International Journal of Food Microbiology 308 (2019) 108290

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

First detection of the plasmid-mediated colistin resistance gene *mcr-1* in virulent *Vibrio parahaemolyticus*

Tao Lei[a,b], Jumei Zhang[a,b], Fufeng Jiang[a,b,c], Min He[a,c,d], Haiyan Zeng[a,b], Moutong Chen[a,b], Shi Wu[a,b], Juan Wang[e], Yu Ding[f], Qingping Wu[a,b,*]

[a] Guangdong Institute of Microbiology, Guangdong Academy of Sciences, Guangzhou, Guangdong Province 510070, China
[b] State Key Laboratory of Applied Microbiology Southern China, Guangdong Provincial Key Laboratory of Microbial Culture Collection and Application, Guangdong Open Laboratory of Applied Microbiology, Guangzhou, Guangdong Province 510070, China
[c] School of Food and Biological Engineering, Shaanxi University of Science and Technology, Xi'an, Shaanxi Province 710021, China
[d] School of Bioscience and Bioengineering, South China University of Technology, Guangzhou, Guangdong Province 510006, China
[e] College of Food Science, South China Agricultural University, Guangzhou, Guangdong Province 510642, China
[f] Department of Food Science & Technology, Jinan University, Guangzhou, Guangdong Province 510632, China

ARTICLE INFO

Keywords:
Antimicrobial resistance
Colistin
Mcr-1
IncX4
Tdh
PMQR

ABSTRACT

The plasmid-mediated colistin resistance gene *mcr-1* has been identified in various *Enterobacteriaceae* species, which poses a great challenge to the public health. The present study aimed to investigate the prevalence of *mcr-1* in *Vibrio parahaemolyticus* isolated from food samples in China, and to conduct a comprehensive analysis on the molecular characterization of *V. parahaemolyticus* isolate carrying *mcr-1*-harboring plasmid. A total of 646 *V. parahaemolyticus* strains isolated from 2531 food samples collected in retail markets in 34 different cities in China were screened for colistin resistance. Of the 646 *V. parahaemolyticus* isolates tested, 25 (2.5%) exhibited colistin resistance. The *mcr-1* gene was detected in one colistin-resistant *V. parahaemolyticus* isolate, VP181, obtained from a shrimp sample collected in Hong Kong. The *mcr-1* gene was located on a transferable IncX4 plasmid with size of ~40 kb. A Class A *β*-lactamase gene, $bla_{CARB-17}$ and the plasmid-mediated quinolone resistance (PMQR) gene *qnrVC5* were detected in the *mcr-1*-positive *V. parahaemolyticus* isolate VP181. Virulence gene assays indicated that *tdh* was detected in VP181 by PCR. This is the first report of the occurrence of plasmid-encoded *mcr-1* in virulent *V. parahaemolyticus* strain. Our findings indicate horizontal transfer of this gene to non-*Enterobacteriaceae* gram-negative bacteria, which warrants further investigation because of the public health threat it poses.

1. Introduction

Antimicrobial resistance is now recognized as one of the most serious threats to global public health (Paterson and Harris, 2016). It is estimated that unless action is taken, the burden of deaths from antimicrobial resistance could balloon to 10 million lives every year by 2050, at a cumulative cost to global economic output of 100 trillion USD (O'Neill, 2016). Colistin (polymyxin E), which belongs to the family of cationic polypeptide antibiotics polymyxin, is the last line of defense against fatal infections caused by multidrug-resistant gram-negative bacteria, such as carbapenem-resistant *Klebsiella pneumoniae* and *Escherichia coli* (Quan et al., 2017; Wang et al., 2017). Although colistin has been used both in human and veterinary medicine for > 50 years, rates of colistin resistance have been relatively low until 2015, due to all reported colistin resistance is caused by chromosomal mutations and has long been thought to be non-transferable by mobile genetic elements (Schwarz and Johnson, 2016). However, a plasmid-mediated colistin-resistance gene, *mcr-1*, was first described by a research group in China in late 2015 (Liu et al., 2016). The plasmid-mediated transfer of the *mcr-1* gene enables a variety of bacteria to be tolerant with the antibiotic polymyxin, which poses a great challenge to the public health. Since its identification, *mcr-1* has been reported in > 30 countries or regions, spanning five continents (Skov and Monnet, 2016; Walsh and Wu, 2016). Although *E. coli* is the main host of *mcr-1*, several other *Enterobacteriaceae*, including *K. pneumoniae*, *Salmonella*, *Shigella somnei*, *Enterobacter aerogenes*, and *Enterobacter cloacae*, have been confirmed to carry this gene (Skov and Monnet, 2016; Walsh and Wu, 2016; Yi et al., 2017). However, to date, there has been no evidence of *mcr-1* in non-*Enterobacteriaceae* bacteria, such as *Vibrio parahaemolyticus*.

* Corresponding author at: Guangdong Institute of Microbiology, No. 100 Central Xianlie Road, Guangzhou, Guangdong Province 510070, China.
E-mail address: wuqp203@163.com (Q. Wu).

https://doi.org/10.1016/j.ijfoodmicro.2019.108290
Received 6 January 2019; Received in revised form 2 July 2019; Accepted 5 August 2019
Available online 07 August 2019
0168-1605/

