Detection and Surveillance of Antibiotic Resistance Genes From Food and Fertilizer Sources Using qPCR Technology

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Abstract

One potential way to acquire antibiotic resistance genes is through the food supply chain. Both livestock and feed may acquire antibiotic resistant bacteria via different mechanisms. Foodstuffs can be exposed to antibiotic resistant bacteria through fertilizer originating from waste-water treatment plants. This, in addition to increasing administration of antibiotics to livestock, can lead to food being a potential source of antibiotic resistance genes. This may lead to horizontal gene transfer to pathogenic enteropathogens and further to drug resistance in humans. Therefore, the surveillance and prevention of antibiotic resistance genes in food is important.

To effectively combat the spread of difficult-to-treat bacterial infections, rapid surveillance methods to detect antibiotic resistance genes are required; in order to monitor both bacterial isolates and metagenomic samples.

Since the gut is known to act as a reservoir for antibiotic resistance genes, a small-scale research study was performed on 5 stool samples isolated from healthy human adults using an antibiotic resistance gene identification PCR array. In addition, the diversity of antibiotic resistance genes in municipal biosolids was determined using an Antibiotic Resistance Genes Microbial DNA qPCR Array with DNA extracted from belt-filter, press-cake sewage samples.

22 antibiotic resistance genes were identified from different resistance classifications. Further studies were performed in beef, chicken, vegetable and pork samples. In conclusion, PCR arrays can be effective tools for detection of antibiotic resistance genes from food samples and potential fertilizer sources.

Antibiotic Resistance Gene Screening Microbial qPCR Array

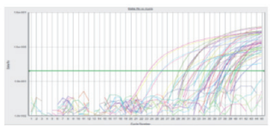
	1	2	3	4	5	6	7	8	9	10	11	12
A	KPC	GES	IMI & NMC-A	SME	IMP-1 group	IMP-2 group	IMP-5 group	IMP-12 group	SFC-1	SHV	SHV (156G)	SHV (156D)
B	SHV (238G240E)	SHV (238S240K)	SHV (238S240E)	SHV (238G240K)	BIC-1	ereB	ermA	ermB	ermC	mefA	msrA	tetA
C	tetB	ccrA	vanB	vanC	CTX-M-1 Group	CTX-M-2 Group	CTX-M-8 Group	CTX-M-9 Group	OXA-2 Group	OXA-10 Group	OXA-18	OXA-45
D	OXA-48 Group	OXA-23 Group	OXA-24 Group	OXA-51 Group	OXA-58 Group	OXA-50 Group	OXA-54	OXA-55	OXA-60	OXA-62	CMY-2 Goup	CMY-10 Group
E	DHA	FOX	MOX	ACT-1 group	ACT 5/7 group	ACC-1 group	ACC-3	MIR	LAT	CFE-1	VIM-1 group	VIM-7
F	VIM-13	NDM	Per-1 group	Per-2 group	VEB	QnrA	QnrB-1 group	QnrB-4 group	QnrB-5 group	QnrB-8 group	QnrB-31 group	QnrC
G	QnrD	QnrS	QepA	AAC(6)-Ib-cr	aphA1	aphA6	aacC1	aacC2	aacC4	aadB	aadA1	BES-1
H	SFO-1	TLA-1	oprj	Pan Bacteria 1	Pan Bacteria 1	Pan Bacteria 1	Pan Bacteria 2	Pan Bacteria 2	Pan Bacteria 2	PPC	PPC	PPC

Layout of antibiotic resistance gene screening microbial qPCR array. The antibiotic resistance gene screening microbial qPCR array allows identification of different antibiotic resistance genes in a single PCR run. Each array contains controls such as Pan-Bacteria 1 and Pan-Bacteria 2 to detect total bacteria and ensure bacterial genomic DNA was added to the array. The positive PCR control (PPC) confirms a positive PCR run and the absence of PCR inhibitors in the sample.

Microbial PCR Array Method

Genomic DNA was purified from *Klebsiella pneumoniae* and stool samples were extracted.

Samples were mixed with microbial qPCR probe mastermix and microbe-free water, and then dispensed into a 96-well PCR plate containing dried-down primers and 5'-hydrolysis probes for each of the antibiotic resistance genes tested.



PCR was performed using a Roche® LightCycler® 480

Raw C_T values were exported to the microbial qPCR analysis software to detect the presence of antibiotic resistance genes. The identification criteria were: C_T <32 = positive, C_T >35 = negative and a C_T >32–<35 = inconclusive. The control assay needed a C_T = 22±2 to show that the PCR instrument and mastermix performed correctly and there were no PCR inhibitors in the sample.

