Antimicrobial resistant bacteria from imported vegetables and spices purchased from niche markets in Saskatoon, Saskatchewan

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Abstract

The role of imported vegetables and spices in the epidemiology of antimicrobial resistance in Canada has been inadequately studied. In this investigation, resistant organisms from imported vegetables and spices in Saskatoon, Saskatchewan were investigated.

A total of 147 vegetable and spice products imported from primarily Asian and African countries, were purchased from international markets in Saskatoon, Saskatchewan. Samples were selectively cultured for a panel of bacteria where resistance is known to be emerging. The proportion of samples positive for each organism were as follows: *E. coli* (n=15, 10.2%), *Salmonella* spp. (n=2, 1.4%), *Enterobacter* spp. (n=10, 6.8%), *K. pneumoniae* (n=2, 1.4%), *Acinetobacter* spp. (n=11, 7.5%), *Pseudomonas* spp. (n=10, 6.8%), *S. aureus* (n=7, 4.8%) and *Enterococcus* spp. (n=68, 46.3%). Antimicrobial susceptibility testing of isolates was conducted by broth micro dilution and agar dilution. Based on the susceptibility of each organism, isolates were then screened for resistance genes (β-lactamases and plasmid mediated quinolones resistance determinants) by PCR.

Multidrug resistant Enterobacteriaceae were identified. Among other organisms, methicillin resistant *S. aureus* (MRSA) were identified. Resistance to the penicillins, cephalosporins, quinolones and aminoglycosides mediated by broad spectrum β-lactamases and plasmid mediated quinolone resistance determinants (PMQR) were identified. Broad spectrum β-lactamase producers were cultured from 6/147 (4.1%) of samples tested. The most common enzymes found were CTX-M-15 (n=4), TEM-1 (n=2), SHV-142 (n=1) and SHV-106 (n=1). The PMQRs (QnrB1, QnrB2, QnrS1 and AAC(6')-Ib-cr) containing isolates were cultured from 2/147 (1.4%) of the samples. While all multi-drug resistant Enterobacteriaceae isolates were susceptible to meropenem and colistin, several non-fermenters were resistant to these drugs. However, none of these organisms produced carbapenemases or possessed mobilized colistin resistance determinants (MCR-1, MCR-2, MCR-3, MCR-4). *Salmonella* spp. were susceptible to the most of tested drugs. With the exception of two MRSA, the *S. aureus* isolates were susceptible to other non-beta lactam drugs. *Enterococcus* spp. isolates were resistant to various drugs but susceptible to ampicillin, penicillin and vancomycin.

It was interesting to observe that all of the multi-drug resistant organisms originated from countries which are recognized to have a high prevalence of resistance. Therefore, further study is required to understand the extent of antimicrobial resistance transmission by imported vegetables and spices from these countries to Canada.

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Lastly, I thank you who are reading this thesis right now. I hope my thesis would be able to provide you interesting scientific findings and benefit your academic works.

To my Mom, my Dad and my grandparents

For your love, patience and sacrifices

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AGISAR	Advisory Group on Integrated Surveillance of Antimicrobial Resistance
AMR	Antimicrobial Resistance
ARG-ANNOT	Antibiotic Resistance Gene-ANNOTation
BG	Brilliant Green
BPW	Buffer Peptone Water
CARD	Comprehensive Antibiotic Resistance Database
CARSS	Canadian Antimicrobial Resistance Surveillance System
CDC	Centers for Disease Control and Prevention
CIPARS	Canadian Integrated Programs for Antimicrobial Resistance Surveillance
CLSI	Clinical and Laboratory Standards Institute
EARS-Net	European Antimicrobial Resistance Surveillance Network
EDTA	Ethylenediaminetetraacetic Acid
EFSA	European Food Safety Authority
ESBLs	Extended Spectrum β-lactamases
EU/EEA	European Union/European Economic Area
EUCAST	European Committee on Antimicrobial Susceptibility Testing
HGT	Horizontal Gene Transfer
IMI	Imipenem hydrolysing β-lactamase
IMP	Imipenem Resistant *Pseudomonas*
KPC	*Klebsiella pneumoniae* Carbapenemase
MCR	Mobilized Colistin Resistance
MDR	Multidrug Resistance

$MgCl_2$	Magnesium Chloride
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin Resistant *Staphylococcus aureus*
MSSA	Methicillin Susceptible *Staphylococcus aureus*
NARMS	National Antimicrobial Resistance Monitoring System
NCBI	National Center for Biotechnology Information
NDM	New Delhi Metallo-β-lactamase
NGS	Next Generation Sequencing
NHFPC	National Health and Family Planning Commission
OXA	Oxacillinase
PBPs	Penicillin Binding Proteins
PCR	Polymerase Chain Reaction
PDR	Pandrug Resistance
PHAC	Public Health Agency of Canada
PMQR	Plasmid Mediated Quinolone Resistance
PYR	Pyrrolidonyl Arylamidase
RND	Resistance Nodulation Cell Division
RV	Rappaport-Vassiliadis
SME	*Serratia marcescens* Enzymes
ST	Sequence Type
VIM	Verona Integron encoded Metallo-β-lactamase
VRE	Vancomycin Resistant Enterococci
WHO	World Health Organization

XDR	Extensive drug Resistance
XLD	Xylose Lysine Deoxycholoate

1. Introduction and Literature Review

1.1. Antibiotic resistance

1.1.1. Definition and the problem of antimicrobial resistance

The introduction of antimicrobials has resulted in a tremendous impact on human and veterinary medicine. Ever since the discovery of antibiotics, antimicrobials have resulted in reduced morbidity and mortality in humans (Andersson & Hughes, 2010). This was associated with clinical benefits such as prevention of bacterial infections in cancer patients receiving chemotherapy and the development of organ transplantation procedures; antimicrobials are estimated to have increased human life expectancy by an estimated eight years between 1944 and 1972 (Gafter-Gvili et al., 2012; Hancock & Knowles, 1998; Kabalin & Kessler, 1989). Antimicrobials also have contributed to improved control of infectious diseases in animals, and their growth promoting effects on livestock has been known since this effect was first reported in poultry in 1946 (Dibner & Richards, 2005; Gustafson & Bowen, 1997; Moore et al., 1946).

Antimicrobials are defined as organic molecules that may be natural, semi-synthetic, synthetic substances that kill or inhibit the growth of microbes (E. D. Brown & Wright, 2016; Julian Davies & Davies, 2010; Giguère, 2013; Hashmi, Strezov, & Varma, 2017). Penicillin was the first discovered antibiotic by Alexander Fleming and the discovery of its antibacterial effect on Staphylococci and *Streptococcus pyogenes* was important breakthrough (Fleming, 1929). Subsequently streptomycin, which was identified in 1943, proved useful for the treatment of *Mycobacterium tuberculosis*; these discoveries brought about the golden era of antibiotics between 1950 and 1960 when a half of currently used compounds were discovered (Comroe, 1978; Davies, 2006).

As pioneering antibiotics have been discovered during the golden era of antibiotics, this breakthrough seemed to bring successful improvements in our quality of life; reduced bacterial infections in human and animal, and animal growth promotion (Andersson & Hughes, 2010; E. D. Brown & Wright, 2016; Dibner & Richards, 2005). However, as Alexander Fleming who discovered penicillin warned that bacteria could become resistant to antibiotics, the organisms became less susceptible to inhibitory action of the drugs (Fleming, 1945). Shortly after the discovery of penicillin, penicillin resistant *S. aureus* was identified in the 1940s (H. F. Chambers,

2001). In response to the resistance, other drugs were developed but resistance to those drugs followed soon after as well (Figure 1.1). What made the resistance follow so quickly were interactions of microorganisms to survive from their hazard including antimicrobial compounds. Moreover, the selective pressure in favour of antibiotic resistance is made worse by their overuse/misuse. Increasingly, multidrug resistance (MDR)/extensive drug resistance (XDR)/pandrug resistance (PDR) has been identified from the hospital patients. *Neisseria gonorrhoea* is one of the extreme cases that has developed their resistance to almost every antibiotic used to treat to this organism (Centers for Disease Control and Prevention, 2013). The exposure of antibiotics has been occurring not only in clinical settings, but also in agriculture and the environment, indicating the scale of selective pressure to microbes by antibiotics are vast and the distribution of resistant organisms is extensive (Hughes, 2014; Le Page, Gunnarsson, Snape, & Tyler, 2017; Sarmah, Meyer, & Boxall, 2006). For example, antibiotic residue in wastewater from healthcare facilities may enter wastewater treatment plant which could be a reservoir of the resistant organisms as the studies from Sweden and the US have reported (Börjesson, Matussek, Melin, Löfgren, & Lindgren, 2010; Rosenberg Goldstein et al., 2012). It may be because wastewater treatment is designed to remove solid waste and microbial contaminants but not antibiotics from untreated water. Resistant organism can also pass through treatment process as described by a study from India where resistant organisms were identified in tap water (Walsh, Weeks, Livermore, & Toleman, 2011). Moreover, antibiotics have been used in agricultural environments extensively as growth promoters for livestock which resulted in selective pressure on the existing microorganisms (Aarestrup, 1995; Bager, Madsen, Christensen, & Aarestrup, 1997; Davies, 2008). Avoparcin, the glycopeptide antibiotic, was used as growth promoter for poultry and pigs since 1975 in Europe (Davies, 2008). In 1990s, Danish studies found that vancomycin resistant *Enterococcus faecium* emerged on farms that used avoparcin as a growth promoter (Aarestrup, 1995; Bager et al., 1997). Even though its use was banned in 1995 in Europe, multiple studies from Europe have found that vancomycin resistant Enterococci were still present on farms (Borgen et al., 2000; Garcia-Migura, Liebana, Jensen, Barnes, & Pleydell, 2007; Ghidán et al., 2008; Novais et al., 2005). Vancomycin resistant *E. faecium* was still found from Danish broiler flocks in 2010 (Bortolaia, Mander, Jensen, Olsen, & Guardabassi, 2015). Although growth promoting applications for antibiotics were recently banned, the resistant bacteria selected for by these practices are still harboured by livestock, which may transmit to

human via food products (Food and Drug Administration, 2013; Larsen et al., 2011; Public Health Agency of Canada, 2016; US Food and Drug Administration, 2017; World Health Organization, 2011). Therefore, the problem of antimicrobial resistance (AMR) has now become one of the biggest threats on public health (World Health Organization, 2014).

Not only has AMR resulted in increased morbidity and mortality, but additional financial costs associated with the requirement for lengthier hospitalizations, additional diagnostic investigations, more expensive treatments and infection control procedures (Gandra, Barter, & Laxminarayan, 2014). Although the human and financial cost of AMR in Canada have not been estimated thoroughly, one study has estimated the annual global incidence of mortality was estimated to be 700,000. It is expected to get worse if AMR is not tackled. The projected cumulative costs from 2014 through 2050 would be between 60-100 trillion US dollars. (O'Neil, 2014; Public Health Agency of Canada, 2017b).

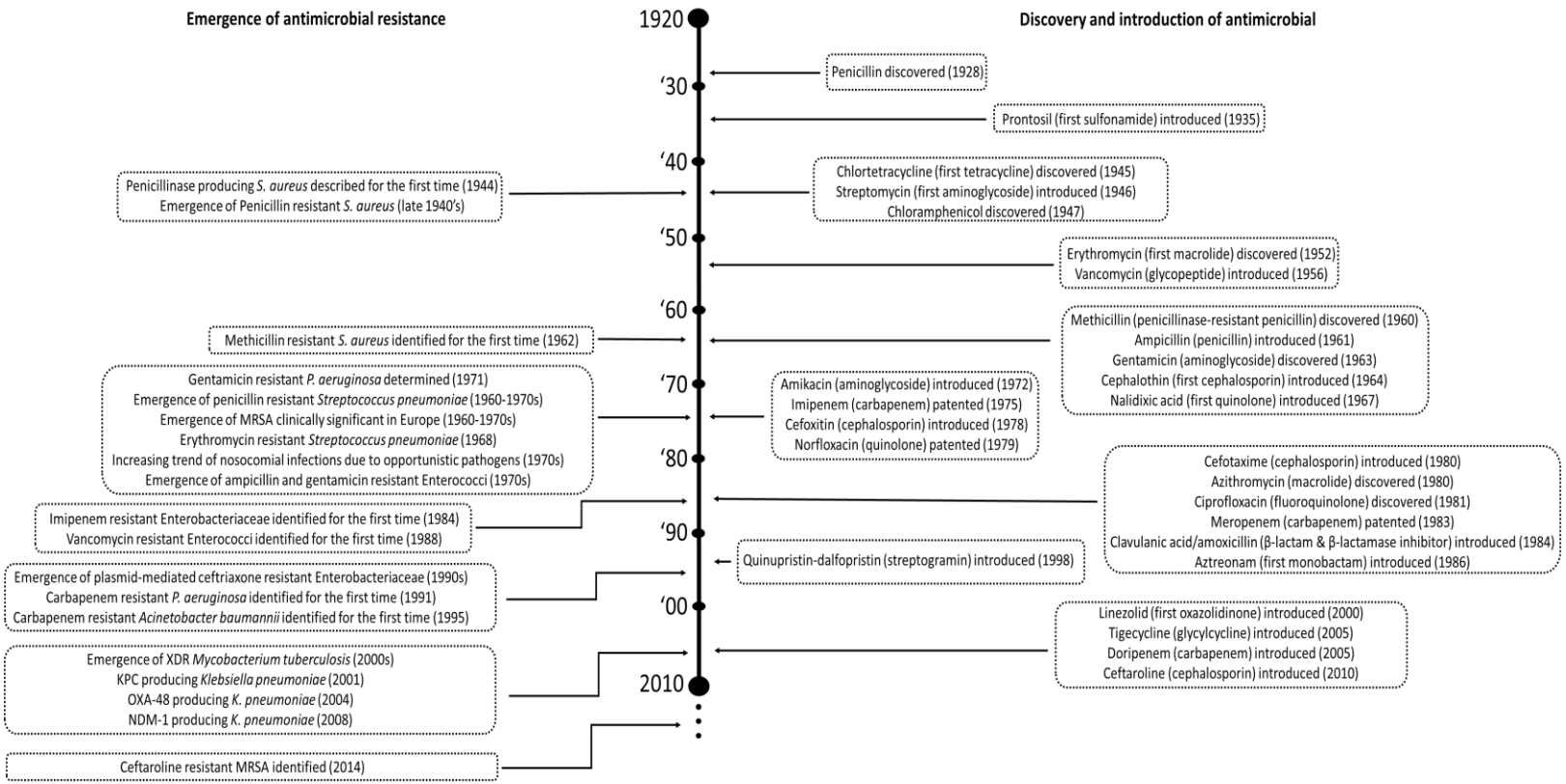

Figure 1. 1. History of antimicrobial development and emergence of antimicrobial resistant organisms. Modified figure from Giguère, 2013. Timeline of the introduction of antimicrobials and the first detection of resistance.

1.1.2. General mechanisms of antimicrobial resistance

To fight back against antibiotics, bacteria have developed several resistance mechanisms to disrupt the action of antibiotics. Resistance has existed since ancient time to overcome actions of naturally-produced antimicrobial compounds in the environment due to their innate abilities. For example, enteric bacteria such as *E. coli* has an efflux pump called AcrAB system to pump out bile salts to survive in human intestine (Thanassi, Cheng, & Nikaido, 1997). This efflux system can also remove antibiotics to ensure bacterial survival at the time of antibitoc exposure. Other factors such as lack of drug targets, poor permeability of the bacterial cell and production of enzymes can all result in bacteria being intrinsically resistant to antibiotic compounds. For example, *Pseudomonas* spp. and *Stenotrophomonas maltophilia* are resistant to β-lactams by low permeability of the outer membrane and chromosomally encoded β-lactamases, respectively (Avison, Higgins, von Heldreich, Bennett, & Walsh, 2001). As this type of resistance occurs naturally without any change by selective pressure, it is termed as intrinsic resistance (Culyba, Mo, & Kohli, 2015). The resistance genes that confer the resistance in the organisms intrinsically can be mobilized by mobile gene elements. This can allow the resistance genes to transmit into the other organisms horizontally (Bennett, 2008). The transmitted genes in recipient organism then would be able to confer resistance to antibiotics which makes the organism non-susceptible anymore. These same resistance mechanisms, when in other bacteria which are not normally resistant, would be classified as acquired resistance. One of the examples is transfer of mobile resistance genes such as extended spectrum β-lactamase or AmpC β-lactamase encoding genes from either resistant organisms or outer environment into susceptible organisms. This could cause emergence of penicillins and cephalosporins resistance among Enterobacteriaceae.

Resistance occurs by several common mechanisms: preventing access of antibiotics into their cell, altering the drugs targets to prevent binding and even enzymatic destruction or alteration as it is shown in Figure 1.2.

Decreased permeability

Bacteria can reduce the penetration of antibiotics into the cell preventing access to drug targets. Many Gram-negative bacteria modify their cell membrane structure or alter porin expression to prevent most of antibiotics including β-lactams and aminoglycosides from crossing

their outer membrane (R. E. W. Hancock & Brinkman, 2002; Pechère & Köhler, 1999; Strateva & Yordanov, 2009). For example, *Pseudomonas aeruginosa* has low outer membrane permeability excluding most of the β-lactams including meropenem (Hancock & Brinkman, 2002; Pechère & Köhler, 1999; Strateva & Yordanov, 2009). Similarly, decreased permeability is recognized among Gram-positive bacteria. Vancomycin intermediate *S. aureus* has been related to decreased entry of the drug into the cell through thickening of cell wall (Howden, Davies, Johnson, Stinear, & Grayson, 2010).

Increased efflux

Another mechanism for Gram-negative bacteria to be resistant are efflux pumps. These efflux pumps can result in higher resistance with decreased permeability synergistically. Resistance nodulation cell division (RND) pumps, the multicomponent pumps located in cytoplasmic membrane in Gram-negative organisms, is one of the examples of efflux activity (Nikaido, 2001; Schweizer, 2012). These pumps exclude a broad spectrum of antibiotic molecules from many resistant Gram-negative organisms and can be acquired by susceptible ones (Schweizer, 2012). For example, Cml and Flo pumps conferring chloramphenicol resistance are found in several Gram-negative organisms such as *E. coli, Enterobacter aerogenes, Klebsiella pneumoniae, Salmonella enterica* serovar Typhimurium, *P. aeruginosa* and *Vibrio cholerae* (Schwarz, Kehrenberg, Doublet, & Cloeckaert, 2004). Among them studies have found *flo* type genes and *cmlA* type genes can be mobilized and confer the resistance in *E. coli* (Poirel, Decousser, & Nordmann, 2003; Poirel et al., 1999). The tetracyclines are the substrates for the TET family of pumps; over 10 different types in both Gram-negative and Gram-positive organisms are described (Poole, 2005). For example, TetA and TetB pumps are reported as the most prevalent Tet pumps in *Acinetobacter baumannii* and their determinants are transferrable to other *Acinetobacter* species (Huys et al., 2005; Mak, Kim, Pham, Tapsall, & White, 2009). Another study reported *E. coli* with *tetM* for the first time in 2004 which was almost identical to *tetM* from *E. faecalis* and also exist in transposons (Bryan, Shapir, & Sadowsky, 2004). This *tetM* gene also has been identified from *Listeria monocytogenes* and *tetM* exchange between two bacteria has been demonstrated *in situ* indicating its transferability among Gram-positives. In other Gram-positives, Tet38 in *S. aureus* is one example of a chromosomally located Tet gene, which is overexpressed when mutated (Truong-Bolduc, Dunman, Strahilevitz, Projan, & Hooper,

2005). QepA which is plasmid-mediated quinolone efflux pump confers resistance to ciprofloxacin and norfloxacin in Gram-negatives (Jacoby, Strahilevitz, & Hooper, 2014).

