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Non-typhoidal *Salmonella* contamination in egg shells and contents from retail in Western Australia: Serovar diversity, multilocus sequence types, and phenotypic and genomic characterizations of antimicrobial resistance

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ABSTRACT (word counts = 335)

In recent years, the number of human salmonellosis cases in Western Australia (WA) has increased more dramatically than in any other Australian state. In 2017, the number of cases in WA was more than double the five-year average, and eggs had emerged as the key culprit for several *Salmonella* foodborne disease outbreaks. To better understand such an epidemiologically intriguing situation, our research goal was to investigate the prevalence, serovar diversity, multilocus sequence types, and antimicrobial resistance of non-typhoidal *Salmonella* contamination in retail eggs produced and sold in WA. A total of 200 visually clean and intact retail egg samples (each containing a dozen eggs) were purchased for one year (2017–2018) from supermarkets in metropolitan Perth, the capital of WA. For each sample, the contents and shells of the 12 eggs were separately pooled and cultured according to standard methods. Overall, *Salmonella* was detected in 11.5% (23/200) of the tested egg samples. *Salmonella* was isolated from 4.5% (9/200) and 3% (6/200) of eggshells and egg contents, respectively. In 4% (8/200) of the samples, *Salmonella* was recovered from both eggshell and egg contents. Isolates from positive retail egg samples were serotyped as either *S.* Typhimurium (52.2% [12/23]) or *S.* Infantis (39.1% [9/23]). Both serotypes were concurrently recovered from two different retail egg samples. We retained a set of both *S.* Typhimurium (n = 29) and *S.* Infantis (n = 12) isolates from all *Salmonella*-positive retail packs (n = 23) for further characterization. Only two (*S.* Typhimurium) isolates showed resistance to ampicillin, of which one carried *β*-lactamase resistance gene $bla_{\text{TEM-1b}}$. The remaining isolates (39/41) were susceptible to all 14 antimicrobials included in the minimum inhibitory concentrations (MICs) testing panel. Multilocus sequence typing and serotyping were perfectly mirrored, as all *S.* Typhimurium isolates were characterized as sequence type (ST)-19, and all *S.* Infantis isolates were ST-32. This study points to the noteworthy *Salmonella* prevalence rate in retail egg samples in WA. Our results illustrate minimal public health risks arising from antimicrobial resistance *Salmonella* from Australian eggs.

Keywords: Perth; baseline survey; table eggs; Salmonellosis; antimicrobial resistance

1. Introduction

Non-typhoidal *Salmonella* are among the most commonly reported enteric pathogens worldwide. It has been estimated that infections and illnesses with non-typhoidal *Salmonella* causes 4.07 million Disability Adjusted Life Years (DALYs) (Kirk et al., 2015). Although the majority of non-typhoidal salmonellosis infections are self-limiting, severe life threatening complications such as bacteremia and death have been reported in infants, elderly and immunocompromised individuals. Another dimension of the public health importance of non-typhoidal salmonellosis is the growing trend of reported antimicrobial resistance in several countries (Akullian et al., 2018). The probability of transferring these resistant strains to humans through the consumption of food of animal origin is a matter of public health concern (Pande et al., 2015).

Eggs and egg-based products have been among the most frequently implicated food categories in a number of non-typhoidal salmonellosis outbreaks worldwide (Moffatt et al., 2016). Contamination of egg contents by *Salmonella* can occur before laying eggs through colonization of this pathogen in cecum and transmission to reproductive organs of layers' hens. Additionally, the outer shell of eggs might become contaminated post-laying from inappropriate sanitary conditions at the layer farms, improper washing, grading and packing operations (Pärn et al., 2017). *Salmonella* Enteritidis (*S.* Enteritidis) is a major cause of eggs-linked foodborne outbreaks in USA, Europe and other parts of the world. However, the situation in Australia is remarkably different where *Salmonella* Typhimurium (*S.* Typhimurium) is the predominant cause of foodborne salmonellosis outbreaks, of which many are frequently linked to consumption of eggs and egg-related products (Chousalkar et al., 2017). From January 2011 to January 2014, 128 outbreaks of *S.* Typhimurium resulted in 2343 cases with 347 hospitalizations reportedly due to

consumption of raw egg and raw egg-related products in different food preparation settings across different states in Australia particularly in New South Wales, Queensland and Victoria (Moffatt et al., 2016).

