Antimicrobial resistance profiles of *Salmonella* spp. from agricultural environments in fruit production systems

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Abstract

Foodborne disease outbreaks involving fresh produce have increased in recent years. The risk of infection from contaminated food is worsened by the increased prevalence of antibiotic resistant strains. This study evaluated the prevalence of antibiotic resistance in *Salmonella* isolates (n=263) from agricultural production systems through to the final packed product. *Salmonella* isolates were preliminarily identified by matrix-assisted laser desorption ionisation-time of flight mass spectroscopy (MALDI-TOF MS) and API 20E and identities confirmed by *invA* gene PCR. Antimicrobial susceptibility was performed with 15 antimicrobial agents using the Kirby Bauer disk diffusion test. Of the 263 *Salmonella* isolates assessed, 59.3% were resistant to one or more antimicrobials. The most frequently detected resistance was against chloramphenicol and kanamycin (46.7%), trimethoprim-sulfamethoxazole (28%) and streptomycin (14%) and less frequently detected resistance was towards ampicillin (1.14%), amikacin (0.76%) and amoxicillin–clavulanic acid (0.38%). Multiple antimicrobial resistance (MAR) (resistance to ≥3 antibiotics) was found in 48.7% (76/156) isolates. The most common MAR phenotype was to chloramphenicol-trimethoprim/sulfamethoxazole-kanamycin (43.6%). Resistance to chloramphenicol, kanamycin or trimethoprim/sulfamethoxazole was only observed in MAR phenotypes. All isolates were susceptible to ceftiofur, cefoxitin, ceftriaxone, ciprofloxacin, nalidixic acid, gentamicin and tetracycline. This study confirms the importance fresh produce production environments as potential reservoirs and fresh produce as carriers of antibiotic resistant *Salmonella* spp. with significant clinical importance. Further studies to evaluate the actual level of health risk from these pathogens should include characterisation of the antibiotic resistance determinant genes among the isolates.

Key words: horticultural production systems, fresh produce, primary production, agricultural irrigation, packhouse environment, antibiotic resistance, health risk.

Introduction

Increased consumption of fresh produce as part of a healthy diet, has led to increased risks associated with foodborne disease outbreaks (Painter *et al*., 2013) of which *Salmonella* has been identified as the most important pathogen in the European Union and the United States (Callejon *et al*., 2015; Scallan *et al*., 2011). Infections with non-typhoid *Salmonella* may result in severe gastroenteritis when infections spread beyond the intestine, especially among the immuno-compromised including infants and the aged (Hohmann, 2001; Su *et al*., 2004). Adding to the complexity of health care, single and multiple drug resistance (MDR) to traditional drugs of choice for treating *Salmonella* infections have been reported (Hohmann *et al*., 2001; Varma *et al*., 2005; Wasfy *et al*., 2002; WHO, 2015). Infections caused by these drug-resistant *Salmonella* strains are associated with increased morbidity and mortality, disease severity, treatment failure, and hospital stays compared to infections with susceptible strains (WHO, 2005).

Studies to evaluate antibiotic resistance in fresh produce, and in pre- and postharvest environments are limited and the results are often conflicting. Some studies report that *Salmonella* isolates of plant origin food are generally susceptible to commonly used antibiotics (Gorski *et al*., 2011; Greene *et al*., 2008; Patchanee *et al*., 2010; Sivapalasingam *et al*., 2003) Conversely, *Salmonella* resistant strains are gradually being reported in fresh produce production systems (Abakpa *et al*., 2015; Learn-Han *et al*., 2009; Li *et al*., 2014; Singh *et al*; 2007; Zhao *et al*., 2003). Any efforts to control antibiotic resistance in the fresh produce chain should therefore systematically evaluate the role of production environments to act as reservoirs or carriers of antibiotic resistant bacteria (Chidamba and Korsten, 2015). In light of the health implications associated with drug-resistant *Salmonella* strains, the emergence of antibiotic-resistant *Salmonella* in the horticultural system is a new and emerging food safety challenge which deserves further study. This study was undertaken to assess antimicrobial resistance profiles of *Salmonella* isolates from commercial horticultural environments.