Altered drug binding sites

Some bacteria can modify the sites in their cell wall which drug molecules target, preventing drug binding and therefore inhibiting antibacterial activity. Some examples include methicillin-resistant *S. aureus* (MRSA), penicillin resistant *Streptococcus pneumoniae*, vancomycin-resistant Enterococci (VRE), and β-lactam resistant *Neisseria gonorrhoeae* (Cherazard et al., 2017; Miller, Munita, & Arias, 2014; Zapun, Contreras-Martel, & Vernet, 2008). MRSA produces structurally modified penicillin binding proteins (PBPs) with reduced affinity of the β-lactams; similarly, *S. pneumoniae* and *N. gonorrhoeae* produce structurally modified PBPs as well which decrease affinity of penicillins and cephalosporins. (Bharat, Demczuk, Martin, & Mulvey, 2015; Cherazard et al., 2017; Cornick & Bentley, 2012; Pantosti, Sanchini, & Monaco, 2007; Smith et al., 2005; Zapun et al., 2008). VRE is resistant to glycopeptide antimicrobials due to its altered structure of peptidoglycan cell wall precursor component (D-alanine-D-alanine). It is this component in susceptible organisms that vancomycin targets to prevent cross-link of peptidoglycan chains and inhibit synthesis of the cell wall thereby having its effect (Miller et al., 2014). Quinolone resistance can also result from alterations in the antibiotic binding site. DNA gyrase and topoisomerase IV which maintain superhelical tension from DNA supercoiling to stabilize DNA synthesis are targeted by quinolones (Correia, Poeta, Hébraud, Capelo, & Igrejas, 2017; G A Jacoby & Medeiros, 1991). QnrA, which is one of plasmid-mediated quinolone resistance (PMQR) determinants, binds to gyrase holoenzyme and its subunits, GyrA and GyrB, to protect DNA gyrase. It also binds to topoisomerase IV holoenzyme and its subunits, ParC and ParE. Once QnrA-gyrase or QnrA-topoisomerase IV complex are formed, quinolone binding is prevented (Tran, Jacoby, & Hooper, 2005a, 2005b).

Enzymatic degradation

Bacteria also can produce enzymes to inactivate antibiotics by either destroying or modify the drug. β-lactamases which hydrolyze the β-lactam ring in penicillins, cephalosporins and carbapenems are well-known examples of this mechanism. These enzymes are the most common mechanism of β-lactam resistance (Bush & Jacoby, 2010). As β-lactams have been widely used clinically, a wide variety of different β-lactamases have emerged. Important examples of these

enzymes in Gram-negatives include the extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases and enzymes with carbapenemase activity. ESBLs include the TEM, SHV (except parental TEM and SHV enzymes) and CTX-M. All ESBLs hydrolyze penicillins but TEM and SHV enzymes conduct limited hydrolyzation of cephalosporins and azteonam. In contrast, CTX-M enzymes hydrolyze all 3rd generation of cephalosporins. AmpC β-lactamases such as CMY-2, hydrolyze an expanded spectrum of cephalosporins and cephamycins, and are not inhibited by β-lactamase inhibitors (clavulanic acid) (Thomson, 2010). Carbapenemases, NDM, VIM, KPC and OXA varieties, hydrolyze carbapenems which have broader spectrum of antibiotic activity than penicillins and cephalosporins (Sekirov et al., 2016; Thomson, 2010). Enzyme-mediated resistance also has been identified in association with aminoglycoside and quinolone resistance. The AAC(6')-Ib-cr enzyme, one of PMQR determinants, acts as an acetyltransferase to acetylate aminoglycosides (amikacin, kanamycin and tobramycin) and fluoroquinolones (ciprofloxacin and norfloxacin) (Jacoby et al., 2014).

Mobility of antimicrobial resistance genes and acquired resistance

In terms of the problem of AMR, mobility of AMR genes plays a major role allowing susceptible bacterial organisms to acquire the resistance mechanisms.

Acquired resistance occurs when previously susceptible organisms (wild type organisms) develop or acquire resistance. Susceptible bacteria can become resistant by either mutating their chromosomal genes (spontaneous mutation, hypermutator and adaptive mutagenesis) or acquiring external genetic determinants from other resistant organisms (horizontal gene transfer) (Džidić, Šušković, & Kos, 2008). Horizontal gene transfer (HGT) is an important mechanism contributing to rapid and broad dissemination of AMR within or between species. HGT can occur by transformation, transduction and conjugation as it is described in Figure 1.2 (Bennett, 2008). First, bacterial cells can take up free resistance genes from extracellular environment, which is called natural transformation (von Wintersdorff et al., 2016). Transformation occurs when the recipient bacteria is exposed to altered environments such as growth conditions, nutrient access, cell density or starvation (Thomas & Nielsen, 2005; von Wintersdorff et al., 2016). Antibiotics can be one of the main contributors on altered extracellular environment. As antibiotics kill or inhibit the growth of the organisms, bacteria have strategies to survive from the stress caused by antibiotics. One of their strategies is to take mobile resistance gene elements from their outer

environment. (Charpentier, Polard, & Claverys, 2012). Bacteriophage mediated HGT, transduction occurs when bacteriophage replicates genomes (chromosomal DNA, plasmid DNA, transposons, gene cassettes) from their previous bacteria host into the phage head and transfers them to new bacteria host (Huddleston, 2014; Schwarz, Loeffler, & Kadlec, 2017). Transfer of this material can then occur following infection of a naïve cell. As bacteriophages have been identified to have broad host range, between not only different species but also different classes, transduction plays a significant role in the dissemination of resistance genes in microbial communities (Jensen et al., 1998; Mazaheri Nezhad Fard, Barton, & Heuzenroeder, 2011). The other HGT mechanism that covers host range between different species and classes is conjugation. Conjugation happens when contact between two bacterial cells is made; cell-to-cell contact is made by a pilus from donor bacterium forming a channel between the donor and recipient, and transfer of genetic elements can happen through the channel. DNA plasmids play a major role when it comes to conjugation for the transmission of important emerging resistance genes. Important examples of conjugative plasmids playing a role in the dissemination of antimicrobial resistance include the ESBL, AmpC β-lactamases, carbapenemases, PMQR determinants and colistin resistance (MCR-1) (Alba et al., 2018; Branger et al., 2018; Yang et al., 2018). HGT has contributed to the rapid dissemination of resistance genes and the emergence of clinically problematic superbugs including broad spectrum β-lactamase producers, methicillin-resistant *Staphylococcus aureus* and vancomycin resistant Enterococci.

Other mobile gene elements, transposons and integrons, also contribute to HGT. Transposons incorporate DNA segments and transport the gene between bacterial chromosome and a plasmid or from a plasmid and another plasmid (Craig, 1997). They can be transferred from Gram-negatives to Gram-positives and vice versa (Bertram, Strätz, & Dürre, 1991). Transposons are known to contribute to the emergence of resistance in Gram-positives; vancomycin resistance genes are located in transposon (Tn1546) carried by plasmid (pIP816) and it can be transferred one *Enterococcus* spp. to other conducting inducible resistance in recipient *Enterococcus* spp (Arthur, Molinas, Depardieu, & Courvalin, 1993).

Integrons also mobilize gene fragments as gene cassettes that are inserted to their recombination site by integron integrase. Gene cassettes can carry resistance genes, and various resistance genes can be carried on gene cassettes in integrons. One of the examples is

carbapenemase resistance genes (*bla*$_{IMP}$, *bla*$_{VIM}$, *bla*$_{OXA}$) which have caused rapid spread of carbapenemase producing Enterobacteriaceae along with plasmid itself (Nordmann & Poirel, 2002).

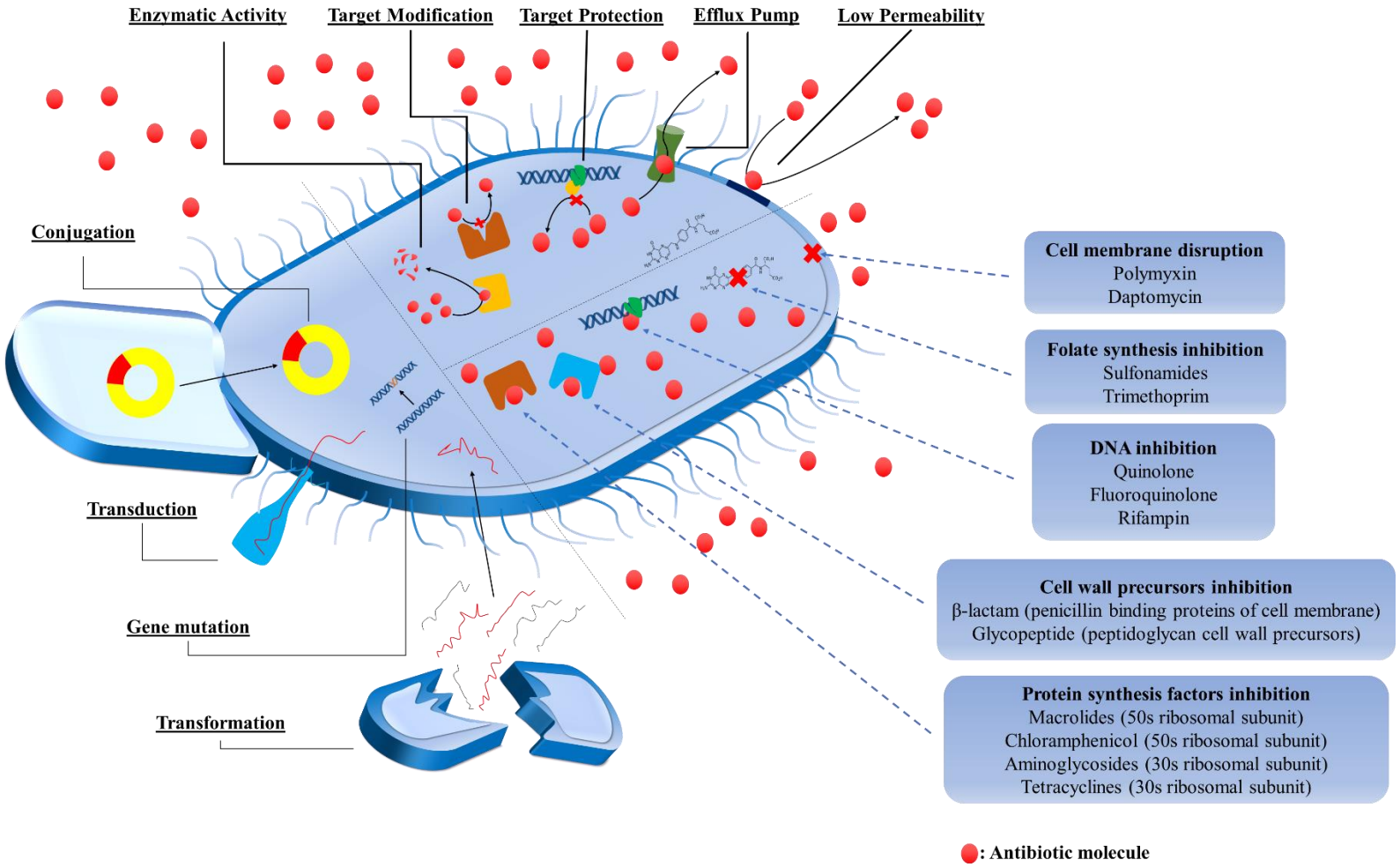

Figure 1. 2. Antimicrobial targets and antimicrobial resistance mechanisms. Antimicrobial targets are highlighted on the right (blue), mechanisms of resistance are highlighted on top (green) and types of resistance transmission are highlighted on the left (grey)

1.1.3. Extended spectrum β-lactamase and carbapenemases

Broad spectrum β-lactamases are one of the major MDR threats in both community and healthcare settings. The spread, among Enterobacteriaceae, of genes encoding for β-lactamases allows for organisms such as *E. coli* and *K. pneumoniae* to become resistant to β-lactams (Karanika, Karantanos, Arvanitis, Grigoras, & Mylonakis, 2016; Reddy et al., 2007; Woerther, Andremont, & Kantele, 2017). The emergence of these enzymes is associated with serious therapeutic challenges since β-lactams are important drugs to treat bacterial pathogens; these drugs constitute 65% of the total antibiotic market worldwide (Thakuria & Lahon, 2013). β-lactams consist of penicillins, cephalosporins, monobactams and carbapenems. The mechanism of action of β-lactams is to bind with PBPs on the cell membrane followed by inhibition of the cell wall synthesis (McDermott, Walker, & White, 2003). To prevent the activity of β-lactams, bacteria produce β-lactamases which hydrolyze the β-lactam ring to inactivate the antibiotics (Džidić et al., 2008).

Under the Ambler classification scheme, β-lactamases are divided into four classes, A, B, C and D, depending on their amino acid motifs (Table 1.1). Class A β-lactamases are characterized by the presence of a serine residue at their active site (Ambler, 1980). Class A includes narrow spectrum β-lactamases (such as TEM-1, 2 and 13, and SHV-1), ESBLs (the other TEM and SHV varieties and CTX-M varieties), and KPC (*K. pneumoniae* carbapenemase), SME (*Serratia marcescens* Enzymes), IMI (IMIpenem hydrolysing β-lactamase) carbapenemases (Jacoby & Medeiros, 1991; Queenan et al., 2000; Yigit et al., 2001). While most of the class A β-lactamases hydrolyze 3rd generation cephlosporins and penicillins, the enzymatic activities of TEM and SHV are only limited to penicillins (Jacoby & Medeiros, 1991). Furthermore, class A carbapenemases including KPC, SME, IMI can hydrolyze monobactam, cephamycin and carbapenems along with penicillins and cephalosporins (Queenan et al., 2000; Yigit et al., 2001). Class B enzymes, metallo-β-lactamases, require bivalent metal ion, usually Zn^{2+}, for their hydrolyzing activity and it includes NDM (New Delhi metallo-β-lactamase), VIM (Verona integron-encoded metallo-β-lactamase), IMP (Imipenem resistant *Pseudomonas*) varieties (Ambler, 1980). These class B β-lactamases can hydrolyze all β-lactams. Class C and D β-lactamases also have serine active site like the class A enzymes, but their functional traits are different. Class C (AmpC) have broader spectrum of activity on cephalosporins because their active site is more opened which makes

them bind to the bulkier side chains of cephalosporins easier than Class A (Jacoby, 2009). Class D includes oxacillinases (OXA varieties) which are more likely to conduct enzymatic activity against isoxazolyl-type penicillins, oxacillin, and carbapenem hydrolyzing oxacillinases such as OXA-48 and OXA-23 (June, Vallier, Bonomo, Leonard, & Powers, 2014; Ledent, Raquet, Joris, Van Beeumen, & Frère, 1993).

The β-lactamase genes can be transmitted from β-lactamase producers to the non-producers thereby confering resistance to susceptible bacteria. Plasmids are one of the mobile gene elements that distribute β-lactamase genes to the susceptible bacteria effectively. The first reported plasmid-mediated β-lactamase was TEM in *E. coli* from Greece (TEM-1) (Patricia A. Bradford, 2001). Soon after this initial discovery reports of mobilization of SHV-1 from *K. pneumoniae* (encoded chromosomally) into *E. coli* (plasmid-mediated) were published (Patricia A. Bradford, 2001). These first reported β-lactamases were narrow spectrum β-lactamases which are not able to hydrolyze the broader spectrum of β-lactams such as the extended-spectrum penicillins or cephalosporins (Patricia A. Bradford, 2001). However, new groups of β-lactamases, ESBL, started emerging soon after broad spectrum penicillins and cephalosporins were introduced in both human and veterinary medicine (Elander, 2003; Shaikh, Fatima, Shakil, Rizvi, & Kamal, 2015; Tal Jasper, Coyle, Katz, & Marchaim, 2015). The first plasmid-mediated ESBL discovered was SHV-2 in *K. pneumoniae* found in Germany in the early 1980s (Kliebe, Nies, Meyer, Tolxdorff-Neutzling, & Wiedemann, 1985). The SHV-2 enzyme could hydrolyze a extended spectrum of β-lactams than could its parental enzyme, SHV-1. In 1987, a plasmid-borne CTX-1 (TEM-3 under current nomenclature) was identified to confer resistance to cefotaxime (3^{rd} generation cephalosporin), which was a broader spectrum of hydrolysis than its parent enzymes, TEM-1 or TEM-2 (Sirot et al., 1987). In contrast, the CTX-M type β-lactamases, which did not evolve from narrow spectrum parental enzymes, can hydrolyze 3^{rd} generation cephalosporins. The first CTX-M was identified in cefotaxime resistant *E. coli* from Japan in 1988, and was called FEC-1 (Matsumoto, Ikeda, Kamimura, Yokota, & Mine, 1988). One year later cefotaxime resistant *E. coli* was also isolated from Germany in 1989 and in this case the identified β-lactamase was called CTX-M-1 (Bauernfeind, Grimm, & Schweighart, 1990). Since then, over 170 CTX-M variants have been identified (Lahey Clinic, 2017). Their hydrolytic spectrum includes penicillins, 2^{nd} and 3^{rd} generation cephalosporins, and they are primarily

identified among Enterobacteriaceae (including *K. pneumoniae, E. coli* and *Proteus mirabilis*) (Tal Jasper et al., 2015).

Plasmid-mediated AmpC (*bla*$_{CMY-2}$) enzymes are also frequently encountered, these genes are typically encoded chromosomally in *Enterobacter, Citrobacter, Serratia* but also can be mobilized. They are now found in other Enterobacteriaceae as well. The first plasmid-mediated AmpC β-lactamase that was characterized was CMY-1 in *K. pneumoniae* isolated from a patient in South Korea (Bauernfeind, Schweighart, & Chong, 1989). The resistance spectrum of CMY-1 includes cephamycin along with penicillins, cephalosporins, which is broader spectrum than ESBLs. Besides CMY-1, other plasmid-mediated AmpC β-lactamases (CMY, FOX, ACT, MOX, ACC, DHA variants) also started to be identified globally (Bauernfeind, Schneider, Jungwirth, Sahly, & Ullmann, 1999; P. A. Bradford et al., 1997; Gaillot, Clément, Simonet, & Philippon, 1997; Gonzalez Leiza et al., 1994; Horii et al., 1993). Since the 1990s, CMY-2 has become the most prevalent plasmid-mediated AmpC β-lactamases (Bauernfeind, Stemplinger, Jungwirth, & Giamarellou, 1996; Denisuik et al., 2013; Philippon, Arlet, & Jacoby, 2002; Sidjabat et al., 2009).

More recently, the emergence of other β-lactamases with broader hydrolytic spectrum including carbapenemases have been identified. These enzymes are particularly problematic because the carbapenems are last-resort drugs with a very broad spectrum of activity. The first identified carbapenemase producing Enterobacteriaceae was *E. cloacae* producing NmcA imipenem-hydrolyzing β-lactamase (IMI-1) in 1984, a year before the clinical use of imipenem was approved (Rasmussen et al., 1996). Shortly after, in 1991, the plasmid-mediated carbapenemase, VIM-1, was identified from *P. aeruginosa* in Japan. This was followed by the discovery of ARI-1 (OXA-23 under current nomenclature) from *A. baumannii* in the UK in 1995 and KPC-1 from *K. pneumoniae* in the US in 2001 (Donald, Scaife, Amyes, & Young, 2000; Scaife, Young, Paton, & Amyes, 1995; Watanabe, Iyobe, Inoue, & Mitsuhashi, 1991; Yigit et al., 2001). Additional types of carbapenemase have subsequently been identified including OXA-48, the most common carbapenemase among OXA types, from *K. pneumoniae* in Turkey in 2004, and NDM-1 from *K. pneumoniae* in Sweden, which originated in India in 2008 (Poirel, Héritier, Tolün, & Nordmann, 2004; Yong et al., 2009). All IMP, VIM, KPC and NDM types successfully hydrolyze carbapenems along with almost all the other classes of β-lactams (Daikos et al., 2014; Qureshi et al., 2012; Tumbarello et al., 2012; Zarkotou et al., 2011). The OXA-48 type

carbapenemaess hydrolyze the penicillins and carbapenems but not the cephalosporins which can make them difficult to be detected *in vitro* (Doi & Paterson, 2015; Poirel, Potron, & Nordmann, 2012).

The dissemination of ESBL and carbapenemase-producing Enterobacteriaceae worldwide has been dramatic. In the US, 140,000 healthcare-associated Enterobacteriaceae infections occur each year, nearly 26,000 (19%) of them are ESBL producers and 9,000 (6%) of them were carbapenem resistant according to the report from Centers for Disease Control and Prevention (CDC) (Centers for Disease Control and Prevention, 2013). In Europe, ESBL and carbapenemase-producing Enterobacteriaceae were reported by the European Antimicrobial Resistance Surveillance Network (EARS-Net). Data are reported as EU/EEA (European Union/European Economic Area) population-weighted mean which is determined by "applying population-based weights to each country's data before calculating the arithmetric mean for all reporting countries" (European Centre for Disease Prevention and Control, 2017). In the 2016 EARS-Net report, based on the highest EU/EEA population-weighted mean, 57.4%, 12.4% and less than 0.1% of *E. coli* isolates were aminopenicillin, 3rd generation cephalosporin and carbapenem resistant respectively. Whereas, 25.7% and 6.1% of *K. pneumoniae* isolates were resistant to the 3rd cephalosporins and carbapenems respectively based on the highest EU/EEA population-weighted mean (European Centre for Disease Prevention and Control, 2017).

