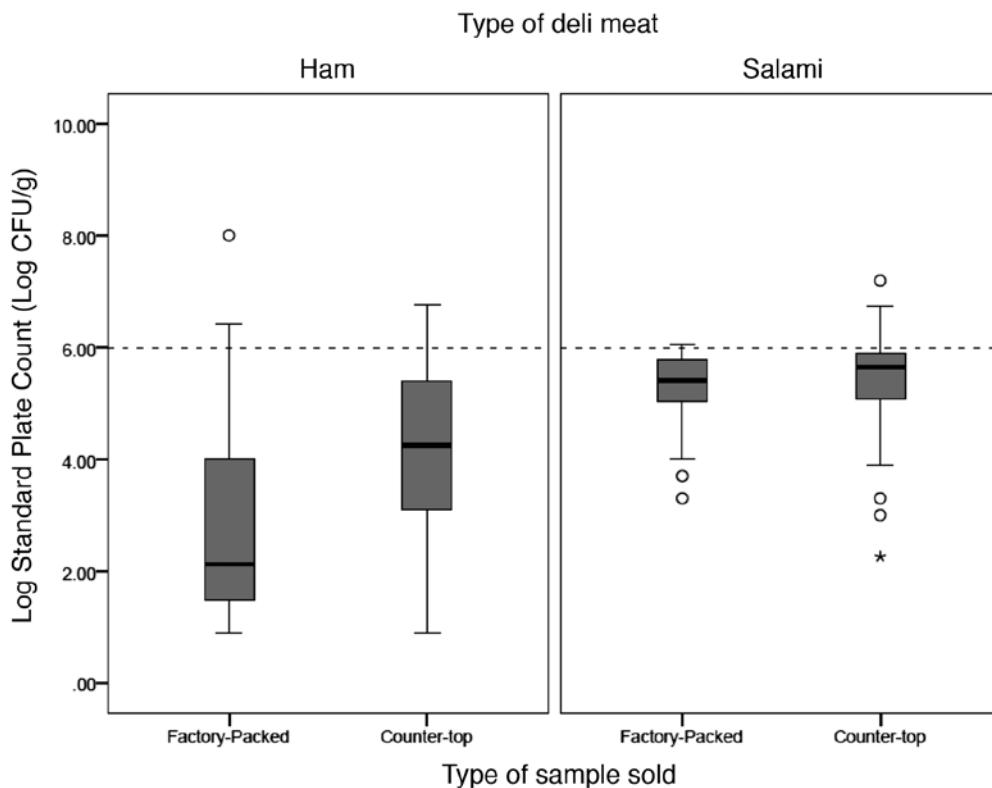


Fig 1-Standard plate counts of deli meats sold in Singapore

Thirty samples of each meat type were randomly selected from markets across Singapore, 25 g of each sample were homogenized and one ml aliquot of diluted homogenized sample was transferred onto a 3M Petrifilm Aerobic Count Plate and incubated for 48 hours at 37°C prior to colony counting. Dark horizontal line denotes median standard plate count level; box represents the interquartile range (IQR); vertical line extends 1.5 times of the IQR from the box edges; circles outside 1.5 IQR were considered as outliers. Dashed horizontal line indicates standard plate count level of 10^6 CFU/g; ready-to-eat meat exceeding this level suggests elevated food safety risk (Singapore Food Agency, 2005).

potentially pathogenic *B. cereus* that can cause diarrhea or nausea and vomiting (Aung *et al*, 2016). The only *Listeria* spp found in the deli meats was the non-pathogenic *L. welshimeri*. This is in contrast with the study conducted in Singapore in 1995 that detected *L. monocytogenes* in 17.6% of deli meat samples (Ng and Seah, 1995). The difference reflects the improvement in food safety technologies in manufacturing and retail in the country during the past years.

Higher level of bacterial contamination in counter-top compared factory-packed hams has been observed in other studies, where deli products sliced at retail shops contain higher microbial loads compared to their factory-sliced counterparts (Kurpas *et al*, 2018). This suggests displaying, slicing and handling of deli ham products on counter-top may introduce more microbiological contamination compared to factory packed ones (Chaitiemwong *et al*, 2014). Thus, education emphasizing hygiene awareness at deli counters would be of value. However, no significant difference was observed in standard plate count between counter-top salami and factory-packed salami, suggesting contamination from handling steps at the counter-top, if any, was insufficient to affect the total aerobic bacteria counts in salamis, which are inherently high, likely due to the use of lactic-acid bacteria in the processing (de Castilho *et al*, 2015).

In summary, this microbiological assessment provides an update on the hygiene status of deli meats sold in Singapore. Although the findings suggest microbiological contamination could be increased during counter-top processing, 91% of tested deli meat samples had standard plate counts within the regulation limit. Since 1995, emphasis on better manufacturing practices, importer

credentials and certifications, import-level screening control efforts for food, as well as hygiene control at the retail level ought to have contributed to better hygiene and safety in deli meats sold in the country. This update on the microbiological quality of deli meats sold in Singapore should be useful to support future policy developments.

ACKNOWLEDGEMENTS

This research was supported by the Reinvestment Fund (RF), Ministry of Finance (MOF), Singapore.

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