Virulence gene, antimicrobial resistance and phylogenetic characterization of *Vibrio parahaemolyticus* in migratory birds, Guangdong

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Research article

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Abstract

Background

Vibrio parahaemolyticus is an intestinal pathogen that is transmitted by contaminated aquatic products.

Migratory birds ate foods infected with *Vibrio parahaemolyticus*, excreting feces on other foods. Other organisms ate contaminated food again, leading to the spread of strains. It were reported previously that *Vibrio parahaemolyticus* was sensitive to most antibiotics except penicillin such as ampicillin and amoxicillin. However, due to the frequent used of antibiotics in recent years, *Vibrio parahaemolyticus* developed resistance to antibiotics. The recommended antibiotics for *Vibrio parahaemolyticus* treatment were tetracycline, β-lactam, quinolone and sulfonamide. In the storage migratory bird, *Vibrio parahaemolyticus* could complete the accumulation of drug resistance, resulting in the generation of drug resistance genes, or the horizontal drug resistance genes transfer from other bacteria, resulting obtain drug resistance genes.

Results

In this study, 122 isolates of *Vibrio parahaemolyticus* were obtained for further pathogenicity identification, antibacterial susceptibility testing of 18 antimicrobial compounds and genetic characterization based on the whole genome sequencing. There is no pandemic serotype isolated between migratory birds, but 37 strains with cholera toxin. Five strains had *col3M* plasmid-moderately resistant drug-resistant strains of quinolone mediated by the *qnrD1* gene. For the first time in China, *Vibrio parahaemolyticus* with col3M plasmid-mediated *qnrD1* gene was isolated from migratory birds.

Conclusion

According to the mutltilocus sequence typing analysis, authors found the bacterial genetic diversity is higher, the clones are less and there is regional aggregation in Shenzhen. And mallards are more susceptible to *Vibrio parahaemolyticus*.

Background

Vibrio parahaemolyticus was a main zoonotic pathogenic enteropathogenic bacteria in seafood [1]. It usually caused acute gastroenteritis after infection, and gastrointestinal bleeding, which was characterized by fever, vomiting, diarrhea and blood in the stool [2]. It could also threaten life safety. The serotype associated with human disease was the O3:K6 pandemic clone, which contain both thermostable direct hemolysin (*tdh*) and *toxRS/new* virulence genes [3, 4]. According to the US Centers for Disease Control and Prevention (CDC), the incidence of *VP* infection had increased significantly since 2001 [5]. In the United States, an average of two hundred and fifteen people were infected with *VP* each year, of which thirty are hospitalized and one or two are fatal [5]. In recent years, some studies shown that serotypes other than the O3:K6 pandemic clone also contain strong virulence genes [6]. For example,

some non-pandemic clone strains of *VP* also had a single *tdh* without a *toxRS*, or some the strains contained thermostable related hemolysin (*trh*) and urease (*ure*) [7]. V. *parahaemolyticus* containing these genes could also cause cholera-like food poisoning. Thermolabile hemolysin gene (*tlh*) was generally considered to be non-pathogenic, but there were experiments [7]. It was indicated that raw seafood infected with *VP* containing the *tlh* could also cause diarrhea and blood in the stool. Some *VP* strains also had effector proteins in the type III secretion system and the type IV secretion system [7–9]. In addition, *VP* forms a viable but non-cultivable (VBNC) state when the external conditions were poor (eg, lower temperature, lower pH, lower nutrient conditions, etc.) [10]. It posed a major threat to food safety in the modern life.

Clinical studies have highlighted the importance of identifying drug resistance and detecting virulence genes. It were reported previously that *VP* was sensitive to most antibiotics except penicillin such as ampicillin and amoxicillin [11]. However, due to the frequent used of antibiotics in recent years, *VP* developed resistance to antibiotics [11–13]. The recommended antibiotics for *VP* treatment were tetracycline, β-lactam, quinolone and sulfonamide [5, 14]. Among them, tetracycline or ciprofloxacin could be used for long-term *VP* treatment too [5, 14].

It was found that *VP* spread by migratory birds as a medium [15]. Migratory birds ate foods infected with *VP*, excreting feces on other foods [16]. Other organisms ate contaminated food again [17]. The amount of bacteria in the feces were rich, and it was easier to transfer the resistance genes between bacteria [18]. Therefore, migratory birds were considered to be important reservoirs of *VP*, forming a fecal-food-mouth route [15]. Therefore, some foods might not be seafood or might contaminate *VP*. In the storage migratory bird, *VP* could complete the accumulation of drug resistance, resulting in the generation of drug resistance genes, or the horizontal drug resistance genes transfer from other bacteria, resulting obtain drug resistance genes.