Table 1. 1. Classification of β-lactamases. Information was adapted from Antunes & Fisher, 2014; Poirel, Walsh, Cuvillier, & Nordmann, 2011; Rubin & Pitout, 2014

Ambler classification	Active site	Enzyme	Examples of enzyme	Spectrum of resistance	Inhibitors
A	Serine	Narrow spectrum of β-lactamase	TEM-1, TEM-2, TEM-13, SHV-1	Penicillins	
		ESBLs	TEM (except TEM-1,2,13), SHV (except SHV-1), CTX-M	Penicillins, cephalosporins, monobactam	Clavulanic acid, tazobactam, sulbactam
		Serine carbapenemase	KPC, SME, IMI	Penicillins, cephalosporins, monobactam, meropenem, cephamycin	
B	Bivalent metal ion (usually Zn^{2+})	Metallo-β-lactamase	NDM, VIM, IMP	All β-lactams	Metal chelators (e.g. EDTA)
C	Serine	AmpC	CMY-2, FOX, ACT, MOX, ACC, DHA	Penicillins, cephalosporins, cephamycins, monobactam	Cloxacillin, Boronic acid
D		OXA type carbapenemase	OXA-48	Penicillins, carbapenems	NaCl (except OXA-23, 40, 163)

1.1.4. Methicillin-resistant *Staphylococcus aureus*

Methicillin-resistant *S. aureus* (MRSA) is another superbug frequently identified from both health and community settings. *S. aureus* is frequently implicated in skin, soft-tissue, respiratory, bone, join and endovascular infection and sepsis (Lowy, 1998; Otto, 2012). Although most of the infections caused by MRSA are not different from the ones by methicillin-susceptible *S. aureus* (MSSA), MRSA is associated with increased mortality rates, increased treatment costs, and prolonged stays in the hospital (Hanberger et al., 2011; Labreche et al., 2013). Soon after the discovery of penicillin, penicillin-resistant *S. aureus* emerged and a year after of methicillin clinical usage began in 1960, MRSA was detected (H. F. Chambers, 2001; Jevons, Coe, & Parker, 1963). MRSA produces the novel PBP (PBP2a) which has lower affinity for β-lactam drugs rendering these isolates resistant to the entire drug class (Hartman & Tomasz, 1981). Methicillin resistance is encoded by the *mec* family of genes (*mec*A, *mec*B, *mec*C and *mec*D); *mec*A and *mec*C are reported to confer the resistance in MRSA (Baba et al., 2009; Katayama, Ito, & Hiramatsu, 2000; Paterson, Harrison, & Holmes, 2014; Schwendener, Cotting, & Perreten, 2017). The *mec* genes are located in staphylococcal chromosomal cassette (SCC*mec*) and the SCCmec is mobilizable by the actions of the *ccr* genes which allow excision and integration (Hartman & Tomasz, 1981; Katayama et al., 2000; Paterson et al., 2014). MRSA started circulating in the hospitals in the UK, Denmark and the US between 1960 and 1970 (Barrett, McGehee, & Finland, 1968; Crisóstomo et al., 2001; Stewart & Holt, 1963). Strains of MRSA found in the hospitals of the US in the 1970s and ‘80s, made their way out of health care settings, disseminating worldwide in both hospitals and the community (Henry F. Chambers & Deleo, 2009). Recent CDC reports from the US indicated that there were 80,461 invasive MRSA infections and 11,285 MRSA infection-related deaths in 2011 in the US (Centers for Disease Control and Prevention, 2013). In Europe, the prevalence of MRSA infection has been reported to range between 1.2% and 50.5% in 2016. Overall, 3.7% of *S. aureus* isolates were MRSA based on the highest EU/EEA population-weighted mean (European Centre for Disease Prevention and Control, 2017).

1.1.5. Vancomycin resistant Enterococci

Enterococcus species are normal inhabitants of the intestinal tract of humans and animals and they are also ubiquitous in the environment (Ahmed & Baptiste, 2018; Ranotkar et al., 2014). Although they are commensal bacteria in the human gastrointestinal tract, they can cause opportunistic infections including urinary tract infections, endocarditis, surgical wound infections, bacteraemia and sepsis (Beukers et al., 2017). *Enterococcus faecalis* and *E. faecium* are the most commonly isolated organisms from clinical infections (Arias & Murray, 2012). They are intrinsically resistant to various drugs including cephalosporins (Arias & Murray, 2012; Kristich, Rice, & Arias, 2014). To overcome MDR *Enterococcus* spp., vancomycin started to be used as alternative to penicillins to treat infections in the US in 1958 (Roland Leclercq, Derlot, Duval, & Courvalin, 1988; Levine, 2006). However, vancomycin resistant *E. faecium* was first reported in England in 1988 and the transferability of the resistance by a plasmid was identified in the US in 1987 (Roland Leclercq et al., 1988; Uttley, Collins, Naidoo, & George, 1988). Subsequently, vancomycin resistant enterococci (VRE) continued to emerge in the US as its percentage among enterococci isolates increased from 0.3% and 0.4% in 1989 to 15.4% and 23.4% in the hospitals and intensive care units by 1997, respectively (Méndez-Alvarez, Pérez-Hernández, & Claverie-Martín, 2000). The spread of VRE is the result of various genes, *vanA, vanB, vanC, vanD* and *vanE* (Kristich et al., 2014). *vanA*, which is the most problematic can be found in *E. faecium* and *E. faecalis* and it imparts high resistance to vancomycin and teicoplanin, the other glycopeptide drug along with vancomycin (Qu et al., 2009). It can be mobilized by mobile genetic elements (plasmid, transposons) and acquired by susceptible enterococci. *vanB* also can be found in *E. faecium* and *E. faecalis* and is transferrable by plasmid and transposons. Unlike *vanA*, it doesn't confer resistance to teicoplanin (Quintiliani & Courvalin, 1994). *vanC, vanD and vanE*, on the other hand, show intermediate resistance. (Fines, Perichon, Reynolds, Sahm, & Courvalin, 1999; R Leclercq, Dutka-Malen, Duval, & Courvalin, 1992; Navarro & Courvalin, 1994; Perichon, Reynolds, & Courvalin, 1997). *vanC* is found on the chromosomes of *E. gallinarum, E. casseliflavus* and *E. flavescens* which are infrequently implicated species in human infections (Leclercq, Dutka-Malen, Duval, & Courvalin, 1992; Navarro & Courvalin, 1994). *vanC* confers low levels of intrinsic resistance in *E. casseliflavus* and *E. flavescens* and high intrinsic resistance in *E. gallinarum* which are infrequently implicated species in human infections (Leclercq et al., 1992; Navarro & Courvalin, 1994). *vanD* and *vanE* are acquired genes

in *E. faecium* and *E. faecalis* respectively but not commonly identified and confer low resistance to vancomycin and teicoplanin (Fines et al., 1999; Perichon et al., 1997).

The incidence of VRE in the US remains high, 30% of enterococcal infection per year are reported to be vancomycin resistant including 77% of *E. faecium* and 9% of *E. faecalis*, resulting in 650 and 200 deaths respectively (Centers for Disease Control and Prevention, 2013). Meanwhile, according to EARS-Net report in 2016 the EU/EEA population-weighted mean percentage for vancomycin resistant *E. faecium* and *E. faecalis* were 11.8% and 30.5% in Europe (European Centre for Disease Prevention and Control, 2017). Alternative treatments for VRE include quinupristin-dalfopristin, linezolid and daptomycin. However, use of these drugs would bring new emergence of resistance and make it even harder to treat VRE (Arias & Murray, 2012).

1.2. Epidemiology of foodborne antimicrobial resistance in Canada

1.2.1. Transferability of antimicrobial resistance from food

Food has been recognized as a potential source of resistant bacteria such as *E. coli, Salmonella, Campylobacter, Vibrio* species since the food can be contaminated with them either directly or indirectly during pre-processing, processing, distribution, food preparation and consumption (Centers for Disease Control and Prevention, 2016, 2018). In Canada, the Canadian Integrated Programs for Antimicrobial Resistance Surveillance (CIPARS) program monitors resistant *E. coli, Salmonella* spp. and *Campylobacter* from farm, abattoir and meat products since the early 2000s. For example, MDR *E. coli* and *Salmonella* spp. resistant to penicillins and cephalosporins have been identified from farm chickens, abattoir chickens and retail chicken meats between 2003 and 2015 in Ontario (Agunos et al., 2018). Other researchers have found MRSA on pork both before and after processing (scalding, skinning, pasteurizing and washing) obtained from commercial pork processing plants across the Canada in 2010 and 2011 (Narvaez-Bravo et al., 2016). All these identified resistant bacteria from meat could be transmitted by complex interactions between and within humans, animals, and the environment. The primary route of meat contamination though is fecal contamination which can include enteric pathogens such as *E. coli, Salmonella* and *Campylobacter* spp. The animal carcass can be exposed to

ingesta, feces and soil, which contain enteric pathogens, during the slaughter process resulting in contamination of the meat if washing during processing is not done properly (Ashby et al., 2003; Yalçin, Nizamlioğlu, & Gürbüz, 2001). Given resistant enteric bacteria from gastrointestinal tracts and feces of food animals are the primary source of contamination for meat, they have been one of the main targets for identifying resistant bacteria in farm surveillance by CIPARS (Government of Canada, 2017).

Furthermore, on the farm, the lack of cleaning/disinfection practices or contact with environmental factors such as dust or water can also contribute to transmission of resistant bacteria (Agunos et al., 2018; Schulz et al., 2016). The contacts between animals and farm personnel, truck drivers during transportation to slaughterhouse and abattoir personnel may also contribute to further disseminaton of resistant organisms (Agunos et al., 2018; Lassok & Tenhagen, 2013; Molla et al., 2012). Use of antibiotics maintains resistance and applies pressure for the continued development of resistance (Holman & Chénier, 2013). Then, the transmission of organisms from one animal to the other in confined environments allows for the resistant organisms to be maintained within the food animal population (McEwen & Fedorka-Cray, 2002).

Food can also be contaminated with the resistant organisms by food handlers during the processing and distribution stages. For example, penicillin and cephalosporin resistant *E. coli* were isolated from migrant food handlers in Qatar (Eltai et al., 2018). Another study from Malaysia showed that MDR *E. coli* and *S. aureus* were isolated from food handlers' hand before, during and after ready-to-eat foods preparation (Tan, Lee, & Mahyudin, 2014). These studies indicate that processing and distribution by food handlers could potentially result in the transmission of resistant organisms to the meat products and that this occurrence is more likely there if hygiene practices are poor.

Less attention has been paid to the identification of antibiotic resistant bacteria from vegetables than in meat. Antimicrobial resistant oxidase-positive Gram-negatives (*P. fluorescens, P. putida, Sphingobacterium* spp. and *A. baumannii*) were isolated from Ontario-grown Romaine lettuce, Savoy spinach and alfalfa sprouts; isolates were resistant to amipicillin, cephalothin, chloramphenicol, streptomycin, nalidixic acid, kanamycin and gentamicin (Bezanson, MacInnis, Potter, & Hughes, 2008). In British Columbia, *E. coli* were isolated from lettuce purchased from farmers markets, 35% were resistant to amikacin, sulfonamides and nalidixic acid (Wood, Chen,

Friesen, Delaquis, & Allen, 2015). Similar investigations have been done in other countries as well. For example, 13% of *E. coli* from fruits and vegetables purchased from local retail markets and wholesale market in Osaka, Japan from 2005 to 2008 were resistant to cephalotin (Wang, Nakamura, Kage-Nakadai, Hara-Kudo, & Nishikawa, 2017). *E. coli* isolated, described as diarrheagenic, from whole, cut and salad nopalitos (a dish made with nopal, a type of cactus) purchased from public markets were identified from Mexico in 2016 (Gómez-Aldapa et al., 2016). The isolates were resistant to amoxicillin/clavulanic acid, colistin and gentamicin (Gómez-Aldapa et al., 2016). A study from Benin identified more *E. coli* were isolated from lettuce, cabbage, great nightshades and carrots purchased from market garden sites during dry season than during the rainy season and that more than 70% of the isolates were resistant to amoxicillin/clavulanic acid, 3rd generation cephalosporins and nalidixic acid (Moussé et al., 2015). The prevalence of resistant bacteria may depend on factors from pre-harvest such as irrigation water or sewage sludge which is used for soil fertilization (Araújo et al., 2017; Rahube et al., 2014, 2016). A study from Portugal demonstrated that irrigation water may transmit resistant organisms to vegetables as the same sequence types of MDR *E. coli* were detected both in the water and on the vegetables (Araújo et al., 2017). Two studies in Canada have experimentally demonstrated that harvested vegetables grown in sewage sludge-treated soil had more types and abundance of resistance genes and mobile genetic elements than the ones grown in soil without sewage sludge treatment (Rahube et al., 2014, 2016).

1.2.2. Role of imported food on antimicrobial resistance transmission

Multiple factors can contribute to the contamination of food sources and products by resistant organisms which can then be transmited to humans (Figure 1.3). Besides domestically sourced AMR, the import and export of foods may play a role in the global distribution of resistant organisms. Because food has been actively imported and exported across the world, it is possible that resistant organisms can be transmited from one country to the other and this phenomenon has been reported by studies from around the world. For example, ESBL producing *E. coli* were identified from chicken imported from Brazil and purchased in Japan (Nahar et al., 2018). In Germany, ESBL and AmpC producing *E. coli* isolates were identified on both legally and illegally imported pork and poultry products from Brazil, Chile, and Egypt (A. Müller et al.,

2018). A study in the US identified *Salmonella* spp. from imported vegetables, fruits, meats and seafood from Taiwan, Indonesia, Vietnam, China, Australia and Pakistan (Bae, Kweon, & Khan, 2016). The isolates were resistant to multiple drugs including ampicillin, ciprofloxacin, nalidixic acid, gentamicin, tetracycline (Bae et al., 2016). In Canada, *E. cloacae* were identified from bivalve mollusks imported from Vietnam harbouring $bla_{\text{NDM-1}}$ along with bla_{TEM} and $bla_{\text{OXA-1}}$ (Janecko et al., 2016). Carbapenemase (OXA-48) producing non-pathogenic bacteria, *Stenotrophomonas* spp., *S. maltophila, Myroides odoratimimus* and *P. putida,* were identified from seafood imported from China and Korea to Canada as well (Morrison & Rubin, 2015).

As all these studies have identified, serious pathogenic bacteria resistant to clinically important antibiotics can be transferred to humans from other countries via imported foods. Furthermore, it may be inevitable that the countries with a low prevalence of resistance will be likely to face an increasing number of unexpected AMR organisms being transferred into the country from the regions with a higher prevalence of the resistance.

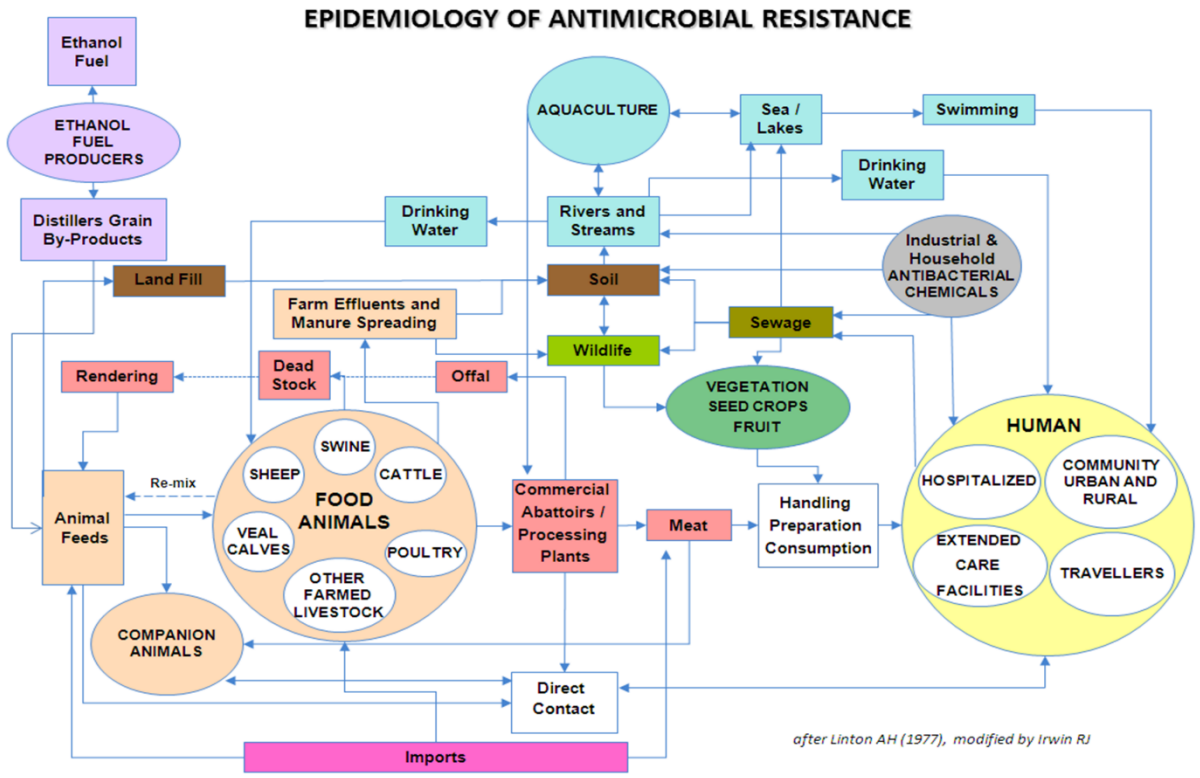

Figure 1. 3. Epidemiology of antimicrobial resistance. Besides its transmission among clinical and community settings, antimicrobial resistance transmission to human occurs via interactions with environment, animals and foods. Among many other factors, direct transmission may occur from water consumption, swimming, contacting companion and wildlife animals and domestic or imported food consumption. The range of the transmission can be extended by travellers and imported goods (e.g. imported foods). Figure is adopted from Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS).

1.3. Antimicrobial resistance studies on imported food in Canada

1.3.1. Current resistance surveillance by CIPARS and studies in Canada

Canada has a national antimicrobial resistance surveillance program called CIPARS which has been monitoring resistant bacterial organisms from farm, abattoir and retail along with human clinical *Salmonella* isolates across the country since its establishment in 2002 (Government of Canada, 2017). British Columbia, the prairie provinces (Alberta, Saskatchewan, Manitoba), Ontario and Quebec have been targeted since these regions are major pork and poultry producers (Government of Canada, 2017). *E. coli*, *Salmonella* spp. and *Campylobacter* spp. are the main enteric pathogens colonized in gastrointestinal tracts of domestic animals and are easily transmited to other animals on farm or at the abattoir by fecal contamination. Therefore, CIPARS is targeting enteric bacteria that may be resistant to the most critically important antimicrobials to human health as identified by the World Health Organization (WHO) including the 3rd and 4th generation cephalosporins and fluoroquinolones (Agunos et al., 2018; Collignon et al., 2016; Government of Canada, 2017). The recent 2015 CIPARS report has focused on ceftriaxone resistance in non-typhoidal *Salmonella* spp. and *E. coli*, and fluoroquinolone resistance in *Campylobacter* spp. This is because 3rd and 4th generation cephalosporins and fluoroquinolones are considered as Category I (very high importance) for the treatment of serious bacterial infections in human by Health Canada (Government of Canada, 2009).

CIPARS identified a decreasing rate of ceftriaxone resistant *E. coli* and *Salmonella* spp. in farm, abattoir and retail chicken since 2013 from all provinces (Government of Canada, 2017). This was identified after the Chicken Farmers of Canada implemented a policy that no longer allows the use of Category I antimicrobials for preventative use in chicks (Government of Canada, 2017). No flocks participating in the CIPARS broiler chicken program in 2015 reported any use of Category I antimicrobials (Government of Canada, 2017). Levels of ciprofloxacin resistant *Campylobacter* spp., isolated from chickens, varied across Canada; interestingly the reason for the persistence of this resistance is unexplained as CIPARS has reported no fluoroquinolone use on broiler chicken farms participating in CIPARS since 2013 (Government of Canada, 2017). CIPARS also identified increasing rates of MDR *Salmonella* spp., resistant up to 6 classes among 7 classes of tested antimicrobials (β-lactams, aminoglycosides, quinolones,

macrolides, phenicols, folate pathway inhibitors and tetracyclines) from cattle (Quebec, Ontario and Alberta) and pigs (Manitoba, Quebec, Ontario) (Government of Canada, 2017).

Overall, the resistance in food animals in Canada has been monitored well and various trends can be identified both nationally and regionally annually. However, one important limitation to the current CIPARS program is the lack of representation from the Atlantic region. In the case of clinical *Salmonella* isolates, another limitation is that repeated submissions from the same farm or animal in disease outbreak events may bias the sample population (Government of Canada, 2017).

