



Antimicrobial resistance not related to 1,2,3 integrons and Superintegron in *Vibrio* spp. isolated from seawater sample of Lima (Peru)

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ABSTRACT

Antimicrobial resistance (AMR) in microorganisms has been attributed to integrons, which have the ability to capture antimicrobial resistance gene cassettes and express them in their hosts. 170 strains of *Vibrio* spp. were isolated from Lima (Peru) seawater samples and identified by biochemical tests and PCR. AMR profiles were generated using 15 standard antibiotics. The presence of class 1, 2 and 3 integrons and Superintegron in these strains were also investigated by PCR. Ten species of *Vibrio* were identified with *V. alginolyticus* the most frequent. All strains were resistant to antibiotics, especially to penicillin group. No resistance to norfloxacin or tetracycline was observed. Class 1, 2 and 3 integrons were not found, only one Superintegron containing the *mutT* gene was identified in *V. cholerae* L22 strain. This indicated that AMR is not related to integrons as mentioned previously and that these strains can be reservoirs of resistance genes in marine environments.

1. Introduction

Some species of the genus *Vibrio* are pathogenic to humans. In addition to *V. cholerae*, *V. parahaemolyticus*, and other less frequent pathogens, such as *V. vulnificus* (Elmor et al., 2007; Bross et al., 2007), *V. mimicus* which produces a enterotoxigenic hemolysin (Mizuno et al., 2009), and *V. metschnikovii* which can rarely be isolated from human clinical samples (Pariante et al., 2008), can be human pathogens. *Vibrio* spp. has two circular chromosomes, for example the species of *V. cholerae* O1 contains a larger (ChrI) and one small (ChrII) chromosome (Schoolnik and Yildiz, 2000). In general, non-cholerae vibrios can cause infections to humans when the microorganism comes into contact with the wounds of the skin and especially by seafood consumption (Elmor et al., 2007). The most notable pathogens are *V. cholerae* O1 and O139 strains, which are etiological agents of the pandemic known as cholera (Vora et al., 2005; Winn et al., 2008). Some species of this genus such as *V. tubiashi*, *V. anguillarum*, *V. splendidus*, and *V. alginolyticus* can affect aquaculture production, producing diseases called bacillary necrosis which affect larval stages of bivalve molluscs from fish farms. This can apparently occur in any process on a farm crop and its presence is associated with improper handling.

Currently, drug-resistance in *Vibrio* spp. is increasing worldwide, and is attributed to mobile genetic elements such as class 1, 2 integrons (Opintan et al., 2008; Adabi et al., 2009). The gene cassettes associated

with these integrons make up approximately 1–3% of the entire genome in *Vibrio* species (Rapa and Labbate, 2013). Integrons or integration elements were discovered and characterized in 1989 by Stokes and Hall as a result of the evaluation of multidrug resistance to antibiotics (Stokes and Hall, 1989). Thus, the integron is a key component of site-specific recombination systems capable of capturing and mobilizing genes that are contained in mobile elements called gene cassettes (Hall and Collis, 1995). These gene cassettes can also be expressed. The essential components of an integron include the integrase gene (*intI*), the binding site (attachment site, *attI*), and the promoter which promotes expression of an integrated gene.

In this work, we aimed to determine the prevalence of the antimicrobial resistance in strains of *Vibrio* spp. isolated of seawater samples from different monitoring points of Lima (Peru) sea and analyze if this resistance profile is due to the presence of resistance integrons.

2. Materials and methods

2.1. Isolation and identification of *Vibrio* strains

Vibrio spp. strains evaluated belong to the culture collection of the Laboratory of Aquatic Microbiology from Aquaculture Research Center “Alexander Von Humboldt” – IMARPE. Between 2006 and 2008, 170 *Vibrio* strains were isolated from seawater samples collected from

