RESEARCH NOTE

SEROTYPE, SEQUENCE TYPE AND ANTIBIOGRAM PROFILE OF *LISTERIA MONOCYTOGENES* ISOLATES FROM RETAIL FOOD IN SINGAPORE (2010 - 2016)

Kyaw Thu Aung[1,2,3,4], Man Ling Chau[1,4], Vijitha Manogaran[1,4], Jia Quan Oh[1,4], Min Yap[1], Lee Ching Ng[1,3] and Ramona Alikiiteaga Gutiérrez[1]

[1]Environmental Health Institute, National Environment Agency, Singapore; [2]School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore, [3]School of Biological Sciences, Nanyang Technological University, Singapore; [4]National Centre for Food Science, Singapore Food Agency, Singapore

Abstract. *Listeria monocytogenes* infection can be acquired through consumption of contaminated food, which can lead to serious adverse outcome, particularly among pregnant women and immunocompromised individuals. There is currently a limited information on risk posed by *Listeria* in food in Singapore, and thus a better understanding of the characteristics (serotype, sequence type and antibiogram profile) of *L. monocytogenes* detected in food would be useful to public health practitioners in assessment of food safety in country. This study aimed to determine the serotypes, sequence types and antimicrobial resistance profiles, and assess the food safety relevance, of *L. monocytogenes* isolated from ready-to-eat retail food in Singapore. A total of 60 *L. monocytogenes* isolates obtained from ready-to-eat retail food between 2010 and 2016 were subjected to conventional serotyping, multi-locus sequence typing and disc diffusion assay against 12 antimicrobial agents. Detection of clinically relevant serotypes, namely, 4b (53.3%) and 1/2b (18.3%) highlighted possible food safety concern. Multi-locus sequence typing showed 98% of the isolates belonged to sequence types that had been reported in clinical cases overseas, suggesting their potential to cause disease. Some 85% of isolates were resistant to at least one antimicrobial agent, including general drugs used for treating invasive listeriosis, such as penicillin and ampicillin. These findings are in line with global growing concerns and reinforce the need to heighten awareness of the emerging risk of antimicrobial resistance in foodborne pathogens. In addition, the data provide baseline information on local foodborne *L. monocytogenes* characteristics that should be of use in comparison with other environmental and human isolates to elucidate contamination and transmission routes of *L. monocytogenes* in Singapore.

Keywords: *Listeria monocytogenes*, serotypes, sequence types, antibiogram, retail food

Correspondence: Aung Kyaw Thu, Environmental Health Institute, National Environment Agency, 11 Biopolis Way, Helios Block #06-05/08 Singapore 138667.
E-mail: AUNG_Kyaw_Thu@sfa.gov.sg

INTRODUCTION

Listeria monocytogenes is a human pathogen ubiquitous in nature. Hence, *L. monocytogenes* can be found in various environmental matrices, such as agricultural soil, and from there be transferred to food and food processing environments. *L. monocytogenes* infection is acquired through consumption of contaminated food products (Jordan and McAuliffe, 2018), with such high risk food items as unpasteurised dairy products, smoked fish and deli meat. Its psychotropic property (ability to grow at low temperatures) is of a particular concern in chilled ready-to-eat food (USFDA, 2012). Although the pathogen is generally known to cause mild and asymptomatic illnesses in healthy adults, it can also lead to severe systemic infection and even death, particularly in high risk population including pregnant women, infants, elderly people, and immunocompromised individuals, resulting in a high case-fatality rate of 20-30% (Kourtis *et al*, 2014; WHO, 2018).

In Singapore, there has been sporadic listeriosis cases reported over the past years (Ministry of Health Singapore, 2018). Listeriosis is not a legally notifiable disease (Singapore Salute Online, 1976), and thus may be under-reported. There is also a general lack of information on local foodborne *L. monocytogenes* strains and their potential to cause disease. In order to provide a better understanding food safety risk associated with *L. monocytogenes* in food, the study determined serotype, sequence type and antibiogram profile of *L. monocytogenes* isolated from retail food in Singapore over a 7-year period. Data gathered should be useful to evaluate potential food safety risk of *L. monocytogenes* in the country, and be compared with data from human isolates for further investigation on the epidemiology of listeriosis.

