Preliminary study on the antimicrobial susceptibility pattern related to the genotype of *Vibrio vulnificus* strains isolated in the north-western Adriatic Sea coastal area

Patrizia Serratore, Emanuele Zavatta, Eleonora Fiocchi, Emanuele Serafini, Andrea Serraino, Federica Giacometti, Giorgia Bignami

Department of Veterinary Medical Science, Alma Mater Studiorum-University of Bologna, Bologna, Italy

Abstract

V. vulnificus is a Gram-negative bacterium, commonly found in estuarine and coastal habitats, that can infect humans through seafood consumption or wound exposure. This study represents the first attempt to correlate the genotype of *Vibrio vulnificus* strains isolated in the north-western Adriatic Sea coastal area, with their antimicrobial susceptibility patterns. On the whole, 40 *V. vulnificus* strains, isolated from shellfish (n=20), different coastal water bodies (n=19), and the blood of a *Carretta carretta* turtle (n=1), were utilized. All strains were positive for the species-specific genes *vvh*A and *hsp*, with high variability for other markers: 55% (22 out of 40) resulted of the environmental (E) genotype (*vcg*E, 16S rRNA type A, CPS2 or CPS0), 10% (4 out of 40) of the clinical (C) genotype (*vcg*C, 16S rRNA type B, CPS1), and 35% (14 out of 40) of the mixed (M) genotype, possessing both E and C markers. The antimicrobial susceptibility was assayed by the diffusion method on agar, according to the Clinical Laboratory Standards Institute (CLSI), utilizing the following commercial disks (Oxoid): ampicillin (AMP), ampicillin-sulbactam (SAM), piperacillin (PRL), cefazolin (KZ), cefotaxime(CTX), ceftazidime(CAZ), imipenem (IPM), meropenem (MEM), amikacin (AK), gentamicin(CN), tetracycline(TE), ciprofloxacin (CIP), levofloxacin (LEV), trimethoprim-sulfamethoxazole (SXT), and chloramphenicol (C). 75% of the strains, (n=30) including all C strains, was sensitive to all the tested antibiotics, whereas E strains showed intermediate sensitivity to AK (2 strains), CIP and CAZ (1 strain), TE (1 strain) and resistance to KZ (1 strain), and 4 M strains showed I to AK.

Introduction

V. vulnificus is a Gram negative bacillus, natural inhabitant of estuarine and marine environments. Even if the optimal environmental conditions for *V. vulnificus* are considered the medium or low salinity, between 5 and 25 ppt, and temperatures between 20 and 30°C (DePaola *et al.*, 2006), it has been recovered from fish, shellfish, water and sediments of a wide range of temperatures and salinities and also in effluent from wastewater treatment plants in Spain (Cañigral *et al.*, 2010) and South Africa (Igbinosa *et al.*, 2009).

V. vulnificus can infect humans through seafood consumption or wound exposure. In the immunocompromised or individuals with pre-existing chronic illnesses e.g. liver disease, chronic alcohol use, cancer, diabetes mellitus, infection often leads to primary or secondary septicaemia respectively (DePaola *et al.*, 2006). Primary septicaemia shows an average mortality rate exceeding 50%, and represents the leading cause of seafood-related death in the US, but wound infection may also be severe, with a fatality rate of 25% (Han *et al.*, 2011).

In Europe *V. vulnificus* infections are not nationally notifiable diseases, therefore today reports are limited to a few cases each year, even if it is expected an increase of incidence in the future, due to raw shellfish consumption, the increasing number of immune-compromised people, and the impact of anthropogenic activity and global warming on the marine environment (Baker-Austin *et al.*, 2010; Martinez-Urtaza *et al.*, 2010).

In Italy, three cases of fatal septicaemia have been documented: one attributed to wound infection in a hemodialyzed patient (Stabellini *et al.*, 1998) and two attributed to the consumption/manipulation of seafood products in individuals with chronic liver disease (Madeddu *et al.*, 2004). Moreover a case of infection with benign outcome, has been documented (Benini *et al.*, 1994) and other cases have been reported by the press in Rimini, (*La Voce di Romagna*, 25 August, 2011), leading to a Public Prosecutor's Office investigation, whose conclusions have not yet been made public.

Actually, three biotypes of *V. vulnificus* are recognized and differentiated by biochemical characteristics: biotype 1, indole positive, which is pathogenic to humans, biotype 2, indole negative, which is pathogenic to eels, and biotype 3, indole positive hybrid of biotypes 1 and 2, which is pathogenic to humans but has only been reported in Israel, representing an emerging form of this species (Thiaville *et al.*, 2011).

