

# Distribution of antimicrobial resistance genes in various types of food including meat, produce (vegetables and fruits) and dairy products in Canada

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## Introduction

Distribution of microorganisms with antimicrobial resistance (AMR) genes in food including meat, produce (vegetables and fruits) and dairy products has been recognized as a growing public health concern worldwide. Food contaminated with microorganisms are responsible for numerous foodborne illnesses, including outbreaks caused by AMR gene containing bacteria. Due to the wide distribution of microbes possessing AMR genes, food contaminated with bacteria containing AMR genes could facilitate the transfer of AMR to humans. Currently AMR gene surveillance in Canada is mainly focused on several pathogenic bacteria in nosocomial environments and certain foodborne bacterial pathogens from meat. In order to understand the distribution of AMR genes in microorganisms in a broader spectrum of food for effective mitigation, this study investigated the presence of microorganisms containing AMR genes in the meat, produce (fruit and vegetables) and dairy products.

## Methods

**Food samples and preparation.** Food samples (n=59), including meat (n=18), produce (n=30, vegetables=21, fruits=9) and dairy (n=11), were randomly purchased at different times from local retailers. Microbes in food were enriched by merging 125-160 grams of food in a universal media (modified Schaedler media) placed in a stomacher bag and grown for 24 hour at 37 °C at 130 RPM using an orbital shaker (MAXQ 4000, Thermo Scientific). Bacteria were concentrated by centrifugation at 9,000 x g (30 minutes, 4°C) followed by collection of bacterial pellets. Genomic DNA was extracted using DNeasy<sup>®</sup> *mericon* Food Kit (QIAGEN) and DNA was quantified using the Qubit 3.0 (Life Technologies).

**AMR gene detection.** AMR genes relevant to human and agriculture were detected using a commercial AMR gene qPCR array kit (Cat# 330261 BAID-1901ZR, QIAGEN Inc.) using ABI 7500 Standard or Fast Real-time PCR system. This kit detects 87 AMR genes belonging to 8 classes (aminoglycoside, β-lactadfadam, erythromycin, fluoroquinolone, macrolide-lincosamide-streptogramin B, tetracycline, vancomycin, and multidrugafasid resistance classifications) (Tables 1a and 1b). Based on the preliminary experiments, 20,000 ng DNA per 96 well plate (208.3 ng/well) was used for each sample. C<sub>T</sub> values were analysed using standard manufacturer's tools. Values from 16 to 34 were evaluated as positive based on manufacturer's instruction.

## Results

**1. AMR genes detected.** Thirty-three AMR genes had at least one occurrence in all three groups with potential resistance against aminoglycoside, fluoroquinolone, tetracycline, macrolide and Class C beta-lactam (Fig 1). Eight AMR genes including erythromycin resistance genes were not detected in any of the food samples. The meat contained the highest number of AMR genes/sample (mean=14, ranged from 2 to 28), followed by produce (mean=10, 1 to 34) and dairy products (mean=7, 1 to 23). Six AMR genes including *vanB* (vancomycin resistance) were only detected in meat samples, 11 and 2 other AMR genes were only detected in produce and dairy products, respectively. The *mecA* gene, encoding a methicillin-resistant *Staphylococcus aureus*-specific penicillin-binding protein, was also detected in produce and dairy products but not in meat (Table 1a&b). Ground meat and produce in direct contact with soil contained the greatest number of AMR genes indicating the importance of various environments as sources of these genes.