MATERIALS AND METHODS

Collection of *L. monocytogenes* isolates

L. monocytogenes isolates from smoked salmon (n = 44), raw salmon (n = 8) and other types of food (n = 8) (chilled cooked chicken, chilled cooked prawn, chilled cooked shrimp, chilled deep-fried soft-shell crab, prepacked chicken pasta salad, raw chicken, roasted chicken, and unagi sushi) were obtained from 2010 and 2016 as part of Singapore National Environment Agency (NEA) surveillance (unpublished) and risk assessment studies (Chau *et al*, 2017).

Serotyping

Conventional serotyping was carried out using O- and H- antigen antisera (Denka Seiken Co Ltd, Tokyo, Japan) as previously described (Chau *et al*, 2017).

Multi-locus sequence typing (MLST)

DNA extraction was performed using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). PCR amplifications were carried out using published primers and protocols (Salcedo *et al*, 2003; Chau *et al*, 2017). In brief, MLST involved PCR amplifications targeting housekeeping genes of *abcZ, blgA, cat, dapE, dat, idh* and *ihkA* (Salcedo *et al*, 2003). Thermocycling was carried out in a GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA) as follows: 98°C for 30 seconds, 35 cycles of 98°C for 10 seconds, 45°C for 30 seconds and 72°C for 30 seconds with a final step of 72°C for 10 minutes. Amplicons were separated by 2% agarose gel-electrophoresis, visualised under UV light following staining with GelRed, purified from gel (Biotium,

Hayward, CA) and sequenced using Applied Biosystems® 3730/3730xl DNA Analyser and BigDye Terminator v3.1 (Axil Scientific, Singapore). Sequences were assembled employing a Lasergene Software version 8.0 (DNASTAR, Madison, WI) and compared with the PubMLST database to construct allelic profiles based on seven housekeeping genes and sequence types (STs).

Antibiogram profiling

Antimicrobial susceptibility test was performed using a disc diffusion method against 12 antimicrobials, namely, amikacin (30 μg, AK30), amoxicillin/clavulanic acid (20/10 μg, (AMC30), ampicillin (10 μg, AMP10), azithromycin (15 μg, AZM15), ciprofloxacin (5 μg, CIP5), ceftriaxone 30 μg (CRO30), chloramphenicol 30 μg (C30), gentamicin 10 μg (CN10), norfloxacin (10 μg, NOR10), penicillin (10 μg, P10), sulfamethoxazole/trimethoprim (23.75/1.25 μg, SXT25), and tetracycline (30 μg, TE30) (Oxoid, Hampshire, UK) (CLSI, 2007). The selection of antimicrobials was based on their availability, relevance in human and veterinary medicine, including the agricultural sector, and on previous overseas antibiogram profiles of *L. monocytogenes* in food and food environment (Reis *et al*, 2011; Mat Issa *et al*, 2011; Jamali and Thong, 2014; Doménech *et al*, 2015; Garedew *et al*, 2015; de Vasconcelos Byrne *et al*, 2016; Du *et al*, 2017; Lotfollahi *et al*, 2017). As there were no susceptibility interpretation criteria specific to *L. monocytogenes* in the Clinical and Laboratory Standard Institute (CLSI) guidelines, data for *Staphylococci* spp. were used to interpret susceptible (S), intermediate (I) and resistant (R) except for SXT25, where EUCAST guideline was used (Conter *et al*, 2009; de Vasconcelos Byrne *et al*, 2016; EUCAST, 2016). *Escherichia coli* ATCC 25922 and S. *aureus* ATCC 25923 were used as control strains. Isolates phenotypically resistant to three or more antimicrobial classes were defined as multi-drug resistant.

RESULTS

Of the *L. monocytogenes* isolates (n = 60), 32 (53%) and 11 (18%) were identified as serotype 4b and 1/2b respectively; these 43 isolates belonged to lineage I based on their respective serotypes. The other 17 isolates belonged to lineage II, serotypes 1/2a (n = 10, 17%), 3a (n = 6, 10%) and 1/2c (n = 1, 2%).

MLST identified eight *L. monocytogenes* sequence types, namely, ST2 (n = 31, 52%), ST7 (n = 6, 10%), ST9 (n = 1, 2%), ST87 (n = 11, 18%), ST155 (n = 3, 5%), ST193 (n = 1, 2%), ST403 (n = 6, 10%), and ST1227 (n = 1, 2%). All *L. monocytogenes* isolates from ready-to-eat food in this study, except for one isolate (ST1227), belonged to sequence types reported in human cases in other countries (Moura *et al*, 2016).

