Examination of Quaternary Ammonium Compound Resistance in *Proteus mirabilis* Isolated from Cooked Meat Products in China

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The aim of this study was to examine the presence of genes responsible for resistance to quaternary ammonium compounds (QACs) and the association of qac genes with class 1 integrons in *Proteus mirabilis* isolated from cooked meat products. A total of 52 *P. mirabilis* isolates (29.2%) were detected from 178 samples, and their minimum inhibitory concentrations (MICs) of benzalkonium chloride (BC) ranged from 4 to >32 μg/mL. The isolates with BC MICs of 24 μg/mL were observed most frequently. PCR assays indicated that *mdfA*, *ydgE/ydgF*, *qacE*, *qacE_1*, *emrE*, *sugE(c)*, and *sugE(p)* were commonly present (32.7%–100%) in these isolates, but *qacH* was less prevalent (3.8%). Five groups of resistance gene cassettes were identified in 10 intI1-positive isolates. An unusual gene cassette array *dfrA32-ereA-aadA2* was found in one foodborne isolate of *P. mirabilis*. Two isolates harbored *qacH*- and *sul3*- associated non-classic integrons: *aadA2-cmlA1-aadA1-qacH-IS*₄₄₀-*sul3* and a new arrangement *dfrA32-ereA1-aadA2-cmlA1-aadA1-qacH-IS*₄₄₀-*sul3*, which is first reported in *P. mirabilis*. Non-classic class 1 integrons were located on conjugative plasmids of 100 kb in two tested isolates. Our data showed that the QAC resistance genes were commonly present among *P. mirabilis* isolates from cooked meats and *qacH* was associated with non-classic class 1 integrons. The creation of transconjugants demonstrated that *qacH*-associated non-classic class 1 integrons were located on conjugative plasmids and therefore could facilitate the co-dissemination of disinfectant and antimicrobial resistance genes among bacteria, an increasing area of concern.

**Keywords:** *Proteus mirabilis*, qac genes, integron, benzalkonium chloride, resistance

**INTRODUCTION**

*Proteus mirabilis*, widely distributed in the natural environment, is a member of Enterobacteriaceae family. As an opportunistic pathogen, *P. mirabilis* can cause urinary tract and wound infections. In addition, it can also contaminate meat (Kim et al., 2005; Wong et al., 2013), vegetables (Uzeh et al., 2009), and seafood (González-Rodríguez et al., 2002), and has been associated with food poisoning (Wang et al., 2010). Previous studies focused on the antimicrobial resistance and the distribution of resistance genes among foodborne *P. mirabilis* (Kim et al., 2005; Wong et al., 2013). Unlike other foodborne bacteria (Zou et al., 2014; Zhang et al., 2016), little data have been reported...
on the disinfectant resistance and molecular mechanisms underlying resistance in foodborne *P. mirabilis*. Disinfectants have the ability to co-select for antimicrobial resistance when these traits are genetically linked (Chapman, 2003). Moreover, much evidence has shown that disinfectant and antimicrobial resistance genes can be co-transferred between bacteria via horizontal gene transfer (Zhao et al., 2009; Call et al., 2010; Sáenz et al., 2010; Partridge et al., 2012), which poses a risk to public health.

Benzalkonium chloride (BC), an important representative of quaternary ammonium compounds (QACs), is used extensively as a disinfectant in the food processing environment to prevent the growth of microorganisms and to ensure the microbiological safety of food products. Its widespread use, however, may impose a selective pressure for resistant strains of bacteria (Cruz and Fletcher, 2012). In the past decades, resistance to BC (resistant breakpoints for BC were >30 µg/mL in Sidhu et al. (2002) and ≥20 µg/mL in Meier et al. (2017)) has been reported in many bacterial isolates from food and food processing plants (Sidhu et al., 2002; Meier et al., 2017).