Few studies involved the isolation of the strains from migratory birds to analyze genetic characteristics, including virulence genes, drug resistance genes to analysis genetic characteristics. The aim of the study was to investigate the strain isolation rates of migratory birds in three provinces, nine cities to found out the potential antibiotic resistance, potential pathogenicity and multilocus sequence typing (MLST). In order to better understand the evolution of *VP* in other hosts, it is better to control the spread of *VP* to ensure food safety.

Results

Epidemiological investigation of migratory birds *VP*

We analyzed 122 *VP* strains, from which 781 of migratory birds feces were found from China as detected by PCR (read Table S1). *VP* was detected in 122 (15.62% ,122/781) samples and the prevalence of *VP* contamination varied geographically between 0% and 71.43% in 9 cities, 3 provinces, China. The mallard feces showed the higher contamination prevalence than others. The isolation rate of *VP* isolated from the migratory bird droppings in Shenzhen was 71.43% , which was higher than other cities in 2019. And the

percent of 2019 in Shenzhen was higher than 2018. The samples from Guangxi and Ningxia provinces were negative for *VP.* In addition, the prevalence of *VP* contamination in Zhaoqing, Guangdong was negative, too. The separation of *VP* was mainly concentrated in Shenzhen (read Table 1). We had two sampling points in Shenzhen, Overseas Chinese Town Wetland Park (OCT) (22°55'50"N/114°02'02" E) and Futian Red Forest reserve (Futian) (22°52'81"N/114°00'93" E). Of the 63 samples collected in Shenzhen, 48 samples were collected in OCT, and 21 samples were collected for the Futian in 2018. The sampling separation rate in OCT was 37.5%, higher than Futian. The separation rate of OCT in 2019 was higher than that in 2018, but the separation rate of Futian in 2019 was lower than that in 2018 (read Table 2). Strains didn't carry *tdh, trh* and *ure.* All strains had conservative hemolysin *tlh,* but they didn't product hemolytic ring.

The minimum inhibitory concentration of migratory birds *VP*

The minimum inhibitory concentration and drug-resistance profiles of 122 *VP* isolates were shown in Table S2. The minimum inhibitory concentration of amikacin against *VP* was concentrated at 6-24mg/mL. However, the MIC value of 9 strains reached more than 16mg/mL , showing moderate resistance. For β-lactam drugs, *VP* had strong resistance to penicillin, of which 36.1% had strong resistance to ampicillin and 35.2% had srong drug resistance to amoxicillin, and the MIC value for 2 strains of ampicillin is up to 256mg/mL, and the MIC value for 1 strain of amoxicillin is up to 256mg/mL. The MIC value of one strian for ampicillin and amoxicillin is up to 256 mg/mL. However, the MIC value is significantly reduced after the addition of the enzyme inhibitor. Except cefotaxime, *VP* is mostly sensitive for other cephalosporins. For Chloramphenicol, Quinolones, Tetracycline and Sulfonamide**,** *VP* is mostly sensitive.

From the comparison of MIC, it was found that the MIC of the two sampling points were different. The Figure 1a shown that MIC value from OCT and Futian for amoxicillin were concentrated at 2-24 mg/mL and 1.5-8 mg/mL. And the Figure 1b shown that MIC value from OCT and Futian for ampicillin were concentrated at 5-32 mg/mL and 2-32 mg/mL. The resistance between strains from mallard, ardeola bacchus and charadriiformes was quite different, too. For strains from ardeola bacchus, the MIC of amoecillin for them were 1.5-12mg/mL, while the MIC of ampicillin were concentrated at 2-32mg/mL. For mallard, MIC of amoxicillin for *VP* was concentrated at 1.5-32mg/mL, while the MIC of ampicillin was concentrated at 6-32mg/mL. For charadriiformes, MIC of amoxicillin for *VP* was concentrated at 1.5-12 mg/mL, while the MIC of ampicillin was concentrated at 6-32mg/mL (Figure 2). From the comparison of MIC, it was found that MIC value of two years were different. The Figure 3a shown that MIC value from 2018 and 2019 for amoxicillin were concentrated at 1-8 mg/mL and 1.5-24 mg/mL. And the Figure 3b shown that MIC value from OCT and Futian for ampicillin were concentrated at 1-32 mg/mL and 8-32 mg/mL.