Screening of Sewage Samples and Gut Microbiota

Gene	Resistance classification	Sewage
AAC(6)-Ib-cr	Aminoglycoside-resistance	+
aacC1	Aminoglycoside-resistance	+
aadA1	Aminoglycoside-resistance	+
aadB	Aminoglycoside-resistance	+
aphA1	Aminoglycoside-resistance	+
GES	Class A beta-lactamase	+
SHV(156G)	Class A beta-lactamase	+
SHV(238G240E)	Class A beta-lactamase	+
TLA-1	Class A beta-lactamase	+
VEB	Class A beta-lactamase	+
IMP-2 group	Class B beta-lactamase	+
VIM-1 group	Class B beta-lactamase	+
CMY-10 group	Class C beta-lactamase	+
MOX	Class C beta-lactamase	+
OXA-10 group	Class D beta-lactamase	+
OXA-2 group	Class D beta-lactamase	+
QnrB-5 group	Fluoroquinolone resistance	+
QnrS	Fluoroquinolone resistance	+
ermB	Macrolide Lincosamide Streptogramin_b	+
mefA	Macrolide Lincosamide Streptogramin_b	+
tetA	Tetracycline efflux pump	+
tetB	Tetracycline efflux pump	+

14 antibiotic resistance genes were present in the metagenomic sample. Verification determined that these genes were present in the sewage sample.

Gene	Resistance classification	1	2	3	4	5
aacC2	Aminoglycoside-resistance					+
aadA1	Aminoglycoside-resistance	+/–				
SFO-1	Class A beta-lactamase	+/–	+/–	+/–	+/–	+/–
ACT-1 group	Class C beta-lactamase		+/–			
ACT 5/7 group	Class C beta-lactamase		+			+/–
MIR	Class C beta-lactamase		+/–			
ermB	Macrolide Lincosamide Streptogramin_b	+	+	+	+	+
mefA	Macrolide Lincosamide Streptogramin_b	+	+	+	+	+
tetA	Tetracycline efflux pump	+	+	+		
tetB	Tetracycline efflux pump	+/–				+

ermB, mefA, and *tetA* were found in all/most of the stool samples, showing possible high prevalence in the gut. These genes are reported to be isolated from bacterial strains originating from food, suggesting a possible origin.

Screening Using Antibiotic Resistance Genes qPCR Array

Species/gene	Antibiotic classification/virulence factor gene description	Sensitivity	Beef	Chicken	Pork	Carrot	Lettuce	Potato
aadA1	Aminoglycoside-resistance	200	+	+		+/–		
CTX-M-1 group	Class A beta-lactamase	50				+/–		
ACC-1 group	Class C beta-lactamase	100	+	+				
ACC-3	Class C beta-lactamase	30		+				
ACT-1 group	Class C beta-lactamase	100				+		+
CFE-1	Class C beta-lactamase	50				+/–		+
FOX	Class C beta-lactamase	100	+	+				
LAT	Class C beta-lactamase	100	+/–					
MIR	Class C beta-lactamase	30				+		
OXA-48 group	Class D beta-lactamase	50				+/–	+/–	
OXA-51 group	Class D beta-lactamase	100				+/–		
QnrB-1 group	Fluoroquinolone resistance	20						+
QnrB-5 group	Fluoroquinolone resistance	40				+		+
QnrB-8 group	Fluoroquinolone resistance	20				+		+
ermB	Macrolide Lincosamide Streptogramin_b	20	+	+	+			
ermC	Macrolide Lincosamide Streptogramin_b	100		+	+			
mefA	Macrolide Lincosamide Streptogramin_b	100	+	+	+			
msrA	Macrolide Lincosamide Streptogramin_b	100	+	+	+		+	+/–
oprm	Multidrug resistance efflux pump	20		+/–				
tetA	Tetracycline efflux pump	40	+	+				
tetB	Tetracycline efflux pump	30	+	+		+		
Staphylococcus aureus	*Staphylococcus aureus*	100	+	+	+	+	+/–	
mecA	Beta-lactam resistance	40	+/–		+			+/–
lukF	Panton-Valentine leukocidin chain F precursor	20						
spa	Immunoglobulin G binding protein A precursor	200	+	+	+	+/–		
	Methicillin-resistant *Staphylococcus aureus*		MSSA	MSSA	HA-MRSA			

Screening of food samples using the Antibiotic Resistance Genes qPCR Array. Bacterial cultures from food were prepared by incubating overnight in TB at 37°C. DNA was extracted and 200 ng of genomic DNA was loaded onto an Antibiotic Resistance Genes qPCR Array. PCR was performed and raw C_T values were imported into the Antibiotic Resistance + MRSA Microbial Identification Data Analysis software. Positive (+), inconclusive (+/–) and negative (blank) result for each antibiotic resistance gene was determined by the data analysis software. The data show that all the tested food samples contained multiple antibiotic resistance genes and *ermB, mefA* and *msrA* were specifically detected in all the meat samples.

Summary

- Microbial qPCR arrays are a collection of sensitive and specific qPCR assays for the detection of antibiotic resistance genes from both bacterial isolates and metagenomic samples.
- Detection of 87 antibiotic resistance genes can be performed simultaneously in one 3-hour PCR run.
- *ermB* and *mefA* were found in all meat and stool samples suggesting that acquisition of these antibiotic resistance genes may come from consumption of meat.
- The Antibiotic Resistance Gene Microbial PCR Array is an effective tool for monitoring potential outbreaks of antibiotic resistant bacteria, detection in food samples and potential sources of fertilizer, and identifying new sources of antibiotic resistance genes.

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