Isolation and Identification of Aerobic Bacteria Carrying Tetracycline and Sulfonamide Resistance Genes Obtained from a Meat Processing Plant

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Abstract: Microbial contamination in food-processing plants can play a fundamental role in food quality and safety. The purpose of this study was to investigate aerobic bacteria carrying tetracycline and sulfonamide resistance genes from a meat processing plant as possible sources of meat contamination. One hundred swab samples from surfaces of conveyor belts, meat slicers, meat knives, benches, plastic trays, gloves, and aprons were analyzed. A total of 168 isolates belonging to 10 genera were obtained, including *Pseudomonas* sp. ($n = 35$), *Acinetobacter* sp. ($n = 30$), *Aeromonas* sp. ($n = 20$), *Myroides* sp. ($n = 15$), *Serratia* sp. ($n = 15$), *Staphylococcus* sp. ($n = 14$), *Enterobacter* sp. ($n = 11$), *Escherichia coli* ($n = 10$), *Lactococcus* sp. ($n = 10$), and *Klebsiella* sp. ($n = 8$). Of the 168 isolates investigated, 60.7% showed resistance to tetracycline and 57.7% to trimethoprim/sulfamethoxazole. The tetracycline resistance genes *tet*L, *tet*A, *tet*B, *tet*C, *tet*E, *tet*M, *tet*S, *tet*K, and *tet*X were found in the frequency of 7.7%, 6.0%, 4.8%, 4.8%, 3.6%, 3.6%, 3.6%, 1.2%, and 0.6%, respectively. Sulfonamide resistance genes *sul*1 and *sul*2 were observed in the frequency of 17.9% and 38.1%, respectively. The tetracycline resistance genes *tet*X was first found in *Myroides* sp. This investigation demonstrated that food contact surfaces in a meat processing plant may be sources of contamination of aerobic bacteria carrying tetracycline and sulfonamide antibiotic resistance genes.

Keywords: aerobic bacteria, antibiotic resistance, tetracycline, sulfonamide, meat processing plant

Practical Application: The occurrence of antibiotic-resistant bacteria in raw meat products and processing environments has been found to be a potential danger to human health. To improve the safety of meat products, it is important to determine the antibiotic resistance, especially to important clinical antibiotics and its antibiotic resistance determinants. In this study, aerobic bacteria isolated from food contact surfaces in a meat processing plant were found to be a potential reservoir of tetracycline and sulfonamide antibiotic-resistance genes and the situation has to be monitored regularly.

Introduction

Long-time extensive and inappropriate use of antibiotics for growth promotion or as therapeutic and preventive treatments in food animals may generate selective pressure for emergence of multiple drug resistant (MDR) bacteria (Barton 2014). The prevalence of MDR bacteria in farm, slaughterhouse environments, and retail foods have been reported in several studies (Lavilla Lerma and others 2013; Novais and others 2013; Casella and others 2015). Microorganisms, including pathogens and commensal bacteria present in animal foods and their processing environment may cause a great challenge for human health in terms of their role as a potential reservoir of antibiotic resistance genes (ARGs) and potential pathogenic power, which may transmit to humans through the food chain (Wang and others 2012). Therefore, studies on the sources of contamination and dissemination routes of ARGs in food chains and processing environments are critically important.

Meat products are quite susceptible to microbial cross-contamination due to their abundant nutrients, which favor microbial growth in meat processing facilities (Schwaiger and others 2012). Various microbial contamination sources can be identified in a meat processing plant, including conveyor belts, knives, benches, and other tools (Martín and others 2014). Although any buildup of microbial contamination on most food contact surfaces during day-to-day operations can be controlled by sanitation practices, some contact surfaces may develop excessively high loads of microbial contamination, which would act as continual sources of contamination for products coming in contact with them (Lavilla Lerma and others 2013). Sublethal concentrations of biocides in food industries may lead to the emergence of tolerance/resistance to various biocides and specific genotypes (resistant) may have the ability to survive routine disinfection procedures (Lavilla Lerma and others 2014). Some species can also become resident in the facilities by forming biofilms on food-processing surfaces (Lindsay and others 1996). In fact, food contact surfaces have been recognized as important sources of microbial contamination and recontamination in the food industry (Khamisse and others 2012; Lavilla Lerma and others 2013; de Candia and others 2015). Several foodborne pathogens and opportunistic pathogens in poultry and bovine slaughterhouses and processing plants have been isolated, such as *Staphylococcus* sp. including methicillin-resistant *Staphylococcus aureus*, extended-spectrum b-lactamase—producing *Escherichia coli*, *Listeria monocytogenes*, *Campylobacter*, *Salmonella*, *Enterococcus* spp., Psychrotrophs including pseudomonas and lactic acid bacteria