Materials and methods

Sampling sites

Three large commercial farms located in there different regions/provinces were selected for this study. The farms were export oriented and GLOBAL G.A.P. certified and had their own packhouses. The types of fruit produced on these farms as well as the exact locations are not given due to confidentiality reasons. Moreover, it was not the purpose of this study to investigate a specific fruit type but rather a horticultural production system. The selected sites represent major fruit production regions with no nearby large-scale cattle farm, feedlot or battery poultry farming. The water used on these farms is extracted from large nearby river systems. The quality of this water is at times compromised due to nearby informal settlements or municipal waste water (De Villiers, 2007; Fatoki *et al*., 2001).

Sample collection

A total of 491 samples comprising fruit (225), agricultural water (140) and packline and hand swabs (126) were collected from the three commercial farms (A, B and C) and associated packhouses during June to September, 2011. Water samples were collected from both pre- and postharvest stages at each farm and at the associated packhouses. Preharvest water samples were collected from river, dam and/or storage tanks. Irrigation water samples were collected as described in ISO 5667-10:1992 from pipes along the drip-line in the same orchards where fruit samples were collected.

Fruit samples included low hanging fruit regularly watered with the micro-irrigation system in the orchard, and fruit (from the same orchard) collected from the harvesting crates before wash and at various points further down the packline including after washing, fungicide treatment, wax application and in the final packed box. Swab samples (Medical Wire and Equipment, Johannesburg) were collected from hands of packhouse personnel handling the fruit and approximately 25 cm^2 conveyer belt surfaces that came into contact with the same batch of fruit by thoroughly swabbing with at least 10 passes vertically and horizontally. All samples were collected aseptically and kept chilled at 4 ^{o}C for no longer than 48 h prior to analysis.

Salmonella detection, isolation and identification

All microbiological media were purchased from Merck (Johannesburg, South Africa) unless otherwise stated. All samples (*n*=491) were analysed for *Salmonella* following the United States Food and Drug Administration (U. S. FDA) Bacteriological Analytical Manual (BAM) protocol for fresh produce (Andrews and Hammack, 2007) with minor modifications. Fruit samples (three fruit per sample) were submerged in1 L sterile 0.1% buffered peptone water supplemented with 0.02% (v/v) Tween 80 (Associated Chemical Enterprises, Johannesburg). Microbial epiphytes on fruit surfaces were dislodged by sonication in a digital heated ultra-sonic cleaner (Eumax, UD200SH-6L, Labotec, Johannesburg) for 5 min at 200W and 50Hz. The fruit microflora washings and 1 L water samples were concentrated by filtration through a 0.45μm pore-size nitro-cellulose membrane (Sartorius, Goettingen, Germany).

Subsequently, membranes and swabs were aseptically transferred into 9 ml tryptone soy broth (TSB) and incubated with agitation for 24 h at 37 ^{o}C. An aliquot (0.1 ml) of the pre-enriched broth was inoculated into 10 ml Rappaport for selective enrichment and isolation of *Salmonella* as previously described (Gorski *et al*., 2011). A total of 263 *Salmonella* were isolated and preliminarily identified by matrix-assisted laser desorption ionisation-time of flight mass spectroscopy (MALDI-TOF MS) (Dieckmann and Malorny, 2011) and API 20E (Holmes *et al*., 1978) and identities confirmed by *invA* gene PCR as previously described (Rahn *et al*., 1992).

Antimicrobial susceptibility testing

Isolates were tested for antimicrobial susceptibility using the Kirby–Bauer disk diffusion method on Muller-Hinton agar (Oxoid, Johannesburg) as previously described (Gorski *et al*., 2011) in accordance with the Clinical and Laboratory Standards Institute guidelines (CLSI, 2011). Fifteen antimicrobial agents (Mast Diagnostics, UK, supplied by Davies Diagnostics, SA) representing eight classes of drugs were tested in this study (Table 1).

TABLE 1. ANTIBIOTIC RESISTANCE PROFILES OF *SALMONELLA* SPP. ISOLATED FROM FRUIT, WATER, AND CONTACT SURFACES IN HORTICULTURAL PRODUCTION ENVIRONMENTS REPRESENTING THREE MAJOR FRUIT PRODUCTION REGIONS