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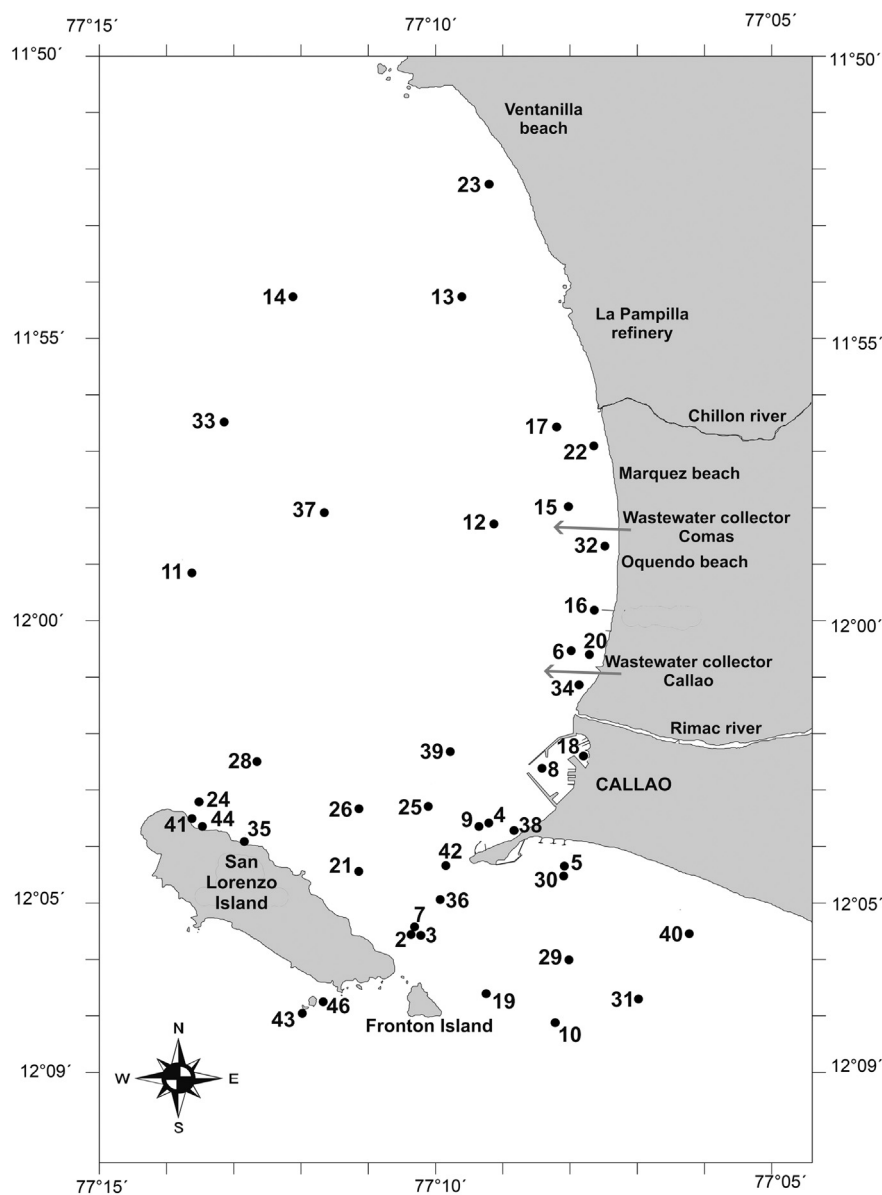


Fig. 1. Monitoring points for the collection of seawater samples for the isolation of *Vibrio* strains in the Lima sea (Peru).

different monitoring points of Lima (Peru) sea (Fig. 1). The latitude and longitude of these monitoring points are listed in the Supplementary Table S1. Water samples were processed following the methods published by the American Public Health Association (APHA, 1989). Traditional biochemical identification was done according to Llop et al. (2001) (sucrose oxidase, lysine, ornithine, lactose, indole, mannitol, sodium chloride at 0, 1, 6, 7 and 10%), it was considered that the reactions were positive or negative after 48 h incubation at 35–37 °C; identification by API 20 NE was done on some strains because they did not present as expected in conventional biochemical tests. Confirmation of identity by PCR was done only with *Vibrio* L22 strain.

2.2. Antimicrobial sensitivity assay

Selected isolates of different *Vibrio* species were tested for antimicrobial sensitivity using the disk diffusion method with MHA culture plates supplemented with 1% NaCl (Zavala-Norzagaray et al., 2015). All procedures and corresponding results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2010). All antimicrobial disks and their concentration used in this study

were: chloramphenicol (C), 30 mg; norfloxacin (Nor), 10 g; amikacin (Ak), 30 mg; kanamycin (K), 30 mg; ampicillin (A), 10 mg; penicillin (P); tetracycline (I), 30 mg; aztreonam (az), 30 mg; ceftazidime (CAZ), 30 mg; gentamicin (Ge), 10 mg; amoxicillin (AMX), 25 mg; nitrofurantoin (Nit), 300 mg; cotrimoxazole (trimethoprim/sulfamethoxazole) (Sxt), 1.25/23.75 mg; nalidixic acid (W), 30 mg; and ciprofloxacin (CIP), 5 mg. All disks were purchased from Oxoid, England. Quality control ranges for disk diffusion susceptibility testing were done with *Escherichia coli* ATCC 25922.

2.3. DNA extraction

Vibrio spp. strains were cultured in TSB 2% NaCl for 6 h at room temperature. Subsequently, 1.5 mL of the culture was placed in microcentrifuge tubes; centrifugation was done at 13,000g for 3 min. The supernatant was removed and 200 µL of 5% Chelex 100 (Bio-Rad Laboratories, SIGMA) was added to the bacterial pellet (De Lamballerie et al., 1992). The pellet was resuspended by vortex and incubated in a water bath at 100 °C for 6–7 min to permit the lysis of the cells. After the bath, the tubes were centrifuged at 13,000g for 5 min, the

Table 1
Primer pairs and conditions of amplification by PCR.