The gaps from CIPARS could be filled by investigator driven research projects. These independent research projects can identify more resistant organisms than CIPARS do and also conduct further characterization. While the targets of CIPARS are *E. coli, Salmonella* spp. and *Campylobacter* spp., independent projects have the flexibility to target additional organisms such as *S. aureus* or *Clostridium difficile* (Narvaez-Bravo et al., 2016; Rodriguez-Palacios, Staempfli, Duffield, & Weese, 2007). Furthermore, the identification of resistance genes or other molecular characterization can be more readily done in the context of independent research than in routine surveillance (Narvaez-Bravo et al., 2016; Rodriguez-Palacios et al., 2007). Other types of food such as vegetables can also be targeted by other independent studies to identify the resistant organisms belonging to taxa (e.g. *Pseudomonas* spp., *Acinetobacter* spp.) which CIPARS has not included (Bezanson et al., 2008; Wood et al., 2015).

1.3.2. Resistance surveillance gap from imported foods

There are gaps within CIPARS with regard to certain resistant bacterial organisms and food sources. Recently, the Canadian Antimicrobial Resistance Surveillance System (CARSS) was created by Public Health Agency of Canada (PHAC) to try to cover the knowledge gaps regarding the AMR problem in Canada by integrating data from various programs. Among the data integrated by CARSS, one focus was to identify carbapenemase producing Enterobacteriaceae from imported foods. Researchers have done screening for carbapenemase producing bacteria from imported foods (retail seafoods, dried spices and dried chicken pet treats) between 2011 and 2015 (Janecko et al., 2016; Public Health Agency of Canada, 2018a).

As a result, two and three bivalve mollusks containing *E. cloacae* harbouring the NDM-1 gene and *E. cloacae* harbouring the IMI-1 gene respectively have been identified. Additionally, *E. cloacae* and *E. aerogenes* harbouring the IMI-1 and IMI-2 genes were identified from imported shrimp (Janecko et al., 2016; Public Health Agency of Canada, 2018a). The VCC-1 gene, a novel carbapenemase gene, from *V. cholerae* was isolated from imported retail shrimp (Mangat et al., 2016; Public Health Agency of Canada, 2018a). *E. coli* harbouring the mobile colistin resistance gene, *mcr*-1, were isolated from two imported seafood products from a research project that spanned from 2010 to 2016 (Public Health Agency of Canada, 2018a). One recent study found nitrofurantoin resistant *E. faecalis* and ciprofloxacin, erythromycin, linezolid, nitrofurantoin, penicillin and tetracycline resistant *E. faecium* in imported herbs, leafy greens and spinach purchased in 2011 (Allen et al., 2013). Although these findings highlight the importance of monitoring antibiotic resistance in imported foods, there is still less information regarding imported foods as reservoirs for resistant organisms. Given that the role of antibiotic resistance coming from imported food has been inadequately investigsted in Canada, more focus on imported food sources and products with broader spectrum of resistant organisms as targets may provide a better understanding of this route of antibiotic resistance transmission to our community.

2. Objectives

Food is a potential reservoir for bacterial organisms and a vehicle for bacteria which are resistant to antimicrobials or their resistance genes to be transmitted into humans. This transmission can be extended by the import and export of food items between countries. International trade could result in the transmission of resistance from high to low prevalence countries, or the introduction of novel resistance genes. In Canada, CIPARS has been main surveillance program to identify antimicrobial resistant organisms from food sources and products. However, core surveillance only focuses on domestically produced food animals and locally available meat products; therefore, missing seafood, dried spices, vegetables and imported non-meat products as possible sources of AMR organisms. Researchers have found carbapenemase-producers from imported seafoods and dried spices between 2011 and 2015, demonstrating these products as an important source of AMR. This thesis focuses on identifying antimicrobial resistant organisms from imported vegetables and spices with two main objectives:

- To identify the presence of antimicrobial resistant organisms in imported vegetables and spices purchased at international markets in Saskatoon, Saskatchewan
- To identify and characterize resistance genes present in foodborne organisms

3. ANTIMICROBIAL RESISTANT BACTERIA FROM IMPORTED VEGETABLES AND SPICES PRODUCTS PURCHASED FROM NICHE MARKETS IN SASKATOON, SASKATCHEWAN

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Conceived and designed the experiments: DJ, JER. Sample preparation: DJ, JER.

Experiments conduct: DJ. Data anaylsis: DJ, JER. Manuscript preparation: DJ

3.1. Introduction

Antibiotic resistance is one of the most problematic public health issues in the world. Antimicrobial resistance (AMR) has disseminated into people, companion and agricultural animals, wildlife, wastewater and food products (Doi, Iovleva, & Bonomo, 2017). Moreover, selective pressure by antibiotic use in both human and veterinary medicine promotes the emergence of antimicrobial resistant organisms. The emergence can be facilitated by horizontal gene transfer (HGT) from other resistant bacteria or mobile gene elements in outer environments being acquired by susceptible bacteria (Bennett, 2008). Among the contributors for both the distribution and emergence, food sources/products can be an effective reservoir for both the resistant organisms and mobile genes. Fecal contamination is the primary route of resistance transmission particularly related to contamination of enteric pathogens such as *E. coli*, *Salmonella* spp. and *Campylobacter* spp. (Ashby et al., 2003). These resistant pathogens from ingesta or feces can contaminate the animal carcasses on farm or at the abattoir (Ashby et al., 2003; Yalçin et al., 2001). Besides food animals, vegetables are exposed to various factors. Dust, soil, irrigation water, sewage sludge treatment, wild animals or birds, and direct contact with farm workers and food handlers with poor hygiene can all play ar role in contamination of vegetables (Araújo et al., 2017; Eltai et al., 2018; Narvaez-Bravo et al., 2016; Rahube et al., 2014, 2016; Schulz et al., 2016; Tan et al., 2014). Furthermore, antibiotic use can lead to the maintenance of resistant organisms within animals or the environment possibly leading to contamination of plant-based foods (Holman & Chénier, 2013; McEwen & Fedorka-Cray, 2002).

Meanwhile, resistance distribution via food can be more complex when it comes to import and export around the world. Exporting countries having a high prevalence of resistant organisms and poor hygiene practices are more likely to contaminate the foods. Following contamination, it is challenging to control the temperature during transit from different countries and within domestic distribution possibly allowing contaminant organisms to multiply (Allen et al., 2013; McKellar, LeBlanc, Lu, & Delaquis, 2012). The contaminant organisms may include the frequently reported foodborne bacteria such as *E. coli, Salmonella* spp. and Gram-positives such as *S. aureus* and *Enterococcus* spp. (Allen et al., 2013; Bae et al., 2016; Anja Müller et al., 2016; Zurfluh et al., 2015).

Emerging multidrug resistance among Enterobacteriaceae are particularly problematic; broad spectrum β-lactamases including TEM, SHV and CTX-M, confer resistance to penicillins and cephalosporins (Kliebe et al., 1985; Sirot et al., 1987; Tal Jasper et al., 2015). CTX-M hydrolyzes an extended-spectrum of penicillins and 3rd generation cephalosporins and plasmid-mediated AmpC β-lactamases (CMY-2) hydrolyze a broader spectrum of cephalosporins and cephamycins (Philippon et al., 2002; Tal Jasper et al., 2015). Moreover, carbapenemases (IMP, VIM, KPC, OXA-48, NDM) hydrolyze carbapenems which have a very wide range of antibiotic activity (Daikos et al., 2014; Doi & Paterson, 2015; Poirel et al., 2012; Qureshi et al., 2012; Tumbarello et al., 2012). Another emerging resistance from Enterobacteriaceae is by PMQR determinants conferring resistance to fluoroquinolones and aminoglycosides. The determinants produce Qnr proteins that bind to holoenzyme and subunits of DNA gyrase and topoisomerase to protect them from quinolones. QepA is another example of PMQR determinants. QepA is an efflux pump conferring resistance to fluoroquinolones (Jacoby et al., 2014). Another PMQR determinant, AAC(6')-Ib, is able to inactivate the aminoglycosides and fluoroquinolones by acetylation (Jacoby et al., 2014).

Multi-drug resistant *S. aureus* and *Enterococcus* spp. also pose important therapeutic challenges. Methicillin resistant *S. aureus* (MRSA) produces PBP2a, an altered penicillin binding protein which confers resistance to all β-lactams (Hartman & Tomasz, 1981). Methicillin resistance of *S. aureus* is conferred by *mecA* and *mecC* genes among other *mec* family genes (*mecB, mecD*) identified from *Macrococcus* spp. (Baba et al., 2009; Katayama et al., 2000; Paterson et al., 2014; Schwendener et al., 2017). The *mecA* and *mecC* genes are found within the staphylococcal chromosomal cassette (SCC*mec*). The SCC*mec* is mobilizable through the action of the *ccr* genes which facilitate excision and reintegrating of the *mec* genes into methicillin susceptible *S. aureus* (MSSA) (Hartman & Tomasz, 1981; Katayama et al., 2000; Paterson et al., 2014). Vancomycin resistant Enterococci (VRE) is resistant to vancomycin by production of altered peptidoglycan cell wall precursor, D-alanine-D-alanine, which the drug originally binds to inhibit linking of peptidoglycan chains and cell wall synthesis (Miller et al., 2014). Resistance is conferred by VanA or VanB and their genes are able to transmit to the susceptible Enterococci via plasmids or transposons (Courvalin, 2006; Kristich et al., 2014).

Antimicrobial resistant bacteria have been identified from food all around the world. These studies have shown that the foodborne bacteria carry resistance genes conferring resistance to the 3rd generation cephalosporins and fluoroquinolones, and penicillins in the case of *S. aureus* and *Enterococcus* spp. Among the resistance genes, CTX-M and CMY-2 genes are increasingly identified from food products. CTX-M type ESBL producing genes and CMY-2 β-lactamase producing gene were identified from *E. coli* isolated from imported meats in Germany (Müller et al., 2018). CTX-M type ESBLs were also identified from Enterobacteriaceae including *E. coli, K. pneumoniae, Enterobacter* spp. and *Salmonella* spp. from imported vegetables and seafood in Switzerland and the United States (Zurfluh et al., 2015 and Bae et al., 2016). Gram-positive organisms including MRSA were identified from imported meats in Germany and penicillin resistant *E. faecium* were identified from imported vegetables in Canada (Müller et al., 2016 and Allen et al., 2013).

Despite the research studies showing AMR in imported food and its transmission to countries having low prevalence of resistance, surveillance programs on foodborne resistant organisms around the world focus on meats available to consumers rather than specifically targeting imported foods. The National Antimicrobial Resistance Monitoring System (NARMS) from the United States and European Food Safety Authority (EFSA) have targeted domestic meats but not imported foods (Centers for Disease Control and Prevention, 2017; European Food Safety Authority & European Centre for Disease Prevention and Control, 2018). In Canada, CIPARS is the main surveillance program for identifying resistant bacteria from foods. However, the program has a couple of limitations. One is that only *E. coli, Salmonella* spp. and *Campylobacter* spp. are included. There are many other bacteria that potentially contaminate meat. For example, *S. aureus,* and *C. difficile* have been identified from food animals and meat products by researchers in Canada. MRSA has been identified from slaughtered pigs and retail pork from abattoirs (Narvaez-Bravo et al., 2016). Levofloxacin and clindamycin resistant *C. difficile* has been identified from ground meat products in Ontario and Quebec (Rodriguez-Palacios et al., 2007). The other limitation of CIPARS is that it only focuses on particular categories of retail products; Canada imports a variety of foods including dairy products, fish and seafood and fresh or processed fruits/vegetables which are not included in routine surveillance (Canadian Food Inspection Agency, 2018). However, between 2011 and 2015, researchers launched projects off of the CIPARS infrastructure and studied imported dried spices and retail

seafoods and found resistant Enterobacteriaceae (Public Health Agency of Canada, 2018a). As a result, NDM, IMI type genes within *E. cloacae* and *E. aerogenes* have been identified from imported seafood and the VCC-1 gene from *V. cholerae* has been identified from imported shrimp (Janecko et al., 2016; Mangat et al., 2016; Public Health Agency of Canada, 2018a). Targeting more imported food and bacterial organisms are necessary as independent research studies have indicated; Morrison and Rubin have found carbapenemase producing *Pseudomonas, Stenotrophomonas* and *Myroides* spp. from imported seafoods (Morrison & Rubin, 2015). Allen et al. as another example, has found resistant *E. faecalis* and *E. faecium* from imported herbs, leafy greens and spinach from Columbia, Dominican Republic, Mexico, Guatemala and the US (Allen et al., 2013). Considering the findings from independent research studies, more different types of food and different bacteria have to be targeted for AMR identification in Canada.

The identification of antibiotic resistant organisms from imported food in Canada is necessary to fill the gap on the antibiotic resistance transmission in Canada. Especially, imported vegetables and spices have been neglected by the national surveillance program and to a lesser extent research studies. In this study, antibiotic resistant organisms have been identified from imported vegetables and spices purchased from local international markets. Characterization of their resistance and species identification followed. This pilot investigation therefore provides fundamental information on transferability of antibiotic resistant organisms via neglected food sources in Canada.

3.2. Method and Materials

3.2.1. Food samples for bacteria isolation

The ten international markets selling imported foods identified in Saskatoon, Saskatchewan were visited, and a convenience sampling of 147 vegetable and spice products were purchased (Figure 3.4). Although samples were selected conveniently, they were chosen to represent the breadth of products available. The samples were imported from 17 countries including Asian countries, India (n=34), China (n=20), Philippines (n=18), Thailand (n=24), Bangladesh (n=8), Vietnam (n=8), Sri Lanka (n=2), Taiwan (n=1) and Pakistan (n=1), African

countries, Cameroon (n=10), Congo (n=6), Togo (n=5), Ghana (n=4), Nigeria (n=4) and Egypt (n=1), and Jamaica (n=1). The samples consist of 77 different types and all samples were either frozen or dried when they were purchased. All samples were stored on the day of purchase; frozen samples were stored at -80℃ and dried samples were stored at room temperature.

3.2.2. Bacteria isolation

All frozen samples were completely thawed and prepared at room temperature before the bacteria isolation process. For isolation of the targeted bacterial organisms, washing of 25 grams of each sample, or the whole sample if ≤25g was available, in a sterile plastic bag with 250 mL of buffer peptone water[F] (BPW) was conducted. After 5 minutes of washing, 50 mL of BPW were aliquoted into an empty sterile bottle and each double strength of selective broth, MacConkey broth[H], modified tryptone broth (10 g/L of tryptone, 75 g/L of sodium chloride, 10 g/L of mannitol and 2.5 g/L of yeast) (Ji, 2007) and Enterococcosel broth[F]. All aliquoted broth and bottles were incubated at 35℃ for 20 hours.

After the incubation, 10µL of MacConkey broth, modified tryptone broth and Enterococcosel broth was inoculated into eosin methylene blue agar[I], mannitol salt agar[F] and Enterococcosel agar[F] respectively (Figure 3.4). Other selective agar plates, CHROMagar ESBL[G] and agar selective for meropenem resistant Gram-negatives (Mueller-Hinton agar[F] with 2µg/ml of meropenem[A]), and other additional selective broth, 10 mL of Rappaport-Vassiliadis (RV) broth[F] and tetrathionate broth[F], were inoculated with 0.1 mL and 1 mL of incubated BPW respectively. All inoculated selective agar plates and tetrathionate broth were incubated at 35℃ for 20 hours, and incubated RV broth were incubated at 42℃ for 20 hours (Figure 3.4).

On the following day, colonies were picked from each selective plate and streaked onto Columbia blood agar with 5% sheep blood[I]. 10µL of RV and tetrathionate broth was inoculated into xylose lysine deoxycholoate (XLD) agar[F] and brilliant green (BG) agar[F]. All inoculated blood agar, XLD agar and BG agar were incubated at 35℃ for 20 hours. If colonies with targeted morphology from XLD agar and BG agar appeared on next day, the colonies were streaked into blood agar and incubated at 35℃ for 20 hours.

If blood agar contained the colonies with single morphology on the next day, colonies were streaked and inoculated into the cryogenic vile which contained 1mL of tryptic soy broth[F] with 15% of glycerol followed by storage in -80℃.

To confirm the isolates from selective media were targeted organisms, biochemical tests were conducted (Table 3.2). Indole[J] and citrate[J] tests were done for *E. coli* identification. Triple Sugar Iron (TSI)[J] and urea[M] tests were done for *Salmonella* spp. identification. CHROMagar ESBL isolates were tested with DrySlide Oxidase test kit[J] to distinguish Enterobacteriaceae from others. Coagulase test by BBL Coagulase Plasma, Rabbit with EDTA[J] was done for the *Staphylococcus* spp. from mannitol salt agar plates and only coagulase positive isolates were included for further processing. Pyrrolidonyl arylamidase (PYR) test[L] was done for isolates from Enterococcosel agar plates to distinguish *Enterococcus* spp.

Table 3. 2. Properties for bacteria identification. Colonies with targeted morphology on selective media were collected. Biochemical tests were conducted to identify if collected bacteria had biochemical characteristics of the targeted bacteria. PYR indicates pyrrolidonyl aminopeptidase, pos indicates positive result, neg indicates negative result.

	Appearance on selective media	Biochemical characteristics	Method for definitive identification
E. coli	Dark colonies with green metallic sheen	Tryptophanase production	Indole (pos), citrate (neg), urea tests (neg)
Salmonella spp.	Red colonies with black centre on XLD agar, pink-white colonies with brilliant red zones on BG agar	Gas production and hydrogen sulfide production	TSI slant test (red slant, yellow butt, gas pos, hydrogen sulfide pos)
ESBL producing Enterobacteriaceae	Dark pink to reddish colonies if *E. coli,* metallic blue colonies if other Enterobacteriaceae	Facultative anaerobes	Oxidase test (neg)
S. aureus	Yellow colonies with yellow zones	Coagulase production	Coagulase test (pos)
Enterococcus spp.	Small translucent colonies with black zones	Pyrrolidonyl Aminopeptidase production (Hippurate hydrolysis + if *E. gallinarum*) (Hippurate hydrolysis - if *E. casseliflavus*)	PYR test (pos) Hippurate test for *E. gallinarum* (pos) and *E. casseliflavus* (neg) identification

3.2.3. Species identification

Polymerase chain reaction (PCR) with universal primers (Table 3. 3), targeting cpn60 or 16s rRNA, was conducted to identify species of the isolates. Crude DNA extracts from isolates were obtained by boil prep. The reactions were conducted with 24 µL of master-mix (distilled water, 10X PCR buffer, dNTPs, primers, 50 mM Magnesium Chloride ($MgCl_2$), Taq polymerase)[Q] and 1 µL of DNA template. Conditions for amplification of 16s rRNA and cpn60 genes by C1000 Touch Thermal Cycler[O] are described in Table 3.4. Amplicons were resolved by gel electrophoresis using 1% agarose gel at 100 volts for 30 minutes. The gels were visualized under UV light of Gel Doc XR+[O] to identify the presence of targeted amplicons. To sequence the amplicons, amplified either cpn60 or 16s rRNA genes of isolates were purified by the Illustra ExoProStar kit[P] and EZ-10 Spin Column PCR purification kit[N]. All purified DNA was quantified by Nanodrop 2000 spectrophotometer[R]. To sequence DNA with high quality, the absorbance ratio of UV light (260:280nm) of 1.8, and a 260:230 ratio of 2.0-2.2 were used. All prepared genes were sent to a commercial lab (Macrogen; Seoul, Rep. of Korea) for sequencing. Sequenced data were assembled and analyzed using Pregap4 and Gap4 software which are part of Staden Package. Contigs were then compared with reference sequences from with National Center for Biotechnology Information (NCBI) GenBank. (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify species of the isolates (Figure 3.4).

To identify serotype of *Salmonella* spp., *Salmonella* spp. isolates were submitted to National Microbiology Laboratory in Guelph, ON for serotyping. Because the reference data from NCBI BLAST for *E. casseliflavus* and *E. gallinarum* were ambiguous, sequenced data were compared to each other to identify the most robust species.