V. parahaemolyticus is the leading cause of foodborne bacterial poisoning in China (Li et al., 2014). This organism bearing the *tdh* gene, which encodes thermostable direct haemolysin (TDH), is generally pathogenic to humans and responsible for the vast majority of clinical cases (Raimondi et al., 1995). Although most cases of *V. parahaemolyticus* infections are self-limiting, antimicrobial therapy is required for severe or prolonged infections (Wong et al., 2012). As a result of excessive use of antibiotics in human healthcare and aquaculture systems, antimicrobial resistance has emerged and evolved in *V. parahaemolyticus* during the past few decades (Cabello, 2006; Elmahdi et al., 2016; Li et al., 2015; Liu et al., 2013; Mazel and Davies, 1999). Surprisingly, extremely high resistance rates to colistin have been observed among *V. parahaemolyticus* isolates collected from both food and human samples in previous studies (Devi et al., 2009; Elmahdi et al., 2016; Lesmana et al., 2001). Since no additional information is available, it *is* unclear whether the high resistance rate was associated with the *mcr-1* gene. The aim of this study is to investigate the prevalence of *mcr-1* in *V. parahaemolyticus* isolated from food samples in China, and to carry out a comprehensive analysis, including *β*-lactamase, plasmid-mediated quinolone resistance (PMQR), and virulence genes analysis, and assessment of transfer of *mcr-1* gene, on the molecular characterization of *V. parahaemolyticus* strains carrying *mcr-1*-positive plasmid.

2. Materials and methods

2.1. Sample collection and bacterial identification

From July 2014 to July 2016, a total of 2531 food samples, including 1685 seafood samples (including 1020 shrimp samples and 665 fish samples) and 846 ready-to-eat food samples (including 576 deli meat samples, 179 cold vegetable dishes or noodles in sauce, and 91 fried rice or noodle samples), were randomly collected from retail markets in 34 different cities in China (Fig. 1). The isolation of *V. parahaemolyticus* was conducted as previously described, with slight modification (Wong et al., 2012). Briefly, all samples were inoculated on thiosulfate-citrate-bile salts-sucrose agar plates (Huankai Co. Ltd., Guangzhou, China) and incubated at 37 °C for 18–24 h. One suspected colony with typical *V. parahaemolyticus* morphology (green or blue-green colonies) and size (2–3 mm diameter) was selected from each sample, identified by a PCR assay targeting the *toxR* gene (Kim et al., 1999), and confirmed by the API 20E identification system (BioMérieux, Marcy l'Étoile, France).

2.2. Antimicrobial susceptibility testing

All *V. parahaemolyticus* isolates were screened for colistin resistance by the broth microdilution method as recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2017). The antimicrobial susceptibility of the *V. parahaemolyticus* isolate harboring *mcr-1* and its corresponding transconjugant to 15 antimicrobial agents was determined using broth microdilution method as recommended by the EUCAST and the Clinical and Laboratory Standards Institute (CLSI, 2010). The following antimicrobials were tested: colistin, ampicillin, piperacillin, cefazolin, cefoxitin, cefotaxime, cefepime, imipenem, meropenem, amikacin, gentamicin, tetracycline, ciprofloxacin, trimethoprim–sulphamethoxazole, and chloramphenicol. Results were interpreted according to the criteria of the CLSI (2010), except for colistin, for which results were interpreted according to EUCAST (2017). *E. coli* ATCC 25922 and *V. parahaemolyticus* ATCC 17802 were used as quality control strains.

2.3. Screening of the mcr-1 gene

Colistin-resistant isolates were screened for the presence of *mcr-1* gene by PCR with the primers described by Liu et al. (2016). The full length of *mcr-1* was amplified with primers MCR-F (ATGATGCAGCATACTTCTGTGTG) and MCR-R (TCAGCGGATGAATGCGGT). The PCR products were purified and sequenced to confirm the gene identity.

2.4. Screening of the β-lactamase, PMQR, and virulence genes tdh and trh

The presence of *β*-lactamase and PMQR genes were determined by PCR assay and confirmed by sequencing as previously described (Chiou et al., 2015; Dallenne et al., 2010; Liu et al., 2013). The virulence genes *tdh* and *trh* in *mcr-1*-positive *V. parahaemolyticus* were detected as described by West et al. (2013). The oligonucleotide primers were synthesized by Sangon Biotech (Shanghai, China) (Tdh-F: CTGTCCCTTTTCCTGCCCCCG, Tdh-R: AGCCAGACACCGCTGCCAT- TG; Trh-F: ACCTTTTCCTTCTCCWGGKTCSG, Trh-F: CCGCTCTCATATG- CYTCGACAKT) (West et al., 2013).

2.5. Plasmid analysis

Conjugation experiments were carried out as previously described (Liu et al., 2013), with slight modification. Briefly, donor (*mcr-1*-positive *V. parahaemolyticus*) and recipient (sodium-azide-resistant *E. coli* strain J53) strains were mixed in a 1:1 ratio, mated on a filter and selected on plates supplemented with colistin (2 mg/L) and sodium azide (100 mg/L) to select transconjugants. PCR-based replicon typing (PBRT) was conducted as described previously (Li et al., 2015). To analyze the location of the *mcr-1* gene, *S1* nuclease pulsed-field gel electrophoresis (*S1*-PFGE) and Southern blot analysis were carried out as described by Li et al. (2015). Plasmid stability was assessed by daily serial passages of culture without antibiotic; the culture was analyzed daily for colistin resistance and the presence of *mcr-1* was confirmed by DNA probing (Liu et al., 2016).

3. Results

3.1. Detection of mcr-1 in V. parahaemolyticus

Of the 2531 food samples, 646 *V. parahaemolyticus* isolates were obtained, including 592 isolates from seafood samples (including 426 from shrimp samples and 166 from fish samples) and 54 isolates from ready-to-eat food samples (including 36 from deli meat samples, 15 from cold vegetable dishes or noodles in sauce, and 3 from fried rice or noodle samples). Of the 646 *V. parahaemolyticus* isolates tested, 25 (2.5%) with a minimal inhibitory concentration (MIC) $\geq$2 mg/L, including 22 from the seafoods and 3 from the ready-to-eat food samples, were defined as colistin-resistant according to EUCAST clinical breakpoints. The *mcr-1* gene was detected in one colistin-resistant *V. parahaemolyticus* isolate, VP181, which was obtained from a shrimp sample collected from Hong Kong, China. Sequences of the *mcr-1* products obtained from VP181 showed that it was 100% identical to that reported by Liu et al. (2016).