Target	Primer	Sequence (5' → 3')	Size (bp)	Reference
<i>int1</i>	INT-1U	GTTGGTCAAGGTTCTG	923	Shi et al. (2006)
	INT-1D	GCCAACCTTCAGCACATG		
<i>int2</i>	inti2F	GCAAATGAAGTGCAACGC	467	Reyes et al. (2003)
	inti2R	ACACGCTTGCTAACGATG		
<i>int3</i>	Inti3F	AGTGGGTGGCGAATGAGTG	600	Goldstein et al. (2001)
	Inti3R	TGTTCTTGATCGGCAGGTG		
<i>int4</i>	INT-4U	GTGTCGCGAATTATGC	936	Shi et al. (2006)
	INT-4D	ACGGGATAATGGGCTTAA		
<i>mrhB</i>	mrhF	GAAGAGTGTTCGTGAAGT	173	This study
	mrhR	CGATAATGATATCCCATACC		
<i>int4</i>	INTI4-1F	GAGTGTATGCGCTTGAGAG	546	This study
	INTI4-2R	CCCTTACCTTGCCAGATT	460	
16S rRNA	27f	AGAGTTTGATCMTGGCTCAG	–	This study
	536r	GWATTACCGCGGCKGCTG		
MutT	Mut-F	GGGTGGTCTATTGATGC	289	This study
	Mut-R	TGTGTAACCTGCCAAATG		
Lipoproteins	Lipo-F	CCCGAATCAGTAAAACC	384	This study
	Lipo-R	AATGCCTCGTTCTACAGTC		
Transposase OrfAB	Trans-F	CTTGATCGCTATGGAACA	751	This study
	Trans-R	GATGGATAAATGGGTTTCG		
HigAB	higAB-F	GTCTTTGCCTATGGATGC	516	This study
	higAB-R	GGCTAACTTGATTGCTACC		
IS5	IS5-F	ATGGCTAATGGTTGTTCG	709	This study
	IS5-R	GGTACAGCTTAAGTGACGAA		
Doc	doc-F	AACCAGGAATGAAAAGGAG	314	This study
	Doc-R	TGCGTATTGTGCGTAGAG		
With Mut-R	VCR-F	CAAGAGGGACTGTCAACG	523	This study
With higAB-R	VCR7-F	TAACAAACGCCTCAAGCG	698	This study
With Trans-F	Vcr2-R-transposase	CGCACTACCACTAACTCAA	1225	This study

supernatant was recovered and transferred to a new tube and stored at -20°C until analysis by PCR. Isolation of the small chromosome (ChrII) or megaplasmid was done only in *V. cholerae* L22 strain using the alkaline lysis method (Sambrook et al., 1989), because this strain has the Superintegron.

2.4. PCR assays

PCR was done to confirm identification of *Vibrio* L22, as well as amplify Class 1 (*int1*), Class 2 (*int2*), Class 3 (*int3*) integrase genes; *int4* gene (integrase of Superintegron) and gene cassettes with virulence or metabolic function described for Superintegron. We used information in GenBank (<http://www.ncbi.nlm.nih.gov/GenBank/>) to select target genes for the PCR assay, some sequence primers were derived from published articles and others were designed using Primer3 software (version 0.4.0) and Prime SeqBuilder (LASERGen). Specificity of the primers was checked by performing nucleotide BLAST with the gene sequences obtained from GenBank. The sequences of the primers used in this study are shown in Table 1. Optimization of PCR assays were performed for each primer pair. A typical 25 μL of PCR reaction mixture contained, 1 μL DNA, 20 pmol of each primer (PCR program are describe in Table 1), 0.2 mM of each deoxynucleotide triphosphate (dNTPs), 1.5 mM MgCl_2 , 1 U *Taq* polymerase (Invitrogen), and 1 \times Buffer [20 mM Tris-HCl (pH 8.4, 50 mM KCl)]. For visualization, 7 μL of amplified product and 3 μL of buffer were separated by electrophoresis in 1% agarose gels with TAE Buffer 1 \times and run at 80 mV for 60 min. The gel was stained with ethidium bromide (1 $\mu\text{g mL}^{-1}$) for 10 min, and the bands were visualized on a transilluminator and photographed. MassRuller DNA Ladder (Fermentas) was used to calculate the molecular weight of amplified product.

VCR primers were designed to confirm if gene cassettes with virulence or metabolic function are present in the genome of Superintegron.

2.5. Sequencing of PCR products of *int4* and 16S rRNA gene from *V. cholerae* L22 strain

DNA sequencing of the amplified fragments obtained by PCR was performed by the company MacroGenUSA (Maryland-USA), the method based on sequencing with fluorescent chain-terminating dideoxynucleotides was used. Sequencing was done using the primers: INT-4D, INTI4-1F, INTI4-2R, INT-4U to *int4* gene and 27f and 536r to 16S rRNA gene (Table 1). Sequences were viewed using the nucleotide BLAST, BioEdit and Mega7 softwares. The nucleotide sequence of these genes was deposited in European Nucleotide Archive (ENA) (<http://www.ebi.ac.uk/ena>).