Table 1
Prevalence of V. *parahaemolyticus* in 3 provinces, China

collection time	Sample collection location	Sample category	V.*parahaemolyticus*(%)	separation rate(%)
2018.1.5	Shenzhen, Guangdong	Mallard feces	18/48 (37.5)	56.52
		Heron feces	6/21(28.57)	
2018.1.8	Zahoqing, Gaungdong	Red-crowned crane feces	0/26 (0)	0
		Cormorant feces	0/23 (0)	
		Heron feces	0/18 (0)	
2018.1.13	Beihai, Guangxi	migratory bird feces	0/65 (0)	0
	Fangcheng, Guangxi	migratory bird feces	0/91 (0)	
2018.1.14	Nanning, Guangxi	migratory bird feces	0/70 (0)	0
2018.1.15	Dongxing, Guangxi	migratory bird feces	0/72 (0)	0
2018.3.29	Zhongxing, Ningxia	Mallard feces	0/100 (0)	0
		Bone cranes feces	0/125 (0)	
2019.3	Shenzhen, Guangdong	Mallard feces	33/18 (100)	71.43
		Charadriiformes feces	44/38 (50)	
2019.3	Zhanjiang, Guangdong	Heron feces	8/31 (16.1)	16.1
2019.3	Leizhou, Guangdong	Heron feces	6/22 (22.7)	25.7
		Charadriiformes feces	6/13 (30.8)	

Table 2
Prevalence of V. *parahaemolyticus* in Shenzhen

collection time	Sample collection location	Sample category	V.*parahaemolyticus*(%)	separation rate(%)
2018.1.5	OCT,Shenzhen	Mallard feces	14/32 (72.7)	66.70
		Heron feces	4/16 (50)	
2018.1.5	Futian, Shenzhen	Mallard feces	4/18 (33.33)	33.33
		Heron feces	2/3(33.33)	
2019.3	OCT,Shenzhen	Mallard feces	36/18 (100)	92.9
		Charadriiformes feces	40/23 (95.7)	
2019.3	Futian, Shenzhen	Charadriiformes feces	4/15 (13.3)	13.3

Virulence gene and drug resistance gene of migratory birds *VP*

We found two kinds of cholera toxin, including ace and zot. All strains didn't have - secretory system and 1-7 pathogenic island. Six kinds of drug resistance genes were we found, including *CARB, qnr, tet, fos, dfrA and sul*. 99.19% of strains contained the *CARB* genes, which was the hydrolase gene [19]. *CARB-33* was distributed more frequently in strains, but it was not found in 2018. *CARB-27* was only found in 2018, it was not found in 2019. Two kinds of *qnr* genes were detected in migratory birds *VP*, one was *qnrD1*, the another was *qnrC* (read Table S2).

Moible components of migratory birds *VP*

We analysis moible components of 37 strains with special drug resistance genes and cholera toxin. We only found immovable super-integron (SI) in all strains and without - integrons. Five strains had *col3M* plasmid-moderately resistant drug-resistant strains of quinolone mediated by the *qnrD1* gene. Only one kind of transposon, Tn7 was found in strains. We screened 17 kinds of insert components by IS finder. Except SH7-2 and SH13-1, the another strains with one completed phage at least (detail in Table S3).

Multilocus sequence typing of migratory birds *VP*

We found that strains evolved faster, with higher genetic diversity leading to ST type failure. Only 24 strains could be used for ST typing. The results showed that the clones higher in Zhanjing and Leizhou than Shenzhen through ST typing. ST3 didn't not exist in our strains (read Table S2 and Table S4).

Discussion

We collected migratory birds feces from Guangdong, Guangxi and Ningxia according to their migration route. *VP* was only positive in Guangdong and negative in other province. As an important entry and the exit ports in China, Guangdong and Shanghai are not only economically developed, but also attract a large number of winter migratory birds because of their pleasant environment [20]. Among them, the mallard feces and OTC, Shenzhen showed the higher contamination prevalence than others. Isolation of strains showed species aggregation and regional aggregation. Through virulence gene screening, it was found that *tdh, trh, ure* and other genes were not isolated. Studies have found that genes including *tdh, trh* and *ure* are introduced as foreign genes when *VP* evolved in response to adverse external environment [21]. At present, the pathogenicity was weak, but it did not rule out that the bacteria would evolve to produce cytotoxin or enterotoxin, when the external pressure reached a certain condition [22,23]. But *tdh* gene was able to integrated to migratory birds *VP.* The probability of them was lower than aquatic products [24].

Cholera toxin was a complex protein including *ace, zot, toxR, toxS, ctxA and ctxB.* We found all strains with *toxR* and *toxS*, among them, 37 strains carried *ace* and *zot*. One strain, SH15-2 with resistance gene, *qnrD1*. SH15-2 was isolated from mallard in Shenzhen. *Ace* and *zot* leaded cell membrane permeability to change of human small intestine, human diarrhea [25]. The same cholera toxin was integrated to non-migratory birds *VP* through VfO3K6 and VfO4K68 in Mexico [26]. But, six strains (1-2, 6-1, 16-2, 33-1, 33-2 and SH7-1) of migratory birds *VP* didn't carry VfO3K6 and VfO4K68. They were all with VCY phage. It was likely that VCY phage also has the ability to carry cholera toxin. It's interesting, *ace* and *zot* showed regional aggregation in Zhanjiang and Leizhou.