Table 3. 3. List of universal targets primers

Genes	Primer sequence (5'->3')	Size	References
Universal targets			
*cpn*60	CGCCAGGGTTTTCCCAGTCACGAC	700bp	(Sahin, Gonzalez, Iizuka, & Hill, 2010)
	AGCGGATAACAATTTCACACAGGA		
16s rRNA	GAGTTTGATCCTGGCTCAG	500bp	(Dorsch & Stackebrandt, 1992)
	GWATTACCGCGGCKGCTG		

Table 3. 4. Temperature conditions of PCR for 16s rRNA and cpn60

PCR reaction process	16s rRNA	*cpn60*
Initial Denaturation	94°C for 3 mins	68°C, 1 min 72°C, 1 min
(Cycles)	(x30)	(x40)
Denaturation	94°C, 30 secs	94°C, 30 secs
Annealing	60°C, 1 min	60°C, 1 min
Extension	72°C, 1 min	72°C, 1 min
Final Extension	72°C for 10 mins	

3.2.4. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done by broth microdilution method using the Sensititre Automated Inoculation system[D]. Two different plate formats were used: the CMV4AGNF plate[E] for Gram-negative bacteria which contains 14 drugs from 7 classes and GPALL1F plate[E] for Gram-positive bacteria which contains 21 drugs from 16 classes. Testing was done according to the manufacturer's instructions. Isolates were sub-cultured on blood agar and incubated at 35℃ overnight. Then, colonies from the plate were suspended in 5mL of demineralized water[D] until its density reached 0.5 McFarland. 30 µL of the suspended water was added to 11 mL of cation-adjusted Mueller-Hinton broth[D]. 50 µL of the inoculated broth was dispensed into each well of designated plates for either Gram-negative or -positive using the Sensititre auto-inoculator. All plates were inoculated at 35℃ for 18-22 hours while being sealed by adhesive film. Minimum inhibitory concentrations (MICs) of each antimicrobial were interpreted after incubation (Figure 3.4).

Because polymyxin drugs were not included in the NARMS plates, colistin susceptibility testing was done by agar dilution method. Colistin sulfate salt[B] were dissolved in distilled water to make different concentrations at 20 mL. Mueller-Hinton agar mixture was boiled, cooled and aliquoted into 18 mL volumes in glass vials. 2 mL of each colistin solution were then inoculated into each 18 mL of Mueller-Hinton agar mixture to make final concentrations ranging from 0.032 to 64 µg/mL. Each different concentration was poured into a plate and solidified. To inoculate the agar plates with isolates, subcultured bacterial colonies on blood agars were prepared and suspended into demineralized water to make 0.5 McFarland suspension. 600 µL of the suspended water were transferred into wells of Cathra replicator[C] and then inoculum from each well was spotted onto each colistin selective agar plate by the replicator (Figure 3.4).

To identify either susceptible or resistant to each drug and non- or intrinsic resistance, Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines were referred (Clinical and Laboratory Standards Institute, 2014; European Society of Clinical Microbiology and Infectious Diseases, 2016) (Figure 3.4). For quality control, *S. aureus* ATCC 29213 and *E. coli* ATCC 25922, *E. faecalis* ATCC 29212 and *P. aeruginosa* ATCC 275853 were used. If meropenem resistant isolates were

identified from Gram-negative isolates, β CARBA test[K] was done to identify the presence of carbapenemases (KPC, OXA-48, NDM, VIM, and IMP).

3.2.5. Resistance genes identification

Isolates determined to be phenotypically resistant were screened for resistance genes including ESBLs (CTX-M, TEM, SHV), plasmid mediated AmpC β-lactamase (CMY-2), carbapenemase (IMP, KPC, NDM, OXA-48, VIM), PMQR determinants (QnrA, QnrB, QnrS, QepA, AAC(6')-Ib), MCR (MCR-1, MCR-2, MCR-3, MCR-4) genes and class I integrons. The primers used are described in Table 3.5. The details PCR reactions are described in Tables 3.6 - 3.11. PCR amplicons were visualized and prepared for sequencing as previously described. The identity of analyzed contigs were then determined by comparison with reference sequences from The Comprehensive Antibiotic Resistance Database (CARD; https://card.mcmaster.ca/) and Lahey database (https://www.lahey.org/Studies/). Antibiotic Resistance Gene-ANNOTation (ARG-ANNOT; http://en.mediterranee-infection.com/article.php?laref=283%26titre=arg-annot) reference sequences were also compared with the contigs if there were more than two genes with same bit-score and identity from CARD (Figure 3.4).

Table 3. 5. List of resistance genes primers

Genes	Primer sequence (5'->3')	Size	References
bla$_{SHV}$	TTATCTCCCTGTTAGCCACC	797bp	(Arlet, Rouveau, & Philippon, 1997)
	GATTTGCTGATTTCGCTCGG		
	CGGCCCTCACTCAAGGATG (*bla*$_{SHV-F}$ sequence primer)	336bp	(Patricia A. Bradford, 1999)
	GCGAGTAGTCCACCAGATCC (*bla*$_{SHV-F}$ sequence primer)		This study
	CTGCTGCAGTGGATGGTG (*bla*$_{SHV-R}$ sequence primer)	350bp	This study
	ATTACCGACCGGCATCTCTC (*bla*$_{SHV-R}$ sequence primer)		
	CGCCGGGTTATTCTTATTTGTCGC (*bla*$_{SHV-UP}$ sequence primer)	1016bp	(Perilli et al., 2002)
	TCTTTCCGATGCCGCCGCCAGTCA (*bla*$_{SHV-LO}$ sequence primer)		
bla$_{TEM}$	GCGGAACCCCTATTTG	964bp	(Olesen, Hasman, & Møller Aarestrup, 2004)
	ACCAATGCTTAATCAGTGAG		
bla$_{CTX-M-U}$	ATGTGCAGYACCAGTAARGTKATGGC	593bp	(Mulvey, Soule, Boyd, Demczuk, & Ahmed, 2003)
	TGGGTRAARTARGTSACCAGAAYCAGC GG		
bla$_{CTX-M-G1}$	GTTGTTAATTCGTCTCTTCC	900bp	(Ghosh, 2017)
	AGTTTCCCCATTCCGTTTC		
	TGGGTRAARTARGTSACCAGAAYCAGC GG (Forward sequence primer)		(Mulvey et al., 2003)
bla$_{CTX-M-G2}$	ACTCAGAGCATTCGCCGCTCA	1000bp	(Ghosh, 2017)
	TTATTGCATCAGAAACCGTG		
bla$_{CTX-M-G8}$	CGCTTTGCCATGTGCAGCACC	307bp	(Pitout, Hossain, & Hanson, 2004)
	GCTCAGTACGATCGAGCC		
bla$_{CTX-M-G9}$	GACCGTATTGGGAGTTTGAG	600bp	(Hammad, Ishida, & Shimamoto, 2009)
	ATCTGATCCTTCAACTCAGC		(Gonsalves, 2011)
bla$_{CMY-2}$	ATGCAACAACGACAATCC	1000bp	(Kim, Kwon, Pai, Kim, & Cho, 1998)

	TTGGCCAGCATGACGATG		(D'Andrea et al., 2006)
bla$_{IMP}$	GAAGGCGTTTATGTTCATAC	587bp	(Pitout et al., 2005)
	GTACGTTTCAAGAGTGATGC		
bla$_{KPC}$	TGTCACTGTATCGCCGTC	900bp	(Yigit et al., 2001)
	CTCAGTGCTCTACAGAAAACC		
bla$_{NDM-1}$	GCAGCTTGTCGGCCATGCGGGC	782bp	(Peirano, Ahmed-Bentley, Woodford, & Pitout, 2011)
	GGTCGCGAAGCTGAGCACCGCAT		
bla$_{OXA-48}$	GCGTGGTTAAGGATGAACAC	438bp	(Poirel, Walsh, Cuvillier, & Nordmann, 2011)
	CATCAAGTTCAACCCAACCG		
bla$_{VIM}$	GTTTGGTCGCATATCGCAAC	382bp	(Pitout et al., 2005)
	AATGCGCAGCACCAGGATAG		
aac(6')-Ib	TTGCGATGCTCTATGAGTGGCTA	482bp	(Park, Robicsek, Jacoby, Sahm, & Hooper, 2006)
	CTCGAATGCCTGGCGTGTTT		
qnrA	ATTTCTCACGCCAGGATTTG	516bp	(Robicsek, Strahilevitz, Sahm, Jacoby, & Hooper, 2006)
	GATCGGCAAAGGTTAGGTCA		
qnrB	GATCGTGAAAGCCAGAAAGG	469bp	
	ACGATGCCTGGTAGTTGTCC		
qnrS	ACGACATTCGTCAACTGCAA	417bp	
	TAAATTGGCACCCTGTAGGC		
qepA	GCAGGTCCAGCAGCGGGTAG	199bp	(J.-H. Liu et al., 2008)
	CTTCCTGCCCGAGTATCGTG		
mcr-1	CGGTCAGTCCGTTTGTTC	309bp	(Y.-Y. Liu et al., 2016)
	CTTGGTCGGTCTGTAGGG		
mcr-2	TGGTACAGCCCCTTTATT	1,617bp	(Xavier et al., 2016)
	GCTTGAGATTGGGTTATGA		
mcr-3	TTGGCACTGTATTTTGCATTT	542bp	(Yin et al., 2017)
	TTAACGAAATTGGCTGGAACA		
mcr-4	ATTGGGATAGTCGCCTTTTT	487bp	(Carattoli et al., 2017)
	TTACAGCCAGAATCATTATCA		
Class I Integron	GGCATCCAAGCAGCAAG	Variable	(Pellegrini et al., 2009)
	AAAGCAGACTTGACCTGA		

Table 3. 6. Temperature conditions of PCR for SHV and TEM genes

PCR reaction process	*bla*$_{SHV}$				*bla*$_{TEM}$
	bla$_{SHV-U}$	*bla*$_{SHV-F}$	*bla*$_{SHV-R}$	*bla*$_{SHV-UP, LO}$	
Initial Denaturation	94°C for 6 mins				
(Cycles)	(x35)	(x30)	(x30)	(x30)	(x30)
Denaturation	94°C, 1 min	94°C, 1 min	94°C, 1 min	94°C, 1 min	94°C, 1 min
Annealing	68°C, 1 min	61.5°C, 1 min	51.5°C, 1 min	68°C, 1 min	54.7°C, 1 min
Extension	72°C, 1 min	72°C, 1 min	72°C, 1 min	72°C, 1 min	72°C, 1 min
Final Extension	72°C for 10 mins				

Table 3. 7. Temperature conditions of PCR for CTX-M and AmpC genes

PCR reaction process	*bla*$_{CTX-M}$					*bla*$_{AmpC}$
	bla$_{CTX-M-U}$	*bla*$_{CTX-M-G1}$	*bla*$_{CTX-M-G2}$	*bla*$_{CTX-M-G8}$	*bla*$_{CTX-M-G9}$	*bla*$_{CMY-2}$
Initial Denaturation	94°C for 6 mins					
(Cycles)	(x35)	(x35)	(x35)	(x35)	(x30)	(x30)
Denaturation	94°C, 1 min	94°C, 1 min	94°C, 1 min	94°C, 1 min	94°C, 1 min	94°C, 1 min
Annealing	59.5°C,	61°C, 1 min	58.3 °C,	64°C, 1 min	61°C, 1 min	50°C, 1 min
Extension	1 min	72°C, 1 min	1 min	72°C, 1 min	72°C, 1 min	72°C, 1 min
	72°C, 1 min		72°C, 1 min			
Final Extension	72°C for 10 mins					

Table 3. 8. Temperature conditions of PCR for carbapenemases genes

PCR reaction process	Carbapenemase genes				
	bla$_{IMP}$	*bla*$_{KPC}$	*bla*$_{NDM-1}$	*bla*$_{OXA-48}$	*bla*$_{VIM}$
Initial Denaturation	95°C for 5 mins				
(Cycles)	(x35)	(x35)	(x40)	(x35)	(x35)
Denaturation	95°C, 45 secs	95°C, 45 secs	95°C, 45 secs	95°C, 45 secs	95°C, 45 secs
Annealing	55.1°C, 45 secs	54.8°C, 45 secs	58.3 °C, 45 secs	54.5°C, 45 secs	60.3°C, 45 secs
Extension	72°C, 1 min	72°C, 1 min	72°C, 1 min	72°C, 1 min	72°C, 1 min
Final Extension	72°C for 8 mins				

Table 3. 9. Temperature conditions of PCR for PMQR determinants genes

PCR reaction process	PMQR determinant genes				
	aac(6')-Ib	*qnrA*	*qnrB*	*qnrS*	*qepA*
Initial Denaturation	94°C for 6 mins			94°C for 3 mins	
(Cycles)	(x35)	(x35)	(x35)	(x35)	(x35)
Denaturation	94°C, 1 min	94°C, 1 min	94°C, 1 min	94°C, 30 secs	94°C, 30 secs
Annealing	68°C, 1 min	53.4°C, 1 min	66.1°C, 1 min	51°C, 30 secs	61°C, 30 secs
Extension	72°C, 1 min	72°C, 1 min	72°C, 1 min	72°C, 30 secs	72°C, 30 secs
Final Extension	72°C for 10 mins				

Table 3. 10. Temperature conditions of PCR for MCR genes

PCR reaction Process	MCR genes			
	mcr-1	*mcr-2*	*mcr-3*	*mcr-4*
Initial Denaturation	94°C for 6 mins			
(Cycles)	(x35)	(x30)		
Denaturation	94°C, 1 min		95°C, 1 min	
Annealing	56°C, 1 min		50°C, 1 min	
Extension	72°C, 1 min		72°C, 1 min	
Final Extension	72°C for 3 mins		72°C for 10 mins	

Table 3. 11. Temperature conditions of PCR for class I integron

PCR reaction process	Class I integron
Initial Denaturation	94°C for 5 mins
(Cycles)	(x30)
Denaturation	94°C, 30 secs
Annealing	68.2°C, 1 min
Extension	72°C, 2 min
Final Extension	72°C for 10 mins

3.2.6. Multilocus sequence typing

Sequence types (STs) of MDR *E. coli*, *K. pneumoniae* and *S. aureus* isolates were determined by MLST using previously published schemes (Diancourt, Passet, Verhoef, Grimont, & Brisse, 2005; M. C. Enright, Day, Davies, Peacock, & Spratt, 2000; Ghosh, 2017; Wirth et al., 2006) (Table 3. 12). Targeted genes for each MLST were amplified by PCR reactions. 7 locus (*adk, fumC, gyrB, icd, mdh, purA, recA*) from *E. coli*, 6 locus (*gapA, infB, mdh, phoE, pgi, rpoB*) from *K. pneumoniae* and 6 locus (*arcC, aroE, glpF, gmk, tpi, pta*) from *S. aureus* were targeted for MLST. Primers that were used and conditions for each PCR reactions are indicated in Tables 3.13 – 3.15. Sequences were analysed using online MLST databased: https://enterobase.warwick.ac.uk/species/ecoli/allele_st_search for *E. coli*, http://bigsdb.pasteur.fr/klebsiella/ for *K. pneumoniae* and http://saureus.mlst.net/ for *S. aureus*.

Table 3. 12. List of primers used in MLST schemes

Genes	Primer sequence (5'->3')	Size	References
E. coli			
adk	ATTCTGCTTGGCGCTCCGGG	536bp	(Wirth et al., 2006)
	CCGTCAACTTTCGCGTATTT		
fumC	TCACAGGTCGCCAGCGCTTC	469bp	
	GTACGCAGCGAAAAAGATTC		
gyrB	TCGGCGACACGGATGACGGC	460bp	
	ATCAGGCCTTCACGCGCATC		
icd	ATGGAAAGTAAAGTAGTTGTTCCGGCACA	518bp	
	GGACGCAGCAGGATCTGTT		
mdh	ATGAAAGTCGCAGTCCTCGGCGCTGCTGGCGG	452bp	
	TTAACGAACTCCTGCCCCAGAGCGATATCTTTCTT		
purA	CGCGCTGATGAAAGAGATGA	478bp	
	CATACGGTAAGCCACGCAGA		
recA	GTGCGTTTATCGATGCTGAA	510bp	(Ghosh, 2017)
	TCTTTTACGCCCAGGTCAAC		
K. pneumoniae			
gapA	TGAAATATGACTCCACTCACGG	501bp	(Diancourt et al., 2005)
	CTTCAGAAGCGGCTTTGATGGCTT		
infB	CTCGCTGCTGGACTATATTCG	318bp	
	CGCTTTCAGCTCAAGAACTTC		
	ACTAAGGTTGCCTCCGGCGAAGC (Forward sequencing primer)		
mdh	CCCAACTCGCTTCAGGTTCAG	477bp	
	CCGTTTTTCCCCAGCAGCAG		
phoE	ACCTACCGCAACACCGACTTCTTCGG	420bp	
	TGATCAGAACTGGTAGGTGAT		
pgi	GAGAAAAACCTGCCTGTACTGCTGGC	432bp	
	CGCGCCACGCTTTATAGCGGTTAAT		
	CTGCTGGCGCTGATCGGCAT (Forward sequencing primer)		
	TTATAGCGGTTAATCAGGCCGT (Reverse sequencing primer)		
rpoB	GGCGAAATGGCWGAGAACCA	501bp	
	GAGTCTTCGAAGTTGTAACC		
S. aureus			
arcC	TTGATTCACCAGCGCGTATTGTC	456bp	(M. C. Enright et al., 2000)
	AGGTATCTGCTTCAATCAGCG		
aroE	ATCGGAAATCCTATTTCACATTC	456bp	

	GGTGTTGTATTAATAACGATATC	
glpF	CTAGGAACTGCAATCTTAATCC	465bp
	TGGTAAAATCGCATGTCCAATTC	
gmk	ATCGTTTTATCGGGACCATC	429bp
	TCATTAACTACAACGTAATCGTA	
tpi	TCGTTCATTCTGAACGTCGTGAA	402bp
	TTTGCACCTTCTAACAATTGTAC	
pta	GTTAAAATCGTATTACCTGAAGG	474bp
	GACCCTTTTGTTGAAAAGCTTAA	
yqiL	CAGCATACAGGACACCTATTGGC	516bp
	CGTTGAGGAATCGATACTGGAAC	

Table 3. 13. Temperature conditions of PCR for *E. coli* MLST genes

PCR reaction	*adk*	*fumC*	*gyrB*	*icd*	*mdh*	*purA*	*recA*
Initial Denaturation	94°C for 6 mins						
(Cycles)	(x30)						
Denaturation	94°C, 1 min						
Annealing	54°C-60°C, 1 min						
Extension	72°C, 1 min						
Final Extension	72°C for 10 mins						

Table 3. 14. Temperature conditions of PCR for *K. pneumoniae* MLST genes

PCR reaction	*rpoB*	*gapA*	*mdh*	*pgi*	*phoE*	*infB*	*tonB*
Initial Denaturation	94°C for 5 mins						
(Cycles)	(x35)						
Denaturation	94°C, 30 secs						
Annealing	50°C-55°C, 30 secs						
Extension	72°C, 30 secs						
Final Extension	72°C for 10 mins						

Table 3. 15. Temperature conditions of PCR for MRSA MLST genes

PCR reaction	*arcC*	*aroE*	*glpF*	*gmk*	*pta*	*tpi*	*yqiL*
Initial Denaturation	95°C for 5 mins						
(Cycles)	(x30)						
Denaturation	95°C, 1 min						
Annealing	50°C-60°C, 1 min						
Extension	72°C, 1 min						
Final Extension	72°C for 5 mins						

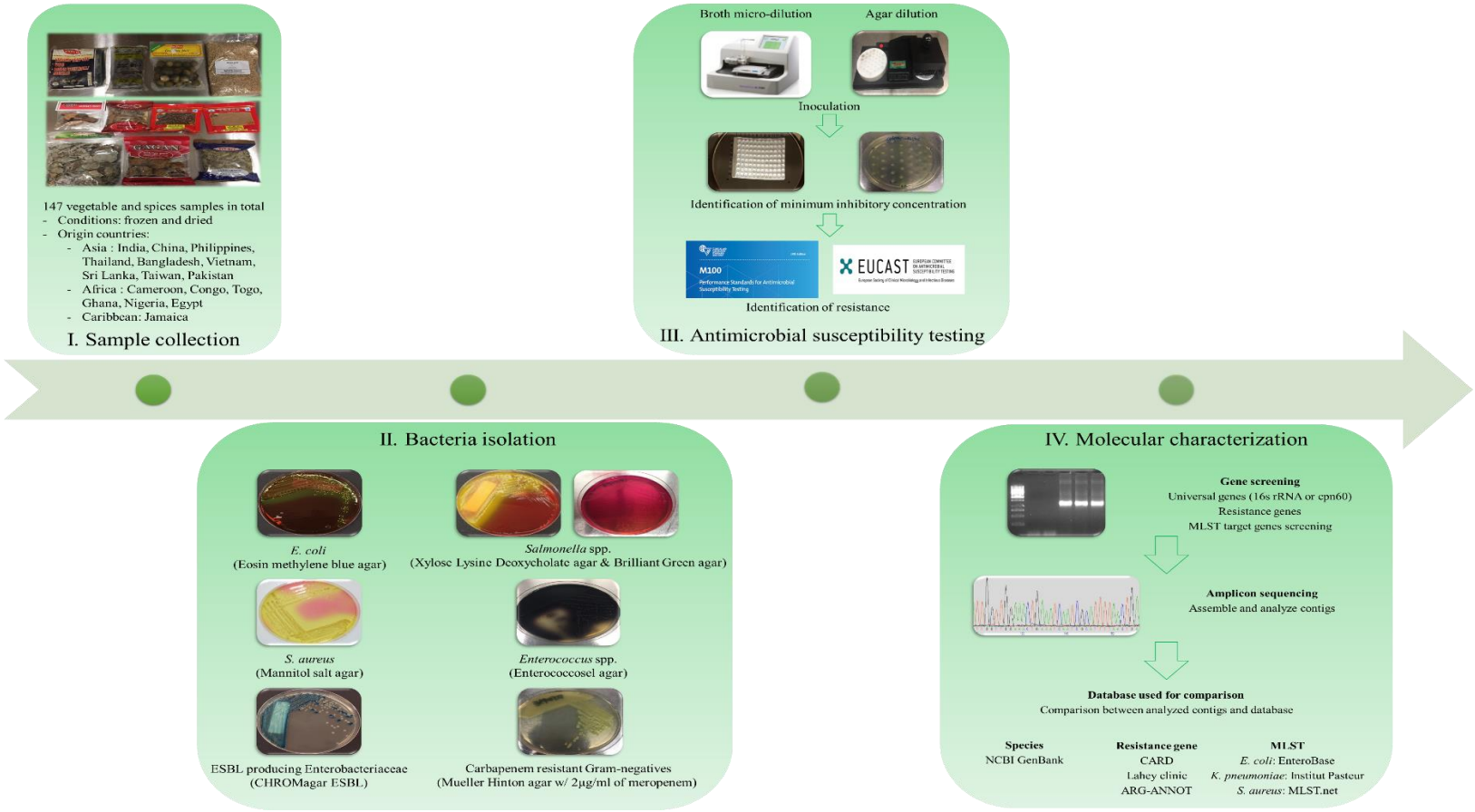

Figure 3. 4. Sample processing and bacterial characterization. 147 vegetables and spices were purchased from international markets in Saskatoon, Saskatchewan. Samples were selectively cultured for *E. coli*, *Salmonella* spp., *S. aureus*, *Enterococcus* spp., ESBL producing Enterobacteriaceae and meropenem resistant Gram-negatives. Antimicrobial susceptibility testing was conducted by broth microdilution and agar dilution and interpreted according to the CIPARS and EUCAST guidelines. Lastly, organisms were characterized through amplification and sequence analysis universal bacterial targets, resistance genes and through previously published MLST schemes.

