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Occurrence of *Staphylococcus aureus* in a commercial poultry plant and poultry farm

A Thesis submitted in partial fulfilment of the requirement for the degree of Master of Food Technology

Massey University

Albany, New Zealand

Cheng QIAN

April, 2015

ABSTRACT

Poultry products are popular due to their healthier image compared to red meats. However, the products are susceptible to contamination by many spoilage microorganisms and pathogens, including *Staphylococcus aureus, Campylobacter* spp.*, Clostridium perfringens*, *Yesinia enterocolitica*, *Pseudomonas* spp. and *Escherichia coli.* In New Zealand (NZ), foodborne outbreaks caused by *S. aureus* infections may be uncommon but serious.

S. aureus can grow in a wide range of pH, temperature and salt concentrations. Some strains of *S. aureus* can produce heat-resistant enterotoxins, while others may be methicillin-resistant which can result in hospital-linked and community-linked infections. Raw (fresh) and frozen poultry products have been associated with *S. aureus* contamination in many countries. The common contamination sources of *S. aureus* in poultry products have been linked to poor hygiene of food handlers, processing equipment and skins of live chickens. The aim of this project was to identify potential contamination sources of products and processing equipment by *S. aureus* from a selected processing plant to the farm in Auckland, New Zealand.

Poultry meat samples were collected from Final Products, Frozen Mechanically Separated Meat (MSM), Frozen Skin, Frozen Skin-on Breast Fillet (SO BF) (further processing plant), Fresh MSM, Fresh Skin, Fresh SO BF (secondary processing plant). Swab samples were collected from the MSM conveyor, inside the Mechanically Deboning Machine (MDM), the Skinner Conveyor (secondary processing plant), Rubber Fingers in Pluckers (primary processing plant), skin and nostrils of live chicken at the farm. Viable cell counts of *S. aureus* were enumerated using Petrifilm™ Staph Express Count Plate to determine the contamination level of the samples. Isolates of *S. aureus* was confirmed by Gram-stain and coagulase-positive test. Six main sampling sites were selected for further investigation which comprised final products, Fresh MSM, Fresh Skin, Fresh SO BF, Rubber Fingers and live chickens. Ten representative *S. aureus* isolates grown on Petrifilms were randomly selected from samples of each of the six main sampling sites. Polymerase Chain Reaction (PCR) and Multilocus Sequence Typing (MLST) were then used to detect the presence of staphylococcal enterotoxins and identify sequence types of the sixty *S. aureus* isolates, respectively. eBURST was used to identify the relatedness of the sequence types. Also, the contamination sources of *S. aureus* in the samples were traced based on the sequence types of the sixty isolates.

In the further processing plant, all final product samples (n=36) were contaminated with *S. aureus*. Frozen MSM had the highest contamination level ranging from 2.00±1.02 to 2.50±0.48 Log_{10} CFU/g. Similarly, *S. aureus* in Fresh MSM from the secondary processing plant contained the highest *S. aureus* cell counts (1.79±0.25 to 2.85±0.51 Log_{10} CFU/g), followed by Fresh SO BF (1.85±0.56 to 2.33±0.50 Log_{10} CFU/g) and Fresh Skin (1.72±0.60 to 2.15 [1.67, 3.37] Log_{10} CFU/g). In primary processing, Rubber Fingers in Plucker 1 had the highest level of *S. aureus* (2.46±0.50 Log_{10} CFU/swab). *S. aureus* counts of chicken skin ranged from 1.00 [0.79, 1.48] to 1.36±0.45 Log_{10} CFU/swab, while nostrils contained 1.00 [0.85, 1.48] to 1.59±0.70 Log_{10} CFU/swab. Cell counts of live chicken increased with the age (first, third, sixth week) of the chicken. Eight different types of enterotoxin genes (*seg*, *sei*, *seh*, *sek*, *sel*, *sem*, *sen*, *seo*) were identified. Of the 60 *S. aureus* isolates, 59 were positive for at least two different staphylococcal enterotoxins. Six different sequence types were identified (ST5, ST2594, ST101, ST83, ST398, ST1). Sequence types of isolates that had at least five identical loci were assigned to a single clonal complex (CC). In this study, ST5, ST83 and ST2594 belonged to CC 5 with ST5 being the clonal ancestor.

MSM had the highest *S. aureus* contamination level due to cross-contamination inside the MDM, therefore, a proper hygiene and regular cleaning routine inside the MDM is recommended. The results suggested that the sources of *S. aureus* contamination in the final poultry products could be Fresh MSM, Fresh Skin, Fresh SO BF (secondary processing), Rubber Fingers in the Pluckers (primary processing) and live chickens at the farm. Chicken skin from live chickens at farm was most likely the origin of contamination of final products and equipment by *S. aureus*. Since not all the identified strains that colonised on the live chickens were traced back to the final products, further investigations on other potential contamination sources such as gloves and knifes used at the processing plant, feeders and drinkers at the farm are recommended.