In Western Australia (WA), a state that occupies the entire western third of Australia, the trend of reported foodborne salmonellosis has increased more dramatically in recent years than that of any other Australian state (OzFoodNet, 2017). In 2017, the number of *Salmonella* cases in WA was more than double the five-year average, and between January 2015 and June 2017, egg dishes were the implicated food in 17 out of 18 point-source *Salmonella* outbreaks in WA (OzFoodNet, 2017). This epidemic is very intriguing, given that *S.* Typhimurium was revealed as the dominant causative serotype in the majority of egg-linked outbreaks in WA. Despite the high incidence of human salmonellosis in Australia in general and in WA in particular, there has been a limited number of studies over the past ten years pertaining to the prevalence of *Salmonella* in retail eggs (Fearnley et al., 2011). To better understand the ongoing situation in WA, we aim in this baseline study to assess the presence of *Salmonella* in and on eggs available through retail supermarkets in metropolitan Perth, the capital of WA. Furthermore, we investigate the serovar diversity, multilocus sequence types, phenotypic patterns, and genomic determinants of antimicrobial resistance among *Salmonella* isolates recovered from retail eggs. The present study fills a gap in knowledge on microbial safety of retail eggs and antimicrobial resistance status of *Salmonella* isolated from retails eggs in the Australian context.

2. Materials and Methods

2.1. Sample size, collection and identification

The sample unit in this study was a pre-packaged retail carton of one dozen (12) eggs; this unit was used to ensure that the sample reflects the most common type of pack purchased by consumers from Australian supermarkets. A limited number of published studies have examined *Salmonella* contamination in Australian retail eggs; the most recent retail survey was conducted in South Australia (SA) using the same sample unit. In that study, it was reported that 3.5% (7/199) of the retail egg samples were positive for *Salmonella* (Fearnley et al., 2011). In the present study, the number of samples was estimated based on an assumed prevalence of ~7% (twice as many eggs as that of the survey done in SA (Fearnley et al., 2011)), with a desired confidence interval (CI) of 95% and accuracy of 5% (Dohoo et al., 2009). Matching these criteria with the capacity and feasibility of sampling and laboratory testing and taking into consideration sampling from different production systems and different months, a total 200 retail egg samples (each containing one dozen eggs) were tested between October 2017 and June 2018. Each month, around 20 retail egg samples (pulled bimonthly) were purchased by the research team from different supermarkets across Perth. The supermarkets belonged to five major retailers across WA (Woolworth, Coles, Aldi, IGA and Spudshed) and were sampled from different branches from across Perth northern and southern suburbs (distanced between two and twenty five kilometers from the testing laboratory). All samples were visually examined to ensure that the eggs to be tested were visibly clean and crack-free. The proportion of samples from each production system was targeted at "approximately" 50% free-range, 30% cage-laid, and 20% barn-laid. Free-range eggs were oversampled in consideration of their growing demand all-over

Australia and in WA in recent years, promoted in accordance with the WA government's plan for a 10-year phase-out of conventional cages for egg-laying hens (Government of WA, 2018).

As part of Australia's Primary Production and Processing Standard for Eggs and Egg Products (FSANZ, 2011), individual eggs inside retail packs are uniquely identifiable by stamp so that they can be traced back to their place of production. For all the collected retail samples in this study, the codes of the egg stamps were recorded and cross-checked against the updated list of egg business registry codes provided by the Food Unit of the WA Department of Health. All of the samples were traced back to ten egg businesses in WA. Two of these businesses were presented at supermarkets with different multiple commercial brands (e.g., their own brands versus supermarkets' brands). The display of the egg packs varied between supermarkets; 83 (41.5%) of the 200 collected egg sample units were displayed in refrigerated supermarket cabinets, while the rest of the samples were laid directly on shelves at their respective supermarket's ambient temperature (all of the visited supermarkets were air-conditioned). After the retail purchase, the samples were transported in insulated containers with ice bricks (6–8°C) to the Veterinary Public Health Research Laboratory at Murdoch University for testing, and all examinations were conducted within 24 h of collection.

2.2. Isolation, identification and serotyping of Salmonella

Eggshells and egg contents of each sample (each containing one dozen eggs) were tested separately according to Musgrove et al. (2005). Briefly, each of the twelve eggs inside each retail pack were aseptically cracked open, using sterile scalpel blade applied on the blunt/wide end (air sac) of eggshell to avoid contaminating the contents with pieces of the shell. Then, contents of the twelve eggs were transferred and pooled in a sterile stomacher bag. The inside of the shells was rinsed using sterile phosphate-buffered saline to remove most of the adhering albumen. An

effort was made to eliminate as much of this material as possible because of the antimicrobial components of albumen (Musgrove et al., 2005). Shells of the twelve eggs from each sample unit were pooled into a sterile stomacher bag and crushed by hand massaging, then the bag with egg crush was placed inside a further two stomacher bags to prevent leakage during further homogenization processing.