3.3. Results

3.3.1. Bacteria isolation and their species identification

E. coli (n=15, 10.2%), *Salmonella* spp. (n=2, 1.4%), *Enterobacter* spp. (n=10, 6.8%), *K. pneumoniae* (n=2, 1.4%), *Acinetobacter* spp. (n=11, 7.5%), *Pseudomonas* spp. (n=10, 6.8%), *S. aureus* (n=7, 4.8%) and *Enterococcus* spp. (n=68, 46.3%) were isolated from 147 imported vegetables and spices (Table 3.16 & 3.17).

Species of *Salmonella* spp. isolates were *Salmonella enterica* subsp. enterica serovar Redlands and unnamed *Salmonella* spp. (*Salmonella* I:4, 12:r:-). ST (Sequence Type) of CTX-M containing *E. coli* were ST189 and ST226. ST of CTX-M containing *K. pneumoniae* were ST101 and ST261. ST of MRSA isolates were ST834 and unknown due to lack of one of the targeted genes for MLST (Table 3.18 - 3.20).

Table 3. 16. Information of all isolates

Isolate number	Name	Country of origin	Condition	*E. coli* (non-ESBL producer)	*Salmonella* spp.	CHROMagar ESBL isolates	Meropenem resistant Gram-negatives	*S. aureus*	*Enterococcus* spp.
DJ001	Curry leaves	India	Dried	-	-	-	-	-	-
DJ002	Cinnamon Sticks flat	India	Dried	-	-	-	-	-	-
DJ003	Sichuan peppercorns	China	Dried	-	-	-	-	-	-
DJ004	Five spice powder	China	Dried	-	-	-	-	-	-
DJ005	Brown rice	India	Dried	-	-	-	-	-	-
DJ006	Spicy lentil chunks (Punjabi Wadi)	India	Dried	-	-	-	-	-	-
DJ007	Chaney root	Jamaica	Dried	-	-	-	-	-	-
DJ008	Dried taro leaves (Dahon ng gabi)	Philippines	Dried	-	-	-	-	-	DJ008D (*E. hirae*)
DJ009	Calamansi fruit	Philippines	Fresh	-	-	-	-	-	-
DJ010	Pentaclethra macrophyl UGBA	Cameroon	Frozen	-	-	-	-	-	-
DJ011	Legume vert ugu ugu/Green vegetable Nigeria	Togo	Frozen	-	-	-	-	-	DJ011D (*E. faecalis*)
DJ012	Liquorice roots	Pakistan	Dried	-	-	-	-	-	-
DJ013	Pan Glori Candy (Sucrerie)	India	Dried	-	-	-	-	-	-
DJ014	Pan Glori Candy (Sucrerie)	India	Dried	-	-	-	-	-	-
DJ015	Calcutta pan	India	Dried	-	-	-	-	-	-
DJ016	Calcutta pan	India	Dried	-	-	-	-	-	-
DJ017	Kokum wet	India	Dried	-	-	-	-	-	-
DJ018	Chili whole kashmiri	India	Dried	-	-	DJ018F (*E. cloacae*)	-	-	-
DJ019	Mint leaves	India	Dried	-	-	-	-	-	-
DJ020	Mint leaves (Tit-bit)	India	Dried	-	-	-	-	-	-
DJ021	Coconut slice	India	Dried	-	-	-	-	-	-
DJ022	Anardana whole (pomegranate seeds)	India	Dried	-	-	-	-	-	-
DJ023	Anardana whole (pomegranate seeds)	India	Dried	-	-	-	-	-	-
DJ024	Noori (Alu Bukhara)	India	Dried	-	-	-	-	-	-
DJ025	Tulsi leaf	India	Dried	-	-	-	-	-	DJ025D (*E. gallinarum*)
DJ026	Tulsi leaf	India	Dried	-	-	-	-	-	DJ026D (*E. gallinarum*)
DJ027	Spice powder mix	China	Dried	-	-	-	-	-	-
DJ028	Spice powder mix	China	Dried	-	-	-	-	-	-
DJ029	Dried hairy basil leaves	Thailand	Dried	-	-	-	-	-	-
DJ030	Dried hairy basil leaves	Thailand	Dried	-	-	-	-	-	-
DJ031	Dried hairy basil leaves	Thailand	Dried	-	-	-	-	-	-
DJ032	Mbinzo	Congo	Dried	-	-	-	-	-	DJ032D (*E. gallinarum*)
DJ033	Mbinzo	Congo	Dried	-	-	-	-	-	DJ033D (*E. faecalis*, *E. gallinarum*)
DJ034	Dilombo lombo	Congo	Dried	-	-	-	-	-	-
DJ035	Dilombo lombo	Congo	Dried	-	-	-	-	-	-
DJ036	Mayebo	Congo	Dried	-	-	-	-	-	-
DJ037	Mayebo	Congo	Dried	-	-	-	-	-	-
DJ038	Soursop leaf-Fine cut leaf	Sri Lanka	Dried	-	-	-	-	-	-
DJ039	Soursop leaf-Fine cut leaf	Sri Lanka	Dried	-	-	-	-	-	-
DJ040	Nchuonwu leaf	Ghana	Dried	-	DJ040B	DJ040F (*A. baumannii*)	-	-	DJ040D (*E. faecium*, *E. hirae*)
DJ041	Nchuonwu leaf	Ghana	Dried	-	-	-	-	-	DJ041D (*E. hirae*)
DJ042	Utazi leaf	Ghana	Dried	-	-	DJ042F (*A. baumannii*)	-	-	DJ042D (*E. gallinarum*)
DJ043	Utazi leaf	Ghana	Dried	-	-	DJ043F	-	-	DJ043D

						(*A. baumannii*)			(*E. casseliflavus, E. hirae, E. gallinarum*)
DJ044	Senna leaf	India	Dried	-	-	-	-	-	-
DJ045	Senna leaf	India	Dried	-	-	-	-	-	-
DJ046	Dried kaffir lime leaves	Thailand	Dried	-	-	-	-	-	-
DJ047	Dried kaffir lime leaves	Thailand	Dried	-	-	-	-	-	-
DJ048	Ginger powder	China	Dried	-	-	-	-	-	-
DJ049	Five spice powder	Taiwan	Dried	-	-	-	-	-	-
DJ050	Five spice powder	China	Dried	-	-	-	-	-	-
DJ051	Ginger	China	Dried	-	-	-	-	-	-
DJ052	Ginger	China	Dried	-	-	-	-	-	-
DJ053	Seasoning powder	China	Dried	-	-	-	-	-	-
DJ054	Dried rose	China	Dried	-	-	-	-	-	-
DJ055	Frozen sadao flower	Bangkok	Frozen	-	-	-	-	-	-
DJ056	Frozen tumeric	Bangkok	Frozen	-	-	-	-	-	-
DJ057	Frozen turmeric	Bangkok	Frozen	DJ057A	-	-	-	-	-
DJ058	Lemon grass	Bangkok	Frozen	-	-	-	-	-	-
DJ059	Lemon grass	Bangkok	Frozen	-	-	-	-	-	-
DJ060	Frozen pandan leaves	Thailand	Frozen	-	-	-	DJ060E (*S. maltophilia*)	-	DJ060D (*E. hirae*)
DJ061	Frozen sadao flower	Bangkok	Frozen	-	-	-	-	-	-
DJ062	Frozen pandan leaves	Bangkok	Frozen	DJ062A	-	-	DJ062E (*S. maltophilia*)	-	DJ062D (*E. gallinarum*)
DJ063	Seasonal vegetable (Kachur lati-mix stolon of taro)	Bangladesh	Frozen	DJ063A	-	-	DJ063E (*S. maltophilia*)	-	DJ063D (*E. faecalis, E. hirae*)
DJ064	Seasonal vegetable (Kachur lati-mix stolon of taro)	Bangladesh	Frozen	-	-	DJ064F (*E. cloacae*)	DJ064E (*S. maltophilia*)	-	DJ064D (*E. faecalis*)
DJ065	Chopped molokhia	Egypt	Frozen	-	-	DJ065F (*A. guillouiae*)	DJ065E (*S. maltophilia*)	DJ065C	DJ065D (*E. faecium*)
DJ066	Seasonal vegetable (Seembichi-hyacinth bean seeds)	Bangladesh	Frozen	DJ066A	-	DJ066F (*E. coli*)	DJ066E (*P. fulva, P. putida*)	-	DJ066D (*E. hirae*)
DJ067	Baby bhindi (baby okra)	India	Frozen	-	-	-	DJ067E (*S. maltophilia*)	-	DJ067D (*E. hirae*)
DJ068	Sheem bichi	Bangladesh	Frozen	DJ068A	-	DJ068F (*E. coli*)	-	-	DJ068D (*E. hirae*)
DJ069	Karela (Bitter Gourd)	India	Frozen	-	-	-	-	-	-
DJ070	Seeds of flat bean (Lablab niger)	Bangladesh	Frozen	DJ070A	-	-	DJ070E (*S. maltophilia*)	-	DJ070D (*E. faecium*)
DJ071	Kochur loti (Clean taro stems)	Bangladesh	Frozen	-	-	-	DJ071E (*P. fluorescens*)	-	DJ071D (*E. hirae*)
DJ072	Stem amaranth	Bangladesh	Frozen	-	-	-	-	-	-
DJ073	Stem amaranth	Bangladesh	Frozen	-	-	-	-	-	-
DJ074	Frozen kachai	Thailand	Frozen	-	-	DJ074F (*E. cloacae*)	-	-	-
DJ075	Horseradish leaves	Philippines	Frozen	-	-	-	-	-	DJ075D (*E. faecalis, E. faecium*)
DJ076	Frozen whole okras	Vietnam	Frozen	-	-	-	-	-	DJ076D (*E. faecalis*)
DJ077	Bittermelon leaves (Ampalaya)	Philippines	Frozen	DJ077A	-	DJ077F (*E. cloacae*)	DJ077E (*P. monteilii*)	-	DJ077D (*E. casseliflavus, E. hirae*)
DJ078	Bittermelon leaves (Ampalaya)	Philippines	Frozen	DJ078A	-	-	DJ078E (*P. fulva*)	-	DJ078D (*E. faecium*)
DJ079	Sweet potato leaves	Philippines	Frozen	-	-	DJ079F (*E. hormaechei*)	-	DJ079C	DJ079D (*E. faecalis*)
DJ080	Pepper leaves	Philippines	Frozen	-	-	DJ080F (*E. aerogenes*)	-	DJ080C	DJ080D (*E. hirae*)
DJ081	Horseradish leaves	Philippines	Frozen	-	-	-	-	-	-
DJ082	Frozen kachai	Thailand	Frozen	-	-	-	-	-	-
DJ083	Frozen whole okras	Vietnam	Frozen	-	-	DJ083F (*E. cloacae, E. hormaechei*)	DJ083E (*P. fluorescens, S. maltophilia*)	-	DJ083D (*E. faecalis*)
DJ084	Pepper leaves	Philippines	Frozen	-	-	-	DJ084E (*P. parafulva*)	DJ084C	DJ084D (*E. hirae*)
DJ085	Sweet potato leaves	Philippines	Frozen	-	-	-	-	DJ085C	-
DJ086	Screwpine leaves	Philippines	Frozen	-	-	-	-	-	DJ086D (*E. hirae*)

DJ087	Frozen whole tumeric	Vietnam	Frozen	-	-	-	DJ087E (*P. putida*)	-	-
DJ088	Frozen tomyum mixed	Thailand	Frozen	-	-	-	DJ088E (*S. maltophilia*)	-	-
DJ089	Screwpine leaves	Philippines	Frozen	-	-	-	-	-	DJ089D (*E. faecium, E. hirae*)
DJ090	Frozen tomyum mixed	Thailand	Frozen	-	-	-	-	-	DJ090D (*E. gallinarum*)
DJ091	Grated Purple yam	Philippines	Frozen	-	-	-	DJ091E (*S. maltophilia*)	-	DJ091D (*E. faecalis*)
DJ092	Squash flower	Philippines	Frozen	-	-	-	DJ092E (*S. maltophilia*)	-	DJ092D (*E. casseliflavus, E. gallinarum*)
DJ093	Squash flower	Philippines	Frozen	-	-	DJ093F (*A. baumannii, A. pittii*)	DJ093E (*P. putida*)	-	DJ093D (*E. gallinarum*)
DJ094	Grated Purple yam	Philippines	Frozen	-	-	-	DJ094E (*S. maltophilia*)	DJ094C	DJ094D (*E. faecalis*)
DJ095	Palm heart	Philippines	Frozen	-	-	-	-	-	-
DJ096	Palm heart	Philippines	Frozen	-	-	-	-	-	-
DJ097	Whole tumeric	Vietnam	Frozen	-	-	-	DJ097E (*S. maltophilia*)	-	-
DJ098	Frozen tamarind fruit	Vietnam	Frozen	-	-	-	-	-	-
DJ099	Frozen Sa-tor	Thailand	Frozen	-	-	-	-	-	-
DJ100	Frozen Sa-tor	Thailand	Frozen	-	-	-	-	-	-
DJ101	Cooked frozen bamboo shoots	China	Frozen	-	-	-	-	-	DJ101D (*E. gallinarum*)
DJ102	Frozen steamed sliced banana	Vietnam	Frozen	-	-	-	-	-	-
DJ103	Frozen mixed chili	Thailand	Frozen	-	-	-	-	-	DJ103D (*E. faecalis*)
DJ104	Frozen mixed chili	Thailand	Frozen	-	-	-	-	-	-
DJ105	Frozen tamarind fruit	Vietnam	Frozen	-	-	-	-	-	-
DJ106	Frozen Cha-om	Thailand	Frozen	-	-	-	DJ106E (*S. maltophilia*)	-	DJ106D (*E. faecalis*)
DJ107	Salted seaweed slice	China	Dried	-	-	-	-	-	-
DJ108	Salted seaweed slice	China	Dried	-	-	-	-	-	-
DJ109	Wolfberry lycium	China	Dried	-	-	-	-	-	DJ109D (*E. mundtii*)
DJ110	Wolfberry lycium	China	Dried	-	-	-	-	-	DJ110D (*E. mundtii*)
DJ111	Organic Goji berry	China	Dried	-	-	-	-	-	-
DJ112	Organic Goji berry	China	Dried	-	-	-	-	-	-
DJ113	Tumeric whole	India	Dried	-	-	DJ113F (*E. cloacae*)	-	-	-
DJ114	Tumeric whole	India	Dried	-	-	-	-	-	-
DJ115	Chili whole regular (with stem)	India	Dried	-	-	-	-	-	-
DJ116	Sounth (dry ginger)	India	Dried	-	-	-	-	-	-
DJ117	Ugu leaves	Togo	Frozen	-	-	-	-	-	DJ117D (*E. faecalis, E. hirae)*
DJ118	Ugu leaves	Togo	Frozen	-	-	-	-	-	DJ118D (*E. faecium, E. hirae*)
DJ119	Okra (Bhindi)	Cameroon	Frozen	-	-	DJ119F (*A. baumannii*)	DJ119E (*P. reactans*)	-	DJ119D (*E. faecalis*)
DJ120	Okra (Bhindi)	Cameroon	Frozen	-	-	DJ120F (*A. baumannii*)	-	-	DJ120D (*E. faecalis, E. hirae*)
DJ121	Ukazi	Cameroon	Frozen	-	-	-	-	-	DJ121D (*E. faecalis*)
DJ122	Ukazi	Cameroon	Frozen	-	-	-	-	-	DJ122D (*E. faecalis, E. hirae*)
DJ123	Gboma	Togo	Frozen	-	-	-	-	-	DJ123D (*E. faecium*)
DJ124	Gboma	Togo	Frozen	-	-	-	DJ124E (*S. maltophilia*)	-	DJ124D (*E. faecium*)
DJ125	Jute leaves	Cameroon	Frozen	-	-	-	-	DJ125C	DJ125D (*E. faecalis*)
DJ126	Jute leaves	Cameroon	Frozen	-	-	-	DJ126E (*A. baumannii*)	-	DJ126D (*E. faecalis, E. gallinarum*)
DJ127	Frozen bamboo shoots	China	Frozen	-	-	-	DJ127E (*A. baumannii*)	-	DJ127D (*E. gallinarum*)

DJ128	Frozen Cha-om	Thailand	Frozen	-	-	-	-	-	DJ128D (*E. faecalis, E. hirae*)
DJ129	Punjabi tinda (Indian baby pumpkin slices)	India	Frozen	-	-	-	-	-	DJ129D (*E. faecalis, E. mundtii*)
DJ130	Sambhar mix	India	Frozen	-	-	DJ130F (*K. pneumoniae*)	-	-	DJ130D (*E. hirae*)
DJ131	Sambhar mix	India	Frozen	-	-	-	-	-	DJ131D (*E. gallinarum*)
DJ132	Punjabi tinda (Indian baby pumpkin slices)	India	Frozen	-	-	-	DJ132E (*A. baumannii, S. maltophilia*)	-	DJ132D (*E. faecium, E. mundtii*)
DJ133	Surti unduiu mix	India	Frozen	-	-	-	-	-	DJ133D (*E. faecalis, E. thailandicus*)
DJ134	Surti unduiu mix	India	Frozen	-	-	-	-	-	DJ134D (*E. gallinarum*)
DJ135	Fenu greek	India	Frozen	DJ135A	-	-	-	-	DJ135D (*E. faecalis*)
DJ136	Fenu greek	India	Frozen	DJ136A	-	-	-	-	DJ136D (*E. faecalis, E. gallinarum*)
DJ137	Frozen steamed sliced banana	Vietnam	Frozen	-	-	DJ137F (*E. hormaechei*)	-	-	-
DJ138	Frozen rhizome	Thailand	Frozen	-	-	-	-	-	-
DJ139	Solanum melangina	Cameroon	Frozen	DJ139A	-	-	-	-	DJ139D (*E. faecalis, E. hirae, E. mundtii*)
DJ140	Solanum melangina	Cameroon	Frozen	-	-	-	-	-	DJ140D (*E. hirae*)
DJ141	Cassava leaves	Cameroon	Frozen	-	-	-	-	-	-
DJ142	Chopped spinach	China	Frozen	DJ142A	-	DJ142F (*E. aerogenes*)	-	-	DJ142D (*E. hirae*)
DJ143	Chopped spinach	China	Frozen	-	-	DJ143F (*K. pneumoniae*)	-	-	DJ143D (*E. hirae*)
DJ144	Peppers	Nigeria	Frozen	-	-	-	-	-	DJ144D (*E. hirae*)
DJ145	Peppers	Nigeria	Frozen	-	-	-	DJ145E (*P. monteilii*)	-	DJ145D (*E. casseliflavus, E. faecalis*)
DJ146	Ukazi	Cameroon	Frozen	-	DJ146B	-	-	-	DJ146D (*E. casseliflavus, E. faecalis, E. hirae*)
DJ147	Ukazi	Cameroon	Frozen	DJ147A	-	DJ147F (*A. baumannii*)	-	-	DJ147D (*E. faecalis, E. hirae*)