3.2. Antimicrobial susceptibility of VP181

Antimicrobial susceptibility tests revealed that VP181 exhibited MIC of 4 mg/L to Colistin (Table 1). The isolate was also shown to be resistant to ampicillin, piperacillin, gentamicin, amikacin, and ciprofloxacin, but was susceptible to other antimicrobials tested, including carbapenems (Table 1).

3.3. Detection of the β-lactamase, PMQR, and virulence genes

β-lactamase and PMQR gene screening and sequencing showed that a Class A β-lactamase gene, $bla_{\text{CARB-17}}$, and the PMQR gene *qnrVC5* were detected in VP181. Virulence gene screening assays indicated that *tdh* was present in VP181.

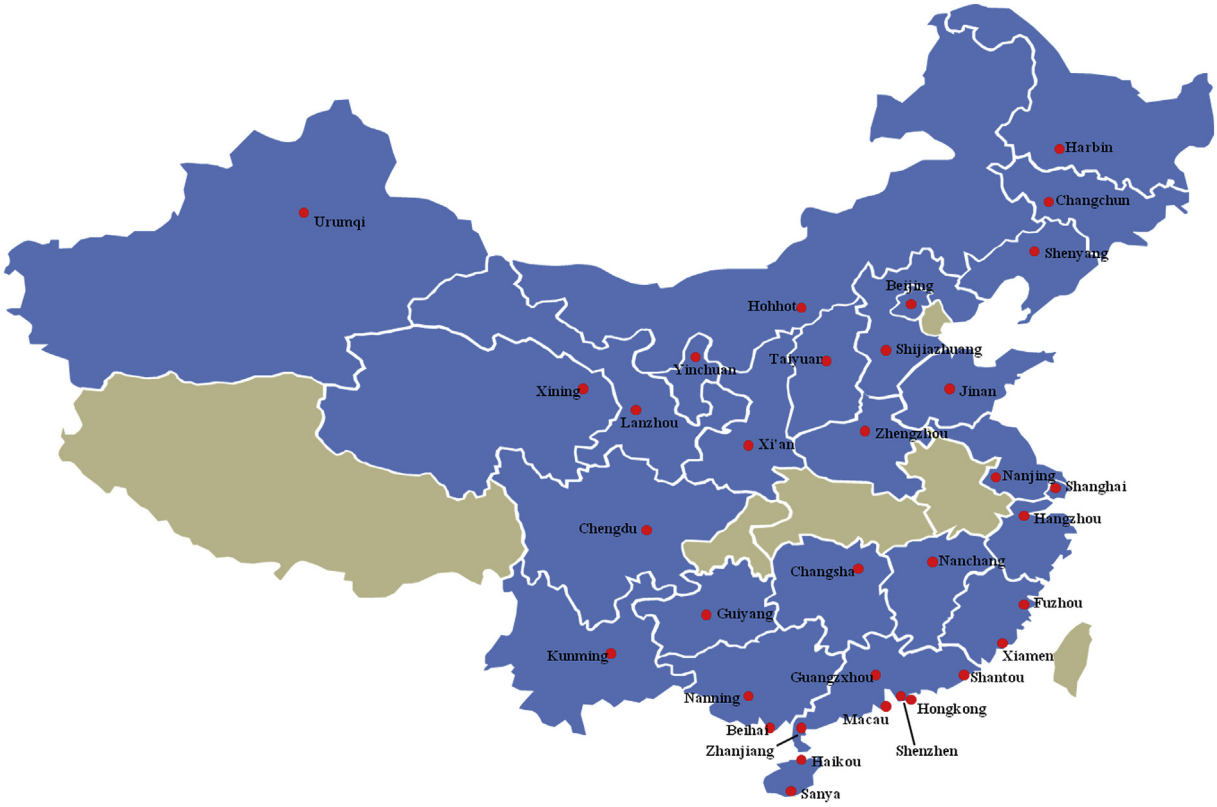

Fig. 1. The sampling sites of foods sample in China.
The sampling 34 cities including Harbin, Changchun, Shenyang, Hohhot, Urumqi, Xining, Lanzhou, Yinchuan, Xi'an, Taiyuan, Beijing, Shijiazhuang, Jinan, Zhengzhou, Nanjing, Shanghai, Hangzhou, Nanchang, Changsha, Guiyang, Chengdu, Kunming, Nanning, Beihai, Zhangjiang, Guangzhou, Shenzhen, Shantou, Xiamen, Fuzhou, Haikou, Sanya, Hongkong, Macau, are denoted with red dots. The provinces included in this study are shaded entirely with blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Antimicrobial resistance profiles of VP181 and its corresponding transconjugant VP181TC.

Antimicrobial agents	MIC (mg/L)		
	VP181	*E. coli* J53	VP181TC[a]
Colistin	4	0.25	4
Ampicillin	256	4	4
Piperacillin	> 512	2	4
Cefazolin	1	8	8
Cefoxitin	4	2	4
Cefotaxime	0.125	0.0625	0.0625
Cefepime	1	< 0.25	< 0.25
Imipenem	< 0.125	< 0.125	< 0.125
Meropenem	< 0.125	< 0.125	< 0.125
Amikacin	128	< 0.5	< 0.5
Gentamicin	64	0.125	0.25
Tetracycline	2	1	1
Ciprofloxacin	16	0.0625	0.125
SXT[b]	1/19	0.125/2.375	0.125/2.375
Chloramphenicol	2	4	4

[a] VP181TC, Transconjugant of VP181.
[b] SXT, trimethoprim–sulphamethoxazole.