Isolation of *Salmonella* was performed according to the ISO 6579-1:2017 standard, with some modifications (Mooijman, 2018). For each sampling unit, the bag containing crushed shells was weighed, and accordingly a corresponding volume of Buffered Peptone Water (BPW) (Oxoid, England) was added to maintain sample-to-diluent ratio at 1:9 (1 in 10). Then, crushed shells and BPW were homogenized in a stomacher for 1 min, this allowed for a maceration of shells and membranes and a thorough mixing of the sample with the diluent. In parallel to that, the bag containing the twelve pooled egg contents of the same sample unit was first blended in a stomacher for 2 min, and subsequently 25 mL of this mixture was homogenized for 1 min with 225 ml of BPW. Both of the homogenized shells crush and contents from each sampling unit were incubated at 37 °C for 48 h. After pre-enrichment of the incubated homogenate, one ml was inoculated into Mueller Kauffman Tetrathionate Novobiocin Broth (MKTTn) (Oxoid, England), and also from the same homogenate 0.1 mL was spotted (3 drops) on the surface of Modified Semi-solid Rappaport Vassiliadis (MSRV) (Oxoid, England). MKTTn was incubated for 24 h at 37 °C, while MSRV was incubated at 41.5 °C and checked after 24 h for a migration zone (turbid, white halo, with radius larger than 10 mm). MSRV plates with no migration zone after 24 h were checked again after 48 h. Streaks from both MKTTn broth and MSRV media were applied on Xylose Lysine Deoxycholate (XLD) agar (Oxoid, England) and Brilliant Green (BGA) agar (Oxoid, England), which were then incubated at 37 °C for 24 h. Presumptive (up to

5) colonies with suspected *Salmonella* morphology were selected from both selective media and transferred into Nutrient Agar (Oxoid, England) plates. After incubating Nutrient Agar plates at 37 °C for 24 h, well isolated colonies were confirmed to species level using Matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS) using the Microflex instrument (Bruker Diagnostics, Germany). All confirmed *Salmonella* isolates (up to 5 isolates per positive sample) were sent for serotyping (Kauffmann-White-Le Minor scheme) by a nationally accredited reference laboratory (PathWest Laboratory, Perth, WA). Isolates from confirmed positive egg samples were stored at –80ºC till further use.

2.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by micro-broth dilution using commercially prepared panels (Sensititre EUVSEC, TREK Diagnostic Systems, Thermofisher Scientific). Inoculation and incubation was carried out per manufacturer's guidelines, with quality control strains *E. coli* ATCC 35218 used throughout the testing. The panel comprised of 14 antimicrobials: ampicillin (1–64 mg/L, AMP), azithromycin (2–64 mg/L, AZT), cefotaxime (0.25–4 mg/L, CTX), ceftazidime (0.5–8 mg/L, CAZ), chloramphenicol (8–128 mg/L, C), meropenem (0.03–16 mg/L, MER), nalidixic acid (4–128 mg/L, NA), ciprofloxacin (0.015–8 mg/L, CIP), tetracycline (2–64 mg/L, TE), tigecycline (0.25–8 mg/L, TI), colistin (1–16 mg/L, COL), gentamicin (0.5–32 mg/L, GN), trimethoprim (0.25–32 mg/L, W) and sulfamethoxazole (8–1024 mg/l, SMX). Minimum inhibitory concentrations (MICs) were interpreted according to the criteria set by European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC), on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2015 (EFSA, 2017). Epidemiologic cutoff values (ECOFF) is

defined as the highest MIC for organisms devoid of phenotypically detectable acquired resistance mechanisms (EFSA, 2017).

2.4. Whole-genome Sequencing (WGS) based identification of multilocus sequence types (ST) and antimicrobial resistance genes

DNA was extracted from a collection of *Salmonella* isolates recovered from positive retail packs using the BIOLINE DNA extraction kit (ISOLATE II, Genomic DNA Kit) according to the manufacturer's instructions. Genomic analysis was conducted in the Antimicrobial Resistance (AMR) and Infectious Diseases Laboratory at Murdoch University. Library preparation was performed using an Illumina NexTera® XT library preparation kit (Illumina) as per manufacturer's instructions. The library preparations were sequenced on an Illumina Nextseq platform using a mid-output 2 × 150 kit. Reads were de novo assembled using SPAdes 3.11.1software (http://cab.spbu.ru/software/spades/).

The contig files were uploaded to the Centre for Genomic Epidemiology (http://www.genomicepidemiology.org/) to screen for multilocus sequence types (MLST 1.8), and to extract AMR genes data (ResFinder 3.0). All read data generated in this study has been deposited in the NCBI Sequence Read Archive, and the 41 whole-genome sequenced *Salmonella* isolate accessions numbers are in a continuous serial between SAMN12097892 to SAMN12097931 (project accession number PRJNA549805).

2.5. Statistical analysis

The variation in the frequency of *Salmonella* detection between shell and contents, across different production systems, egg businesses and in relation to the display conditions at supermarkets were compared by means of the Chi-square and the two-tailed Fisher's exact test.

The tests were carried out using STATA software (version 11.0; StataCorp, College Station, Texas, USA).