For the identification of *V. vulnificus*

biotype 1 at the species level, two genetic markers are commonly employed: *vvh*A (Neogi *et al.*, 2010) and *hsp* (Tarr *et al.*, 2007), and others are commonly utilized to differentiate clinical (C) from environmental (E) genotypes, among which: the *vcg* gene, with the two variants clinical (*vcg*C) and environmental (*vcg*E) (Han *et al.*, 2011); the 16S rRNA gene, with the variant type A being significantly correlated with environmental strains, and the variant type B or AB significantly correlated with clinical strains (Nilsson *et al.*, 2003); the CPS operon group 1, referred to as allele 1 (CPS1) and allele 2 (CPS2), the first more frequent among clinical strains (87%) and the latter more frequent among environmental strains (87%) (Chatzidaki-Livanis *et al.*, 2006), even if strains not typeable for CPS markers (CPS0) have also been described (Thiaville *et al.*, 2011).

The relevance of these factors has been examined in various *in vivo* and *in vitro* models, but unique virulence markers that are present exclusively in virulent strains have not yet been identified, and all clinical and environmental isolates resulted equally virulent in animal and cell culture patho-

genesis models (Mala *et al.*, 2014). Consequently, at present, according to the Food and Agricultural Organization of the United Nations and the World Health Organization (FAO/WHO, 2005), all *V. vulnificus* strains may be considered virulent, and there is a broad consensus on the need for monitoring presence and characteristics of this waterborne pathogen worldwide.

For a long time vibrios were considered to be susceptible to all antibiotics except ampicillin (Zanetti *et al.*, 2001), but more recent studies on *V. vulnificus* evidenced an increase of resistance to different classes of antibiotics (Ottaviani *et al.*, 2001; Han *et al.*, 2007; Backer-Austin *et al.*, 2009; Shaw *et al.*, 2014). In any case, in contrast to the extensive antibiotic resistance investigation in other pathogens such as *Vibrio cholera* and *Salmonella* spp., the awareness of antibiotic resistance of *V. vulnificus* and *V. parahaemolyticus* is considered not well documented (Elmahdi *et al.*, 2016). Particularly, only a few studies have been conducted in Italy on antibiotic susceptibility of pathogenic vibrios (Ottaviani *et al.*, 2001; Zanetti *et al.*, 2001).

The aim of the present study was to compare the prevalence of *V. vulnificus* in different coastal water bodies and shellfish of the Emilia-Romagna region, and to determine the relationship between the genotype and the antimicrobial susceptibility patterns of the environmental isolates.

Materials and Methods

For the present study, 21 strains of *V. vulnificus* belonging to our collection were utilized together with 19 newly isolates from different water bodies (n=67) of which 32 from the beach area of Cesenatico, 32 from the brackish waters of Cesenatico (a channel flowing into the sea and its inland branch), 3 from the beach area of Rimini.

Among the 21 collection strains, 20 were isolated from shellfish (*Ruditapes philippinarum*) harvested in the Sacca di Goro lagoon at the Po River delta, and 1 strain was isolated from the blood of a turtle (*Carretta carretta*) stranded in the same area. All isolates were characterized by means of genotype and antimicrobial susceptibility patterns.

Study area and sampling plan

The study area is represented in Figure 1. Surface water samples (50 cm) were collected from June to November, from various water bodies of the coastal area of the Emilia Romagna region: seawater of Cesenatico at the beach area not directly influenced by terrestrial water discharges (SWC1 and SWC2); brackish water of Cesenatico, partially influenced by terrestrial water discharges (a channel flowing into the sea, indicated as BWC3 and its inland branch, indicated as BWC4); seawater of Rimini at the beach area (SWR) periodically influenced by overflow pipes dumping of

combined wastewater exceeding the capacity of the municipal depuration plant; *Ruditapes philippinarum* harvesting area at Goro. All samples were transported to the laboratory in isothermal boxes then stored at 6-8 °C, to avoid viable but nonculturable conditions of *Vibrio* spp., and processed within 24 h.

Genotyping of isolates

The strains belonging to our collection, and utilized in the present study, were already phenotypically characterized and genotyped as reported elsewhere, and the newly isolates were characterized following the same protocol, targeting the genes *vvh*A, *hsp*, *vcg*, 16S-rRNA, CPS, by the polymerase chain reaction (PCR) (Passalacqua *et al.*, 2016).

Moreover, reference strains of *V. vulnificus* were utilized as positive controls: DSM 11507, DSM 10143 (ex ATCC 27562), NCTC 11066 (ex ATCC 29306), NCTC 11067 (ex ATCC 29307). Reference strains of *V. parahaemolyticus* were utilized as negative controls: ATCC 17802 and ATCC 43996.