3.4. Plasmid characterization

Conjugation experiments showed that the MIC of colistin against VP181TC (transconjugant of VP181) was 4 mg/L, which was equal to the MIC value of colistin against the donor VP181 and 16-fold higher than that of the recipient strain *E. coli* J53 (0.25 mg/L) (Table 1). These results indicated colistin resistance was transferrable from the VP181 to the recipient strain *E. coli* J53. However, there were no differences between the MICs of other 14 antimicrobials tested in this study against VP181TC and recipient strain *E. coli* J53, which demonstrated no other resistance was co-transferred (Table 1). The analysis of S1-PFGE revealed that VP181 contained two plasmids of ~40 kb and ~100 kb, respectively, but VP181TC only contained the ~40 kb plasmid, which suggested only the ~40 kb plasmid was transferrable to the recipient strain (Fig. 2A). PBRT results showed that the ~40 kb transmissible plasmid belonged to the IncX4 type plasmid, while the ~100 kb plasmid was untypeable. Southern Blotting hybridization with *mcr-1*-specific probe showed the *mcr-1* gene was located on the IncX4 plasmid of ~40 kb in both VP181 and its corresponding transconjugant VP181TC, which confirmed the *mcr-1* gene was transferrable to the recipient strain by conjugation (Fig. 2B). While southern Blotting hybridization with *qnrVC5*-specific probe showed the *qnrVC5* was only detected on the plasmid of ~100 kb, which demonstrated the *qnrVC5* was unable to transfer to the recipient strain by conjugation (Fig. 2C). The $bla_{CARB-17}$ gene was not detected on any of the two plasmids (data not shown). The plasmid carrying *mcr-1* was stable in both VP181 and its corresponding transconjugant VP181TC for up to 14 days of passage in the absence of colistin.

4. Discussion

V. parahaemolyticus is one of the important zoonoses that pose a threat to public health globally (Zhang et al., 2016). Although most cases of *V. parahaemolyticus* infections are self-limiting, the use of antimicrobials, such as tetracycline, quinolones, and third-generation cephalosporins, are necessary for the treatment of severe infections (Elmahdi et al., 2016). However, increasing antimicrobial resistance in *V. parahaemolyticus*, due to excessive use of antimicrobials in clinical

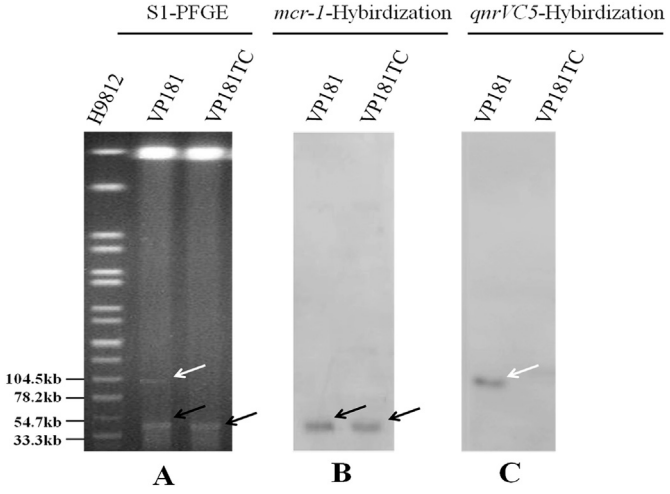

Fig. 2. S1-PFGE and Southern hybridization of VP181and its corresponding transconjugant VP181TC. A) Plasmid size determination by S1 nuclease-PFGE. B) Southern Blotting hybridization with *mcr-1*-specific probe. C) Southern Blotting hybridization with *qnrVC5*-specific probe. *Salmonella* H9812 served as the DNA marker. Plasmid carrying and transferring *mcr-1* is noted by the black arrow. Plasmid carrying *qnrVC5* is noted by the white arrow.

treatments and aquaculture production, has been observed in several countries, including the USA, Italy, Brazil, India, Mexico, and China (de Jesús Hernández-Díaz et al., 2015; Devi et al., 2009; Elmahdi et al., 2016; Melo et al., 2011; Ottaviani et al., 2013; Shaw et al., 2014; Xie et al., 2017). Both clinical and foodborne *V. parahaemolyticus* isolates are commonly resistant to ampicillin, penicillin, and tetracycline, and some isolates even show resistance to streptomycin, kanamycin, and trimethoprim/sulfamethoxazol (de Jesús Hernández-Díaz et al., 2015; Devi et al., 2009; Elmahdi et al., 2016; Melo et al., 2011; Ottaviani et al., 2013; Shaw et al., 2014; Xie et al., 2017). Surprisingly, extremely high resistance rates to colistin have been observed among *V. parahaemolyticus* isolates obtained from food (100%), environmental (100%), and clinical sources (97%) in previous studies (Devi et al., 2009; Elmahdi et al., 2016; Lesmana et al., 2001). In contrast to these reports, our study revealed a very low rate of colistin resistance in *V. parahaemolyticus* isolates recovered from food samples (2.5%) in China. It is noteworthy that one of the 25 colistin-resistant *V. parahaemolyticus* isolates in the present study, the resistance was attributed to the *mcr-1*-mediated mechanism, which suggests that plasmid-mediated colistin resistance is not even the main source of colistin resistance in *V. parahaemolyticus*. While resistance to colistin for the other 24 colistin-resistant *V. parahaemolyticus* isolates is likely mediated by chromosomally located genes (e.g. *pmrAB*, *phoPQ* and its negative regulator *mgrB*) (Liu et al., 2016).