Efflux pumps, QacE, QacEΔ1, QacF, QacG, QacH/I, and SugE(p), which contribute for BC resistance, have been identified in Gram-negative bacteria (Zou et al., 2014). They are members of the small multidrug resistance (SMR) family and are generally located on mobile genetic elements, such as integrons and plasmids (Zou et al., 2014). *qac* are closely associated with class 1 integrons. *qac*E is located in the 3′-conserved segment (CS) of class 1 integrons and *qac*EΔ1, a deletion derivative of *qac*E, confers increased resistance to BC (Kazama et al., 1998). *qac*F shows a high degree of similarity (67.8% identity) with *qac*E. Class 1 integrons carrying *qacG* have been found in Gram-negative bacteria (Chu et al., 2001; Partridge et al., 2012). *qac*H, which was first identified in *Staphylococcus* (Heir et al., 1998), has been observed frequently in Gram-negative bacteria (Hegstad et al., 2010) and it confers a broader resistance phenotype compared with *qacG* (Heir et al., 1998). *qac*H is usually recognized as an important component of class 1 integron that lacks the normal 3′-CS region (i.e., a “non-classic class 1 integron”), which has been detected in many species of Enterobacteriaceae (Antunes et al., 2007; Chang et al., 2009, 2011; Sáenz et al., 2010; Farkas et al., 2016). Several studies found *qac*H gene and β-lactamase genes (*bla*IMP–15, *bla*GES–1, *bla*GES–5, and *bla*OXA–2) linked to class 1 integrons from *Pseudomonas aeruginosa* clinical isolates (Garza-Ramos et al., 2008, 2010; Viedma et al., 2009). *qac*H-aadB has been reported from environmental bacteria, including *Paracoccus versutus*, *Brevundimonas diminuta*, *Brachymonas denitrificans*, *Stenotrophomonas acidaminiphila*, and *Psychrobacter* spp. (Li et al., 2009) and *qac*H-aadA8 from *Vibrio cholerae* (Ceccarelli et al., 2006). Additionally, *qac*H located on a novel transposon Tn6188 was found in *Listeria monocytogenes* (Müller et al., 2013). In several studies, *qac*H in Enterobacteriaceae was renamed as *qac*I to distinguish it from *qac*H from *Staphylococcus* (Naas et al., 2001; Curiao et al., 2011). For this paper, we still use the gene name "*qac*H". *qac*H exhibits 91.6% similarity with the sequence of *qacF*. Finally, SugE(p) is frequently present on multidrug resistance plasmids, that have been reported in *Escherichia coli* and *Salmonella* (Zhao et al., 2009; Call et al., 2010).

Five chromosome-encoded efflux pump genes (*sugE(c)*, *emrE*, *mdfA*, and *ydgE/ydgF*) have been reported to confer resistance to BC (Bay and Turner, 2009; Zou et al., 2014). In addition to *mdfA* encoding a multidrug resistance efflux pump belonging to the major facilitator superfamily (MFS), the remaining genes encode the SMR family efflux pumps.

The aims of this study were to assess the BC resistance and investigate the presence of disinfectant resistance genes and the association of *qac* genes with class 1 integrons among the *P. mirabilis* isolates from cooked meat products in China.

### MATERIALS AND METHODS

#### P. mirabilis Isolates

Between January and September 2015, 178 samples (250 g) of cooked meat products, including roasted meats (*n* = 67) and sauced meats (*n* = 111), were purchased from supermarkets and cooked meat shops in Xinxiang, a city of Henan. Six local supermarkets and ten cooked meat shops were randomly selected and each site was visited once. Thirteen samples were collected from each supermarket and ten samples from each cooked meat shops. Samples were transported to the laboratory in an icebox and were processed immediately for bacterial isolation. According to the previous study, the traditional *Salmonella* isolation protocol was modified to isolate *P. mirabilis* (Wong et al., 2013). Briefly, a rinse was performed by adding 25 g of sample to 225 mL of buffer peptone water (BPW; Huankai Ltd., Guangzhou, Guangdong, China) in sterile lateral filter bags with thorough mixing by using a homogenizer (BagMixer lab blender 400; Interscience, Saint-Nom-La-Bretèche, France). These samples were then incubated at 37°C for 18 h. Pre-enriched sample (1 mL) was inoculated into 10 mL of tetrathionate broth base (TTB; Huankai) and incubated at 42°C for 24 h. A loop of inoculum was streaked onto xylose lysine deoxycholate agar (XLD; Huankai) and incubated for 24 h at 37°C. Three to four *Salmonella*-like colonies (pink with or without black center on XLD; and yellow with or without black center colonies on XLD were also considered as suspected colonies) were picked and re-streaked on nutrient agar (NA). The isolates with swarming phenotype were identified by using the API 20E bacterial identification system (BioMerieux, Marcy l’Etoile, France). All isolates designated as *P. mirabilis* were additionally analyzed by PCR-based 16S rDNA sequencing using a pair of universal primers 27F/1492R (Table 1). Only one isolate from each sample was selected for further characterization.