Sample sources				Antibiotic resistance										
				AK	S	C-K	AP-C-K	C-K-AUG	C-TS-K	C-AP-TS-K	C-TS-K-AK	C-TS-S-K		
Region	Production area	Type	Source	1[a]	1	2	3	3	3	4	4	4	Susceptible[b]	Total
Site A	Orchard	Water	Irrigation water	1	2	7	—	—	12	—	—	1	10	33 (12.5)
		Fruit	Low hanging	—	—	—	—	—	—	—	—	—	20	20 (7.6)
	Packhouse	Swab	Conveyor swab	—	—	2	1	—	46	1	1	3	—	54 (20.5)
Site B	Packhouse	Water	Chlorine spray water	—	5	—	—	—	—	—	—	—	16	21 (8)
		Fruit	After chlorine wash	—	6	—	—	—	—	—	—	—	11	17 (6.5)
			After warm bath	—	7	—	—	—	—	—	—	—	18	25 (9.5)
			After wax	—	4	—	—	—	—	—	—	—	7	11 (4.2)
			Final pack	—	5	—	—	—	—	—	—	—	5	10 (3.8)
Site C	Orchard	Water	Dam	—	3	—	—	—	—	—	—	—	20	23 (8.7)
		Fruit	Low hanging	—	—	35	—	—	—	—	—	—	—	35 (13.3)
	Packhouse		Final pack	—	—	3	—	1	10	—	—	—	—	14 (5.3)
			Total (%)	1 (0.4)	32 (12.2)	47 (17.9)	1 (0.4)	1 (0.4)	68 (25.9)	1 (0.4)	1 (0.4)	4 (1.5)	107 (40.7)	263

[a]Number of antibiotics to which isolates were resistant.
[b]Other antibiotics to which all isolates were susceptible to included tetracycline, nalidixic acid, ciprofloxacin, cefotaxime, cefoxitin, ceftazidime, ceftriaxone, and gentamicin.
AK, amikacin; AP, ampicillin; AUG, amoxicillin–clavulanic acid; K, kanamycin; C, chloramphenicol; S, streptomycin; TS, trimethoprim–sulfamethoxazole.

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Genotypic determination and serotyping of *Salmonella* isolates

All 263 isolates confirmed as *Salmonella* spp. were typed with the primer sets BOX-A1R and (GTG)-$_5$ as previously described (Versalovic et al. 1994; Rademaker and de Bruijn 1997). A total of 39 representative isolates were systematically selected to represent various antimicrobial resistance and rep-PCR (BOX-A1R and GTG-$_5$) profiles, sources and sample types. Selected isolates were serotyped at the Onderstepoort Veterinary Institute Bacteriology Laboratory, Pretoria as previously described (Grimont and Weill, 2007).

Statistical analysis

Statistica 10 (Stat soft, USA) was used for all the statistical analyses. Data on antibiotic resistance of each bacterial isolate were reported either as the inhibition diameter (in millimetres) or as susceptible or resistant (based on CLSI, 2011 breakpoints). To avoid overestimation of resistance, all isolates that showed intermediate resistance were reclassified as susceptible (Ta *et al*., 2014). Multiple antimicrobial resistance index (MAR index) defined as a/b whereby 'a' is the number of antibiotics to which a particular isolate is resistant to and 'b' is the total number of antimicrobials tested (Krumperman, 1983) was determined for each of the isolates tested. Electrophoretic gels were analysed as previously described (Chidamba and Bezuidenhout, 2012).

Results

A total of 26 (5.3%) of the 491 samples analysed were positive for *Salmonella* and 263 isolates were recovered. At site A *Salmonella* was detected from three orchard water (33 isolates), two orchard fruit (20 isolates) and nine packhouse conveyer belt swab (54 isolates) samples. At Site B *Salmonella* was detected from four packhouse fruit samples; after wash (16 isolates), warm bath (25 isolates), waxing and packed fruit (11 isolates each), and two packhouse wash water (21 isolates) samples. At site C *Salmonella* was detected from one dam water (23 isolates), four orchard fruit (35 isolates) and one packed fruit (14 isolates) sample. All isolates were preliminarily identified with MALDI-TOF-MS as *Salmonella* spp. with score values ranging from 2.292 to 2.537 and harboured the *invA* gene. The data on antibiotic resistance profiles to a panel of 15 antibiotics is shown in Table 1. Antibiotic resistance was observed against three aminoglycosides (amikacin (0.76%), kanamycin

(47.1%) and streptomycin1 (13.7%), two β-lactams (ampicillin; 0.76% and amoxicillin-clavulanic acid (0.38%), phenicols (chloramphenicol (47%) and folate pathway inhibitors (trimethoprim-sulfamethoxazole (28.1%). All the isolates tested were susceptible to tetracyclines (tetracycline), quinolone (nalidixic acid), fluoroquinolones (ciprofloxacin), cephalosporins (cefoxatime, cefoxitin, ceftazidime and ceftriaxone) and one aminoglycoside (gentamicin).