Multiple studies have confirmed that *mcr-1*-positive *Enterobacteriaceae* strains, such as *E. coli* and *Salmonella*, usually also carry other resistance genes (Sun et al., 2016; Yang et al., 2016; Zheng et al., 2016). Accordingly, the Class A β-lactamase gene $bla_{\text{CARB-17}}$ and the PMQR gene *qnrVC5* were detected in the *mcr-1*-positive *V. parahaemolyticus* isolate VP181. The $bla_{\text{CARB-17}}$ gene may contribute to the resistance of VP181 to ampicillin and piperacillin, whereas *qnrVC5* is likely responsible for the resistance to ciprofloxacin. Although these two genes were not located on the conjugative plasmid harboring *mcr-1*, the co-existence of *qnrVC5* and *mcr-1* in *mcr-1*-positive *V. parahaemolyticus* highlights the need to limit its further dissemination, due to ciprofloxacin is a major agent of choice for the treatment for *V. parahaemolyticus* infections.

The prevalence of *mcr-1* on plasmids harbored by different bacterial species highlights its potential horizontal transfer. To date, *mcr-1* has been found on diverse plasmids belonging to the IncI2 (*E. coli*, *Salmonella*, *E. Sakazakii*), IncX4 (*E. coli*, *Salmonella*), IncHI2 (*E. coli*, *Salmonella*), IncP (*E. coli*, *Salmonella*), IncFII (*E. coli*), and IncF (*E. coli*) types (Campos et al., 2016; Falgenhauer et al., 2016; Gu et al., 2016; Li et al., 2016; Liu et al., 2016; Liu et al., 2017; Malhotra-Kumar et al., 2016; Webb et al., 2016; Zhao et al., 2017; Zhi et al., 2016). Among these, IncX4, which is associated with the spread of multiple resistance genes, has been widely identified in *E. coli*, *K. pneumoniae*, and *Salmonella enterica* from different sources and countries (Chen et al., 2013; Ho et al., 2013; Lo et al., 2014; Stokes et al., 2013). In the present study, the *mcr-1* gene was found to be located on an IncX4 plasmid in VP181, which strongly suggests that IncX4 plasmids harboring *mcr-1* have already circulated between *V. parahaemolyticus* and *Enterobacteriaceae* species.

V. parahaemolyticus is a gram-negative, halophilic bacterium that exists naturally in estuarine and marine environments, and is frequently isolated from aquatic products and seafoods (Makino et al., 2003). In this research, the *mcr-1*-positive *V. parahaemolyticus* isolate VP181 was obtained from a shrimp sample collected from Hong Kong, where *V. parahaemolyticus* is the leading cause of foodborne illnesses owing to the high rate of seafood consumption (Liu et al., 2013). Interestingly, a previous study showed that the plasmid-mediated colistin resistance gene *mcr-1*was detected in an ESBL producing *E. coli* ST10 strain retrieved from seawater at a public beach in Norway (Jørgensen et al., 2017). Furthermore, the *mcr-1* in VP181 was located on the IncX4 plasmid, which was also found in the *mcr-1*-positive *E. coli* ST10 strain isolated from seawater in Norway (Jørgensen et al., 2017). Although the relationship between the two isolates is unclear, together, these results indicate a potential transfer of the *mcr-1* gene from *E. coli* or other *Enterobacteriaceae* species to non-*Enterobacteriaceae* gram-negative bacteria, which needs close attention and warrants further investigation.

V. parahaemolyticus is the leading cause of seafood-associated bacterial gastroenteritis in many countries, including the USA and China (Elmahdi et al., 2016; Li et al., 2014). The pathogenesis and virulence of *V. parahaemolyticus* are commonly associated with two genes, *tdh* and *trh*, which encode thermostable direct haemolysin (TDH) and TDH-related haemolysin (TRH), respectively (Honda and Iida, 1993; Xie et al., 2017). According to West et al. (2013), *tdh*-positive isolates are more virulent than *trh*-positive ones. It is disconcerting that *tdh* was detected in the *mcr-1*-positive *V. parahaemolyticus* isolate VP181 in the present study, which suggests that the *mcr-1* gene is likely to spread into virulent *V. parahaemolyticus* strains.

5. Conclusion

In conclusion, we here report the first detection of the plasmid-mediated colistin resistance gene *mcr-1* in *V. parahaemolyticus*. To the best of our knowledge, this is also the first report of *mcr-1* in a non-*Enterobacteriaceae* species worldwide. Our study highlights a potential transfer of the *mcr-1* gene from *E. coli* or other *Enterobacteriaceae* species to non-*Enterobacteriaceae* gram-negative bacteria, and suggests the gene is likely to spread rapidly into virulent *V. parahaemolyticus* strains, which may pose a significant threat to public health and warrants further investigation.

Acknowledgements

This work was supported by the National Key Research and Development Program of China (2017YFC1600100), the Natural Science Foundation of Guangdong Province (2016A030310316, S2012030006235), the Science and Technology Planning Project of Guangdong Province (2017B020207007, 2017A070702018), and GDAS' Special Project of Science and Technology Development (2017GDASCX-0201).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2019.108290.

References

Cabello, F.C., 2006. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environ. Microbiol. 8, 1137–1144. https://doi.org/10.1111/j.1462-2920.2006.01054.x.

Campos, J., Cristino, L., Peixe, L., Antunes, P., 2016. MCR-1 in multidrug-resistant and copper-tolerant clinically relevant *Salmonella* 1, 4,[5], 12: i:-and S. Rissen clones in Portugal, 2011 to 2015. Eurosurveillance 21, 30270. https://doi.org/10.2807/1560-7917.es.2016.21.26.30270.