Migratory birds *VP* without common plasmids, but catched a kind of plasmid named *col3M.* The strains with plasmids all contained *qnrD1*, so it was most likely that the plasmid played a significant role in transfering *qnrD1* gene [27,28]. The MIC values of quinolone to migratory birds *VP* was low. And the value of strains with other drug resistance genes was low, too. The drug resistance genes showed implicit expression. Except *qnrD1*, other drug resistance genes didn't located in moible components that we found. Only strain 34-1 without *CARB* genes, the rest of 99.19% Migratory birds *VP* with *CARB* genes. Recent studies shown that non-migratory birds *VP* with *CARB* from NCBI. And *CARB-17* could be integrated into other bacteria through type 1 integrons, such as: *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, et al [29,30]. *CARB* genes were inherent gene in *VP,* probably. They are not host specific.

CARB genes with diverse evolution in migratory birds *VP.* A strain with the *CARB-46* achieved MIC of 256mg/mL to ampicillin and amoxicillin. Not only every strains contained one *CARB* genes, some strains contain two or more *CARB* genes. Isolated 3 strains with *CARB-17* from charadriiformes and mallard feces in 2019 and these strains contained *CARB-20*. They didn't have type 1 integron. These three strains had strong resistance to ampicillin and amoxicillin, but it was not obvious resistance to other β-lactam drugs. 40% of the strains had strong resistance to ampicillin, but only three strains had *CARB-17*. we counldn't be sure that *CARB-17* caused ampicillin resistance. 36 strains contained the *CARB-33*, and ten of them were strong resistant to ampicillin in 2019. Four of strains containing the *CARB-27,* and the MIC

ranged from 1.5-12mg/mL to ampicillin. Degree of evolution for *CARB* genes might be less related to drug resistance.

Except *CARB* genes, other resistance genes isolated from Shenzhen, one strain, and one strain, L26-1 with *sul2* and *tet(59)* was isolated from Leizhou. It was probablely, the higher population density and pollution was more serious of Shenzhen than other places. Compared with the species with special resistance genes, only two strains (34-1, L26-1) were isolated from Charadriiformes, and the rest of these were isolated from mallard. The isolation rate of *VP* in mallard was higher than that from Charadriiformes, which leaded to more strains containing drug resistance genes.

MIC value was different to different kinds of migratory birds. This maybe ardeola bacchus migrated for a shorter distance or did not migrate, while mallard and charadriiformes was a migratory bird [31,32]. During long-term migration, the accumulation of antibiotics in the body leads to an increase of MIC, leading to *VP* micro-evolution in mallard [33-35]. Thus, the exogenous resistance gene was obtained and a drug resistant strain was produced [36]. For other antibiotics, *VP* showed a more sensitive state, which was not much different from the reported results [11-14]. It was only resistant to the first generation cephalosporin of penicillin. However, by observing the MIC to *VP*, it was found that, for the third-generation cephalosporin and the fourth-generation cephalosporin, MIC began to increase, indicating that *VP* was stimulated by drugs such as cephalosporin. And over time, the MIC value of *VP* for penicillin antibiotics began to rise. Resistance was to develop, perhaps there were other epistasis expressions within the genome, resulting in a drug-resistant phenotype which was not very obvious [37,38].

The MIC of the drug was found to be at a relatively low level for *VP*, but the overall trend was an upward trend, and all strains contained the *CARB* genes. Some of them were mediated by plasmids. Then the quinolone resistance gene *qnrD1* was obtained, indicating that the resistance of *VP* would be a serious problem. In addition, the resistance of *VP* was related to the species of migratory birds, but it has no obvious relationship with the collection site and MLST classification.

ST3 were not found in these strains, lower risk of disease. Most strains were typed, Only 24 strains could be used for ST typing. The clones were higher in Leizhou and Zhanjiang than Shenzhen. The population density of Shenzhen was higher than Leizhou and Zhanjiang. And lots of natural landscape in Leizhou and Zhanjiang, in contrast, lots of parks were builded in Shenzhen. The migratory birds ate complicated food in Shnezhen, they only ate worms, fishes and shrimps in Leizhou and Zhanjiang.

Migratory birds, as a new storage of *VP*, have not received the attention of researchers [39]. Migratory birds *VP* was a kind of conditioned pathogen, some of them caused diarrhea. *VP* had a strong adaptability and evolution fastly [40]. Migratory birds, a kind of migratory animal, could spread bacteria and viruses of their intestine to the migration path and cause unnecessary harm [41].