All *L. monocytogenes* isolates were susceptible to C30, CIP5, CN10, NOR10, TE30, or SXT25; 85% resistant to at least one of the antimicrobial agents, with 67% to P10, 52% to CRO30 and 43% to AMP10 and 2% to AMC30, AMP10 or AZM15. One isolate (ST1227) was considered MDR (resistant to four classes of antimicrobials).

DISCUSSION

Foodborne listeriosis remains a risk worldwide, especially with the increase in popularity chilled ready-to-eat food products. Analysis of *L. monocytogenes* serotypes from a variety of chilled ready-to-eat food items in Singapore over a 7-year period revealed more than 80% of isolates from smoked salmon belonged to serotypes 4b and 1/2b (lineage I).

Globally, serotypes 4b and 1/2b are associated with severe human illnesses and outbreaks, while serotypes 1/2a, 1/2c and 3a (lineage II) (in the minority of food items) are more commonly detected in food and the environment (Orsi *et al*, 2011). Studies in other countries reported 6.7-22.4% detection of serotypes 4b and 1/2b in smoked fish (Gambarin *et al*, 2012; Tocmo *et a l*, 2014; Acciari *et al*, 2017). In addition, MLST analysis revealed that 98% of isolates in the present study belonged to sequence types reported in human cases in overseas countries, suggesting their potential of causing human illness.

L. monocytogenes is generally susceptible to a broad range of antimicrobial classes used in treatment of Gram-positive bacterial infection (Hof *et al*, 1997; Troxler *et al*, 2000; Walsh *et al*, 2001; Hansen *et al*, 2005; Thønnings *et al*, 2016). Antimicrobial resistance in microorganisms can occur as a result of mutations conferring resistance when exposed to drug pressure (Richardson, 2017). For instance, the presence of antimicrobials at low levels in the food and agricultural sectors may contribute to a conditioning environment that allows selection of microorganisms evolved to become resistant to higher levels of antimicrobial pressure (Lungu *et al*, 2011). Antimicrobial resistance also can be mediated by horizontal transfer of genetic elements associated with resistance to antimicrobials but also to heavy metals present in seafood and disinfectants used in food processing (Seiler and Berendonk, 2012; Singer *et al*, 2016).

Although 85% of *L. monocytogenes* isolates characterized in the present study were resistant to at least one antimicrobial agent tested, nearly 50% showed intermediate or resistance to ceftriaxone, a cephalosporin. Cephalosporin resistance is known to be associated with stress response and pathogenesis in *L. monocytogenes* (Krawczyk-Balska and Markiewicz, 2016), a factor of significance to clinical management. Broad-spectrum cephalosporin antibiotics, due to their low therapeutic toxicity and ability to penetrate blood-brain barrier, are often used for treatment of invasive bacterial infections of unknown or yet-to-be diagnosed etiology (Rota *et al*, 1996; Troxler *et al*, 2000). In addition, up to two-thirds of *L. monocytogenes* isolates were resistant to ampicillin or penicillin, two antimicrobials recommended for treating invasive listeriosis (Swaminathan and Gerner-Smidt, 2007). Ampicillin- or penicillin-resistant was reported in various types of food, livestock and human samples in Brazil, China, Ethiopia, Iran, Malaysia, and Spain (Rahimi *et al*, 2010; Mat Issa *et al*, 2011; Reis *et al*, 2011; Jamali and Thong, 2014; Doménech *et al*, 2015; Garedew *et al*, 2015; de Vasconcelos Byrne *et al*, 2016; Du *et al*, 2017; Lotfollahi *et al*, 2017). Extensive use of these two antimicrobials in human and veterinary medicine is believed to be a key factor of the emergence of resistance to these antimicrobials (Harakeh *et al*, 2009; Du *et al*, 2017).

In conclusion, the 7-year survey of *L. monocytogenes* in ready-to-eat food items available in Singapore reveals a relatively high proportion of isolates with pathogenic predisposition and phenotypic resistance against several clinically important antimicrobials used in treating listeriosis. These findings should serve as a useful reference baseline in the country surveillance programs to detect both known and emerging pathogens, to identify significant shifts in types and antibiogram profiles over time, and to develop and evaluate current and future

risk managements measures in food and food environment. Further investigations are recommended on the understanding of antimicrobial resistance, from farm to hospital, and to formulate public health strategies to prevent emergence and control spread of antimicrobial-resistant pathogens in the country.

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