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LIST OF ABBREVIATIONS

AOAC	=	Association of Official Analytical Chemists
ATCC	=	American Type Culture Collection
BFG	=	Bovine Fibrinogen Agar
BHI	=	Brain Heart Infusion
BLAST	=	Basic Local Alignment Search Tool
BPA	=	Baird-Parker Agar
CC	=	Clonal Complex
CCPs	=	Critical Control Points
ClO_2	=	Chlorine Dioxide
DNA	=	Deoxyribonucleic Acid
eBURST	=	Based Upon Repeat Sequence Types
EDTA	=	Ethylenediaminetetraacetic Acid
ELISA	=	Enzyme-linked Immuno-sorbent Assay
ELISA-M	=	Enzyme-linked Immuno-sorbent Assay Membrane
ELISA-T	=	Enzyme-linked Immuno-sorbent Assay Tube
ESR	=	Environmental Science and Research
FSMS	=	Food Safety Management System
HACCP	=	Hazard Analysis and Critical Control Points
HOCI	=	Hypochlorite
ISO	=	International Organization for Standardization
MAP	=	Modified Atmosphere Packaging
MAS	=	Microbial Assessment Scheme
MDCM	=	Mechanically Deboned Chicken Meat
MDM	=	Mechanically Deboning Machine
MRSA	=	Methicillin-resistant *Staphylococcus aureus*
MSA	=	Mannitol Salt Agar
MSM	=	Mechanically Separated Meat
NaCl	=	Sodium Chloride
PSE	=	Pale Soft Exudative
PSE	=	Petrifilm Rapid Staph Express Count

PCR	=	Polymerase Chain Reaction
MLST	=	Mutillocus Sequencing Typing
MPN	=	Most Probable Number
RPFA	=	Rabbit Plasma Fibrinogen Agar
RPLA	=	Reverse Phase latex agglutination
SEs	=	Staphylococcal Enterotoxins
SEA (*sea*)	=	Staphylococcal Enterotoxin A
SEB(*seb*)	=	Staphylococcal Enterotoxin B
SEC(*sec*)	=	Staphylococcal Enterotoxin C
SED(*sed*)	=	Staphylococcal Enterotoxin D
SEE(*see*)	=	Staphylococcal Enterotoxin E
SEG(*seg*)	=	Staphylococcal Enterotoxin G
SEH(*seh*)	=	Staphylococcal Enterotoxin H
SEI(*sei*)	=	Staphylococcal Enterotoxin I
SEJ(*sej*)	=	Staphylococcal Enterotoxin J
SEK(*sek*)	=	Staphylococcal Enterotoxin K
SEL(*sel*)	=	Staphylococcal Enterotoxin L
SEM(*sem*)	=	Staphylococcal Enterotoxin M
SEN(*sen*)	=	Staphylococcal Enterotoxin N
SEO(*seo*)	=	Staphylococcal Enterotoxin O
SEP(*seo*)	=	Staphylococcal Enterotoxin P
SEQ(*seq*)	=	Staphylococcal Enterotoxin Q
SER(*ser*)	=	Staphylococcal Enterotoxin R
SET-EIA	=	Staphylococcal Enterotoxin Enzyme-linked Immuno-sorbent Assay
SET-RPLA	=	Staphylococcal Enterotoxin Reverse Phase Latex Agglutination
SEU(*seu*)	=	Staphylococcal Enterotoxin U
SFP	=	Staphylococcal Food Poisoning
SLVs	=	Single-locus variants
ST	=	Sequencing Type
UK	=	United Kingdom
USA	=	United States
UV	=	Ultraviolet

1. Introduction

The origin of domesticated chickens dates back to thousands of years ago in Southeast Asia (Daghir, 2008; West & Zhou, 1988). Domestication of chickens is a process which humans raise chickens aimed to enhance desired traits, nowadays mostly for commercial trade due to huge demand of poultry meat products (Wiren et al., 2009). Poultry products, such as eggs, fresh chicken and roasted turkey, have become sources of daily meals worldwide (Davies & Board, 1998). In 2007, eggs and chickens processed products were produced by about 145,615 farms in America (EPA, 2013). In New Zealand, poultry production increased from 111,884 to 173,263 tonnes between 2000 and 2012. Chicken products remain the most favourite meat-based meals in New Zealand (PIANZ, 2010).

Chicken products are susceptible to contamination by many pathogens or spoilage microorganisms. Farmers raise chickens on litter floors which have higher risks of being contaminated with Enterobacteriaceae, such as *Salmonella, Escherichia coli* and *Yesinia enterocolitica* (Davies & Board, 1998). Waldroup (1996) reported the incidence of common pathogens on raw poultry products including *Campylobacter* spp*., Clostridium perfringens*, *Staphylococcus aureus*, *Yesinia enterocolitica* and *Pseudomonas*. *Campylobacter*, *Salmonella, Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus* are human pathogens which means that the contaminations are mainly attributed to poor food handling or cross-contamination during food processing (Davies & Board, 1998). The obsence of *S. aureus* is an indicator of good hygiene and correct handling practice (Jacxsens et al., 2011)

S. aureus strains are Gram-positive cocci and facultative anaerobes (Roberts & Greenwood, 2002). They however, grow better in the presence of adequate oxygen than anaerobic environment (Ministry of Primary Industry, 2001). *S. aureus* can grow in a wide range of pH (4.2 to 9.3), temperature (7°C to 48.5°C) and salt concentrations up to 15% (Bhatia & Zahoor, 2007; Kérouanton et al., 2007). The optimum temperature for their growth is 37°C. Low water activity is not an inhibiting factor for *S. aureus* to grow and produce staphylococcal