The bacterial was induced to evolution in in the intestinal tract of migratory birds by eating water and food contaminated with antibiotics [42]. Although migratory birds *VP* were only strong resistance to

ampicillin and amoxicillin, resistance genes of recommended antibiotics for *VP* treatment were found in strains.

Conclusions

At present, the isolation rate of strains carrying *tdh* was lower than in aquatic products. Because of migratory birds as a new carrier of VP, and it was still adapting to the intestines of migratory birds. Leaded to genes deletion, and the chance of obtaining *tdh, trh* and *ure* was not high. Only Tn7 transposon was found in migratory birds *VP*, and other transposons could not be found. These strains maybe had better capture of Tn7 transposon. We only found one plasmid named *col3M*, and *qnrD1* was found in the strains where the plasmid was found. But for other drug resistance genes, no related moving elements have been found. And there maybe unknown moving elements. It showed that strains with strong resistance to ampicillin and amoxicillin, and it was not recommended to use penicillins to treat *VP* infection. For recommended antibiotics for *VP* treatment, the MIC value was low. But Some strains had obtained resistance genes. Although these strains had not formed a clear phenotype, we still couldn't ignore the drug resistance of *VP*. Incidence of contamination for *VP* in mallards showed a higher than ardeola bacchus isolates, and the drug resistance was relatively high. All strains contained a conserved virulence gene *tlh*, indicating that *VP* was genetically relatively conserved in pathogenicity. The strains evolved rapidly, with high genetic diversity, fewer clones, and no geographical aggregation. *Col3M* Plasmid-mediated quinolone-resistant *VP* was located scattered in phylogenetic trees and had multiple ancestors. The strains were found by comparing *VP* from non-migratory bird source to be severely spread. It was worth noting that the evolutionary trend of *VP* to high drug resistance and high pathogenicity highlights the potential health risks to human life. Since the contamination and spread of *VP* due to excretion of manure during migration of migratory birds indicated significant food safety and public health problems, Chinese food safety and veterinary agencies should consider more effective measures to control the use of antibiotics. Due to the diffusivity and evolution of *VP*, the prevention of *VP* not only should stay on aquatic products, but also integrate into all aspects of life.

Methods

VP isolation and identification

Regarding migratory bird faeces sampling, a total of 781 samples including mallards (n=166), heron (n=92), Red-crowned crane (n=26), Cormorant (n=23), Bone cranes (n=125), Charadriiformes (n=51) and other migratory birds (n=298) from different cities (Shenzhen, Zahoqing, Beihai, Fangcheng, Nanning, Dongxing, Zhongxing, Zhanjiang, Leizhou) was randomly collected. All samples were transported to local laboratories within 3h at 4°C in a proper container for *VP* analysis. The isolation and identification of *VP* was conducted based on the method described by National Food Safety Standards of China (GB 4789.7-2013) [43]. Breifly, 25g faece samples were homogenized with 225mL of phosphate-buffered saline (PBS) for 15–30s in a 4mL centrifuge tube followed by incubation at 37°C for 16h. 100μL aliquots were plated on both CHROM agar Vibrio plates (CHROM, Paris, France). They were elementarily identified as

purple colored colonies by CHROM agar. Isolates were transferred onto Thiosulfate-citrate-biliary-sucrose agar (TCBS, Qingdao Haibo,China) and cultivated at 37℃ for 16 h. Biochemical tests were directed using Biochemical identification strip (HBI, Qingdao Haibo, China) to assure the phenotypical identity of the isolates. Prior to each experiment, the strains were activated by transferring from strains which stored at -80 ℃ to CHROM agar and incubated overnight at 37℃. After 16 h incubation a single colony was taken from each plate and inoculated into 5 mL brain heart liquid (Qingdao Haibo, China), then they incubated overnight at 37℃ in shaking incubator at 220 rpm. Pick 2 single colonies on each plate for further PCR identification (Table 3) [43,44].

Table 3
Identification primers of V. *parahaemolyticus* .

Primer	Sequence (3' to 5')	Target gene	Annealing temperature (℃)	Amplicon (bp)
toxR-F	TGTACTGTTGAACGCCTAA	*toxR*	55	503
toxR-R	CACGTTCTCATACGAGTG			
tdh-F	GTAAAGGTCTCTGACTTTTGGAC	*tdh*	60	270
tdh-R	TGGAATAGAACCTTCATCTTCACC			
trh-F	TTGGCTTCGATATTTTCAGTATCT	*trh*	60	500
trh-R	AACAAACATATGCCCATTTCCG			
tlh-R	AAAGCGGATTATGCAGAAGCACTG	*tlh*	60	450
tlh-F	GCTACTTTCTAGCATTTTCTCTGC			