### Determination of Minimum Inhibitory Concentrations (MICs) for BC

The MICs of BC for *P. mirabilis* were determined using the agar dilution method recommended by the Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2012). BC was tested in concentration range of 4–32 µg/mL.
TABLE 1 | Primers used in this study.

| Target gene(s) or region | Primer | Sequence (5′–3′) | Annealing temperature (°C) | Amplicon size (bp) | Reference |
|--------------------------|--------|------------------|--------------------------|------------------|-----------|
| 16S rDNA                | 27F    | AGAGTTTGATCCTGCGGCTAG | 55                        | 1466             | Moreno et al., 2002 |
| qacE                     | qacE-F  | AGGCCCATCCTAACGAG | 55                        | 194              | Gillings, 2010 |
| qacE                     | qacE-R  | ACGCTTGCCCTTCCGCGC | 49                        | 140              | Jiang et al., 2017 |
| qacEΔ1                   | qacEΔ1-F | AGATACTGCAACAATCCG | 52                        | 228              | Jiang et al., 2017 |
| qacF                     | qacF-F  | TTCTCTCCGTGTAATGGTG | 52                        | 133              | Jiang et al., 2017 |
| qacG                     | qacG-F  | GTGCCTGACACTCAAATCG | 52                        | 140              | Jiang et al., 2017 |
| qacH                     | qacH-F  | TTTGTGAGTGGTCGCGA | 54                        | 162              | Jiang et al., 2017 |
| qacH                     | qacH-R  | GCGACGCAAACAAAGCAT | 54                        | 162              | Jiang et al., 2017 |
| sugE(p)                  | sugE(p)-F | CAATCGCCCAGACAACTT | 51                        | 103              | Jiang et al., 2017 |
| sugE(c)                  | sugE(c)-F | CTGCTGGAAGTGGTATGGG | 55                        | 226              | Zou et al., 2014 |
| emrE                     | emrE-F  | CCTGTTATGCGCGTGACAG | 54                        | 310              | Jiang et al., 2017 |
| emrE                     | emrE-R  | TTCGTGCTCACCTTCCCTT | 54                        | 596              | Jiang et al., 2017 |
| mdfA                     | mdfA-F  | GTCAGGGCTTTACTAAGC | 54                        | 632              | Jiang et al., 2017 |
| ydgE                     | ydgE-F  | GCGACGCAAGATCCAAACC | 54                        | 184              | Jiang et al., 2017 |
| ydgF                     | ydgF-F  | ATACCTGGTTGACGGACAG | 49                        | 139              | Jiang et al., 2017 |
| intI1                    | intI1-F | ACAGAGCGAAGATCCAAACC | 50                        | 565              | Li et al., 2006 |
| qacE1-sul1 region        | qac-F  | TAAGCGAGGCTTTACTAAGC | 55                        | 141              | Jiang et al., 2017 |
| variable region          | InF    | GCGCAGCAGGCAAACGGAA | 52                        | 208              | Jiang et al., 2017 |
| aadA2                    | aadA2-F | CATCCGGTGCGGTATATCC | 56                        | 370              | Jiang et al., 2017 |
| cmIA1                    | cmIA1-F | CGCCACGACGCTTGTGACGTTAT | 57                        | 694              | Jiang et al., 2017 |
| cmIA1                    | cmIA1-R | TTGGCTGCGGCTATTAGTAC | 58                        | 141              | Jiang et al., 2017 |
| aadA1                    | aadA1-F | CTTGAACGTTGATGAAACAA | 52                        | 299              | Jiang et al., 2017 |
| IS440                    | IS440-F | TATCCGGTGGACACCTT | 53                        | 299              | Jiang et al., 2017 |
| sul3                     | sul3-F  | CGAGATTTACGTCGGGCC | 50                        | 208              | Jiang et al., 2017 |
| sul3-R                   | sul3-R  | TTGGCTGCTTTAGTGGAGC | 50                        | 208              | Jiang et al., 2017 |