Table 3. 17. Summary of isolated bacteria

Organism	Number (%) of samples (n=147)	Species/serotype	Number (%) of samples (n=147)
Escherichia coli	15 (10.2%)		
Salmonella spp.	2 (1.4%)	Unnamed serotype (*Salmonella* I:4, 12:r:-)	1 (0.7%)
		S. enterica subsp. *enterica* Redlands	1 (0.7%)
Enterobacter spp.	10 (6.8%)	*E. cloacae*	6 (4.1%)
		E. aerogenes	2 (1.4%)
		E. hormaechei	3 (2.1%)
Klebsiella pneumoniae	2 (1.4%)		
Acinetobacter spp.	11 (7.5%)	*A. baumannii*	10 (6.8%)
		A. guillouiae	1 (0.7%)
		A. pittii	1 (0.7%)
Pseudomonas spp.	10 (6.8%)	*P. fulva*	3 (2.1%)
		P. fluorescens	2 (1.4%)
		P. monteilii	2 (1.4%)
		P. parafulva	1 (0.7%)
		P. putida	3 (2.1%)
		P. reactans	1 (0.7%)
Stenotrophomonas maltophilia	16 (10.9%)		
Staphylococcus aureus	7 (4.8%)		
Enterococcus spp.	68 (46.3%)	*E. faecalis*	28 (19.0%)
		E. faecium	10 (6.8%)
		E. hirae	28 (19.0%)
		E. gallinarum	16 (10.9%)
		E. casseliflavus	5 (3.4%)
		E. mundtii	5 (3.4%)
		E. thailandicus	1 (0.7%)

Table 3. 18. MLST profile of *E. coli* isolates

	ST	Allele of housekeeping genes						
		adk	*fumC*	*gyrB*	*icd*	*mdh*	*purA*	*recA*
DJ066F	ST189	10	27	5	10	12	8	49
DJ084C	ST226	10	27	5	8	8	7	2

Table 3. 19. MLST profile of *K. pneumoniae* isolates

	ST	Allele of housekeeping genes						
		infB	*mdh*	*pgi*	*phoE*	*rpoB*	*tonB*	*gapA*
DJ130F	ST101	6	1	5	4	1	6	2
DJ084C	ST261	1	1	1	4	27	12	2

Table 3. 20. MLST profile of MRSA isolates

	ST	Allele of housekeeping genes						
		arcC	*aroE*	*glpF*	*gmk*	*pta*	*tpi*	*yqiL*
DJ079C	Unidentified	151	-	215	34	175	180	169
DJ084C	ST834	3	124	1	1	1	1	40

3.3.2. Phenotypic resistance of isolates

MDR *E. coli, Enterobacter* spp., *K. pneumoniae, Acinetobacter* spp. and *Pseudomonas* spp. were identified (Table 3.21). Among *E. coli* isolates, ampicillin, nalidixic acid, trimethoprim/sulfamethoxazole, sulfisoxazole, tetracycline and gentamicin resistant *E. coli* were isolated from one sample. Two samples had ampicillin, ceftriaxone and sulfisoxazole resistant *E. coli* isolates. Among *Enterobacter* spp. isolates, ceftriaxone, nalidixic acid, ciprofloxacin, trimethoprim/sulfamethoxazole, sulfisoxazole, tetracycline and chloramphenicol resistant *E. cloacae* were isolated from two samples. One of the samples also had *E. aerogenes* also resistant to ceftriaxone, nalidixic acid, ciprofloxacin, trimethoprim/sulfamethoxazole, sulfisoxazole, tetracycline and chloramphenicol. *K. pneumoniae* were isolated from two samples; the isolate from one sample was resistant to ceftriaxone, trimethoprim/sulfamethoxazole and sulfisoxazole and the other isolate from other sample were resistant to ceftriaxone, trimethoprim/sulfamethoxazole, sulfisoxazole, tetracycline and gentamicin. Among *Acinetobacter* spp. isolates, *A. baumannii* isolate from one sample was resistant to meropenem, ciprofloxacin, gentamicin, sulfisoxazole and colistin and the isolates from two samples were resistant to meropenem, ciprofloxacin, gentamicin and colistin. Another species of isolate, *A. guillouiae*, from one sample was resistant to meropenem and gentamicin.

Among the *S. aureus* isolates, penicillin resistant strains were isolated from six samples including two which contained MRSA. All other *S. aureus* were susceptible to all drugs tested. Phenotypic resistance of *Enterococcus* spp. isolates is indicated in Table 3.22. Chloramphenicol, rifampin, tetracycline, ciprofloxacin and levofloxacin resistance were identified from *E. faecalis* isolates. Rifampin, quinupristin/dalfopristin, ciprofloxacin, levofloxacin and nitrofurantoin resistance were identified from *E. faecium* isolates. Chloramphenicol, rifampin, quinupristin/dalfopristin, tetracycline and nitrofurantoin resistance were identified from *E. hirae* isolates. Rifampin, tetracycline, ciprofloxacin and levofloxacin resistance were identified from *E. gallinarum* isolates. Quinupristin/dalfopristin and tetracycline resistance were identified from *E. casseliflavus* isolates. However, resistance of *Enterococcus* spp. isolates to ampicillin, penicillin and vancomycin was not identified.

Table 3. 21. Resistance profile of Enterobacteriaceae isolates and meropenem resistant Gram-negative isolates

Organism	Isolate ID	Origin country	Sample	ST	Antimicrobial Resistance Profile	β-lactamase Genes	PMQR Determinants Genes	MCR Genes	Class I Integron
E. coli									
	DJ142A	China	Chopped spinach	NA	AMP+NAL+SXT+FIS+TET+GEN	-	-	NA	*dfrA12*
	DJ066F	Bangladesh	Seasonal vegetables (Seem bichi-hyacinth bean seeds)	ST189	AMP+AXO+FIS	$bla_{CTX-M-15}$	-	NA	-
	DJ068F	Bangladesh	Sheem bichi	ST226	AMP+AXO+FIS	$bla_{CTX-M-15}$, bla_{TEM-1}	-	NA	-
E. cloacae									
	DJ064F	Bangladesh	Seasonal vegetable (Kachur lati-mix stolon of taro)	NA	AXO+NAL+CIP+SXT+FIS+TET+CHL	$bla_{CTX-M-15}$, bla_{TEM-1}	*qnrB1, aac(6')-Ib-cr*	NA	*aadA1*
	DJ142F	China	Chopped spinach	NA	AXO+NAL+CIP+SXT+FIS+TET+CHL	$bla_{CTX-M-27}$	*qnrB2, qnrS1, aac(6')-Ib-cr*	NA	-
E. aerogenes									
	DJ142F	China	Chopped spinach	NA	AXO+NAL+CIP+SXT+FIS+TET+CHL	$bla_{CTX-M-27}$	*aac(6')-Ib-cr*	NA	-
K. pneumoniae									
	DJ130F	India	Sambhar mix	ST101	AXO+SXT+FIS	$bla_{CTX-M-15}$+ $bla_{SHV-106}$	-	NA	-
	DJ143F	China	Chopped spinach	ST261	AXO+SXT+FIS+TET+GEN	$bla_{CTX-M-14}$+ $bla_{SHV-142}$	-	NA	-
A. baumannii									
	DJ126E	Cameroon	Jute leaves	NA	MERO+CIP+GEN+SXT+COL	-	-	-	NA
	DJ127E	China	Frozen bamboo shoots	NA	MERO+CIP+GEN+COL	-	-	-	NA
	DJ132E	India	Punjabi tinda (Indian baby pumpkin slices)	NA	MERO+CIP+GEN+COL	-	-	-	NA
A. guillouiae									
	DJ65F	Egypt	Chopped molokhia	NA	MERO+GEN	-	-	NA	NA
P. fluorescens									
	DJ71E	Bangladesh	Kochur loti (clean taro stems)	NA	MERO+GEN+COL	-	-	-	NA
	DJ83E	Vietnam	Frozen whole okras	NA	MERO+GEN+COL	-	-	-	NA
P. monteilii									
	DJ77E	Philippines	Bittermelon leaves	NA	MERO	-	NA	NA	NA
P. putida									
	DJ87E	Vietnam	Frozen whole turmeric	NA	MERO	-	NA	NA	NA
	DJ93E	Philippines	Squash flower	NA	MERO	-	NA	NA	NA
P. reactans									
	DJ119E	Cameroon	Okra	NA	MERO	-	NA	NA	NA

Ampicillin (AMP), ceftriaxone (AXO), meropenem (MERO), nalidixic acid (NAL), ciprofloxacin (CIP), gentamicin (GEN), trimethoprim/sulfamethoxazole (SXT), sulfisoxazole (FIS), tetracycline (TET), chloramphenicol (CHL) and colistin (COL). NA indicates not applicable.

Table 3. 22. Antimicrobial resistance profile of *Enterococcus* spp. isolates

Antibiotics	Number (%) of food samples (n=147) having antimicrobial resistant *Enterococcus* spp. isolates						
	E. faecalis n=28 (19.0%)	*E. faecium* n=10 (6.8%)	*E. hirae* n=28 (19.0%)	*E. gallinarum* n=16 (10.9%)	*E. casseliflavus* n=5 (3.4%)	*E. mundtii* n=5 (3.4%)	*E. thailandicus* n=1 (0.7%)
Chloramphenicol	1 (3.6%)	0	4 (14.3%)	0	0	0	0
Rifampin	23 (82.1%)	10 (100%)	27 (96.4%)	16 (100%)	5 (100%)	0	0
Quinupristin/dalfopristin	NA	6 (60.0%)	4 (96.4%)	NA	NA	0	1 (100%)
Tetracycline	9 (32.1%)	0	10 (35.7%)	4 (25.0%)	1 (20%)	0	0
Ciprofloxacin	12 (32.1%)	1 (10.0%)	0	2 (12.5%)	0	0	0
Levofloxacin	2 (7.2%)	1 (10.0%)	0	1 (6.3%)	0	0	0
Nitrofurantoin	0	6 (60.0%)	2 (7.1%)	0	0	0	0
Ampicillin	0	0	0	0	0	0	0
Penicillin	0	0	0	0	0	0	0
Vancomycin	0	0	0	NA	NA	0	0

NA indicates intrinsic resistance. The percentage of resistance to each antimicrobial indicates the resistance among same species of isolates.

3.3.3. Resistance gene identification

Identified resistance genes from resistant Gram-negative organisms are listed in Table 3.20. Among broad spectrum β-lactamase genes, TEM (1.4%), SHV (1.4%) and CTX-M (4.8%) genes were identified, no carbapenemase genes were found. *qnrB* (1.4%), *qnrS* (0.7%), *aac(6')-Ib-cr* (2.0%) among PMQR determinants genes, and class I integrons which harboured *dfrA* (0.7%) and *aad* (0.7%) genes were identified.

Among penicillin and oxacillin resistant *S. aureus* isolates, *mecA* gene from oxacillin resistant *S. aureus* isolates from two different samples was identified confirming MRSA.

3.4. Discussion

In this study, antimicrobial resistant organisms were identified from 147 imported vegetables and spices and their resistance was characterized.

All foods having *E. coli* were frozen and all of the samples were plant-based vegetables. Among other samples, three samples from Asia had *E. coli* which harboured mobile resistance genes; *dfrA12, bla*$_{\text{TEM-1}}$ and *bla*$_{\text{CTX-M-15}}$. Two bean type samples imported from Bangladesh had ESBL producing *E. coli* isolates. The isolates from both samples harboured *bla*$_{\text{TEM-1}}$ and *bla*$_{\text{CTX-M-15}}$ and all were resistant to ampicillin and ceftriaxone along with sulfisoxazole. These isolates were ST189 and ST226. Interestingly, ST189 has been reported to be isolated from poultry and associated with ESBL/plasmid mediated AmpC β-lactamases; German isolates producing CTX-M-1 and Columbian isolates producing SHV-5/CMY-2 have been previously reported (Belmar Campos et al., 2014; Castellanos et al., 2017; Hussein et al., 2013; Solà-Ginés et al., 2015). (Belmar Campos et al., 2014; Castellanos et al., 2017). ST226 has been reported to be isolated from human patients, broiler chickens and wild boars and associated with ESBL; Chinese isolates producing CTX-M-65, and Nigerian and Algerian isolates producing CTX-M-15 have been reported. (Bachiri et al., 2017; Chah et al., 2018; Chen et al., 2014). Considering distribution of this sequence type in human, food animal and wildlife animal, contamination from human or wildlife to the bean products from this study may have occurred. Furthermore, to the best of our

knowledge, that these sequence types of *E. coli* harbouring ESBL can be transferred across the continents is a new finding.

An *E. coli* resistant to trimethoprim/sulfamethoxazole and sulfisoxazole along with gentamicin, nalidixic acid, sulfisoxazole and ampicillin from a chopped spinach product imported from China harboured *dfrA12* in a class I integron. Disinfectant resistance has also previously been shown to be conferred by qacEΔ1 originally conserved in class I integron indicating possible dissemination of another type of resistance (Gillings, 2014).

Salmonella spp. isolates were identified from two leaf-type samples imported from Ghana and Cameroon respectively. Among Enterobacteriaceae isolates, *Salmonella* spp. is tolerant of low-moisture which might be the reason for the *Salmonella* spp. isolate to have survived in the dried Ghanaian leaves product. (Mattick, Jørgensen, Legan, Lappin-Scott, & Humphrey, 2000). Its serotype is unnamed, however characterization by another technique such as whole genome sequencing might be possible. The other *Salmonella* spp. isolate, which is *Salmonella* Redlands, from a Cameroonian product has been reported from at least one human infection and the environment (surface water and reptiles) (Cherry et al., 1972; Dondero, Thomas, Khare, Timoney, & Fukui, 1977; Lapage, Taylor, Nicewonger, & Phillips, 1966; Riemann & Cliver, 2006).

K. pneumoniae isolates were isolated from Sambhar mix (vegetable mix including tomatoes, Indian vegetable drumstick, eggplant, onion, okra, bottle gourd, green chillies and curry leaves) and chopped spinach imported from India and China respectively. The isolate from Sambhar mix harboured $bla_{\text{CTX-M-15}}$ and $bla_{\text{SHV-106}}$ and was resistant to ceftriaxone, trimethoprim/sulfamethoxazole and sulfisoxazole. This *K. pneumoniae* isolate was ST101, a strain frequently identified with human infections in Asia (Lee et al., 2012; Low et al., 2017; Ma, Lu, Siu, & Hsieh, 2013; Yu et al., 2017). Previous studies reported *K. pneumoniae* ST101 producing ESBLs and other types of broad spectrum β-lactamases (narrow spectrum β-lactamases, ESBL, plasmid mediated AmpC β-lactamases, carbapenemases). However, there is no study reporting this sequence type from any types of food or environmental settings. The isolate from Chinese chopped spinach harboured $bla_{\text{CTX-M-14}}$ and $bla_{\text{SHV-142}}$ and was resistant to ceftriaxone, trimethoprim/sulfamethoxazole, sulfisoxazole, tetracycline and gentamicin. This isolate was ST261 and it has been rarely identified from Asia. Only one study from South Korea

identified CTX-M-14 producing *K. pneumoniae* ST261 from a human infection (Ko et al., 2010). To our best knowledge, this strain only has been identified in meat products (Eibach et al., 2018). ESBL producing *K. pneumoniae* ST261 was identified from locally produced meats in Ghana (Eibach et al., 2018). However, ST261 has not been identified from imported food. Therefore, these MDR *K. pneumoniae* isolates from imported sambhar mix and chopped spinach indicate imported vegetables as a novel route of resistance transmission across the continents.

Enterobacter spp. isolates were identified from stolon of taro and chopped spinach imported from Bangladesh and China respectively. *Enterobacter* spp. isolates harboured more mobile resistance genes (ESBL, PMQR determinants and class I integron) than the rest of Enterobacteriaceae isolates did. *E. cloacae* was isolated from stolon of taro and harboured *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{TEM-1}}$, *qnrB1, aac(6')-Ib-cr* and class I integron with *aadA1*. These resistance genes may explain the phenotypic resistance including ceftriaxone, nalidixic acid, ciprofloxacin, trimethoprim/sulfamethoxazole and sulfisoxazole. The other sample, chopped spinach, had *E. cloacae* and *E. aerogenes*; *E. cloacae* isolate harboured *bla*$_{\text{CTX-M-27}}$, *qnrB2, qnrS1* and *aac(6')-Ib-cr*, and *E. aerogenes* isolate harboured *bla*$_{\text{CTX-M-27}}$ and *aac(6')-Ib-cr*. Interestingly, although the *E. aerogenes* was fluoroquinolone resistant, no PMQR genes were identified. All *Enterobacter* spp. isolates were resistant to ampicillin, amoxicillin/clavulanic acid, cefoxitin and ceftriaxone which plasmid mediated AmpC β-lactamase was expected. However, none of *Enterobacter* spp. isolates had *bla*$_{\text{CMY-2}}$. A previous Dutch study identified 3$^{\text{rd}}$ generation cephalosporin and quinolone resistant *Enterobacter* spp. isolates from culinary herbs imported from Southeast Asia (Veldman et al., 2014). *E. cloacae* complex isolates from holy basil and parsley from Malaysia and Vietnam respectively and *Enterobacter* spp. isolate from houttuynia leaf from Vietnam were identified (Veldman et al., 2014). Their *Enterobacter* spp. isolates harboured broad spectrum β-lactamase genes (*bla*$_{\text{TEM-1}}$ *bla*$_{\text{TEM-1b}}$ *bla*$_{\text{OXA-1}}$, *bla*$_{\text{CTX-M-40}}$, *bla*$_{\text{CTX-M-15}}$) and PMQR genes (*qnrS1, qnrB1, qnrB26, aac(6')-Ib-cr*). The findings by Veldman et al. and this study suggest more clear evidence of the transfer of resistant Enterobacteriaceae via food from Asia to different continents.

All meropenem resistant isolates were *A. baumannii, A. guillouiae, P. fluorescens, P. monteilii* and *P. putida.* Interestingly, none of the isolates harboured any carbapenemase producing genes, *bla*$_{\text{NDM}}$, *bla*$_{\text{VIM}}$, *bla*$_{\text{IMP}}$, *bla*$_{\text{KPC}}$ and *bla*$_{\text{OXA-48}}$ and all were negative for carbapenemase production by the β-carba test. Furthermore, although isolates of *P. fluorescens*

(n=2) and *A. baumannii* isolates (n=2) were colistin resistant, no mobile colistin resistance genes, *mcr-1, mcr-2, mcr-3* and *mcr-4*, were identified. Their meropenem and colistin resistance may be due to mutated outer membrane proteins (OMPs), upregulated efflux pump systems and/or lipopolysaccharide change (Chalhoub et al., 2016; Clausell et al., 2007; Estepa, Rojo-Bezares, Torres, & Sáenz, 2015; Fernández-Cuenca et al., 2003; Hawkey et al., 2018; Huang, Sun, Xu, & Xia, 2008; Moffatt et al., 2010; Moskowitz et al., 2012; Mussi, Limansky, & Viale, 2005; Rojo-Bezares et al., 2014). However, unlike carbapenemase and MCR, all these resistance mechanisms are only identified from *Acinetobacter* spp. and *Pseudomonas* spp. and do not contribute to acquired resistance of carbapenem and polymyxin. Therefore, the findings from *Acinetobacter* spp. and *Pseudomonas* spp. are less significant when it comes to resistance distribution.

There were MRSA isolates from two samples, sweet potato leaves (DJ079C) and pepper leaves (DJ084C), both imported from Philippines. The closest STs of DJ079C were identified with six housekeeping genes with single variant gene (*aroE*): ST1667, ST2196, ST2483 and ST2250 (Enright, 2018). DJ084C was assigned to ST834. The first identified ST834 from Australia in 2006 was also MRSA according to the MLST database (M. Enright, 2018). Since then, all identified ST834 isolates in Norway, Saudi Arabia and Kuwait were also MRSA (Monecke, Aamot, Stieber, Ruppelt, & Ehricht, 2014; Monecke et al., 2012; Udo & Sarkhoo, 2010). These results indicate that ST834 is globally distributed. One of these studies indicated that their identified MRSA ST834 was from patients with a history of travel to the Philippines (Monecke et al., 2014). Although the finding by Monecke et al. might be an indicator, this investigation was designed to identify resistant organisms rather than determining the country of origin of this strain. Furthermore, similar to Enterobacteriaceae isolates, all MRSA isolates may have originated from human as all were STs which were previously reported to have been isolated from human patients.