3. Result

3.1. Prevalence and characterization of Salmonella in retail table egg samples in WA

Twenty-three (11.5% [95% CI: 7.4, 16.7]) of the 200 retail egg samples tested positive for *Salmonella*. Among these (n = 23), *Salmonella* was detected in in the shells of nine samples, the contents of six samples and in both the shell and content of eight samples (Table 1). *Salmonella* was detected in 6.4% (6/93) of the free-range retail egg samples, which was significantly lower ($P < 0.05$) than the detection rate of the barn-laid (17.9% [7/39]) and cage-laid (14.7% [10/68]) samples (Table 1).

Multiple isolates from each positive sample were serotyped according to standard procedures. The results of the serotyping revealed that *S.* Typhimurium (52.2% [12/23]) and *S.* Infantis (39.1% [9/23]) were the only serovars present among the isolates retained from the positive egg samples (Table 2). Interestingly, both serotypes (*S.* Typhimurium and *S.* Infantis) were concurrently detected in two retail egg samples, of which one was cage-laid and the other was barn-laid. The results in Table 2 reveal that *S.* Infantis was more commonly detected ($P < 0.05$) on the eggshells (55.6% [5/9]) than in the egg contents (11.1% [1/9]). On the other hand, *S.* Typhimurium was detected more commonly in the egg contents (41.7% [5/12]); however, this was not statistically different from its recovery rate (33.3% [4/12]) from the eggshells.

In this study, the tested retail egg samples (n = 200) were identified and traced back to ten egg businesses in WA. Egg samples that were detected positive for *Salmonella* (n = 23) were attributed to only five out of those ten businesses. Of the five businesses that had positive samples, some variability was evident in the frequency of the *Salmonella* detection; notably, the proportion of *Salmonella*-positive samples from the two major egg producers in WA (**C**: 10.8%

[11/101] and **D**: 12.5% [5/40]) was significantly lower ($P < 0.05$) than that of the remaining three businesses that had *Salmonella*-positive samples (**A**: 20.0% [2/10], **E**: 21.4% [3/14], and **F**: 20.0% [2/10]). The businesses' identification letters were assigned arbitrarily.

With regard to the supermarket display conditions, there were no significant differences in *Salmonella* detection between the egg samples collected from the refrigerated cabinets (10.8% (9/83)) and those sampled from the ambient temperature displays (11.9% [14/117]).

3.2. Phenotypic and genomic characterization of antimicrobial resistance and MLST of Salmonella isolates

Realizing that the 12 eggs in each of the positive sample units were pooled together, we retained multiple isolates (n= 41) from each of the positive sample units for further characterization (Table 3). Among 41 *Salmonella* isolates screened for susceptibility to 14 antimicrobials by MIC panel, only two were resistant to ampicillin while the rest of isolates (n= 39) were susceptible to all other antimicrobials (Table 4). Both of the ampicillin-resistant isolates were *S.* Typhimurium, and no antimicrobial resistance has been found in any *S.* Infantis isolates (n= 12). The resistance genes profile revealed that 40 out the 41 characterized isolates carried aminoglycoside (*aac(6')-Iaa*) resistance gene. However, none of these isolates exhibited phenotypic resistance (based on MIC panel) to gentamicin, as a representative of aminoglycoside group. Added to that, *β*-lactamase resistance gene $bla_{\text{ACT-7}}$ and fosfomycin resistance gene *fosA* were both detected in one *S.* Typhimurium isolate, yet without expressing any phenotypic resistance to the corresponding antimicrobials present in the MIC panel. On the other hand, *β*-lactamase resistance gene $bla_{\text{TEM-1b}}$ was detected in one of the two *Salmonella* isolates displayed phenotypic resistance to ampicillin.

Sequence types (STs) were extracted from the WGS data. Only two STs were identified among the 41 *Salmonella* isolates retained from positive retail egg samples. ST-19 was identified in all (29/29) of the isolates serotyped as *S.* Typhimurium, and the same was found between ST-32 in all (12/12) of the isolates denoted as *S.* Infantis.

4. Discussion

As Australia moves toward a national strategy to reduce foodborne illnesses caused by *Salmonella*, baseline data on *Salmonella* in the food vehicles that cause the illness are important for monitoring and surveillance; thus, they can be used to provide evidence for effective control measures (Food Regulation Secretariat, 2018). Eggs and egg-containing foods were the most common cause of outbreaks in Australia from 2001–2016, leading to significant morbidity (Ford et al., 2018b). Moreover, since 2017, a dramatic increase in reported cases has become evident in WA, with eggs emerging as the key culprit for several *Salmonella* foodborne disease outbreaks (OzFoodNet, 2017). To the best of our knowledge, this is the first published baseline study on *Salmonella* in retail eggs in WA. The key findings arising from this study provide a new level of knowledge on *Salmonella* characterization among retail eggs in WA. This study also provides the first published analysis of multilocus sequence types, phenotypic patterns, and genomic determinants of antimicrobial resistance among *Salmonella* isolates recovered from eggs sampled at the retail level in Australia. The present study could be used as a benchmark for surveys undertaken by industries and controlling authorities in other jurisdictions and states across Australia, and it is hoped that the study will also aid in the implementation of control measures across the supply chain to attempt to reduce the public health burden of egg-related salmonellosis.