P. mirabilis suspensions were adjusted to a turbidity equivalent to that of a 0.5 McFarland standard with sterilized saline solution (0.9%) and delivered to the Mueller–Hinton (MH; Huankai) agar containing different concentrations of BC (Aladdin Biochemical Technology Co., Ltd., Shanghai, China). The plates were incubated at 37°C for 24 h. The lowest concentration of BC that prevented growth was considered as the MIC. Each of the tests was done in triplicate. In cases in which not all three replicates had the same results, the MICs were determined once more. Escherichia coli ATCC 10536 (a gift from Lijun Zhou, Navy General Hospital, Beijing, China) was used as a quality control strain (the MIC of this strain for BC was 16 μg/mL).

Detection of QAC Resistance Genes

All isolates were screened by PCR for the presence of qac genes, including qacE, qacΔA, qacF, qacG, qacH, sugE(p), sugE(c), emrE, mdfA, and ydgE/ydgF (Table 1). Colonies were transferred to an Eppendorf tube filled with water and boiled to prepared DNA template (Wang et al., 2008). The PCR mixture consisted of 2.5 μL of boiled lysate DNA, 0.6 μM (each) primer, 200 μM deoxynucleoside triphosphate (Takara Bio Inc., Otsu, Shiga, Japan), 1× PCR buffer (Takara), 0.5 U Taq DNA polymerase (Takara) in a total volume of 25 μL. The PCR conditions were as follows: initial denaturation at 94°C for 5 min followed by 30 cycles of denaturation at 94°C for 30 s, annealing at different temperatures (between 49 and 55°C depending on the primer set).
for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 7 min. The purified PCR products were sequenced and DNA sequence data were analyzed using the BLAST program.

Characterization of Class 1 Integrons
All isolates were screened for intI1 (Table 1). Because the occurrence of sulfonamide resistance gene (sul1) and quaternary ammonium compounds resistance gene (qacEAc1) is often associated with classic class 1 integrons, presence of qacEAc1-sul1 region in all intI1-positive isolates was tested using the primers qac-F and sul1-R (Table 1). The presence of gene cassettes in variable regions were characterized by PCR using specific primers (Table 1). Primers InF and InB were used for classic class 1 gene cassettes amplifications; and primers InF and aadA2-R were used for non-classic class 1 integron amplifications. To determine the genetic structure of non-classic class 1 integrons, a wide variety primers were designed. PCR “primer-walking” strategy was used to amplify overlapped individual fragments using Takara LA Taq DNA polymerase to get the complete arrangement. All the obtained amplicons were sequenced on both strands.

Genetic Locations of qacH-Associated Class 1 Integrons
Plasmid DNA was isolated from two qacH-positive isolates using TIANpure Mini Plasmid Kit (TIANGEN Biotech Co., Ltd., Beijing, China). To determine the number and size of plasmids, genomic DNA from qacH-positive isolates was performed by S1 nuclease (Takara) digestion prior to PFGE. S1-PFGE fragments were transferred onto membranes (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom) by Southern blot and hybridized with specific probes of intI1 and qacH genes. The probes were made with the DIG-High Prime DNA Labeling and Detection Starter Kit I (Roche Applied Science, Mannheim, Germany).

Conjugation Experiments
The transfer of plasmids carrying qacH-associated class 1 integrons was studied by performing conjugation experiments as described previously (Koo and Woo, 2011). E. coli J53Az (a sodium azide resistant strain) was used as the recipient and isolates containing non-classic class 1 integron severed as donors. Briefly, donor and recipient cells were mixed with each other at 1:10 ratio and incubated at 37°C overnight. Transconjugants were selected on trypticase soy agar (TSA; Huankai) plates containing sodium azide (150 µg/mL; Sinopharm Chemical Reagent Co., Ltd., Shanghai, China), streptomycin (50 µg/mL; Sigma–Aldrich, St. Louis, MO, United States) and chloramphenicol (16 µg/mL; Sigma–Aldrich). PCR was used to confirm that the transconjugants carried the same resistance genes as their donors.

Statistical Analysis
The statistical package SPSS 15.0 (SPSS Inc., Chicago, IL, United States) was used, and the two-tailed paired Student’s t-test was applied to determine the significance of differences. A P-value < 0.05 was considered statistically significant for comparisons.