3. Results

3.1. Strain identification

The identification of all the strains was done using biochemical and API 20 NE tests. Identification by sequencing of 16S rRNA gene by PCR only was done only to *Vibrio* L22 strain. The *Vibrio* spp. identified were from a total of 170 strains, the percentage of each species is shown in Table 2.

Table 2
Identified strains of *Vibrio* spp.

Species of <i>Vibrio</i>	No.	% (n = 170)
<i>V. alginolyticus</i>	121	71.18
<i>V. vulnificus</i>	17	10
<i>V. parahaemolyticus</i>	7	4.12
<i>V. carchariae</i>	5	2.94
<i>V. damsela</i>	5	2.94
<i>V. cincinnatiensis</i>	5	2.35
<i>V. fluvialis</i>	4	2.35
<i>V. metschnikovii</i>	4	2.35
<i>V. cholerae</i>	1	1.18
<i>V. mimicus</i>	1	0.59

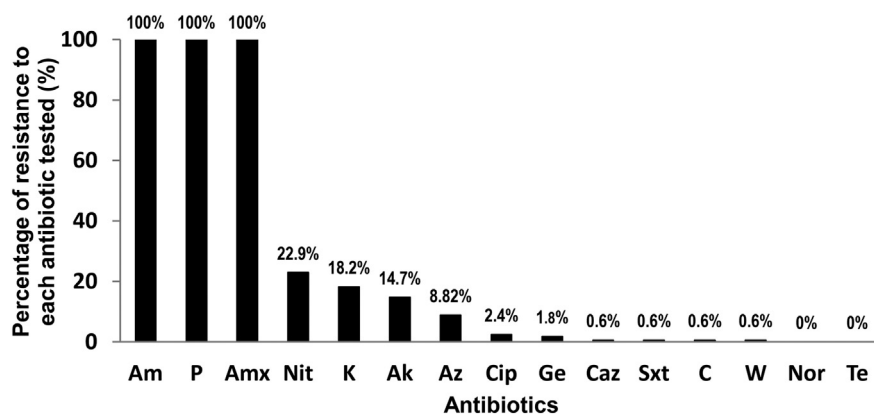


Fig. 2. Percentage of resistant *Vibrio* spp. isolates to tested antibiotics. A: ampicillin, Amx: amoxicillin, P: penicillin, Nit nitrofurantoin, K: kanamycin, Ak: amikacin, Az: aztreonam, Cip: ciprofloxacin, Ge: gentamicin, Caz: ceftazidime, Sxt: cotrimoxazole, C: chloramphenicol, W: nalidixic acid, Nor: norfloxacin, Te: tetracycline.

Identification by sequencing of 16S rRNA gene from *Vibrio* L22 strain was done because this strains could not be identified by conventional biochemical tests and also because this strain was the only one to have an integron (Superintegron) in its genome (see item 3.4). Sequencing of the 16S rRNA gene of this strain showed 99% identical with the 16S rRNA gene of *Vibrio cholerae*. The nucleotide sequence of this gene was deposited in ENA with accession number LT853892. So, *Vibrio* L22 strain was named *V. cholerae* L22; Mazel et al., 1998; Clark et al., 2000; Fluit and Schmitz, 2004; reported the presence of Superintegron only in *V. cholerae*.

3.2. Antimicrobial resistance

All the strains were resistant to several antibiotics, 100% were resistant to ampicillin, penicillin and amoxicillin; 22.9% to nitrofurantoin, 18.2% to kanamycin, 14.7% to amikacin, 8.82% to aztreonam, 2.4% to ciprofloxacin, 1.8% to gentamicin, 0.6% to cotrimoxazole, 0.6% to ceftazidime, 0.6% to chloramphenicol, 0.6% to nalidixic acid. None of the strains presented resistance to norfloxacin or tetracycline (Fig. 2).

We found 25 profiles of resistance, with A, Amx, P (53.53%) and A, Amx, P, Nit (15.29%) the most frequent (Table 3). All strains in this study were resistant to several antibiotics, which clearly show the need for periodic evaluations of the antimicrobial spectrum of *Vibrio* genus.

3.3. Presence of plasmids in *V. cholerae* L22 strain

Extraction of plasmids was done only in *V. cholerae* L22 strain, because it carrying the Superintegron. Were identified a small plasmid of approximately 1400 bp and of one larger molecular weight which is beyond the marker used. This plasmid was the megaplasmid or small chromosome (ChrII), found only in *Vibrio* species (Fig. 3).