On average, 59.3% (156/263) of the *Salmonella* isolates were resistant to at least one or more antimicrobials tested in this study. Resistance towards ampicillin, amikacin and amoxicillin–clavulanic acid were observed in small proportions (1.14, 0.76 and 0.38%, respectively).

A comparison of resistance levels of isolates from different types/sources of samples across sampling sites revealed that isolates from conveyer belt swabs for Site A and fruit from the orchard and final packs at Site C were the most resistant and exhibited 100% resistance to chloramphenicol and kanamycin. Although resistance to chloramphenicol, trimethoprim-sulfamethoxazole, kanamycin and ampicillin were observed for isolates from Site A and C, no such resistance was observed for isolates from Site B. Resistance to amoxicillin-clavulanic acid was only observed in one isolate from final pack fruit at Site C. Similarly, ampicillin resistance was only detected in conveyer swabs at Site A.

All chloramphenicol-resistant isolates also exhibited resistance to kanamycin. Antibiotic resistance phenotypes observed in the current study included single, dual and multiple antibiotic resistances (up to four different antibiotics) (Table 1). Of the 156 antibiotic resistant isolates, 48.7% (76/156) exhibited MAR (≥3) distributed among six different patterns (AP-C-K; C-K-AUG; C-TS-K; C-AP-TS-K; C-TS-K-AK and C-TS-S-K) with MAR indices ranging from 0.2 (3/15) to 0.27 (4/15) (Table 1). The most common MAR phenotypes included combinations of three antimicrobials (44.9%; 70/156) and to a lesser extent, four antimicrobials (3.8%; 6/156). Single AR phenotypes were limited to S and AK whereas the only dual AR phenotype observed was C-K.
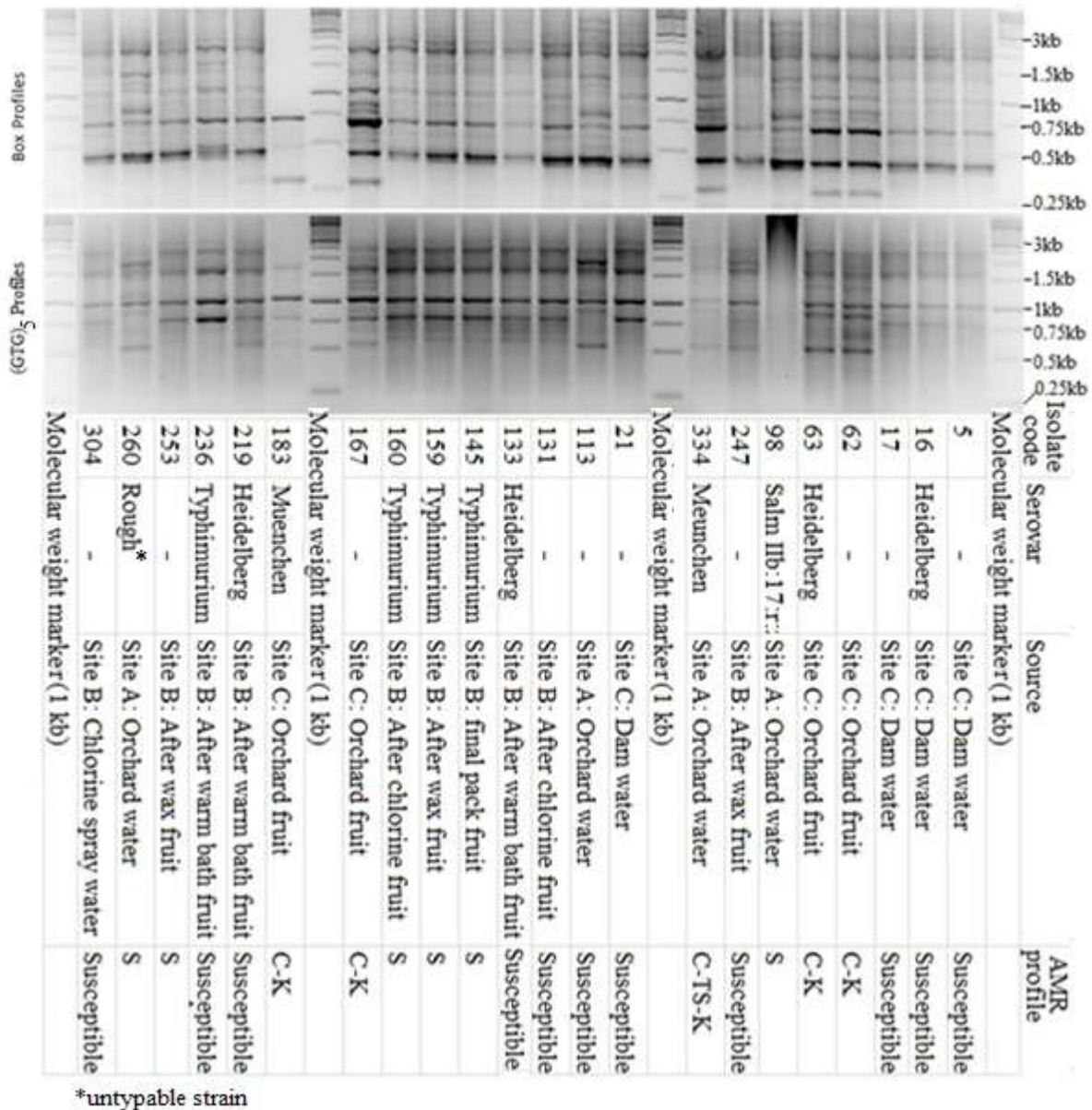
Isolate code	Serovar	Source	AMR profile
Molecular weight marker(1 kb)			
5	–	Site C: Dam water	Susceptible
16	Heidelberg	Site C: Dam water	Susceptible