Enterococcus spp. was isolated from more samples than the other genera. There were MDR *Enterococcus* spp. isolates, including ciprofloxacin, nitrofurantoin, tetracycline, quinupristin/dalfopristin and chloramphenicol. However, none of them were resistant to penicillin or vancomycin. Quinupristin/dalfopristin are streptogramins for alternative treatment of VRE infection. VatD, transposon mediated acetyltransferase, is known to confer streptogramin A resistance (Rende-Fournier, Leclercq, Galimand, Duval, & Courvalin, 1993). Macrolide,

lincosamide and streptogramin (MLS) resistance can be conferred by Erm family of 23s rRNA methyltransferases (Roberts et al., 1999). These methyltransferases encoded by *erm* genes (e.g. *ermA, ermB*) are found on plasmid transposons (Hammerum, Flannagan, Clewell, & Jensen, 2001; Roberts et al., 1999). Although theses resistance genes were not screened in this study, it implies that VRE may acquire MLS resistance from these resistant *Enterococcus* spp. if these isolates were exposed to VRE. Furthermore, in reverse, VRE can transfer their *van* genes to MLS or nitrofurantoin resistant *Enterococcus* spp. from these foods, which would lead to the emergence of serious AMR.

Most of MDR bacteria were isolated from frozen samples. Although the origin of these organisms in these foods is not known, feces, irrigation water, sewage sludge in soil or people handling the product may have been the source of contamination (Araújo et al., 2017; Ashby et al., 2003; Eltai et al., 2018; Rahube et al., 2014, 2016; Tan et al., 2014; Yalçin et al., 2001). Fluctuation of temperature during long-distance transit may have allowed the contaminants to survive longer in frozen foods than in dried foods (Allen et al., 2013; McKellar et al., 2012). Especially, texture of leafy vegetables (chopped spinach, pepper leaves, sweet potato leaves) from this study may have become much softer due to thaw by temperature fluctuation allowing the contaminants to infiltrate into these foods and survive better (Brown, 1977). For dried foods, foodborne bacteria (e.g. *E. coli, Salmonella* spp.) are reported to survive in low-moisture foods (e.g. powder, seeds, peanuts) (Beuchat et al., 2013). However, it is hard for most of bacteria to survive in dried foods with long-term low moisture during transportation from country of origin to Canada,

Overall, this study identified and characterized AMR from imported vegetables and spices in Canada and provided new insight on these items as a source of AMR. The results emphasize the need of up-to-date epidemiological information from countries of origin for better AMR tracking. This study, therefore, will provide fundamental data on antimicrobial resistance surveillance policy to alleviate resistance transmission from overlooked sources.

4. General Discussion and Conclusion

4.1. Summary of this study

AMR has become one of the important public health problems and is emerging in humans, animals (companion and agricultural) and the environment (wildlife, wastewater) (Doi et al., 2017). Food has been reported to harbour resistant organisms and mobile resistance genes. In Canada, CIPARS monitors resistance in enteric bacteria including, *E. coli, Salmonella* spp. and *Campylobacter* spp. from food animals and meat products. Unfortunately, other potential contaminant bacteria have been not targeted at all in routine surveillance. For example, a couple of studies has reported MRSA and levofloxacin and clindamycin resistant *C. difficile* in domestically produced meats (Narvaez-Bravo et al., 2016; Rodriguez-Palacios et al., 2007). Another limitation of CIPARS is it focuses on locally available meats without specifically targeting those products which include imported contents. Independent studies have identified resistant organisms and mobile resistance genes from other types of food such as both domestic and imported vegetables and seafood (Allen et al., 2013; Bezanson et al., 2008; Janecko et al., 2016; Mangat et al., 2016; Morrison & Rubin, 2015; Wood et al., 2015). MDR have been identified on imported products including penicillin resistant *E. faecium* from imported vegetables and carbapenemase producing *Pseudomonas, Stenotrophomonas* and *Myroides* spp. from imported seafoods (Allen et al., 2013; Morrison & Rubin, 2015). However, considering that food can be imported from the countries with a high prevalence of AMR, monitoring through the national surveillance program and additional targeted studies by independent researchers are needed for understanding the risk of AMR through imported foods. Furthermore, there has been no study specifically targeting resistant bacteria from imported vegetables and spices in Canada. This study identified and characterized resistant Enterobacteriaceae, meropenem resistant Gram-negatives, *S. aureus* and *Enterococcus* spp. isolated from imported vegetables and spices as a pilot investigation to address this gap.

Emerging multidrug resistant Enterobacteriaceae (*E. coli, Enterobacter* spp. and *K. pneumoniae*) conferred by ESBL and PMQR determinants were identified. The foods containing these resistant bacteria were all imported from China, Bangladesh and India. Most of meropenem and colistin resistant *Acinetobacter* spp. and *Pseudomonas* spp. were also identified from the foods imported from China, Bangladesh, India, Vietnam and the Philippines. All MRSA were

isolated from the foods imported from the Philippines. Interestingly, most of foods that had these important MDR bacteria were imported from Asian countries which have the highest rate of AMR in the world (Chereau, Opatowski, Tourdjman, & Vong, 2017; World Health Organization, 2016b; Yezli & Li, 2012). This suggests that imported foods can be one of the routes for transfer of resistance from high prevalence of countries to low prevalence of countries. Even though this study is not aiming to discuss the surveillance policies in high use of drugs and prevalence of resistance countries, the situation in country of origins will be discussed briefly.

4.2. Trend of resistance and counteraction from countries of origin

Although this investigation was not designed to compare the frequency of resistance between countries, it was interesting that resistant organisms were most commonly isolated from products of China. Future studies focused on identifying significant epidemiological relationships between geographic regions and the presence of resistant organisms should be done.

China is the second biggest producer and consumer of antibiotics, and antibiotic use for treatment in humans and animal and growth promotion in animals is vast (Van Boeckel et al., 2014; Zhang, Ying, Pan, Liu, & Zhao, 2015). Consequently, large quantities of antibiotics released into the environment through manure, and via rivers through sewage effluent are thought to have contributed to the rapid emergence of resistance (Li, Shi, Gao, Liu, & Cai, 2013; Xu et al., 2016; Zhang, Luo, Wu, Huang, & Christie, 2015; Zhou et al., 2013). To reduce the overuse of antimicrobials and emergence of resistance, collaboration between institutions from China and Sweden (The Sino-Swedish integrated multi-sectorial partnership for antibiotic resistance containment) has made to identify antibiotic use and resistance in rural area of China from 2014 to 2018 (Cars et al., 2016). China has also entered into an agreement with the UK to establish bilateral fund for tackling AMR in 2016 and they are currently aiming to take an action in 2019 (Global and Public Health Group, 2017). On a national level, the Chinese Government announced a national action plan to act against AMR in 2016 (National Health Commission of the People's Republic of China, 2016; Xiao & Li, 2016). While their plan is designed to prevent overuse of antibiotics, resistance control and novel antimicrobial development, this is a complex problem and challenges still exist. Even though National Health Commission (NHFPC) is currently working on a five-year plan (covering 2016 to 2020) no interim reports have been

published, making evaluation of progress impossible (National Health Commission of the People's Republic of China, 2016). Furthermore, since animal agriculture in China is a huge industry, there will undoubtedly be a lot of challenges to regulate antimicrobial use practices from industry. Similar to resistance surveillance systems in North America, the Chinese action plan focuses on identifying the presence of resistant organisms in animal origin food products. (National Health Commission of the People's Republic of China, 2016). As the current study identified ESBLs, PMQRs and class I integrons, we suggest that other types of food need to be targeted to prevent their domestic and international spread.

Bangladesh is also a country of high prevalence of AMR. Challenges in the regulation of appropriate antimicrobial use in Bangladesh including the availability of antimicrobials over the counter, are widely thought to have contributed to the high prevalence of AMR (Fahad, Matin, Shill, & Asish, 2010; Lina, Rahman, & Gomes, 2007). Overcrowding, lack of basic sanitary facilities in rural areas (ex. the use of unsanitized latrines, lack of effective wastewate treatment, living with livestock in same house) and a mobile population are also reported to contribute to resistance dissemination in Bangladesh (Bakshi, Mallick, & Ulubaşoğlu, 2015; Lina et al., 2007). Even though independent researchers have done studies to identify resistance, there is no national surveillance data available in Bangladesh (World Health Organization, 2014). Although researchers in Bangladesh have found important resistance including carbapenemase producers (NDM-1, OXA-48, OXA-181, KPC, IMP, VIM), a systematic surveillance system to tackle AMR problem has yet to be established (Begum & Shamsuzzaman, 2016). Although serious resistance such as carbapenemase was not identified from this study, it indicates that other important resistance determinants, ESBL, PMQR and class I integrons, exist in domestically consumed and exported vegetable products.

Philippines was another country from which foods containing meropenem resistant *P. monteilii* and MRSA were isolated. Although there is a surveillance program and studies that identified carbapenem resistant *P. aeruginosa* from the Philippines, no study has identified carbapenem resistant *P. monteilii* (Antimicrobial Resistance Surveillance Reference Laboratory, 2018; Tesalona, Lagamayo, & Cabal, 2017; Tesalona, Lagamayo, Evangelista, et al., 2017). As *P. monteilii* is usually found in the environment, the meropenem resistant isolate from this study may lead to new investigations to improve our understanding of the distribution of

carbapenemases producers in environmental settings that can lead to human exposure. MRSA isolated from vegetables imported from the Philippines also suggests that there is a need for broader investigations besides human clinical settings for the detection of MRSA. MRSA isolates were identified from samples that are processed and packaged in Philippines. Even though STs of MRSA isolates from this study are not reported from the Philippines, they may be circulating in their environment. Furthermore, considering that these STs were isolated from human patients around the world, these MRSA isolates might have traveled to the Philippines via international travellers.

Although not many resistant bacteria were identified from products originating in India, MDR bacteria isolated from two samples are reported to be infamous superbugs, which were ESBL producing *K. pneumoniae* and meropenem and colistin resistant *A. baumannii*. India is reported to be the largest producer and consumer of antibiotics in the world (Van Boeckel et al., 2014). Along with overuse of antimicrobials including penicillins, cephalosporins and quinolones, multiple factors including high burden of disease, and poor public health infrastructure may have accelerated emergence of the resistance in India (Global Antibiotic Resistance Partnership - India Working Group, 2011; Laxminarayan & Chaudhury, 2016). A national action plan to tackle their severe resistance problem was developed in 2017 by the Government of India with an implementation period of 2017 through 2021 (Government of India, 2017). While the plan is systematic and includes surveillance targetting fruits and vegetables, improvements in the surveillance infrastructure may need to be made before the full potential of this program can be realized (Das, Chaudhuri, Srivastava, Nair, & Ramamurthy, 2017; Government of India, 2017). India previously had an Antimicrobial Resistance Surveillance and Research Network as a main surveillance program which was launched in 2013 (Das et al., 2017). However, gaps caused by inadequate financial resources for the participating health institutions and difficulty coordinating health systems have been crucial obstacles for efficient surveillance in India (Das et al., 2017; Lakshminarayanan, 2011). More frequent international collaboration also needs to be done for intervening the international level of resistance transmission via travellers (e.g. human, animals) and import/export goods (e.g. foods).

Other foods imported from Vietnam, Egypt and Cameroon contained meropenem and colistin resistant oxidase positive bacteria which had no carbapenemase or mobile colistin

resistance determinants. There has been no national resistance surveillance program in Cameroon. Studies by independent researchers have been limited to human patients (World Health Organization, 2014). International action to establish an effective surveillance program in Cameroon among the other countries in Africa and Asia has been ongoing; Cameroon was funded by WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) for investigation on resistant foodborne pathogens and establishment of national surveillance in 2010 and 2012 (World Health Organization, 2018). The plans by AGISAR are promising since they are developing surveillance targeting not only humans but also the food chain and the environment (World Health Organization, 2016a). However, problematic factors including high burden of disease, poor infrastructure of the health care system and wars in Cameroon are huge obstacles to implementing the AGISAR plans (Kesah & Payne, 2013). Therefore, more support is needed at an international level to overcome these challenges. There also needs to be cooperation between researchers from Cameroon and other countries with well-established surveillance systems.

All MDR organisms that harboured transferrable resistance genes (ESBL, PMQR determinants genes) were isolated from the foods imported from the countries that have a high prevalence of AMR. As Canada is recognized to have a relatively of low prevalence of AMR, transmission of organisms from the countries with a high prevalence of AMR may cause increase of the prevalence in Canada. As these organisms harboured transferrable resistance genes, the genes could be transferred to other organisms. Further investigation, such as quantification of the resistant organisms from imported foods, should be done to determine the possibility of infection and transfer of resistance. As we also lack the information on prevalence of AMR in domestic vegetables and spices, investigation of the prevalence in domestic foods are needed. These results then need to be compared with imported foods to provide a better understanding how impactful AMR transmission via imported foods is on the prevalence of AMR in Canada. Furthermore, international research collaboration between Canada and the exporting countries would give better understanding of AMR transmission and bring better prevention methods in international level.

4.3. Limitation of the study and future directions

Because it was a pilot study, there are several recognized limitations of this investigation; the number of food samples, countries of origin, and timeline of the investigation. 147 samples were only a small portion of imported vegetables and spices that are distributed in the international markets in Saskatoon. Our collection of imported vegetables and spices may not be representative of all the products which are distributed across Canada or even our region. There is the possibility that a larger more representative sampling would have revealed more MDR bacteria especially the critical MDR organisms such as carbapenemase producers, MCR genes and VRE. Countries of origin were mostly Asian and African countries and there were no countries of origin from Central and South America, Europe and Oceania. It is possible that VRE might exist in vegetables imported from European countries as their studies reported VRE is still being identified in broiler flocks (Bortolaia et al., 2015). Considering the possibility of contamination from broiler to vegetables, VRE could be identified from their vegetables and transmitted into Canada through imported products. The results could also have been impacted if the timeline of the study was longer. It would also be useful to have had data regarding the seasons in which the food was harvested. An investigation from Benin identified seasonality (dry vs. rainy season) as a factor impacting the prevalence of resistance detection (Moussé et al., 2015).

Furthermore, the laboratory approaches used to isolate bacteria did not allow for a holistic view of the microbial community from the food samples. First, it was not possible to quantify bacterial load because selective broths were used indicating that contamination level of the food with MDR isolates may have been very low (Lipstich & Samore, 2002). Next, although we endeavoured to characterize a wider variety of organisms than are included in current national level surveillance programs, however, culture-based investigations are always limited to a set of bacterial species. It is possible that our methodological approach which excluded *Campylobacter* spp., *Clostridium difficile* or other clinically relevant organisms may have under estimated the true prevalence of resistance.

Another limitation is missing unculturable organisms as vegetables and spices, especially root-types, which were likely to be in contact with the soil that has diverse microbiome. Not only were these organisms unable to be isolated by any selective media, but also it is impossible to

identify and characterize their resistance. The possible solution for this may be next generation sequencing (NGS). NGS is state-of-the-art technology able to sequence entire genomes of the microbiome and analyze specific DNA or RNA of interests (metagenomics, transcriptomics) (Angers-Loustau et al., 2018; Behjati & Tarpey, 2013; Tavares et al., 2013). However, it is limited to predict the phenotypic resistance based on genotype analysis. The utility of NGS approaches in identifying the presence of resistance requires further study, the agreement between this approach and phenotypically derived MICs has not been adequately investigated in the context of microbal communities.

4.4. Future plans and conclusions

This study highlighted gaps in our knowledge which should be addressed by future investigations.

As the information of the presence of AMR in domestically produced vegetables and spices is lacking, future studies targeting AMR in domestically produced plant-based foods are encouraged. A comparison of domestically produced and imported foods is required for a complete understanding of the epidemiology of foodborne resistance.

Another potential plan would be developing NGS tools for better identification of AMR. Although it is possible to identify whole chromosomal genome and mobile gene elements with NGS, it may be challenging to predict transferability of resistance genes due to the difficulty of linking mobile gene elements to genomes of individual organisms in whole microbiome (Goordial & Ronholm, 2018). While NGS approaches may not reliably predict phenotypic reistance (MIC) in an individual organism, when used in combination with classical approaches, they do have the ability to add information about the presence of resistance genes in unculturable members of the microbial community (Bishara et al., 2018; Goordial & Ronholm, 2018).

Lastly, partnering with our national surveillance program would make improvements to surveillance in Canada. Along with the past studies by Canadian researchers, the findings from this study will provide evidence that clinically relevant MDR bacteria can be also identified from foods other than domestic meat (Allen et al., 2013; Bezanson et al., 2008; Morrison & Rubin, 2015). This is very crucial since national surveillance programs are able to regularly and

systematically monitor resistance to provide accurate estimates of population prevalence, which is challenging for independent researchers. Although not a CIPARS activity, government scientists have recently worked to target imported foods; interestingly carbapenemase producers including VCC-1 producing *V. cholerae* was identified from imported shrimp (Public Health Agency of Canada, 2018a). In the CARSS report, the possibility of resistance transmission through imported food was recognized, highlighting the importance of additional collaboration between independent researchers and the national surveillance program (Public Health Agency of Canada, 2018a).

In conclusion, imported vegetables and spices are a source for transmitting clinically important MDR bacteria including ESBL, PMQR determinants within Enterobacteriaceae and MRSA. MDR was identified primarily from foods imported from the countries having a high prevalence of AMR. Future research to identify resistance from a larger number of imported vegetables and spices and to compare resistance trends between imported and domestically produced vegetables and spices needs to be conducted. There are clearly outstanding questions regarding the epidemiology of resistance at the national level. Continued, enriched collaboration between independent researchers and federal agencies is essential to generate evidence for the development of effective intervention strategies to prevent AMR transmission.

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Appendix

List of Companies

Antibiotics

A. Sandoz Canada Inc. (Boucherville, Quebec, Canada)

Meropenem

B. Sigma-Aldrich (St. Louis, Missouri, USA)

Colistin Sulfate Salt

Antimicrobial Susceptibility testing

C. Automed Inc. (St. Paul, Minnesota, USA)

Cathra Replicator

D. Trek Diagnostics (Cleveland, Ohio, USA)

Dimineralized water

Cation-adjusted Mueller-Hinton broth

Sensititre Automated Inoculation System

E. ThermoFisher Scientific (Waltham, Massachusetts, USA)

CMV4AGNF plate for Gram-negative

GPALL1F plate for Gram-positive

Bacteria growth media

F. Beckton Dickson (Sparks, Maryland, USA)

BBL™ Enterococcosel™ Agar

BBL™ Enterococcosel™ Broth

BBL™ Mannitol Salt Agar

BBL™ Mueller Hinton II Agar

BBL™ Tetrathionate Broth Base

BD™ Rappaport Vassiliadis Broth

BD™ Tryptic Soy Broth

Difco™ Brilliant Green Agar

Difco™ Buffered Peptone Water

Difco™ XLD Agar

G. CHROMagar (Paris, France)

CHROMagar™ ESBL

H. Sigma-Aldrich (St. Louis, Missouri, USA)

MacConkey Broth

I. Thermo Fisher Oxoid (Basingstoke, Hampshire, England)

Columbia Blood Agar with 5% Sheep Blood

Thermo Scientific™ Eosin Methylene Blue agar (Levine)

Biochemical test

J. Beckton Dickson (Sparks, Maryland, USA)

Bacto™ Tryptone (for indole)

BBL™ Coagulase Plasma, Rabbit with EDTA

BBL™ DrySlide™ Oxidase

BBL™ Simmons Citrate Agar Slants

BBL™ Sodium Hippurate Broth

BBL™ TSI Agar Slants

K. Bio-Rad (Hercules, California, USA)

β CARBA test

L. Hardy Diagnostics (Santa Maria, California, USA)

PYR Test Kit

M. Thermo Fisher Oxoid (Basingstoke, Hampshire, England)

Urea Agar Base

PCR

N. Bio Basic Canada Inc. (Markham, Ontario, Canada)

EX-10 Spin Column PCR Purification kit

O. Bio-Rad (Hercules, California, USA)

C1000 Touch Thermal Cycler

Gel Doc XR+

P. GE Healthcare (Little Chalfont, Buckinghamshire, England)

Illustra ExoProStar kit

Q. Invitrogen (Carlsbad, California, USA)

Taq DNA Polymerase, recombinant

R. ThermoFisher Scientific (Waltham, Massachusetts, USA)

Nanodrop 2000 Spectrophotometer