3.4. Presence of class 1, 2, 3 integron and Superintegron

Presence of resistance integrons in all *Vibrio* strains was studied by amplification of the integrase gene by PCR. Class 1 (*intI1*), Class 2 (*intI2*) and Class 3 (*intI3*) integrase gene were not found. Of all the strains studied, only the *V. cholerae* L22 strain showed to have the *intI4* gene of Superintegron in total and plasmid DNA (Fig. 4) with an amplified of 936 bp. Using the INT-4U, INT-4D, INTI-1F, INTI-2R primers, fragments of 546 and 460 bp were amplified for sequencing, each one showing homology between 98 or 99% with *intI4* gene. The nucleotide sequence of this gene also was deposited in ENA with accession number: LT853893. So, the occurrence of integrons in all *Vibrio* strains in this study was 0.61%.

Table 3

Profile of resistance to antibiotics of the *Vibrio* spp.

Antibiotype	Profile of resistance	Number of isolates	% of resistance
3	A, Amx, P	91	53.53
4	A, Amx, P, Nit	26	15.29
6	A, Amx, P, Nit, K, Ak	8	4.71
4	A, Amx, P, K	8	4.71
4	A, Amx, P, Ak	7	4.12
5	A, Amx, P, Nit, K	4	2.35
5	A, Amx, P, Nit, Ak	4	2.35
7	A, Amx, P, Nit, K, Ak, Az	4	2.35
6	A, Amx, P, Nit, Az, Cip	2	1.18
7	A, Amx, P, K, Ak, Cip, Nit	1	0.59
5	A, Amx, P, K, Ak	1	0.59
6	A, Amx, P, Nit, K, Az	1	0.59
6	A, Amx, P, Nit, K, Cip	1	0.59
5	A, Amx, P, Nit, Az	1	0.59
5	A, Amx, P, Nit, Cip	1	0.59
5	A, Amx, P, Nit, C	1	0.59
6	A, Amx, P, Nit, Az, Ge	1	0.59
6	A, Amx, P, Nit, Az, Caz	1	0.59
6	A, Amx, P, Nit, Sxt, W	1	0.59
5	A, Amx, P, K, Az	1	0.59
4	A, Amx, P, Az	1	0.59
5	A, Amx, P, Az, Cip	1	0.59
5	A, Amx, P, Az, Ge	1	0.59
5	A, Amx, P, Ak, Cip	1	0.59
4	A, Amx, P, Ge	1	0.59

A: ampicillin, Amx: amoxicillin, O: penicillin, Nit nitrofurantoin, K: kanamycin, Ak: amikacin, Az: aztreonam, Cip: ciprofloxacin, Ge: gentamicin, Caz: ceftazidime, Sxt: cotrimoxazole, C: chloramphenicol, W: nalidixic acid.

3.5. PCR of genes found in Superintegron on megaplasmid of *V. cholerae* L22 strain

PCR for the search of gene cassettes with virulence or metabolic function described for Superintegron, as well as Mannose-resistant hemagglutinin, Mutt, OrfAB Transposase, Insertion Sequences IS5, lipoproteins, and *HigAB* and *doc* loci were done only in *V. cholerae* L22 strain, carrying of the Superintegron. Presence of *MutT* gene, transposase OrfAB and *HigAB* were confirmed (Fig. 5).

3.6. Confirmation of the presence of genes with virulence or metabolic function described for Superintegron

PCR was performed with VCR-F, VCR7-F and VCR2-R-transposase primers; only the gene related to the MutT family protein was related with Superintegron found in megaplasmid from *V. cholerae* L22 (Fig. 6).

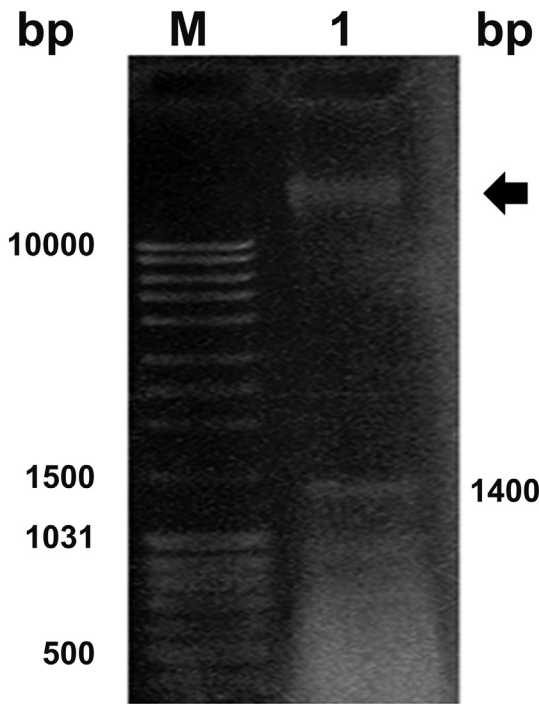


Fig. 3. Representation of megaplasmid isolated in *V. cholerae* L22. M: Molecular Weight Marker MassRuller (Fermentas). 1: Megaplasmid of *Vibrio cholerae* L22 (arrow).