17	–	Site C: Dam water	Susceptible
62	–	Site C: Orchard fruit	C-K
63	Heidelberg	Site C: Orchard fruit	C-K
98	Salm IIb:17:r::	Site A: Orchard water	S
247	–	Site B: After wax fruit	Susceptible
334	Meunchen	Site A: Orchard water	C-TS-K
Molecular weight marker (1 kb)			
21	–	Site C: Dam water	Susceptible
113	–	Site A: Orchard water	Susceptible
131	–	Site B: After chlorine fruit	Susceptible
133	Heidelberg	Site B: After warm bath fruit	Susceptible
145	Typhimurium	Site B: final pack fruit	S
159	Typhimurium	Site B: After wax fruit	S
160	Typhimurium	Site B: After chlorine fruit	S
167	–	Site C: Orchard fruit	C-K
Molecular weight marker(1 kb)			
183	Muenchen	Site C: Orchard fruit	C-K
219	Heidelberg	Site B: After warm bath fruit	Susceptible
236	Typhimurium	Site B: After warm bath fruit	Susceptible
253	–	Site B: After wax fruit	S
260	Rough*	Site A: Orchard water	S
304	–	Site B: Chlorine spray water	Susceptible
Molecular weight marker (1 kb)			

*untypable strain

Figure 1. Representative agarose gel images for *Salmonella* genotyping with Box and (GTG)$_5$ primers showing the selected representative isolates with respect to source, antibiotic resistance and serotypes.

Genotyping of the 263 *Salmonella* isolates with rep-PCR (BOX-A1R and (GTG)-$_5$ primers) resulted in a diversity of profiles (Figure 1). These together with the observed antimicrobial resistance for isolates from different sources and sample type resulted in the systematic selection of 39 representative isolates (Figure 2). Serotyping showed these isolates to belong to the serovars *S*. Muenchen (13/39; 33.3%), *S*. Typhimurium (12/39; 30.8%), *S*. Heidelberg (8/39; 20.5%). *S*. Bsilla (3/39; 7.7%), Salm IIb 17: r: z (2/39; 5.1%) and one untypable rough biotype. Cluster analysis showed the isolates to group relative to their serovars, although other clusters were composed of different serovars. The genotype grouping showed the