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Table 1–Primers used in this study for PCR detection of genes associated with resistance to tetracycline and sulfonamide antibiotics.

Gene	Primers	Sequence(5′-3 ′)	Size (bp)	Reference
*tet*A	tetA-F	GCTACATCCTGCTTGCCTTC	210	Wu and others (2010)
	tetA-R	CATAGATCGCCGTGAAGAGG		
*tet*B	tetB -F	GCCAGTCTTGCCAACGTTAT	975	Koo and others (2011)
	tetB -R	ATAACACCGGTTGCATTGGT		
*tet*C	tetC-F	CTTGAGAGCCTTCAACCCAG	418	Wu and others (2010)
	tetC-R	ATGGTCGTCATCTACCTGCC		
*tet*E	tetE-F	GTTATTACGGGAGTTTGTTGG	278	Wu and others (2010)
	tetE-R	AATACAACACCCACACTACGC		
*tet*G	tetG-F	GCTCGGTGGTATCTCTGCTC	468	Wu and others (2010)
	tetG-R	AGCAACAGAATCGGGAACAC		
*tet*K	tetK-F	TCGATAGGAACAGCAGTA	169	Wu and others (2010)
	tetK-R	CAGCAGATCCTACTCCTT		
*tet*L	tetL-F	TCGTTAGCGTGCTGTCATTC	267	Wu and others (2010)
	tetL-R	GTATCCCACCAATGTAGCCG		
*tet*A/P	tetA/P-F	CTTGGATTGCGGAAGAAGAG	676	Wu and others (2010)
	tetA/P-R	ATATGCCCATTTAACCACGC		
*tet*M	tetM-F	ACAGAAAGCTTATTATATAAC	171	Wu and others (2010)
	tetM-R	TGGCGTGTCTATGATGTTCAC		
*tet*O	tetO-F	AACTTAGGCATTCTGGCTCAC	515	Ng and others (2001)
	tetO-R	TCCCACTGTTCCATATCGTCA		
*tet*Q	tetQ-F	AGAATCTGCTGTTTGCCAGTG	169	Wu and others (2010)
	tetQ-R	CGGAGTGTCAATGATATTGCA		
*tet*S	tetS-F	CATAGACAAGCCGTTGACC	667	Wu and others (2010)
	tetS-R	ATGTTTTTGGAACGCCAGAG		
*tet*W	tetW-F	GAGAGCCTGCTATATGCCAGC	168	Wu and others (2010)
	tetW-R	GGGCGTATCCACAATGTTAAC		
*tet*T	tetT-F	AAGGTTTATTATATAAAAGTG	169	Wu and others (2010)
	tetT-R	AAGGTTTATTATATAAAAGTG		
*tet*X	tetX -F	CAATAATTGGTGGTGGACCC	468	Wu and others (2010)
	tetX -R	TTCTTACCTTGGACATCCCG		
*sul*1	sul1-F	TCACCGAGGACTCCTTCTTC	433	Hammerum and others (2006)
	sul1-R	CAGTCCGCCTCAGCAATATC		
*sul*2	sul2-F	CCTGTTTCGTCCGACACAGA	293	Hammerum and others (2006)
	sul2-R	GAAGCGCAGCCGCAATTCAT		
*sul*3	sul3-F	TCAAAGCAAAATGATATGAGC	787	Hammerum and others (2006)
	sul3-R	TTTCAAGGCATCTGATAAAGAC		
Tn916	Tn-F	CTCTCCTTTCGTGGAAGCG	2000	Poyart (2000)
	Tn-R	GTACTACTAAGCAACAAGACGC		
IntI1	IntI-1U	CTTATGTCCACTGGGTTCGT	565	In this study
	IntI-1D	GGCTTCGTGATGCCTGCTTG		

(Schwaiger and others 2012; Giombelli and others 2014; Martín and others 2014; Normanno and others 2015; Pacholewicz and others 2015). However, commensal bacteria, considered as a hidden reservoir of ARGs, have not been thoroughly analyzed.