Nucleotide Sequence Accession Numbers
The nucleotide sequences of the qacH-carrying integrons have been submitted to GenBank under accession numbers KY662007 for aadA2-cmlA1-aadA1-qacH-IS440-sul3 and KY426918 for dfrA32-ereA1-aadA2-cmlA1-aadA1-qacH-IS440-sul3.

RESULTS
Isolation of P. mirabilis
Colony characteristics of P. mirabilis on XLD agar were similar to those of Salmonella. In this study, Salmonella-like colonies were found in 88 samples (Supplementary Table S1). Among these samples, fifty-six samples were positive for isolates with swarming phenotype (Supplementary Table S1). These suspected P. mirabilis isolates were identified by the biochemical tests and PCR-based 16S rDNA sequencing. Fifty-two samples were positive for P. mirabilis, five samples were positive for P. vulgaris, and one sample was positive for Salmonella (Supplementary Table S1).

Susceptibility of P. mirabilis Isolates to BC
A total of 52 (29.2%) P. mirabilis isolates were recovered from 178 cooked meat samples (Supplementary Table S1). For these isolates, the MICs of BC ranged from 4 to >32 µg/mL (Figure 1 and Supplementary Table S2). The isolates with BC MICs of 24 µg/mL (n = 17, accounting for 32.7% of all isolates) were

![Figure 1](http://www.ncbi.nlm.nih.gov/BLAST/)

**FIGURE 1 |** Distribution of the MICs of benzalkonium chloride for 52 Proteus mirabilis isolates.
TABLE 2 | Different resistance gene combinations in Proteus mirabilis isolates.

| Gene combination                                      | Number of isolates with MIC (µg/mL) as follows | Total |
|-------------------------------------------------------|-----------------------------------------------|-------|
| qacE-qacEΔ1-mdfA-ydgE/ydgF                            | 4 1 9                                        | 14    |
| mdfA-ydgE/ydgF                                        | 2 3 2                                        | 7     |
| sugE(p)-qacE-qacEΔ1-emrE-mdfA-ydgE/ydgF              | 1 1 4                                        | 6     |
| sugE(c)-sugEp-qacE-qacEΔ1-emrE-mdfA-ydgE/ydgF         | 2 2 2                                        | 4     |
| sugE(c)-qacE-qacEΔ1-emrE-mdfA-ydgE/ydgF              | 3 1 1                                        | 4     |
| emrE-mdfA-ydgE/ydgF                                   | 2 1 1                                        | 4     |
| sugE(c)-sugEp-mdfA                                    | 1 3 1                                        | 3     |
| sugE(c)-emrE-mdfA-ydgE/ydgF                          | 2 1 3                                        | 3     |
| sugE(c)-sugEp-emrE-mdfA-ydgE/ydgF                     | 2 2 2                                        | 2     |
| sugE(c)-mdfA-ydgE/ydgF                               | 1 1 1                                        | 2     |
| sugE(c)-qacH-mdfA-ydgE/ydgF                          | 1 1 1                                        | 2     |
| sugE(c)-sugEp-qacH-mdfA                               | 1 1 1                                        | 2     |

Our results showed that qacE always occurred simultaneously with qacEΔ1, but the MICs of BC were not significantly different (P > 0.05) between qacE-qacEΔ1-positive and -negative isolates. The presence of sugE(p) was significantly associated with the higher MICs of BC (P < 0.05). Among the 17 sugE(p)-positive isolates, 82.4% (n = 14) had the MICs of ≥32 µg/mL. High MICs of BC (>32 µg/mL) were also observed in the two isolates that carried qacH.

Genetic Structure of Class 1 Integrons

The 52 P. mirabilis isolates were subjected to the PCR screening for the expected integrase gene, and the 565-bp corresponding amplicon was detected in 10 isolates, consistent with the presence of the class 1 integrase gene. Five groups of resistance gene cassettes, named as type I-V, were identified in these isolates (Figure 2), including: dfrA17-aadA5, dfrA5, dfrA1-orfC, dfrA32-ereA1-aadA2, and aadA2 (Table 3).