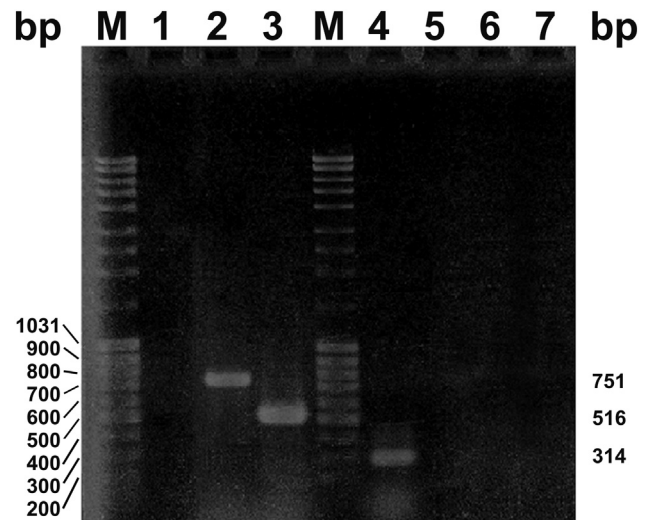


Fig. 5. Amplifications of MutT, OrfAB transposase and HigAB genes. M: Molecular Weight Marker MassRuller (Fermentas). 1: Hemagglutinin mannose resistant. 2: OrfAB transposase. 3: HigAB. 4: MutT. 5: IS5 insertion sequences. 6: lipoproteins. 7: doc.

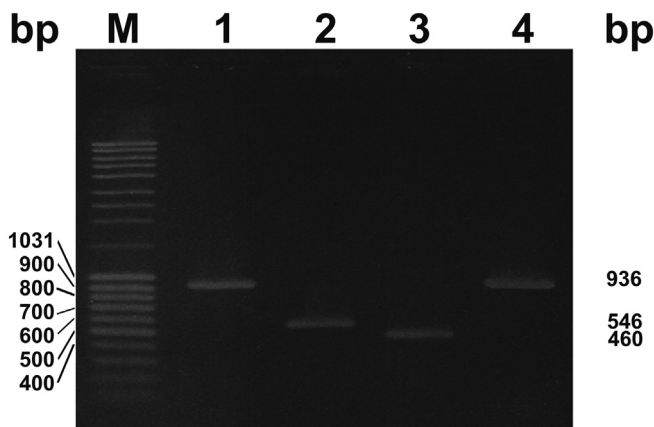


Fig. 4. PCR of *intI4* gene. M: Molecular Weight Marker MassRuller (Fermentas). 1: Amplification of *intI4* gene in total DNA. 2 and 3: Amplifications of *intI4* gene for sequencing. 4: Amplification of *intI4* gene in megaplasmid.

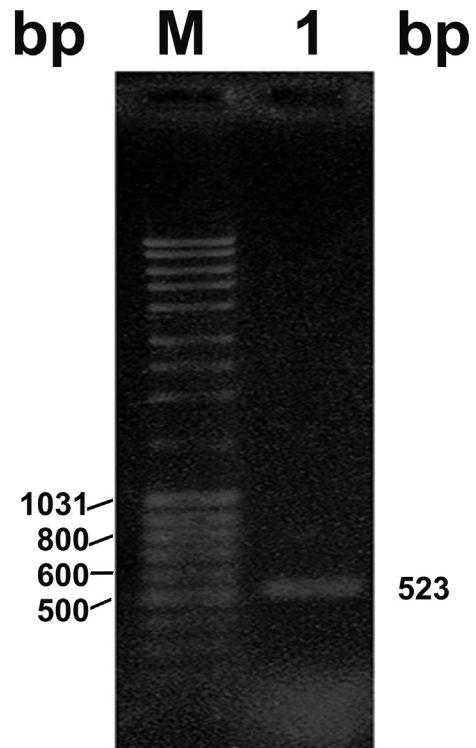


Fig. 6. Presence of the *MutT* gen in the Superintegron. M: Molecular Weight Marker MassRuller (Fermentas). 1: *MutT* gen hosted at Superintegron.

3.7. Relationship between the resistances to antibiotics with the presence of integrons

After susceptibility testing, it was observed that all strains exhibited the same patterns of resistance to antibiotics, especially to penicillins, so any direct relationship between resistance to antibiotics with integrons were not found.

4. Discussion

The genus *Vibrio* is widely distributed in coastal waters; we isolated strains of *V. cholerae* as well as *V. parahaemolyticus*, *V. vulnificus*, *V. mimicus*, and *V. alginolyticus* (Eiler et al., 2006). All of these species are also recognized as human pathogens. In this work, of all 170 strains studied at the beginning of this work, only two were *V. cholerae*. The low presence of *V. cholerae* is likely due to the temperature of the

seawater from Lima coastline, which is 16–17 °C on average which may impede the proliferation of this species. Although, temperature is not the only essential factor for its viability in the environment. Several studies show that this species is normally distributed in tropical and temperate seas; their distribution is also affected by several abiotic factors such as pH, salinity, UV exposure, and others (Gil et al., 2004; Goh et al., 2017). The presence of *V. cholerae* O1 in Peru has endemic characteristics and the occurrence of the bacteria in the Peruvian coast is influenced by environmental factors, favored by El Niño Southern Oscillation (ENSO), which also increased cases of cholera (Ibarra et al., 2000; Gil et al., 2004).

