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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 (Pariente 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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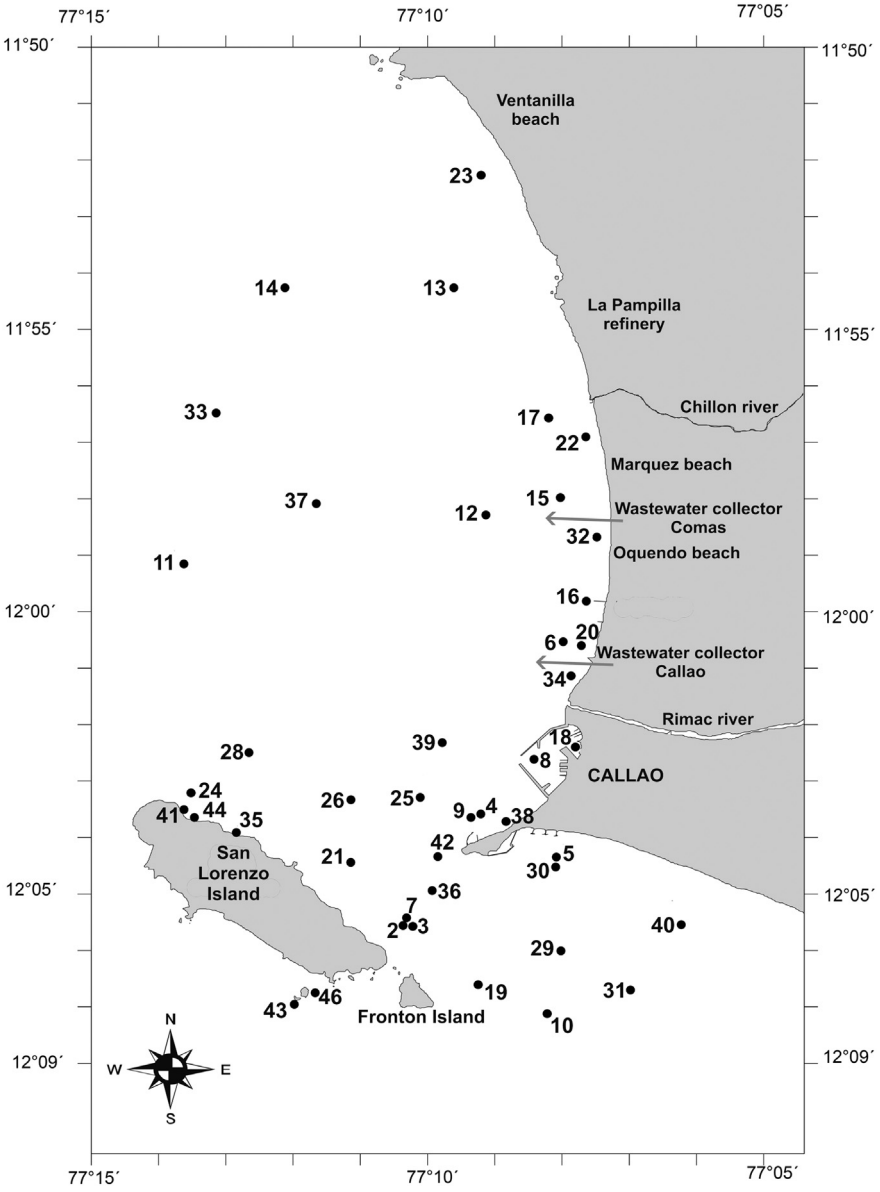

Fig. 1. Monitoring points for the collection of seawater samples for the isolation of *Vibrio* strains in the Lima sea (Peru).

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 13,000*g* 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 13,000*g* for 5 min, the