Chen, L., Chavda, K.D., Fraimow, H.S., Mediavilla, J.R., Melano, R.G., Jacobs, M.R., Bonomo, R.A., Kreiswirth, B.N., 2013. Complete nucleotide sequences of bla_{KPC-4}- and bla_{KPC-5}-harboring IncN and IncX plasmids from *Klebsiella pneumoniae* strains isolated in New Jersey. Antimicrob. Agents Chemother. 57, 269–276. https://doi.org/10.1128/AAC.01648-12.

Chiou, J., Li, R., Chen, S., 2015. CARB-17 family of β-lactamases mediates intrinsic resistance to penicillins in *Vibrio parahaemolyticus*. Antimicrob. Agents Chemother. 59, 3593–3595. https://doi.org/10.1128/aac.00047-15.

Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; approved guideline. In: CLSI (Ed.), CLSI document M45-A2, 2nd ed. Clinical and Laboratory Standards Institute, Wayne, PA.

Dallenne, C., Da Costa, A., Decré, D., Favier, C., Arlet, G., 2010. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in *Enterobacteriaceae*. J. Antimicrob. Chemother. 65, 490–495. https://doi.org/10.1093/jac/dkp498.

de Jesús Hernández-Díaz, L., Leon-Sicairos, N., Velazquez-Roman, J., Flores-Villaseñor, H., Guadron-Llanos, A.M., Martinez-Garcia, J.J., Vidal, J.E., Canizalez-Roman, A., 2015. A pandemic *Vibrio parahaemolyticus* O3: K6 clone causing most associated diarrhea cases in the Pacific Northwest coast of Mexico. Front. Microbiol. 6, 221. https://doi.org/10.3389/fmicb.2015.00221.

Devi, R., Surendran, P.K., Chakraborty, K., 2009. Antibiotic resistance and plasmid profiling of *Vibrio parahaemolyticus* isolated from shrimp farms along the southwest coast of India. World. J. Microb. Biot. 25, 2005–2012. https://doi.org/10.1007/s11274-009-0101-8.

Elmahdi, S., DaSilva, L.V., Parveen, S., 2016. Antibiotic resistance of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in various countries: a review. Food Microbiol. 57, 128–134. https://doi.org/10.1016/j.fm.2016.02.008.

EUCAST, 2017. The European committee on antimicrobial susceptibility testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 7.1. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_7.1_Breakpoint_Tables.pdf.

Falgenhauer, L., Waezsada, S.E., Yao, Y., Imirzalioglu, C., Käsbohrer, A., Roesler, U., Michael, G.B., Schwarz, S., Werner, G., Kreienbrock, L., Chakraborty, T., 2016. Colistin resistance gene *mcr-1* in extended-spectrum β-lactamase-producing and carbapenemase-producing Gram-negative bacteria in Germany. Lancet Infect. Dis. 16, 282–283. https://doi.org/10.1016/s1473-3099(16)00009-8.

Gu, D.X., Huang, Y.L., Ma, J.H., Zhou, H.W., Fang, Y., Cai, J.C., Hu, Y.Y., Zhang, R., 2016. Detection of colistin resistance gene *mcr-1* in hypervirulent *Klebsiella pneumoniae* and *Escherichia coli* isolates from an infant with diarrhea in China. Antimicrob. Agents. Ch. 60, 5099–5100. https://doi.org/10.1128/aac.00476-16.

Ho, P.L., Cheung, Y.Y., Lo, W.U., Li, Z., Chow, K.H., Lin, C.H., Chan, J.F., Cheng, V.C., 2013. Molecular characterization of an atypical IncX3 plasmid pKPCNY79 carrying bla_{KPC-2} in a *Klebsiella pneumoniae*. Curr. Microbiol. 67, 493–498. https://doi.org/10.1007/s00284-013-0398-2.

Honda, T., Iida, T., 1993. The pathogenicity of *Vibrio parahaemolyticus* and the role of the thermostable direct haemolysin and related haemolysins. Rev. Med. Microbiol. 4, 106–113. https://doi.org/10.1097/00013542-199304000-00006.

Jørgensen, S.B., Søraas, A., Arnesen, L.S., Leegaard, T., Sundsfjord, A., Jenum, P.A., 2017. First environmental sample containing plasmid-mediated colistin-resistant ESBL-producing *Escherichia coli* detected in Norway. APMIS 125, 822–825. https://doi.org/10.1111/apm.12720.

Kim, Y.B., Okuda, J., Matsumoto, C., Takahashi, N., Hashimoto, S., Nishibuchi, M., 1999. Identification of *Vibrio parahaemolyticus* strains at the species level by PCR targeted to the *toxR* gene. J. Clin. Microbiol. 37, 1173–1177.

Lesmana, M., Subekti, D., Simanjuntak, C.H., Tjaniadi, P., Campbell, J.R., Oyofo, B.A., 2001. *Vibrio parahaemolyticus* associated with cholera-like diarrhea among patients in North Jakarta, Indonesia. Diagn Mic. Infec. Dis. 39, 71–75. https://doi.org/10.1016/s0732-8893(00)00232-7.

Li, Y.H., Xie, X., Shi, X.L., Lin, Y.M., Qiu, Y.Q., Mou, J., Chen, Q.C., Lu, Y., Zhou, L., Jiang, M., Sun, H.H., Ma, H.W., Cheng, J.Q., Hu, Q.H., 2014. *Vibrio parahaemolyticus*, southern coastal region of China, 2007–2012. Emerg. Infec. Dis. 20, 685–688. https://doi.org/10.3201/eid2004.130744.

Li, R., Lin, D., Chen, K., Wong, M.H.Y., Chen, S., 2015. First detection of AmpC β-lactamase bla_{CMY-2} on a conjugative IncA/C plasmid in *Vibrio parahaemolyticus* of food origin. Antimicrob. Agents Chemother. 59, 4106–4111. https://doi.org/10.1128/AAC.05008-14.