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detection of similar strains among different packhouse samples even though no similar strains could be detected between packhouse and field samples (Figure 2). Although similar serovars were detected between orchard water and packhouse samples, consideration of their antibiotic resistance showed them to be different strains.
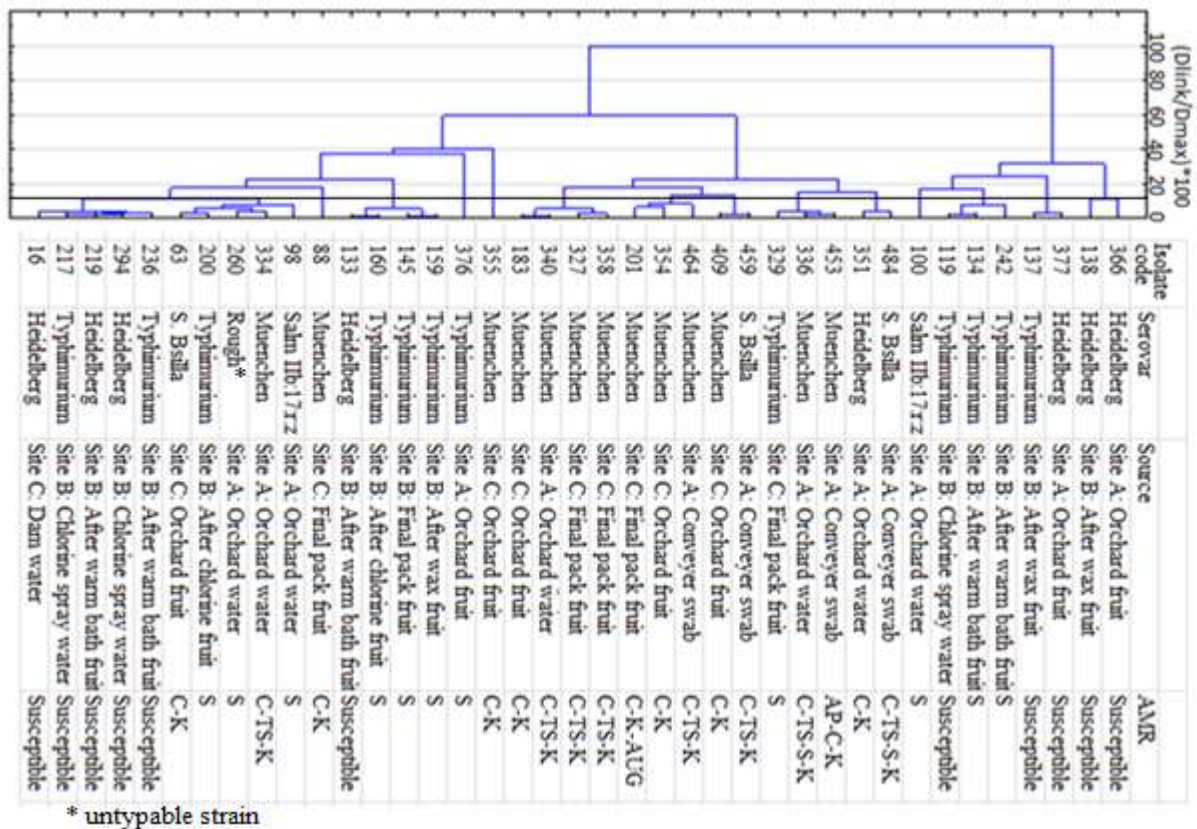
Isolate code	Serovar	Source	AMR
16	Heidelberg	Site C: Dam water	Susceptible
217	Typhimurium	Site B: Chlorine spray water	Susceptible
219	Heidelberg	Site B: After warm bath fruit	Susceptible
294	Heidelberg	Site B: Chlorine spray water	Susceptible
236	Typhimurium	Site B: After warm bath fruit	Susceptible
63	S. Bsilla	Site C: Orchard fruit	C-K
200	Typhimurium	Site B: After chlorine fruit	Susceptible
260	Rough*	Site A: Orchard water	C-TS-K
334	Muenchen	Site A: Orchard water	C-K
98	Salm IIb:17:r:z	Site A: Orchard water	S
88	Muenchen	Site C: Final pack fruit	C-K
133	Heidelberg	Site B: After warm bath fruit	Susceptible
160	Typhimurium	Site B: After chlorine fruit	S
145	Typhimurium	Site B: Final pack fruit	S
159	Typhimurium	Site B: After wax fruit	S
376	Typhimurium	Site A: Orchard fruit	S
355	Muenchen	Site C: Orchard fruit	C-K
183	Muenchen	Site C: Orchard fruit	C-K
340	Muenchen	Site A: Orchard water	C-TS-K
327	Muenchen	Site C: Final pack fruit	C-TS-K
358	Muenchen	Site C: Final pack fruit	C-K-AUG
201	Muenchen	Site C: Final pack fruit	C-TS-K
354	Muenchen	Site C: Orchard fruit	C-K
464	Muenchen	Site A: Conveyer swab	C-TS-K
409	Muenchen	Site C: Orchard fruit	C-K
459	S. Bsilla	Site A: Conveyer swab	C-TS-K
329	Typhimurium	Site C: Final pack fruit	S
336	Muenchen	Site A: Orchard water	C-TS-S-K
453	Muenchen	Site A: Conveyer swab	AP-C-K
351	Heidelberg	Site A: Orchard water	C-K
484	S. Bsilla	Site A: Conveyer swab	C-TS-S-K
100	Salm IIb:17:r:z	Site A: Orchard water	S
119	Typhimurium	Site B: Chlorine spray water	Susceptible
134	Typhimurium	Site B: After warm bath fruit	S
242	Typhimurium	Site B: After warm bath fruit	Susceptible
137	Typhimurium	Site B: After wax fruit	Susceptible
377	Heidelberg	Site A: Orchard fruit	Susceptible
138	Heidelberg	Site B: After wax fruit	Susceptible
366	Heidelberg	Site A: Orchard fruit	Susceptible