Despite the high incidence of human *Salmonellosis*, there are no published studies in almost 10 years on baseline prevalence *Salmonella* in retail eggs in Australia. The concluded prevalence rate of *Salmonella* in the present study (11.5% (23/200)) is three times higher than the rate noted in the last published Australian retail egg survey, conducted in 2008 in South Australia (SA) (Fearnley et al, 2011), where 3.5% (n= 199 egg samples, each of one dozen eggs) were positive

for *Salmonella* on the outside of the egg only. Despite the similarity in both of the sampling setting (retail level) and units of laboratory testing (dozen eggs) between our current study in WA and the retail survey done in SA (Fearnley et al, 2011), a direct comparison should be treated with caution. The setting of the survey in SA was quite different compared to ours, where approximately 80% of eggs available in SA were from "interstate sources" at the time of the survey (Fearnley et al, 2011). On the other hand, the results of our survey are purely reflective of the situation in WA supply chain, and should not be generalized as the situation through-out Australia; given that all of the tested retail eggs in our study were stamped and could be successfully traced to five producers in WA (Table 3). Our results also report a higher *Salmonella* prevalence compared to the rate identified in a retail survey in neighboring New Zealand, identifying *Salmonella* on the outside of 9 of 514 (1.8%) egg samples (retail packs of at least six eggs) (Wilson, 2007). Interestingly, our results align well with both of the above studies in SA (Fearnley et al, 2011) and New Zealand (Wilson, 2007) in relation to a lower rate of *Salmonella* recovery from free-range laid retails eggs compared to other production systems (Table 1). Nevertheless, investigations into the effects of different egg production systems on the prevalence of *Salmonella* in layers and eggs continue to yield conflicting and inconsistent results (Van Hoorebeke et al., 2011; Jones et al., 2015; Parisi et al., 2015). The collective decision to expand more in non-cage laid systems and to move away from conventional cages to enriched cages in Australia should prompt further studies to better predict if and how *Salmonella* contamination of layer facilities, flocks or eggs will change.

Relating the present study results to other surveys conducted in Australia and other countries, it could be concluded that there is a noteworthy level of *Salmonella* contamination present in retail eggs in WA. Several surveys as in UK (Little et al., 2008), Ireland (Murchie et al., 2007) and

Korea (Park et al., 2015) continue to show low *Salmonella* prevalence (<1%). In Sri Lanka, a recent study reported that *Salmonella* contamination was 15% in retail raw table eggs (Kalupahana et al., 2017), and in India a study reported 7.7% contamination of individually tested eggs (n= 492) collected from retail (Suresh et al., 2006). Higher prevalence of a pathogen at retail level implies a higher likelihood of consumer risk; however, a quantitative exposure assessment study would be needed to evaluate further the egg-attributed salmonellosis risk to consumers in WA. Meanwhile, reduction of *Salmonella* rate in retail eggs requires measures to be implemented at farms and packing stations to further reduce the prevalence of contamination, and precautions are required by retailers and caterers to minimize spread, including adequate cooking of eggs and prevention of cross-contamination.

Of the twenty-three retail egg samples that were tested positive in the present study, *Salmonella* was recovered either from eggshells only or from egg contents only, and in some samples it was recovered from both eggshells and egg contents together (Table 2). External contamination of eggshells may arise from infection of the lower reproductive tract of the hen or faecal contamination from hens with gastrointestinal infection with *Salmonella*. Further shell contamination may occur from the environment into which the eggs are laid (De Reu et al., 2006). Experimental results show that salmonellae could survive for a month or more on the shell of whole eggs, indicating capacity to survive from point-of-lay to point-of-consumption (Okamura et al., 2001). External contamination of eggshells presents a risk to humans either directly through contamination of hands and utensils by *Salmonella*, or by the introduction of *Salmonella* into foods when breaking eggs (Little et al., 2007).