Li, X.P., Fang, L.X., Song, J.Q., Xia, J., Huo, W., Fang, J.T., Liao, X.P., Liu, Y.H., Feng, Y., Sun, J., 2016. Clonal spread of *mcr-1* in PMQR-carrying ST34 *Salmonella* isolates from animals in China. Sci. Rep. 6, 38511. https://doi.org/10.1038/srep38511.

Liu, M., Wong, M.H.Y., Chen, S., 2013. Molecular characterisation of a multidrug resistance conjugative plasmid from *Vibrio parahaemolyticus*. Int. J. Antimicrob. Agents 42, 575–579. https://doi.org/10.1016/j.ijantimicag.2013.08.014.

Liu, Y.Y., Wang, Y., Walsh, T.R., Yi, L.X., Zhang, R., Spencer, J., Doi, Y., Tian, G.B., Dong, B.L., Huang, X.H., Yu, L.F., Gu, D.X., Ren, H.W., Chen, X.J., Lv, L.C., He, D.D., Zhou, H.W., Liang, Z.S., Liu, J.H., Shen, J.Z., 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect. Dis. 16, 161–168. https://doi.org/10.1016/S1473-3099 (15)00424-7.

Liu, B.T., Song, F.J., Zou, M., Hao, Z.H., Shan, H., 2017. Emergence of colistin resistance gene *mcr-1* in *Cronobacter sakazakii* producing NDM-9 and in *Escherichia coli* from the same animal. Antimicrob. Agents. Ch. 61https://doi.org/10.1128/aac.01444-16. (e01444-16).

Lo, W.U., Chow, K.H., Law, P.Y., Ng, K.Y., Cheung, Y.Y., Lai, E.L., Ho, P.L., 2014. Highly conjugative IncX4 plasmids carrying blaCTX-M in *Escherichia coli* from humans and food animals. J. Med. Microbiol. 63, 835–840. https://doi.org/10.1099/jmm.0.074021-0.

Makino, K., Oshima, K., Kurokawa, K., Yokoyama, K., Uda, T., Tagomori, K., Iijima, Y., Najima, M., Nakano, M., Yamashita, A., Kubota, Y., Kimura, S., Yasunaga, T., Honda, T., Shinagawa, H., Hattori, M., Iida, T., 2003. Genome sequence of *Vibrio parahaemolyticus*: a pathogenic mechanism distinct from that of *V cholerae*. Lancet 361, 743–749. https://doi.org/10.1016/S0140-6736(03)12659-1.

Malhotra-Kumar, S., Xavier, B.B., Das, A.J., Lammens, C., Butaye, P., Goossens, H., 2016. Colistin resistance gene *mcr-1* harboured on a multidrug resistant plasmid. Lancet Infect. Dis. 16, 283–284. https://doi.org/10.1016/s1473-3099(16)00012-8.

Mazel, D., Davies, J., 1999. Antibiotic resistance in microbes. Cell. Mol. Life Sci. 56, 742–754. https://doi.org/10.1007/s000180050021.

Melo, L.M.R.D., Almeida, D., Hofer, E., Reis, C.M.F.D., Theophilo, G.N.D., Santos, A.F.D.M., Vieira, R.H.S.D.F., 2011. Antibiotic resistance of *Vibrio parahaemolyticus* isolated from pond-reared Litopenaeus vannamei marketed in Natal, Brazil. Braz. J. Microbiol. 42, 1463–1469. https://doi.org/10.1590/s1517-83822011000400032.

O'Neill, J., 2016. Tackling drug-resistant infections globally: final report and recommendations. In: The Review on Antimicrobial Resistance, . http://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf.

Ottaviani, D., Leoni, F., Talevi, G., Masini, L., Santarelli, S., Rocchegiani, E., Susini, F., Montagna, C., Monno, R., D'Annibale, L., Manso, E., Oliva, M., Pazzani, C., 2013. Extensive investigation of antimicrobial resistance in *Vibrio parahaemolyticus* from shellfish and clinical sources, Italy. Int. J. Antimicro. Ag. (2), 191–193. https://doi.org/10.1016/j.ijantimicag.2013.05.003.

Paterson, D.L., Harris, P.N., 2016. Colistin resistance: a major breach in our last line of defence. Lancet Infect. Dis. 16, 132–133. https://doi.org/10.1016/S1473-3099(15)00463-6.

Quan, J., Li, X., Chen, Y., Jiang, Y., Zhou, Z., Zhang, H., Sun, L., Ruan, Z., Feng, Y., Akavo, M., Yu, Y., 2017. Prevalence of *mcr-1* in *Escherichia coli* and *Klebsiella pneumoniae* recovered from bloodstream infections in China: a multicentre longitudinal study. Lancet Infect. Dis. 17, 400–410. https://doi.org/10.1016/S1473-3099(16)30528-X.

Raimondi, F., Kao, J.P.Y., Kaper, J.B., Guandalini, S., Fasano, A., 1995. Calcium-dependent intestinal chloride secretion by *Vibrio parahaemolyticus* thermostable direct hemolysin in a rabbit model. Gastroenterology 109, 381–386. https://doi.org/10.1016/0016-5085(95)90324-0.

Schwarz, S., Johnson, A.P., 2016. Transferable resistance to colistin: a new but old threat. J. Antimicrob. Chemother. 71, 2066–2670. https://doi.org/10.1093/jac/dkw274.

Shaw, K.S., Goldstein, R.E.R., He, X., Jacobs, J.M., Crump, B.C., Sapkota, A.R., 2014. Antimicrobial susceptibility of *Vibrio vulnificus* and *Vibrio parahaemolyticus* recovered from recreational and commercial areas of Chesapeake Bay and Maryland coastal bays. PLoS One 9, e89616. https://doi.org/10.1371/journal.pone.0089616.