Tetracycline and sulfonamide are broad spectrum antimicrobials and are most popularly used in livestock farming in China due to the efficacy, low cost, and lack of side effects (Cheng and others 2013). Tetracycline (*tet*) and sulfonamide (*sul*) resistance genes are associated with integrons, transposons, or plasmids, and can transfer with other resistance genes located in these mobile elements among bacterial species (Robert 2005), leading to multidrug resistance. *Tet* and *sul* genes have been reported as the most frequently detected ARGs in livestock farms, animal manures, and waste water (Cheng and others 2013; Kim and others 2013; Zhu and others 2013). However, few studies have been conducted to evaluate the diversity of *tet* and *sul* genes in the processing environment, which facilitates the transfer of ARGs from indigenous environmental bacteria to foodborne microorganisms through contamination.

This study demonstrated that aerobic bacteria carrying *tet* and *sul* genes can be isolated from food contact surfaces in a meat processing plant during normal operations and may be a potential reservoir of antibiotic resistance genes. This study provides valuable information to reduce the sources of contamination with resistant bacteria during food processing and thus to minimize the risk for the consumer.

Materials and Methods

Sample collection

The present research was performed with 100 samples collected from a local large-scale pork processing plant (Xiamen, China). This pork-processing plant was representative of the region and received pigs from more than100 farms in 9 cities of Fujian province. Samples were taken from contact surfaces during ongoing production early in the mornings on our 4 visits in 2013. Surface samples were taken with sterile cotton swabs from the sites of 100 cm^2 in the selected processing line surfaces that the raw pork was in contact with, including conveyor belts (4 samples each time), meat slicers (4 samples each time), meat knives (4 samples each time), benches (2 samples each time), plastic trays (3 samples each time), gloves (4 samples each time), and aprons (4 samples each time). The samples were transported to the laboratory in an icebox within 2 hours and were used immediately for bacterial isolation.

Bacterial isolation and identification

The cotton swabs were immersed in 10 mL of sterile Brain Heart Infusion (BHI) broth and incubated at 30 ± 1 °C for 24 h. After revivification, 100 μL of each sample were serially diluted and plated on BHI agar and the plates were incubated at 30 ± 1 °C for up to 48 h. After incubation, morphologically different isolates were streaked onto a nutrient agar plate for purification and enrichment at 30 ± 1 °C for 18 to 24 h. The isolates were identified by 16S rRNA gene sequencing and comparison with sequences in GenBank using BLAST. All isolates were stored in BHI broth containing 15% glycerol at −80 °C until use.

Antimicrobial susceptibility testing

All isolates were tested for their susceptibility to tetracycline (30 μg) and trimethoprim/sulfamethoxazole (23.75/1.25 μg) (Sigma-Aldrich, St. Louis, Mo., U.S.A.) using the Kirby–Bauer disk diffusion method. The resistance level was defined as described by the Clinical and Laboratory Standards Inst. (CLSI 2012). *Staphylococcus aureus* ATCC25923, *E. coli* ATCC 25922 were used as quality control organisms.

Detection of *tet and sul genes, Tn 916, and class 1 integrons*

Genomic DNA was extracted from the isolates as previously described (Wang and others 2006). To encompass a broad range of genetic determinants, 15 *tet* genes including 7 efflux pump genes (*tet*A, *tet*C, *tet*E, *tet*G, *tet*K, *tet*L, *tet*A/P), 7 ribosomal protection proteins (RPPs) genes (*tet*M, *tet*O, *tet*Q, *tet*S, *tet*T, *tet*W, *tet*B), and 1 enzymatic modification gene (*tet*X) and 3 *sul* genes (*sul*1, *sul*2, and *sul*3) were screened by PCR using primers and protocols described previously (Table 1). *Tet* and *sul* genes associated mobile genetic elements—transposon Tn*916* and class 1 integron were also screened (Table 1).