Minimum inhibitory concentration determination

The MIC determination of selected strains was determined by macro-broth dilution method. Strains were tested in flat containing 25mm thick MH solid medium, reference strain was ATCC25922 [45]. Eighteen antibiotics were tested in this study (Table 4). Strains adjusted to calculate starting OD 500 by Meishi turbidity (*Box15969)* and spread in MH liquid medium. The zones of antibiotic dilution were measured according to the guidelines of Clinical and Laboratory Standards Institute [46]. Starting concentration of 256mg/mL, serial dilution of 18 gradients to 0.0625mg/mL, determined MIC values by Etest strips. Most of the antimicrobials tested in this study were used in agriculture and aquaculture fields, as well as in the treatment of *vibrio* infections. Then mediums were incubated at 37℃ for 16 h [47].

Table 4
Antimicrobial susceptibility of V. *parahaemolyticus* isolates.

Number	Antibiotic type	Antibiotics
01	Aminoglycosides	Amikacin(AK)
		Gentamicin(GM)
02	Carbapenem	Meropenem(MEM)
		Imipenem(IP)
03	β-lactam	Cefotaxime(CTX)
		Cefepime (FEP)
		Ceftazidime(CAZ)
		Aztreonam(ATM)
		Amoxicillin(AC)
		Ampicillin(AMP)
		Amoxicillin/clavulanic acid(AMC)
		Ampicillin/sulbactam(SAM)
04	Chloramphenicol	Chloramphenicol(C)
05	Quinolones	Ciprofloxacin (CIP)
		Levofloxacin(LVX)
		Moxifloxacin (MXF)
06	Tetracycline	Tetracycline(TE)
07	Sulfonamide	Trimethoprim/sulfamethoxazole(SXT)

Extraction of genome DNA and library construction

Genomic DNA was extracted with Bacterial Genomic DNA Extraction Kit (Omega). The harvested DNA was detected by Qubit® 2.0 Fluorometer (Thermo Scientific). A total amount of 1μg DNA per sample was used as input material for the DNA sample preparations. Sequencing libraries were generated using NEBNext® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) following manufacturer's recommendations and index codes were added to attribute sequences to each sample. Briefly, the DNA sample was fragmented by sonication to a size of 350bp, then DNA fragments were end-polished, A-tailed, and ligated with the full-length adaptor for Illumina sequencing with further PCR amplification. At last, PCR products were purified (AMPure XP system) and libraries were analysed for size distribution by

Agilent2100 Bioanalyzer and quantified using real-time PCR. The whole genome of VP was sequenced using Illumina NovaSeq PE150 at the Beijing Novogene Bioinformatics Technology Co., Ltd.

Genome assembly

Illumina PCR adapter reads and low quality reads from the paired-end were filtered by the step of quality control using readfq(vision 10). All good quality paired reads were assembled using the SOAP denovo[48,49] (http://soap.genomics.org.cn/soapdenovo.html) SPAdes[50] http://cab.spbu.ru/software/spades/ and ABySS[51] http://www.bcgsc.ca/platform/bioinfo/software/abyss into a number of scaffolds. Then the filter reads were handled by the next step of the gap-closing.

Readfq command line arguments: –rq1 input_1.fq, –rq2 input_2.fq,–oq1 out_1.fq,–oq2 out_2.fq,–adp1 adapter_1.lst,–adp2 adapter_2.lst,– Q QUAL,PERCENT,–C QUAL,PERCENT,–N PERCENT,–alen INT,–amis INT,–dup,–gz,–check1 read1.check,–check2 read2.check

Genome Component prediction and gene function

For bacteria, we used the RAST(https://rast.nmpdr.org/) program to annotation genome. The IS finder (http://issaga.biotoul.fr/ISsaga2/issaga_index.php)was used for the insert element. The PHAST [52] was used for the prophage prediction (http://phast.wishartlab.com/).

Bioinformatics analysis.

Antimicrobial resistance genes in the genome of *VP* were predicted by the ARDB (http://ardb.cbcb.umd.edu/)[53]. Virulence factors in the genome of *VP* were identified using the VFDB (http://www.mgc.ac.cn./VFs/main.htm)[54]. Finally, the PlasmidFinder 1.3 (ftp://ftp.ncbi.nih.gov/genomes/Plasmids/) was used to predict the presence of any antimicrobial genes or virulence genes contained in the genome of plasmid.

Multilocus sequence typing

Seven restriction enzyme cutting sites (DnaE, gyrB, recA, dtds, pntA, pyrC and tnaA) in the genome of *VP* were identified by MLST(https://pubmlst.org/databases.shtml)

Abbreviations

VP: Vibrio parahaemolyticus; PCR: Polymerase chain reaction; MLST: Multilocus sequence typing; OCT: Overseas Chinese Town; ARDB: Antibiotic Resistance Genes Database; VFDB: virulence factor database; MIC: minimum inhibitory concentration.