* untypable strain

Figure 2. Ward and Euclidean distance Clustering of selected *Salmonella* representative isolates based on Box and (GTG)$_5$ Rep PCR profiles and their associated serotypes, sources and antibiotic resistance profiles.

Considering the observed antibiotic resistance profiles, the serotype *S*. Muenchen had the highest antibiotic resistance observed with five antimicrobial resistance profile types (Figure 2). Among these the antimicrobial resistance profile C-K and C-TS-K were the most prevalent. Serovar Bsilla had three AMR profile types. The rough type and Salm IIb: 17: r: z had single antimicrobial resistance profile type, while *S*. Typhimurium and Heidelberg having two phenotypes each. The highest MAR was observed for the C-TS-S-K combination in *S*. Bsilla and *S*. Muenchen. Serovar Heidelberg and *S*.Typhimurium were the only serovars with isolates susceptible to all antibiotics tested.

Discussion

Antimicrobial resistance among *Salmonella* spp. isolates from fresh produce is a serious public health concern given that these products are mostly consumed raw or just minimally processed. Although the finding of AR *Salmonella* (59.3%) in this study is high, it is relatively lower than previous findings which reported high levels (82.9%) of antimicrobial resistance in *Salmonella* isolated from market vegetables in Northern India (Singh *et al*., 2007). However, the *Salmonella* isolates were collected at the market-end and did not include any isolates from the primary production environments. In contrast, the majority of the *Salmonella* isolates recovered from tomato farm environments in the Mid-Atlantic region of the U.S (Micallef *et al*., 2012) and crop production watersheds in North Carolina (Patchanee *et al*., 2010), were susceptible to most of the antibiotics tested. Differences in antimicrobial use patterns and associated selection pressures could possibly explain the observed variation in *Salmonella* resistance profiles reported from different countries or regions.

Frequent resistance observed for chloramphenicol, kanamycin and trimethoprim/sulfamethoxazole in this study could be due to their use in human and veterinary medicine for many years (Li *et al*., 2014). Our findings were inconsistent with results from Duffy *et al*. (2005), Learn-Han *et al*. (2009), Gorski *et al*. (2011) and Micallef *et al*. (2012), who reported low or no resistance to these drugs. On the other hand, Osterblad *et al*. (1999) noted a chloramphenicol resistance rate of 12% among *Enterobacteriaceae* isolated from vegetables. Although the use of chloramphenicol as a growth promoter in animal production has been prohibited in developed countries owing to induced anaemia in humans, this drug has for long been on the list of first-line drugs to treat numerous human infections in Sub-Saharan Africa (Okeke and Sosa, 2003). Furthermore, the presence of trimethoprim/sulfamethoxazole resistant *Salmonella* isolates is a cause of concern since this drug is widely used to treat systemic *Salmonella* infections and typhoid fever in humans (WHO/UNAIDS, 2000).