Table 2–Isolates from food contact surfaces in a meat processing plant.

Species	No. of isolates	TET[a]	SXT[b]
Acinetobacter sp.	30	18	8
Aeromonas sp.	20	11	10
Enterobacter sp.	11	0	1
Escherichia coli	10	8	6
Klebsiella sp.	8	0	1
Lactococcus sp.	10	8	10
Myroides sp.	15	2	14
Pseudomonas sp.	35	30	32
Serratia sp.	15	13	3
Staphylococcus sp.	14	12	12
Total	168	102	97

[a]TET, tetracycline.
[b]SXT, trimethoprim/sulfamethoxazole.

Table 3–Distribution of tetracycline and sulfonamide resistance genes in bacteria isolated from food contact surfaces in a meat processing plant.

Resistance genes	Resistant	Susceptible	Total
Tetracycline			
No. of isolates	102	66	168
*tet*A	9	1	10
*tet*B	8	0	8
*tet*C	5	3	8
*tet*E	6	0	6
*tet*L	4	9	13
*tet*M	6	0	6
*tet*S	6	0	6
*tet*K	2	0	2
*tet*X	1	0	1
Sulfonamide			
No. of isolates	97	72	168
*sul*1	11	19	30
sul2	19	45	64

Results

Bacterial species

A total of 168 strains were isolated in this study (belonging to 8 genera), comprising 35 *Pseudomonas* sp. (20.8%), 30 *Acinetobacter* sp. (17.9%), 20 *Aeromonas* sp. (11.9%), 15 *Myroides* sp. (8.9%), 15 *Serratia* sp. (8.9%), 14 *Staphylococcus* sp. (8.3%), 11 *Enterobacter* sp. (6.5%), 10 *E. coli* (6.0%), 10 *Lactococcus* sp. (6.0%), and 8 *Klebsiella* sp. (4.8%) (Table 2).

Antimicrobial susceptibility

Of the 168 isolates, 102 (60.7%) and 97 (57.7%) exhibited resistance to tetracycline and trimethoprim/sulfamethoxazole, respectively. Fifty-three (31.5%) showed resistance to these 2 kinds of antibiotics simultaneously. Concerning antibiotic resistant bacteria, 85.7% (30/35) of *Pseudomonas* sp., 85.7% of *Staphylococcus* sp. (12/14), 86.7% (13/15) of *Serratia* spp., 80% (8/10) of *Lactococcus* sp., 80% (8/10) of *E. coli*, 60.0% (18/30) of *Acinetobacter* sp., and 55.0% (11/20) of *Aeromonas* sp. showed resistance to tetracycline. All *Enterobacter* sp. and *Klebsiella* sp. strains were sensitive to tetracycline. Regarding trimethoprim/sulfamethoxazole resistant bacteria, 100% (10/10) of *Lactococcus* sp., 93.3% (14/15) of *Myroides* sp., 91.4% (32/35) of *Pseudomonas* sp., 85.7% (12/14) of *Staphylococcus* sp., 60% (6/10) of *E. coli*, 50% (10/20) of *Aeromonas* sp., and 26.7% (8/30) of *Acinetobacter* sp. were resistant. It is noteworthy that all species isolated showed resistance to trimethoprim/sulfamethoxazole (Table 2).