In the present study, *Salmonella* was recovered from egg contents at a rate that was similar to recovery rate from eggshells (Table 2). Compared to our results, *Salmonella* have not been

isolated from egg contents in previous surveys testing eggs from retail and farm levels in Australia and New Zealand (Wilson, 2007; Chousalkar et al., 2010; Fearnley et al., 2011; Gole et al., 2014c). In a Japanese survey of egg contents, higher *Salmonella* prevalence rate was reported (13% *Salmonella*-positive) compared to ours (Table 1), but it must be noted that the tested samples were purchased from retail as pooled, liquid eggs (Murakami et al., 2013). Penetration of *Salmonella* from shell into the egg contents has been largely attributed to the existence of numerous factors comprising penetration ability of different *Salmonella* serovars (De Reu et al., 2006; Gast, 2007; Gast et al., 2007), negative pressure due to temperature differences at the time of laying when the egg's cuticle is immature or during storage of the eggs (Miyamoto et al., 1998), load of bacteria on eggshell (Miyamoto et al., 1998), eggshell quality (Gole et al., 2014a) and cuticle deposition (Gole et al., 2014b). It has been shown that a number of different *Salmonella* serotypes can penetrate the shells of eggs but some serotypes appear to survive poorly in the albumen (Whiley and Ross, 2015). If invading *Salmonella* manages to migrate to the yolk or the yolk membrane breaks down, it could multiply in the egg contents at temperatures ≥7°C. The rate of multiplication increases with increased storage temperature (Smadi et al., 2012). Thus, the presence of *Salmonella* in the contents of almost half of the positive samples (Table 2), the long shelf life of retail eggs (up to a month), and the variable levels of refrigerated displays adopted in WA supermarkets could all lead to an interplay that may add to the overall risk to egg consumers in WA.

Our study reveals that two serovars were present in retail table eggs, of which *S.* Typhimurium was more prevalent (52.1%) compared to *S.* Infantis (39.1%). This finding is in accordance with other studies in Australia where *S.* Typhimurium and *S.* Infantis were the predominant serovars detected in the egg production chain (Chousalkar and Roberts, 2012; Pande et al., 2015), as well

as from retail supermarkets (Fearnley et al., 2011). Unlike in Europe and the United States where *S.* Enteritidis is more prevalent in eggs, *S.* Typhimurium has been frequently reported in egg products-associated outbreaks in Australia (Chousalkar et al., 2017). Eggs and egg-containing foods, were the identified food vehicle in 238/476 (50%) of *Salmonella* spp. outbreaks between 2001 and 2016. Added to that, *S.* Typhimurium was the responsible serotype in 95% (226/238) of these outbreaks (Ford et al., 2018a). *S.* Typhimurium caused most (84%) of foodborne or suspected foodborne *Salmonella* spp. outbreaks in Australia, while *S.* Infantis was attributed to only 1% (8/778) of the reported outbreaks between 2001 and 2016 (Ford et al., 2018a). It appears that pathogenicity of *Salmonella* can vary between serotypes although this is not yet predictable. An Australian study showed that the pathogenicity of four strains of *S.* Typhimurium and six other (non-Enteritidis) serotypes (all isolated from layer farms) was variable, but overall the Typhimurium strains exhibited the greatest invasion of human intestinal cell lines and were the only serotypes to cause disease in mice (McWhorter and Chousalkar, 2015). The increasing trend of egg-linked outbreaks in Australia, together with comparatively high and increasing *Salmonella* spp. notification rates (Ford et al., 2016), emphasizes the continued importance of monitoring the pattern of *Salmonella* serotypes and/or genotypes in order to provide evidence for food regulation strategies and control measures.

In recent years, the widespread application and imprudent use of antimicrobials in both humans and animals led to an increase in the pattern of antimicrobial resistance of *Salmonella* in different areas of the world (Anjum et al., 2011; Pande et al., 2015). Results from the present study indicate that antimicrobial resistance among *Salmonella* isolated from retail eggs in WA is considerably low, with 95.2% (39/41) of isolates were fully susceptible to 14 antimicrobials (Table 4). The current study provides the first phenotypic and genomic characterization of

antimicrobial resistance in *Salmonella* isolated from retail eggs in WA. Compared to our study, Pande et al. (2015) reported that 91.72% (133/145) of the *Salmonella* isolates originating from 13 Australian layer farms, in New South Wales (NSW) and SA, were susceptible to all 12 tested antimicrobials. Resistance patterns in *S.* Typhimurium and *S.* Infantis isolates from retail egg samples in the present study, together with previous research on characterizing several *Salmonella* serotypes from commercial layer flocks in NSW and SA, point to an absence of resistance to fluoroquinolones or extended spectrum cephalosporins which are commonly used for the treatment of human salmonellosis (Cheng et al., 2012). The favorable antimicrobial resistance status observed among *Salmonella* isolates from retail eggs (Table 4) is consistent with previous studies on *Salmonella* isolates from egg laying birds (fecal samples) (Pande et al., 2015). Low levels of antimicrobial resistance in general and absence of resistance to clinically critically important antimicrobials in the egg supply chain is a reflection of the quarantine restrictions, regulations and controlled usage for antimicrobials in food producing animals in Australia, compared to the other countries around the world (Snow et al., 2007; Cheng et al., 2012; Obeng et al., 2012). The low antimicrobial resistance among *Salmonella* from eggs also demonstrates minimal public health risks associated with multi-drug resistant *Salmonella* from Australian eggs.