Declarations

Ethics approval and consent to participate

All migratory bird stool samples were collected under the supervision by the Wild Animal Sources and Diseases Inspection Station, National Forestry and Grassland Bureau, and did not cause any harm to the animals. The experimental protocol was approved by the Laboratory Animal Welfare and Ethics Committee of the Key Laboratory of Jilin Province for Zoonosis Prevention and Control.

Consent for publication

Not Applicable.

Availability of data and material

All data generated or analysed during this study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

PC, XJG conceived, directed, and carried out the study. LWZ, YW, JJ and BL prepared samples for sequence analysis; XJ, JYG and LZ acquired samples, and other people analyzed the data. All authors have read and approved the final manuscript.

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Figures

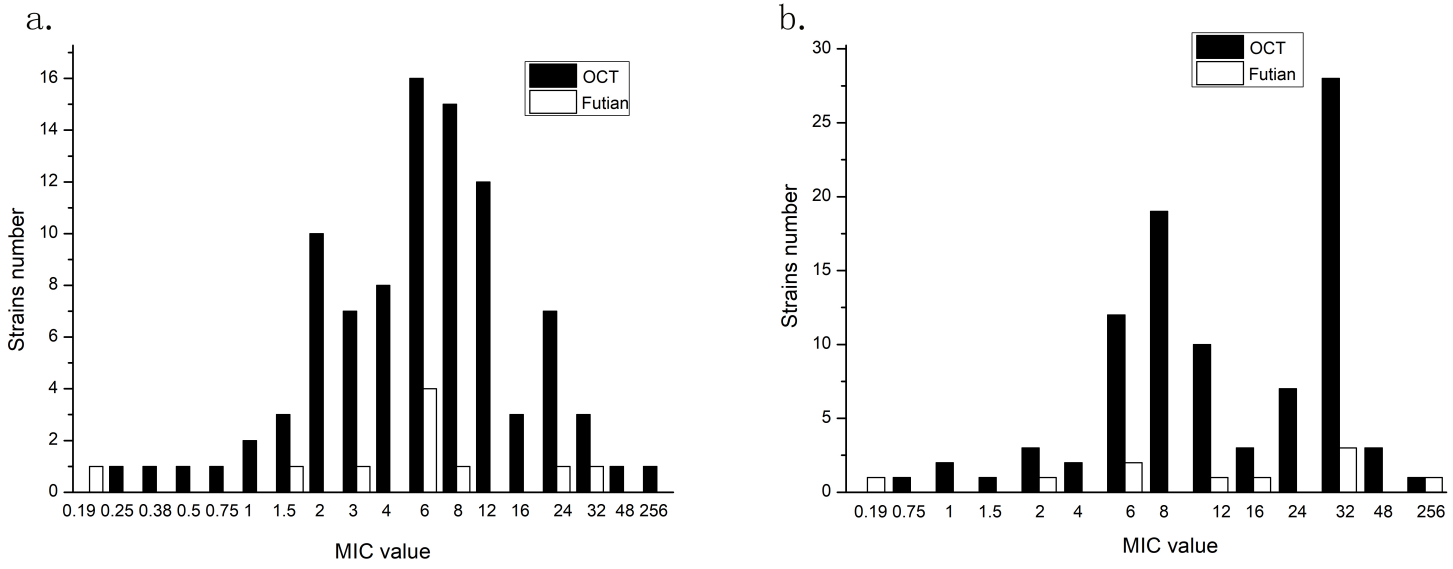

Figure 1

Enrichment comparison of MIC value between two sampling points. The a is MIC value for amoxicillin and b is MIC value for ampicillin. The black map is OCT and white map is Futian.