Occurrence of *tet* and *sul* genes

Among the 15 *tet* genes evaluated in this study, *tet*L (7.7%) was found in the highest frequency followed by *tet*A (6.0%), *tet*B (4.8%), *tet*C (4.8%), *tet*E (3.6%), *tet*M (3.6%), *tet*S (3.6%), *tet*K (1.2%), and *tet*X (0.6%). *tet*G, *tet*A/P, *tet*O, *tet*Q, *tet*T, and *tet*W were not detected in any of the bacteria evaluated (Table 3 and 4). One isolate of *Klebsiella* sp., *E. coli* and *Aeromonas* sp. carried 2 *tet* genes, *tet*A+*tet*L, *tet*A+*tet*C, and *tet*E+*tet*L, respectively (Table 4). Of the 3 *sul* genes detected, *sul*2 was observed at a higher frequency (38.1%) than *sul*1 (17.9%) in these isolates, no *sul*3 was detected. Overall, all of the genera were found to carry 1 or more resistance genes; irrespective of whether the isolates were phenotypically resistant or susceptible (Table 4). No class 1 integron and Tn*916* was detected among these isolates by our PCR method.

Discussion

Meats products are easily contaminated from self and environmental contamination during processing. To understand the strategy of development and spread of antibiotic-resistant bacteria via food chain to human, more information about the various sources of contamination and resistance determinants in microorganisms in the meat processing industry is needed.

Results of this study confirmed that food contact surfaces in a meat processing environment may serve as important sources of meat contamination of antibiotic-resistant bacteria and revealed that these bacteria were a reservoir of ARGs. In this study, most

Table 4–Resistance profile of representative bacteria from food contact surfaces in a meat processing plant.

Genotype	Identified AR gene carriers
*tet*A	*Aeromonas* sp., *E. coli*, *Klebsiella* sp., *Serratia* sp.
*tet*B	*Acinetobacter* sp., *Aeromonas* sp., *Enterobacter* sp., *E. coli*, *Klebsiella* sp., *Serratia* sp.
*tet*C	*E. coli*, *Enterobacter* sp., *Serratia* sp.
*tet*E	*Aeromonas* sp., *Enterobacter* sp., *Lactococcus* sp.
*tet*L	*Aeromonas* sp., *Enterobacter* sp., *Klebsiella* sp., *Myroides* sp.
*tet*M	*Acinetobacter* sp., *Aeromonas* sp., *E. coli*, *Klebsiella* sp., *Pseudomonas* sp., *Serratia* sp.
*tet*S	*Lactococcus* sp., *Serratia* sp.
*tet*K	*Staphylococcus* sp.
*tet*X	*Myroides* sp.
*sul*1	*Acinetobacter* sp., *Aeromonas* sp., *Enterobacter* sp., *E. coli*, *Lactococcus* sp., *Klebsiella* sp., *Serratia* sp.
*sul*2	*Acinetobacter* sp., *Aeromonas* sp., *Enterobacter* sp., *E. coli*, *Lactococcus* sp., *Klebsiella* sp., *Myroides* sp., *Serratia* sp.
*tet*A, *tet*C	*E. coli*
*tet*A, *tet*L	*Klebsiella* sp.
*tet*E, *tet*L	*Aeromonas* sp.
*sul*1, *sul2*	*Enterobacter* sp.
*tet*C, *sul*2	*Serratia* sp.
*tet*L, *sul*1	*Enterobacter* sp., *Klebsiella* sp.
*tet*L, *sul*2	*Aeromonas* sp., *Enterobacter* sp., *Klebsiella* sp.
*tet*M, *sul*1	*Acinetobacter* sp., *E. coli*, *Klebsiella* sp.
*tet*M, *sul*2	*Acinetobacter* sp., *E. coli*, *Serratia* sp.
*tet*S, *sul*2	*Lactococcus* sp.
*tet*A, *tet*L, *sul*1	*Klebsiella* sp.
*tet*L, *sul*1, *sul*2	*Enterobacter* sp.

of aerobic species isolated were opportunistic pathogens or commensals. The majority of isolates were resistant to tetracycline and trimethoprim/sulfamethoxazole. All genera were found to harbor *tet* or *sul* genes. The resistance genes carried by these bacteria may have the potential to transfer into the human flora and pathogens through mobile genetic elements, although further study is required to understand this in the context of the isolates described in this study.