WGS is a valuable tool for prediction of antimicrobial resistance profiles and genes in a variety of pathogens including non-typhoidal *Salmonella* (Nair et al., 2016; Harb et al., 2018). In the present study, two *S.* Typhimurium isolate were not susceptible to ampicillin, of which one was found to harbor the $bla_{\text{TEM-1b}}$ gene. On the other hand, one isolate harbored $bla_{\text{ACT-7}}$ and *fos*A genes but was not susceptible to ampicillin or fosfomycin. More evident mismatch was found in relation to absence of phenotypic resistance to aminoglycoside among 39 isolates with

aminoglycoside acetyltransferase *aac(6')-Iaa* variant gene. Such a phenomenon could occur due to the presence of silent, non-expressing genes or existence of non-integrated gene cassettes as described for several genes in *Salmonella* isolates (Anjum et al., 2011). Beside the insight of WGS on antimicrobial resistance determinants, it has become relatively straightforward to extract MLST profiles directly from WGS datasets. Unlike serotyping, MLST recognizes evolutionary groupings and it has been recommended that *Salmonella* classification by serotyping should be replaced by MLST or its equivalents (Achtman et al., 2012). In the present study, MLST and serotyping were perfectly matching; all *S*. Typhimurium were denoted as ST-19 and all *S*. Infantis were characterized as ST-32. Our results are in agreement with previous research highlighting a good correlation between multilocus STs and *Salmonella* serotypes from clinical human salmonellosis cases, food and animal samples (Cai et al., 2016; Zhao et al., 2017; Harb et al., 2018). A transition to the routine use of MLST, supplemented where appropriate by even more discriminatory genomes-based typing methods, will provide a clearer picture of long-term transmission routes of *Salmonella*, facilitate data transfer and support national control strategies.

5. Conclusion

This study points to the noteworthy *Salmonella* prevalence rate in retail egg samples in WA, a state facing an intriguing increase in the number of human salmonellosis cases in recent years that is greater than the increase in any other state in Australia. The work described here highlights the low rates of antimicrobial resistance of *Salmonella* isolated from retail eggs in WA and is in accordance with previous investigations concluding similar findings at layer farms in different Australian states. The present survey data could aid in developing a better exposure risk assessment for *Salmonella* in eggs consumed by WA consumers. However, development of such

exposure risk assessment will require more data on the frequency of raw egg consumption or under-processing (estimated) versus egg consumption using fully cooked (home or commercially) eggs. To complement the present study, future research is needed to investigate the epidemiology of *Salmonella* at the farm level. The potential of *Salmonella* to contaminate eggs could be examined further through environmental sampling at layer farms. In addition, a separate study is recommended to determine the relationships (if any) between the results of environmental surveys at layer farms and the prevalence of *Salmonella* on and in eggs.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Table 1

Frequency of *Salmonella* detection from eggshells and contents of 200 retail egg samples (each containing one dozen eggs) collected from different supermarkets in Perth, Western Australia.

Production system	No (%) of examined samples	No (%) of positive samples			Total (%) of positive samples
		Shell only	Contents only	Shell and contents	
Cage-laid	68 (34.0)	5 (7.3)	1 (1.4)	4 (5.9)	10 (14.7)
Barn-laid	39 (19.5)	2 (5.1)	2 (5.1)	3 (7.7)	7 (17.9)
Free-range	93 (46.5)	2 (2.1)	3 (3.2)	1 (1.1)	6 (6.4)
Total	200 (100)	9 (4.5)	6 (3.0)	8 (4.0)	23 (11.5)

Table 2

Distribution of *Salmonella* serovars detected in eggshells and contents of 23 positive retail egg samples from different production systems.

Production system	*Salmonella* serovars*					
	***S.* Typhimurium (sole serovar detected in 12 egg samples)**			***S.* Infantis (sole serovar detected in 9 egg samples)**		
	Shell only	**Contents only**	**Shell and Contents**	**Shell only**	**Contents only**	**Shell and contents**
Cage-laid	2	1	1	3	0	2
Barn-laid	0	2	1	2	0	1
Free-range	2	2	1	0	1	0
Total	4 (33.3%)	5 (41.7%)	3 (25.0%)	5 (55.6%)	1 (11.1%)	3 (33.3%)

* In two egg samples (one cage and one barn) both serovars were concurrently detected together in eggshells and egg contents.

Table 3

Distribution of 41 *Salmonella* isolates from 23 positive retail egg samples characterized for their multilocus STs, antimicrobial resistance phenotype and genotype.