Similar to our findings, Micallef *et al*. (2012) also reported very low resistance percentages for amoxicillin/ clavulanic acid and ampicillin. According to the Veterinary Drug Directorate, Health Canada, amoxicillin/clavulanic acid is classified in category 1 (very high importance

in human medicine) (Mainali *et al*., 2014). All *Salmonella* isolates were susceptible to cefoxitin, ceftriaxone, ceftazidime, cefoxatime, nalidixic acid, ciprofloxacin, gentamicin and tetracycline. These findings are similar to those reported by Metcallf *et al*. (2012) who found no resistance towards ciprofloxacin, ceftriaxone, gentamicin and nalidixic acid but observed resistance towards cefoxitin and tetracycline in *Salmonella* isolates from Mid-Atlanta tomato farms. It is important to note that most of their *Salmonella* isolates were recovered from irrigation ponds at large-scale farms located close to poultry production facilities.

Although tetracycline and ampicillin are widely used both in veterinary and human medicine, none of our isolates presented resistance to tetracycline while only one isolate was resistant to ampicillin. A lack of resistance to tetracycline and ampicillin in the present study may imply that the isolates were not of animal origin since these drugs are widely used in livestock farming as feed additives in many countries. and no animal farm was situated near the sampling sites in this study (Geonaras *et al*., 2001; Henton *et al*., 2011; Mainali *et al*., 2014). With the exception of streptomycin and kanamycin, resistance towards other members of the aminoglycosides tested in this study (gentamicin and amikacin) was not observed or insignificant. Use of antibiotics in this class, apart from streptomycin which is mostly used to treat tuberculosis patients, has been reduced in many countries (Goni-Urriza *et al*., 2000) and this may account for the low resistance levels observed in this study.

Resistance to chloramphenicol, kanamycin and trimethoprim/sulfamethoxazole was only observed in multiple resistant isolates and never as single antibiotics, mostly at Site A and Site C. High resistances observed for isolates from site A and C may be because the isolates were from pre-harvest stages (storage dam water, orchard irrigation water and orchard fruit) which are exposed to the outside environment compared to site B isolates which all came from the packhouse environment with limited pathogen dispersal. Associations among antibiotic resistances in particular isolates can be ascribed to the presence of linked genes in mobile genetic elements such as plasmids, transposons, or integrons that harbour one or more resistant genes, each encoding a single antibiotic resistance phenotype (Kelly *et al.,* 2009; Mather *et al*., 2013; Singh *et al*., 2005).

Due to the nature of horticultural production systems assessed in this study, large-scale commercial farms are mostly in rural areas away from poultry plants or feedlots. In certain cases, informal settlements can be found around upstream areas of the river catchment which serve as a source of irrigation water. It has been well documented that municipalities in these areas are the major contributor to agricultural water pollution (CSIR, 2010). Consequently, the most likely source of AR *Salmonella* in this scenario is municipal polluted rivers used by the farmers in this study. Future studies should focus on source tracking to show the human, animal, sewage, river, crop, food link.

Although antibiotic inhibition patterns between isolates from water, fruit and swab samples, suggests irrigation water to be the source of *Salmonella* contamination in packhouses, consideration of rep-PCR genotypic profiles suggest otherwise. There is evidence of spread of the same *Salmonella* strains within the packhouses but no link could be made to the *Salmonella* observed in the field or irrigation water samples. However, the absence of some genotypes and AR phenotypes in some samples at the same farm and packhouse may be a result of failing to pick the representative isolates during the random selection. Hence, the results of this study point to contamination of agricultural production environments by a wide diversity of AR resistant *Salmonella* spp. most likely contributed by polluted river water used for irrigation or packers in the packhouses.

Conclusions

This study confirms the importance of fresh produce production environments as potential reservoirs and fresh produce as carriers of antibiotic resistant *Salmonella* spp. The antimicrobial resistance towards commonly used antimicrobials demonstrated in our study is a cause for concern. However, it is encouraging that all isolates were susceptible to the more clinically important drugs (third generation cephalosporins, quinolones and fluoroquinolones) used for treating life-threatening infections caused by *Salmonella*. Nevertheless, on-going monitoring of antimicrobial resistance is necessary to evaluate overall resistance pools and trends, and to explore risks and risk mitigation strategies in horticultural supply chains.

Acknowledgements

Disclosure Statement: There was no conflict of interests

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