Table 1a. List of target AMR genes and genes detected in three groups of food

Species (NCBI Tax ID) /Gene	Target genes AMR Gene Classification	Meat (n=18)		Produce (n=30)		Dairy (n=11)	
		Total genes detected <sup>1</sup>	Frequency (%) <sup>2</sup>	Total genes detected	Frequency (%)	Total genes detected	Frequency (%)
AAC(6)-Ib-cr	Phenoxymethylpenicillin resistance	0	0.00	333	1.1	909	8.3
aacC1	Aminoglycoside resistance	0	0.00	0	0.00	0	0.00
aacC2	Aminoglycoside resistance	6	33.33	2	6.67	0	0.00
aacC4	Aminoglycoside resistance	5	27.78	0	0.00	2	18.18
aadA1	Aminoglycoside resistance	12	66.67	11	36.67	1	9.09
aphA6	Aminoglycoside resistance	1	5.56	2	6.67	0	0.00
BES1	Class A beta-lactamase	0	0.00	1	3.33	0	0.00
BIC1	Class A beta-lactamase	1	5.56	2	6.67	0	0.00
CTX-M-1 Group	Class A beta-lactamase	3	16.67	7	23.33	0	0.00
CTX-M-8 Group	Class A beta-lactamase	0	0.00	0	0.00	0	0.00
CTX-M-9 Group	Class A beta-lactamase	2	11.11	1	3.33	0	0.00
GES	Class A beta-lactamase	1	5.56	1	3.33	2	18.18
IMP-6/NMC-A	Class A beta-lactamase	0	0.00	4	13.33	0	0.00
KPC	Class A beta-lactamase	1	5.56	1	3.33	0	0.00
Pev-1 group	Class A beta-lactamase	0	0.00	1	3.33	0	0.00
Pev-2 group	Class A beta-lactamase	0	0.00	2	6.67	0	0.00
SFC-1	Class A beta-lactamase	0	0.00	1	3.33	0	0.00
SFO-1	Class A beta-lactamase	9	50.00	4	13.33	0	0.00
SHV	Class A beta-lactamase	13	16.67	2	6.67	0	0.00
SHV(156D)	Class A beta-lactamase	0	0.00	0	0.00	1	9.09
SHV(156G)	Class A beta-lactamase	4	22.22	3	10.00	1	9.09
SHV(28G240E)	Class A beta-lactamase	3	16.67	1	3.33	0	0.00
SHV(28G240K)	Class A beta-lactamase	0	0.00	0	0.00	0	0.00
SHV(28S240E)	Class A beta-lactamase	1	5.56	0	0.00	0	0.00
SHV(28S240K)	Class A beta-lactamase	0	0.00	0	0.00	0	0.00
SME	Class A beta-lactamase	0	0.00	0	0.00	2	18.18
TLA-1	Class A beta-lactamase	1	5.56	3	10.00	2	18.18
VFB	Class A beta-lactamase	0	0.00	1	3.33	1	9.09
ccrA	Class B beta-lactamase	2	11.11	2	6.67	1	9.09
DMP-1 group	Class B beta-lactamase	0	0.00	0	0.00	0	0.00
DMP-2 group	Class B beta-lactamase	0	0.00	0	0.00	0	0.00
DMP-3 group	Class B beta-lactamase	0	0.00	1	3.33	3	27.27
DMP-5 group	Class B beta-lactamase	0	0.00	0	0.00	0	0.00
NDM	Class B beta-lactamase	1	5.56	0	0.00	0	0.00
VM-1 group	Class B beta-lactamase	2	11.11	1	3.33	0	0.00
VM-13	Class B beta-lactamase	0	0.00	1	3.33	0	0.00
VM-7	Class B beta-lactamase	1	5.56	2	6.67	0	0.00

<sup>1</sup> Total genes detected = total number of one type gene detected in all samples of each group of food; <sup>2</sup> Frequency (%) = Total number of genes detected / number of samples x 100%

## Reference

Dione et al. 2016. A quasi-universal medium to break the aerobic/anaerobic bacterial culture dichotomy in clinical microbiology. Clin Microbiol Infect. 22:53-58.

## Acknowledgement

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## Results

Table 1b. List of target AMR genes and genes detected in three groups of food (continued from Table 1a)