Analysis of Plasmids Carrying qacH-Associated Class 1 Integrons

According to the bands obtained by S1-PFGE of qacH-positive isolates, the number and size of their plasmids were determined...
TABLE 3 | Characteristics of isolates carrying integrons.

| Isolate | Size (bp) | Gene cassette | Type a | qacE1-sul1 | Classic or non-classic b |
|---------|-----------|---------------|--------|------------|-------------------------|
| PM1     | 1664      | dfrA17-aadA5  | I      | +          | Classic                 |
| PM4     | 2900      | dfrA32-ereA-aadA2 | IV     | −          | Non-classic             |
| PM7     | 721       | dfrA5         | II     | +          | Classic                 |
| PM9     | 1664      | dfrA17-aadA5  | I      | +          | Classic                 |
| PM13    | 1664      | dfrA17-aadA5  | I      | +          | Classic                 |
| PM19    | 792       | aadA2         | V      | −          | Non-classic             |
| PM21    | 1163      | dfrA1-orfC    | III    | +          | Classic                 |
| PM24    | 721       | dfrA5         | II     | +          | Classic                 |
| PM35    | 1664      | dfrA17-aadA5  | I      | +          | Classic                 |
| PM39    | 1664      | dfrA17-aadA5  | I      | +          | Classic                 |

aType I-V represented five different integron structures in our study (Figure 2).
bIn this study, class 1 integrons with qacE1 and sul1 genes in their 3′-CS were named as classic class 1 integrons. Accordingly, class 1 integrons without the normal 3′-CS were named as non-classic class 1 integrons.

DISCUSSION

In the present study, fifty-two isolates of P. mirabilis were isolated from cooked meat samples. According to Wong et al. (2013), the rate of P. mirabilis in raw chicken carcass samples in Hong Kong was 86.2% (50/58), which was much higher than that (29.2%) in our study. Cooking procedures kill most microorganisms that colonize raw meats, however, survival of microorganisms due to improper processing or cross-contamination of food after cooking may occur. This could be an explanation for the lower incidence of P. mirabilis in cooked meat products compared to raw meat samples.

Proteus mirabilis isolates in our study showed the MICs of BC ranging from 4 to >32 µg/mL, with MICs of 24 µg/mL most frequently. As there is no standard resistant breakpoint of BC for P. mirabilis, it is difficult to classify the isolates as BC susceptible, intermediate or resistance in this study. Although data on the BC susceptibility in P. mirabilis were scarce, several studies have reported the MICs of BC in Enterobacteriaceae isolates from different sources (Sidhu et al., 2002; Aarestrup and Hasman, 2004; Zhang et al., 2016). In the study of Zhang et al. (2016), E. coli isolated from retail meat showed the MICs of BC in the range of 16–64 µg/mL. Aarestrup and Hasman (2004) have shown that the MICs of BC for Salmonella (n = 156) and E. coli (n = 202) from food animals ranged from 64 to 256 µg/mL and from 16 to 128 µg/mL, respectively. Compared with the studies mentioned above, P. mirabilis isolates in our study showed relatively lower MICs to BC. In another survey of Enterobacteriaceae from food, almost half of the isolates exhibited the MICs of BC with <10 µg/mL (Sidhu et al., 2002). Actually, the user concentrations of BC in food industry are usually 200–1000 µg/mL (Møretrø et al., 2017), which are much higher than the MICs of P. mirabilis in this study. In practical application, BC is commonly rinsed from surfaces (equipment, machines, floor etc.) with water after

(Figure 3). PM4 contained a plasmid of 100 kb and PM19 contained two large plasmids of 100 and 150 kb. Both specific probes for intI1 and qacH genes hybridized with the plasmids of 100 kb in the two isolates (Figure 3). Two transconjugants named PM4T and PM19T were obtained after conjugation experiments. Both of them exhibited resistance to streptomycin and chloramphenicol. However, increased MICs of BC were not observed in transconjugants (data not shown). PCR experiments confirmed that the transconjugants harbored the same gene structure of integrons as their donors (PM4T with dfrA32-ereA-aadA2-cmlA1-aadA1-qacH-IS440-sul3 and PM19T with aadA2-cmlA1-aadA1-qacH-IS440-sul3). All the above indicated results confirmed that qacH-associated class 1 integrons in the two studied isolates were located on conjugative plasmids of 100 kb.
disinfection. This rinsing step, however, is not sufficient to
remove all BC residues from surfaces and consequently, bacteria
are likely to be exposed to diluted, sub-lethal BC concentrations
(Buffet-Bataillon et al., 2012). Repeated exposure to sub-lethal
BC concentrations may facilitate the development of resistance
(Buffet-Bataillon et al., 2012). Therefore, it was not surprising that
P. mirabilis isolates in the present study exhibited low-level of BC
MICs.