Psychrotrophs (mainly Gram-negative bacteria) and pseudomonads have been reported as the most commonly detected microbial groups obtained from slaughterhouse surfaces samples (Lavilla Lerma and others 2013). In this study, meat spoilage organisms, including *Pseudomonas* sp., *Acinetobacter* sp., and *Aeromonas* sp. were the most frequently detected bacteria in the samples. Overall, most of the bacterial genera isolated in this study are found naturally in meat and the environment (soil, water, and sewage), with some strains classified as opportunistic human pathogens causing a variety of infectious diseases in animals and humans. Concerning resistance to antibiotics, all species isolated were found resistant to tetracycline and trimethoprim/sulfamethoxazole except all *Enterobacter* sp. and *Klebsiella* sp. strains, which were found sensitive to tetracycline. High percentage of *Staphylococcus* sp., *Pseudomonas* sp., and *Lactococcus* sp. isolates showed resistance to both tetracycline and trimethoprim/sulfamethoxazole. Approximately half of *Aeromonas* sp. isolates were resistant to tetracycline and trimethoprim/sulfamethoxazole. Most of *Acinetobacter* sp. and *Serratia* sp. isolates were just resistant to tetracycline, and most *Myroides* sp. species were resistant to trimethoprim/sulfamethoxazole. Most of *E. coli* isolates were resistant to tetracycline and half of them resistant to trimethoprim/sulfamethoxazole. High resistance rate to tetracycline and trimethoprim/sulfamethoxazole has been reported in China, which was related to the use of those antimicrobials in feed as additives and veterinary practices (Zhu and others 2013; Jiang and Shi 2013). The resistance may also be a result of the genetic transfer of resistance determinants potentiated by the use of sub-inhibitory disinfectants employed as daily sanitation practices. In this sense, further studies are needed to elucidate whether there is a cross resistance or a coresistance between antibiotics and disinfectants.

Previous studies reported various percentages of different *tet* and *sul* genes in ready-to-eat food, food-producing animals, environment, and human bacteria (Schwaiger and others 2010; Schwaiger and others 2012; Jiang and others 2013; Liu and others 2015). Despite the overall occurrence of *tet* and *sul* genes in our isolates being lower than that of previous studies, the range of species known to carry these genetic determinants is increased (Table 4). All genera isolated in this study were found to be *tet* or *sul* carriers (Table 4). To the best of our knowledge, *tet*X was first found in a species of *Myroides* sp. (KT033506). The occurrence of efflux pump genes (*tet*L, *tet*A, *tet*B, and *tet*C) in our isolates is not surprising because these genes are the most commonly found *tet* genes in Gram-negative genera (Robert, 2005). Compared with *tet* genes, both *sul1* and *sul2* were observed at higher frequencies. High frequency of *sul* genes found in sensitive isolates indicated that these genes are probably not expressed, or the genes lose function because of mutation or deletion (Brenciani and others 2007; Yan and others 2010). This phenomenon merit attention as these genes may be potentially expressed under regular exposure to sublethal concentrations of disinfectants or antibiotics.

Conclusions

This study demonstrated that aerobic bacteria on food contact surfaces may act as a reservoir of ARGs and potentially spread to human via food chain. It is necessary to screen a wide range of commensal and common environmental bacteria for carriage of resistance genes from various sources. Further studies are needed to identify the possibility of more organisms and associated ARGs and mobile genetic elements, such as plasmid-mediated conjugation and transposon, and to characterize horizontal dissemination mechanisms for antibiotic resistance among different genera and species. To avoid the adaptation and evolution of antibiotic resistance of strains on food contact surfaces, good hygienic practice should be implemented. Hygienic equipment with no recesses

and crevices, no stagnating water, and other hard or impossible-to-clean places can be designed in food industry to help limit the contamination and persistence of antibiotic-resistant bacteria on food contact surfaces.

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Authors' Contributions

L. Li participated in the execution of the analysis and collaborated in the interpretation of the results and the preparation and writing of the manuscript. L. Ye edited the research paper and was responsible for the financial support offered by Fundamental Research Funds for the central Universities (2015ZM063). S. Zhang collaborated in the sample collection. H. Meng supervised the entire study and for the preparation, writing, and editing of the research paper. All authors critically reviewed the manuscript and approved the final version.

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