The factor that contributes greatly to the identification of the strains is the method used. Conventional biochemical methods should be complemented with other methods of identification, in this case using the method of molecular identification by PCR for *V. cholerae* L22 strain, because with biochemical method this strain was identified as *V. cincinnatiensis*, but with molecular methods we concluded that it was a *V. cholerae* carrier of the Superintegron. A significant number of *V. alginolyticus* strains (71.18%) were isolated and this was identified as predominant species under the climatic conditions of coastal and marine areas of the Lima coast. Only 4.12% of all strains were *V. parahaemolyticus*, this is because this species, like other *Vibrio* species of clinical importance, are related to filtering organisms of commercial importance. So too, this is the first work reporting the population of *Vibrio* in Lima's sea.

All the strains showed multi-resistance especially to ampicillin, amoxicillin and penicillin. Studies about antimicrobial resistance in *Vibrio* species were done primarily with *V. cholerae*. In 1991 this organism appeared causing the cholera outbreak in Peru and resistance to antibiotics was not found, as mentioned in an article published that year by Guevara et al. (1991). Subsequently, Tolmos (1992) mentioned 1.2% of resistance to ampicillin, in 1993, Guevara et al. (1994) reported 8.4% of resistance to ampicillin, Ibarra et al. (2000) reported in *V. cholerae* strains isolated from clinical samples in 1998, resistance to penicillin of 3.1%. Guevara et al. (2000) also report ampicillin resistance in 24.7% of *V. cholerae* strains from clinical origin. Dabanch et al. (2009) did not find any strain of *V. parahaemolyticus* susceptible to ampicillin in an infectious outbreak in Chile. So, antibiotic resistance appears to be increasing, in this work 100% of the strains had resistance to the penicillin group. In environmental strains, Stabili et al. (2010) reported *Vibrio* spp. AO1 had high resistance to ampicillin and streptomycin; Vaseeharan et al. (2005) also reported a 100% ampicillin resistance in *Vibrio* spp. isolated from pond water. Penicillin resistance in *Vibrio* is mediated by production of β -lactamases (Ferreira et al., 1992; Fluit and Schmitz, 2004) localized on chromosome or plasmid or a resistance transposon. In this work, multi-drug resistance was not linked with any resistance integron. As all strains showed resistance to the antibiotics of the penicillin family; it was assumed that such resistance genes were hosted in some type of integron, so we intended to examine the relationship between resistance profiles and the presence of integrons. Resistance to other antibiotics is mediated by production of membrane proteins (porins) as carrier proteins such as VA2212 (*fadI*), conferring resistance to kanamycin and nalidixic acid in *V. alginolyticus* (Xiong et al., 2010).

In relation to the group of antibiotics associated with the surveillance of antimicrobial resistance (ampicillin, chloramphenicol, cotrimoxazole, tetracycline) according to Sacaquispe and Velásquez (2002), strains have shown resistance to ampicillin, but can be sensitive to other antibiotics of this group; in addition, they mentioned that *V. cholerae* strains tend to have a natural resistance to penicillin, which is also corroborated with others species of *Vibrio* in our results.

The *V. cholerae* L22 strain has a megaplasmid (small chromosome), present in the genus *Vibrio*, which was confirmed like a megaplasmid by presence of HigAB loci by PCR, according to Heidelberg et al. (2000), Christensen-Dalsgaard and Gerdes (2006) and Budde et al. (2007). This loci is found in the megaplasmid and would help to maintain it in the host; thus, this plasmid has metabolic and virulence importance.

Presence of class 1 (*intI1*) and Class 2 (*intI2*) integrase gene in total strains was not observed, which is not consistent with reports that these integrons are found, especially in *V. cholerae* from clinical and environment samples (Ceccarelli et al., 2006; Shi et al., 2006; Opintan et al., 2008). The class 3 integron was not observed; actually there are no reports confirming the presence of this integron in Vibrionaceae family. Presence of integrase (*intI4*) gene from Superintegron was observed, this integron is reported for this genus (Clark et al., 2000; Rowe-Magnus et al., 2001). Such strains of *Vibrio* were isolated from seawater sample and our results showed that the occurrence of integrons in