Skov, R.L., Monnet, D.L., 2016. Plasmid-mediated colistin resistance (*mcr-1* gene): three months later, the story unfolds. Euro. Surveill. 21, 30155. https://doi.org/10.2807/1560-7917.ES.2016.21.9.30155.

Stokes, M.O., Abuoun, M., Umur, S., Wu, G., Partridge, S.R., Mevius, D.J., Coldham, N.G., Fielder, M.D., 2013. Complete sequence of pSAM7, an IncX4 plasmid carrying a novel $bla_{CTX-M-14b}$ transposition unit isolated from *Escherichia coli* and *Enterobacter cloacae* from cattle. Antimicrob. Agents Chemother. 57, 4590–4594. https://doi.org/10.1128/AAC.01157-13.

Sun, J., Yang, R.S., Zhang, Q., Feng, Y., Fang, L.X., Xia, J., Li, L., Lv, X.Y., Duan, J.H., Liao, X.P., Liu, Y.H., 2016. Co-transfer of bla_{NDM-5} and *mcr-1* by an IncX3–X4 hybrid plasmid in *Escherichia coli*. Nat. Microbiol. 1, 16176. https://doi.org/10.1038/nmicrobiol.2016.176.

Walsh, T.R., Wu, Y., 2016. China bans colistin as a feed additive for animals. Lancet Infect. Dis. 16, 1102. https://doi.org/10.1016/s1473-3099(16)30329-2.

Wang, Y., Tian, G.B., Zhang, R., Shen, Y., Tyrrell, J.M., Huang, X., Zhou, H., Lei, L., Li, H., Doi, Y., Fang, Y., Ren, H., Zhong, L., Shen, Z., Zeng, K., Wang, S., Liu, J., Wu, C., Shen, J., 2017. Prevalence, risk factors, outcomes, and molecular epidemiology of *mcr-1*-positive *Enterobacteriaceae* in patients and healthy adults from China: an epidemiological and clinical study. Lancet Infect. Dis. 17, 390–399. https://doi.org/10.1016/S1473-3099(16)30527-8.

Webb, H.E., Granier, S.A., Marault, M., Millemann, Y., den Bakker, H.C., Nightingale, K.K., Bugarel, M., Ison, S.A., Scott, H.M., Loneragan, G.H., 2016. Dissemination of the *mcr-1* colistin resistance gene. Lancet Infect. Dis. 16, 144–145. https://doi.org/10.1016/s1473-3099(15)00538-1.

West, C.K.G., Klein, S.L., Lovell, C.R., 2013. High frequency of virulence factor genes *tdh*, *trh*, and *tlh* in *Vibrio parahaemolyticus* strains isolated from a pristine estuary. Appl. Environ. Microbiol. 79, 2247–2252. https://doi.org/10.1128/aem.03792-12.

Wong, M.H.Y., Liu, M., Wan, H.Y., Chen, S., 2012. Characterization of extended-spectrum-β-lactamase-producing *Vibrio parahaemolyticus*. Antimicrob. Agents Chemother. 56, 4026–4028. https://doi.org/10.1128/AAC.00385-12.

Xie, T., Wu, Q., Zhang, J., Xu, X., Cheng, J., 2017. Comparison of *Vibrio parahaemolyticus*

isolates from aquatic products and clinical by antibiotic susceptibility, virulence, and molecular characterisation. Food Control 71, 315–321. https://doi.org/10.1016/j.foodcont.2016.06.046.

Yang, Y.Q., Zhang, A.Y., Ma, S.Z., Kong, L.H., Li, Y.X., Liu, J.X., Davis, M.A., Guo, X.Y., Liu, B.H., Lei, C.W., Xiang, R., Wang, H.N., 2016. Co-occurrence of *mcr-1* and ESBL on a single plasmid in *Salmonella enterica*. J. Antimicrob. Chemother. 71, 2336–2338. https://doi.org/10.1093/jac/dkw243.

Yi, L., Wang, J., Gao, Y., Liu, Y., Doi, Y., Wu, R., Zeng, Z., Liang, Z., Liu, J.H., 2017. *mcr-1*-harboring *Salmonella enterica* Serovar typhimurium sequence type 34 in pigs, China. Emerg. Infec. Dis. 23, 291–295. https://doi.org/10.3201/eid2302.161543.

Zhang, Q., Dong, X., Chen, B., Zhang, Y., Zu, Y., Li, W., 2016. Zebrafish as a useful model for zoonotic *Vibrio parahaemolyticus* pathogenicity in fish and human. Dev. Comp. Immunol. 55, 159–168. https://doi.org/10.1016/j.dci.2015.10.021.

Zhao, F., Feng, Y., Lü, X., McNally, A., Zong, Z., 2017. IncP plasmid carrying colistin resistance gene *mcr-1* in *Klebsiella pneumoniae* from hospital sewage. Antimicrob. Agents. Ch. 61https://doi.org/10.1128/aac.02229-16. (e02229-16).

Zheng, B., Dong, H., Xu, H., Lv, J., Zhang, J., Jiang, X., Du, Y., Xiao, Y., Li, L., 2016. Coexistence of MCR-1 and NDM-1 in clinical *Escherichia coli* isolates. Clin. Infec. Dis. 63, 1393–1395. https://doi.org/10.1093/cid/ciw553.

Zhi, C., Lv, L., Yu, L.F., Doi, Y., Liu, J.H., 2016. Dissemination of the *mcr-1* colistin resistance gene. Lancet Infect. Dis. 16, 292–293. https://doi.org/10.1016/s1473-3099(16)00063-3.