In this study, the presence of QAC resistance genes was
investigated. Our results showed that mdfA and ydgE/ydgF were
the most prevalent among P. mirabilis, which was in agreement
with the similar studies of E. coli (Zou et al., 2014; Zhang et al.,
2016). Among the isolates tested in our study, qacE always
occurred simultaneously with qacEΔ1. It was noted that the
presence of sugE(p) was significantly associated with the higher
MICs of BC (P < 0.05). Two qacH-positive isolates also exhibited
relatively high MICs of BC (>-32 μg/mL). In previous research,
the higher MICs of BC were associated with plasmid-encoded
genomes (Zou et al., 2014). Because each isolate harbored more than
one QAC resistance gene, it is difficult to assess what level of BC
resistance was contributed by each QAC resistance gene.

A previous study reported that aadA1 gene cassette was
observed most commonly among the P. mirabilis isolates from
retail meat products (Kim et al., 2005). In contrast, our results
showed that dfrA17-aadA5 was the most common cassette array,
which is similar to other reports of foodborne P. mirabilis
isolates in China (Shen et al., 2011). Interestingly, an uncommon
integron gene cassette array dfrA32-ereA1-aadA2 was found in
one P. mirabilis isolate in this study. To the best of our knowledge,
this is the first report of dfrA32-ereA1-aadA2 in foodborne
P. mirabilis. The integron gene cassette array dfrA32-ereA1-aadA2 in our study showed 99.6% identity to that of Salmonella
enterica (Krauland et al., 2010; GenBank accession number
GU067642), 99.7% identity to that of Laribacter hongkongensis
(Feng et al., 2011; GenBank accession number GU726907) and
99.7% identity to that of Aeromonas hydrophila (unpublished;
GenBank accession number KJ543558). Recently, this cassette
array was detected in clinical P. mirabilis isolates in Zhejiang
Province of China (Wei et al., 2014), which had 99.6% identity
to our sequence. The high similarity of ereA1 and pm19 with
unnamed (unpublished; Antunes et al., 2007; Sáenz et al., 2010). Pal et al.
(2015) also found that plasmids with co-selection potential for
resistance to disinfectants and antimicrobials tended to be large
and conjugative. Conjugation experiments confirmed that the
disinfectant and antimicrobial resistance genes of non-classic
class 1 integrons in PM4 and PM19 were co-existent on the
same conjugative plasmids and could be co-transferred to E. coli.
When these isolates are exposed to QACs, selection pressure
from disinfectants could increase risks for the spread of QAC
and antimicrobial resistance genes among the bacteria. Notably,
two transconjugants containing qacH showed the same MICs
of BC as the recipient in this study. Similar observations that
the presence of QAC resistance genes didn’t increase the MICs
of BC in transconjugants has also been reported in a previous
study (Zhang et al., 2016). There was the possibility that the
agar dilution method used for susceptibility testing was not
sensitive enough to detect the differences of BC MICs between
the recipient and transconjugants.

**CONCLUSION**

Quaternary ammonium compounds resistance genes, including
mdfA, ydgE/ydgF, qacE, qacEΔ1, emrE, sugE(p), and sugE(p)
were found in foodborne P. mirabilis isolates in this study. Our
data demonstrated the presence of non-classic class 1 integrons
with the gene structure qacH-IS440-sul3 among the isolates.
Moreover, qacH-associated non-classic class 1 integrons were
located on conjugative plasmids and therefore could constitute an effective way for co-dissemination of antimicrobial and disinfectant resistance genes.

**AUTHOR CONTRIBUTIONS**

TY, HW, and LS designed and supervised the study. XJ, LL, YL, and KZ performed the experiments. XJ analyzed data. XJ and TY drafted the manuscript.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2017.02417/full#supplementary-material
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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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