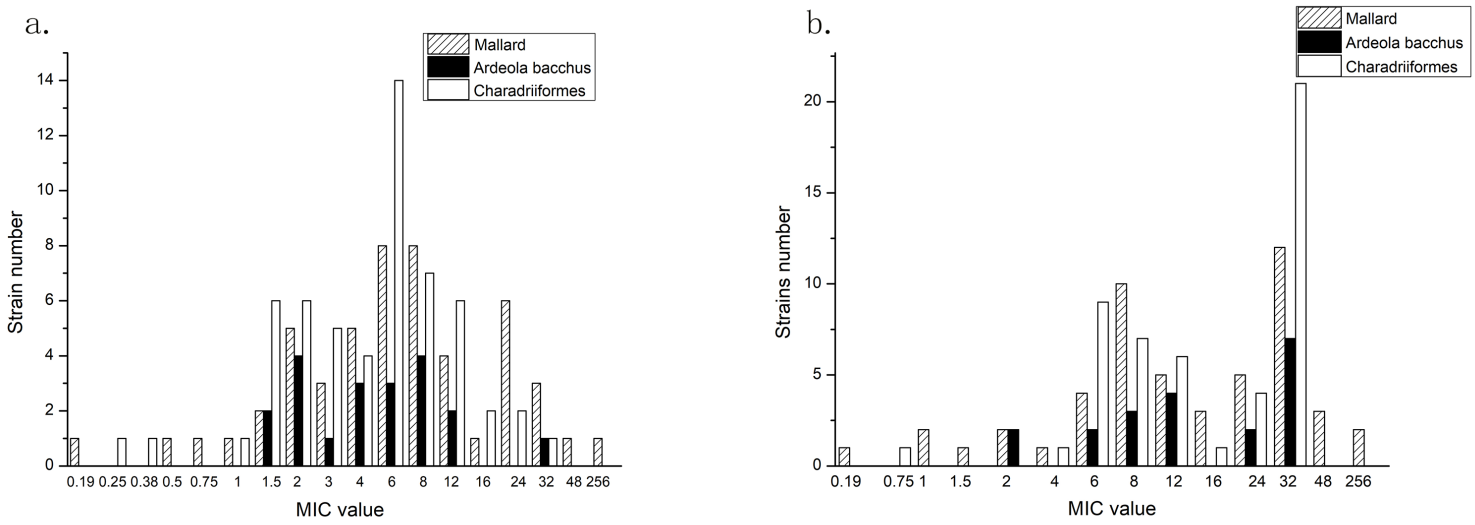

Figure 2

Enrichment comparison of MIC value between three kinds of migratory bird. The a is MIC value for amoxicillin and b is MIC value for ampicillin. The white map with black strip is mallard, the black map is ardeola bacchus and white map is charadriiformes.

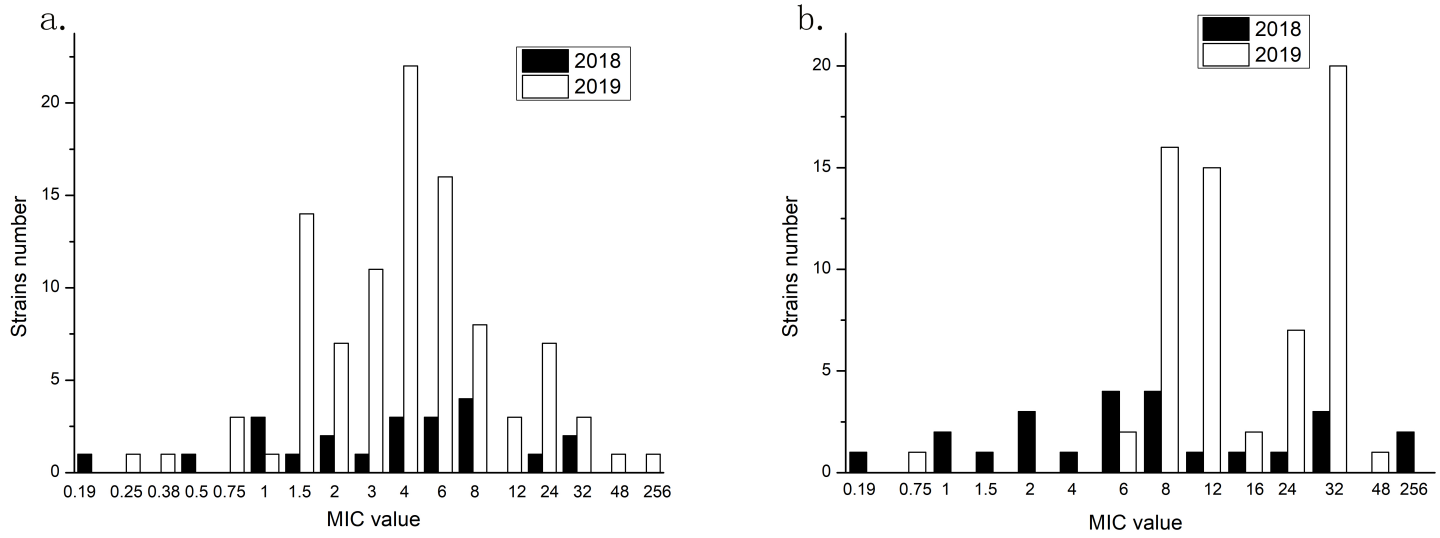

Figure 3

Enrichment comparison of MIC value between two years. The a is MIC value for amoxicillin and b is MIC value for ampicillin. The black map is 2018 and white map is 2019.

Supplementary Files

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