Species (NCBI Tax ID) /Gene	AMR Gene Classification	Meat (n=18)		Produce (n=30)		Dairy (n=11)	
		Total gene detected	Frequency (%)	Total genes detected	Frequency (%)	Total genes detected	Frequency (%)
ACC-1 group	Class C beta-lactamase	17	94.44	8	26.67	4	36.36
ACC-3	Class C beta-lactamase	15	83.33	10	33.33	4	36.36
ACT-5/7 group	Class C beta-lactamase	7	38.89	12	40.00	3	27.27
ACT-1 group	Class C beta-lactamase	3	16.67	19	63.33	3	27.27
ACT-11 group	Class C beta-lactamase	2	11.11	6	20.00	4	36.36
CMY10 Group	Class C beta-lactamase	2	11.11	1	3.33	0	0.00
DHA	Class C beta-lactamase	2	11.11	0	0.00	1	9.09
FOX	Class C beta-lactamase	10	55.56	2	6.67	0	0.00
LAT	Class C beta-lactamase	9	50.00	10	33.33	1	9.09
MIR	Class C beta-lactamase	7	38.89	7	23.33	3	27.27
MOX	Class C beta-lactamase	8	44.44	5	16.67	1	9.09
OXA-10 Group	Class D beta-lactamase	0	0.00	3	10.00	2	18.18
OXA-18	Class D beta-lactamase	1	5.56	2	6.67	0	0.00
OXA-2 Group	Class D beta-lactamase	1	5.56	2	6.67	1	9.09
OXA-23 Group	Class D beta-lactamase	0	0.00	3	10.00	3	27.27
OXA-24 Group	Class D beta-lactamase	1	5.56	2	6.67	3	27.27
OXA-45	Class D beta-lactamase	0	0.00	2	6.67	1	9.09
OXA-48 Group	Class D beta-lactamase	1	5.56	2	6.67	0	0.00
OXA-50 Group	Class D beta-lactamase	0	0.00	7	23.33	0	0.00
OXA-51 Group	Class D beta-lactamase	2	11.11	2	6.67	1	9.09
OXA-54	Class D beta-lactamase	1	5.56	1	3.33	1	9.09
OXA-55	Class D beta-lactamase	2	11.11	2	6.67	2	18.18
OXA-58 Group	Class D beta-lactamase	1	5.56	0	0.00	0	0.00
OXA-60	Class D beta-lactamase	1	5.56	1	3.33	2	18.18
eryB	Erythromycin resistance	0	0.00	0	0.00	0	0.00
QepA	Fluoroquinolone resistance	0	0.00	1	3.33	0	0.00
QnrA	Fluoroquinolone resistance	0	0.00	0	0.00	0	0.00
QnrB-1 group	Fluoroquinolone resistance	5	27.78	8	26.67	4	36.36
QnrB-31 group	Fluoroquinolone resistance	0	0.00	1	3.33	0	0.00
QnrB-4 group	Fluoroquinolone resistance	1	5.56	1	3.33	3	27.27
QnrB-5 group	Fluoroquinolone resistance	7	38.89	10	33.33	2	18.18
QnrB-8 group	Fluoroquinolone resistance	6	33.33	10	33.33	2	18.18
QnrC	Fluoroquinolone resistance	1	5.56	0	0.00	0	0.00
QnrD	Fluoroquinolone resistance	3	16.67	3	10.00	2	18.18
QnrE	Fluoroquinolone resistance	6	33.33	2	6.67	2	18.18
ermA	Macrolide-Lincosamide-Streptogramin B	6	33.33	0	0.00	0	0.00
ermB	Macrolide-Lincosamide-Streptogramin B	13	72.22	13	43.33	4	36.36
ermC	Macrolide-Lincosamide-Streptogramin B	4	22.22	1	3.33	2	18.18
ermE	Macrolide-Lincosamide-Streptogramin B	14	77.78	10	33.33	4	36.36
ermF	Macrolide-Lincosamide-Streptogramin B	4	22.22	5	16.67	0	0.00
orfJ	Multidrug resistance-efflux pump	0	0.00	6	20.00	0	0.00
opm	Multidrug resistance-efflux pump	0	0.00	7	23.33	0	0.00
tetA	Tetracycline efflux pump	12	66.67	12	40.00	2	18.18
tetB	Tetracycline efflux pump	10	55.56	14	46.67	2	18.18
vanB	Vancomycin resistance	1	5.56	0	0.00	0	0.00
vanC	Vancomycin resistance	4	22.22	3	10.00	1	9.09
Staphylococcus aureus	Staphylococcus aureus	1	5.56	0	0.00	0	0.00
mecA	Beta-lactam resistance Penicillin-Vancomycin-resistant penicillin-binding chain F precursor	0	0.00	6	20.00	1	9.09
hskF	Beta-lactam resistance Penicillin-Vancomycin-resistant penicillin-binding chain F precursor	0	0.00	1	3.33	1	9.09
gpa	β-glucuronidase G binding protein A precursor	1	5.56	5	16.67	3	27.27

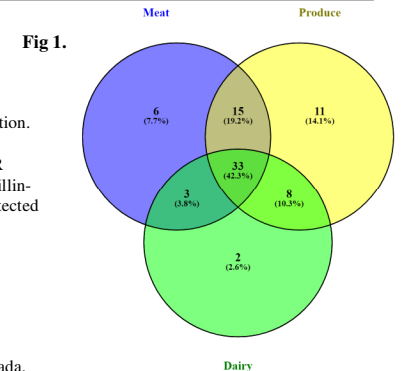


Fig 1.

## 2. Comparison of AMR genes in 3 groups of food (Fig 1).

Comparative studies revealed that some AMR genes had food specific distribution. Six AMR genes including *vanB* (vancomycin resistance) were only detected in meat. Eleven AMR genes were exclusively detected in the produce and 2 AMR genes were detected only in dairy samples. The *mecA* gene, encoding a methicillin-resistant *Staphylococcus aureus*-specific penicillin-binding protein, was also detected in produce and dairy products but not in meat.

## Summary

- AMR genes are present in microorganisms from all three food types in Canada.
- Different types of food may acquire microorganisms containing certain AMR genes from different sources.
- Further studies are necessary to confirm the results of the present preliminary investigation.
- The commercial AMR gene qPCR array kit could be a useful tool for surveillance of multiple AMR genes in microbes in food.
- The information in this study may have impact on the policy making regarding the mitigation measures to prevent or reduce AMR genes or microorganisms containing AMR genes in various sources of food.