Positive egg samples	Producers	Brands	Production systems	No. of retained isolates/+ve sample	No. of isolates from eggshell/serotype	No. of isolates from egg contents/serotype
1	A	A	Free-range	3	2/ *S.* Typhimurium	1/ *S.* Typhimurium
2	A	A	Free-range	2	2/ *S.* Typhimurium	-
3	C	C1	Cage laid	2	2/ *S.* Typhimurium	-
4	C	C1	Cage laid	1	1/ *S.* Typhimurium	-
5	C	C1	Cage laid	2	1/ *S.* Infantis	1/ *S.* Infantis
6	C	C2	Free-range	1	1/ *S.* Typhimurium	-
7	C	C2	Free-range	1	-	1/ *S.* Infantis
8	C	C3	Barn laid	2	-	2/ *S.* Typhimurium
9	C	C3	Barn laid	4	2/ *S.* Typhimurium	2/ *S.* Typhimurium
10	C	C4	Barn laid	1	-	1/ *S.* Typhimurium
11	C	C5	Barn laid	1	1/ *S.* Infantis	-
12	C	C5	Barn laid	1	1/ *S.* Infantis	-
13	C	C14	Cage laid	4	2/ *S.* Typhimurium	2/ *S.* Typhimurium
14	D	D1	Free-range	2	-	2/ *S.* Typhimurium
15	D	D1	Free-range	3	-	3/ *S.* Typhimurium
16	D	D2	Cage laid	1	1/ *S.* Infantis	
17	D	D3	Barn laid	2	1/ *S.* Typhimurium	1/ *S.* Infantis
18	D	D3	Barn laid	2	1/ *S.* Infantis	1/ *S.* Infantis
19	E	E	Cage laid	2	-	2/ *S.* Typhimurium
20	E	E	Cage laid	2	1/ *S.* Infantis	1/ *S.* Typhimurium
21	E	E	Cage laid	1	1/ *S.* Infantis	-
22	F	F	Cage laid	1	-	1/ *S.* Infantis
23	F	F	Cage laid	1	-	1/ *S.* Infantis

Table 4

MICs of *Salmonella* isolates (n=41) from retail table egg samples from different supermarkets in Perth, Western Australia.

Antimicrobial	Range of dilutions tested (mg/liter)	ECOFF value (mg/liter)	No. of isolates with the following MIC (mg/liter):														No. (%) of Resistant Isolates to each serovars	
			0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64	*S.*Typhimurium (n= 29)	*S.* Infantis (n= 12)
Sulfamethoxazole	8-1024	>256	-	-	-	-	-	-	-	-	-	-	-	34	7	-	0 (0)	0 (0)
Trimethoprim	0.25-32	>2	-	-	-	-	39	2	-	-	-	-	-	-	-	-	0 (0)	0 (0)
Gentamicin	0.5-32	>2	-	-	-	-	-	41	-	-	-	-	-	-	-	-	0 (0)	0 (0)
Ampicillin	1-64	>8	-	-	-	-	-	-	24	13	2	-		2	-	-	2 (6.7)	0 (0)
Ciprofloxacin	0.015-8	>0.06	11	30	-	-	-	-	-	-	-	-	-	-	-	-	0 (0)	0 (0)
Nalidixic Acid	4-128	>16	-	-	-	-	-	-	-	-	38	3	-	-	-	-	0 (0)	0 (0)
Tetracycline	2-64	>8	-	-	-	-	-	-	-	41	-	-	-	-	-	-	0 (0)	0 (0)
Tigecycline	0.25-8	>1	-	-	-	-	28	13	-	-	-	-	-	-	-	-	0 (0)	0 (0)
Meropenem	0.03-16	>0.12	-	41	-	-	-	-	-	-	-	-	-	-	-	-	0 (0)	0 (0)
Ceftazidime	0.5-8	>2	-	-	-	-	-	41	-	-	-	-	-	-	-	-	0 (0)	0 (0)
Cefotaxime	0.25-4	>0.5	-	-	-	-	41	-	-	-	-	-	-	-	-	-	0 (0)	0 (0)
Azithromycin	2-64	>16	-	-	-	-	-	-	-	-	13	28	-	-	-	-	0 (0)	0 (0)
Colistin	1-16	>2	-	-	-	-	-	-	41	-	-	-	-	-	-	-	0 (0)	0 (0)
Chloramphenicol	8-128	>16	-	-	-	-	-	-	-	-	-	41	-	-	-	-	0 (0)	0 (0)

Highlights

- We present the first report on *Salmonella* in retail eggs in Australia since almost 10 years
- *Salmonella* was detected in 11.5% (23/200) of the tested egg samples.
- *S*. Typhimurium and *S*. Infantis were the only detected serovars.
- Most (39/41) of *Salmonella* isolates were susceptible to a panel of 14 antimicrobials.
- We applied WGS to analyze a set of 41 recovered *Salmonella* isolates.