bacteria of environmental origin is low with the presence of class 4 integrons of in all *Vibrio* strains from this study was 0.59%. Results obtained by Laroche et al. (2009) using *E. coli* strains isolated from estuaries, found a frequency of 11% for the presence of *intI2*, *intI1* genes but *intI3* gene was not detected. Ozgumus et al. (2007) found a frequency of 2.5% in the presence of class 1 integron, in *E. coli* strains isolated from spring or public use water. In *Vibrio*, Bakhshi et al. (2009) reported a 5.4% incidence of class 1 integron strains of *V. cholerae* non-O1 and non-O139 isolated from aquatic environments; Taviani et al. (2008) reported 26.3% of *Vibrio* strains isolated from seawater and river samples carry class 1 integron. These reports confirm the presence of integron strains of environmental origin. There are numerous studies describing the finding of integrons from isolates obtained from natural environments such as rivers, lakes, soils, seas and other places without known antibiotic exposure (Ozgumus et al., 2007; Taviani et al., 2008; Bakhshi et al., 2009; Laroche et al., 2009; Di Conza and Gutkind, 2010).

A relationship between antibiotic resistances and these four types of integrons was not found in our study. The finding of multiresistance to antibiotics was an indication of the presence of some integron carrying resistance genes, which would have been consistent with previous reports by Fluit and Schmitz (2004) and Rajpara et al. (2009), who reported that several bacterial groups are more resistant to antibiotics and such resistance is significantly associated with the presence of integrons in the microorganism.

Trucksis et al. (1998) and Rowe-Magnus et al. (2002), report the presence of *intI4* gene in the smallest chromosome (megaplasmid), similar to that obtained in this work. This Superintegron had a significant coevolution with the host genome making it highly sedentary (Rowe-Magnus et al., 2002). This Superintegron is hosted in megaplasmid and cannot be removed from this genome, because this megaplasmid has genes of importance for the viability of the bacteria; additionally, the size of megaplasmid would make it very difficult for horizontal transfer to other recipient strains to occur. Functions of megaplasmid would be related to the Superintegron, Rowe-Magnus et al. (2002), reported that gene cassettes of *V. cholerae* reveals a variety of adaptive functions including metabolic activities, virulence factors and determining potential antibiotic resistance, allowing a quick adaptation to new ecological niches.

Due to virulence factor mannose-fucose-resistant hemagglutinin (MFRHA) which was located in gene cassettes linked to VCRs in *V. cholerae* Superintegron (Carattoli, 2001), nine VCRs associated with locus determining resistance hemagglutination fucose and mannose were reported (Barker et al., 1994; Franzon et al., 1993; Clark et al., 1997). Presence of this locus in *V. cholerae* L22 by PCR tests was not detected, although there are reports that associate the presence of this gene with Superintegron. The absence of this virulence factor is likely because the strain L22 was isolated from an environmental sample (seawater), as strains isolated from sources of environmental origin tend not to have pathogenic genes. Núñez et al. (2009), showed that genes found in Pathogenicity Islands of *V. parahaemolyticus* are only found in strains of clinical isolates but not from environmental samples. Zhang et al. (2004), also reports that *tdh* and *trh* pathogenicity genes are preferably present in *V. parahaemolyticus* from hospital waste, the presence of these genes is negative in strains from environmental origin. Several reports indicate a low frequency of resistance integrons in strains of *Vibrio* from environmental origin (Laroche et al., 2009; Taviani et al., 2008).

Presence of Doc, insertion sequences IS5, lipoproteins genes were not observed, as these are also genes pathogenicity and in this case the strain of environmental origin would not need to retain these genes on chromosome or megaplasmid. The presence of the transposase OrfAB was observed, but additional tests showed this transposase is not located in the Superintegron, this should be by the ability of translocation of this genetic material and therefore this would be at another portion of megaplasmid. The HigAB loci or also called Toxin-antitoxin (TA) loci, is present in the megaplasmid but not in the Superintegron, which

is contrary to data obtained by Budde et al. (2007) and Heidelberg et al. (2000). Results obtained by Christensen-Dalsgaard and Gerdes (2006) showed that such loci contribute to the maintenance and genetic stability of the plasmid, this result should be taken into account with obtained by us, HigAB could help in stability of megaplasmid in the *V. cholerae* L22 strain; this HigAB was found in the megaplasmid or chromosome 2, a result comparable with that obtained by Budde et al. (2007), Christensen-Dalsgaard and Gerdes (2006), Heidelberg et al. (2000).

A gene related to the protein family MutT was located in the Superintegron, similar to the results obtained by Heidelberg et al. (2000); this gene has the function of hydrolyzing mutagenic analogues as 8oxo-dGTP and 8oxo-GTP, eliminating bases oxidized of these nucleotides, and preventing their incorporation in DNA and RNA synthesis (Kamiya et al., 2004; Rotman and Kuzminov, 2007) so it would be important to the strain of *V. cholerae* L22.

In this case, in relation to the Superintegron, *V. cholerae* L22 strain maintains gene cassettes with metabolic activities rather than pathogenic (the antibiotic resistance) because this strain is only resists to the penicillins tested and such resistances are linked to other genetic material compared to other studies in *V. cholerae*.

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Conflict of interest

None declared.

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