Antimicrobial susceptibility testing

Antimicrobial susceptibility of isolates were tested by the disk diffusion method on Mueller-Hinton agar (Oxoid) according to the Clinical Laboratory Standards Institute (CLSI, 2006), utilizing *Escherichia coli* ATCC 25922 as control. Particularly the following antimicrobial (OXOID) were uti-
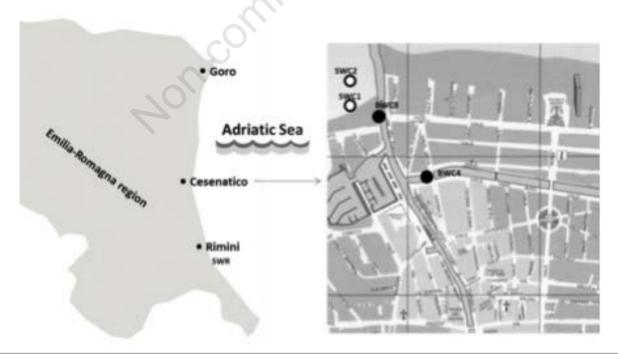

Figure 1. Study area of the Emilia-Romagna region and sampling stations. Goro, clams production area; Cesenatico, beach area (SWC1 and SWC2, white points); Cesenatico, brackish waters (channel flowing into the sea, SWC3, and its inland branch, SWC4, black points); Rimini, beach area (SWR).

Table 1. Interpretative criteria of the antimicrobial susceptibility test following the Clinical Laboratory Standards Institute (CLSI, 2006).

Antimicrobial class and acronym	Antimicrobial agent (disk content)	Zone diameter (mm) interpretive criteria		
		S	I	R
Penicillins and beta-lactam/beta-lactamase inhibitor combinations				
AMP	Ampicillin (10 μg)	≥17	14-16	≤13
SAM	Ampicillin-sulbactam (10/10 μg)	≥15	12-14	≤11
PRL	Piperacillin (100 μg)	≥21	18-20	≤17
Cephems				
KZ	Cefazolin (30 μg)	≥18	15-17	≤14
CTX	Cefotaxime (30 μg)	≥23	15-22	≤14
CAZ	Ceftazidime (30 μg)	≥18	15-17	≤14
Carbapenems				
IPM	Imipenem (10 μg)	≥16	14-15	≤13
MEM	Meropenem (10 μg)	≥16	14-15	≤13
Aminoglycosides				
AK	Amikacin (30 μg)	≥17	15-16	≤14
CN	Gentamicin (10 μg)	≥15	13-14	≤12
Tetracyclines				
TE	Tetracycline (30 μg)	≥19	15-18	≤14
Quinolones				
CIP	Ciprofloxacin (5 μg)	≥21	16-20	≤15
LEV	Levofloxacin (5 μg)	≥17	14-16	≤13
Others				
C	Chloramphenicol (30 μg)	≥18	13-17	≤12
Folate pathway inhibitors				
SXT	Trimethoprim-sulfamethoxazole (1.25/23.75 μg)	≥16	11-15	≤10

lized: ampicillin (AMP, 10 μg), ampicillin-sulbactam (SAM, 10/10 μg), piperacillin (PRL, 100 μg), cefazolin (KZ, 30 μg), cefotaxime (CTX, 30 μg), ceftazidime (CAZ, 30 μg), imipenem (IPM, 10 μg), meropenem (MEM, 10 μg), amikacin (AK, 30 μg), gentamicin (CN, 10 μg), tetracycline (TE, 30 μg), ciprofloxacin (CIP, 5 μg), levofloxacin (LEV, 5 μg), trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 μg), and chloramphenicol (C, 30 μg). The interpretative criteria, according to the Clinical Laboratory Standards Institute (CLSI, 2006), are listed in Table 1.

Table 2. Environmental conditions at the time of sampling: mean values of temperature, salinity and dissolved oxygen.

Station	Temperature (°C)	Salinity (ppt)	Dissolved O_2 (mg/L)
SWC1	23.5	39.5	5.8
SWC2	22.9	37.9	6.1
BWC3	23.1	31	5.5
BWC4	23.4	33.4	4.3
SWG	23.7	28.8	7.4
SWR	23.2	35.2	5.2

SWC1-SWC2, seawater beach area of Cesenatico; BWC3, brackish water of Cesenatico (channel flowing into the sea); BWC4, Cesenatico inland branch; SWG, seawater of Goro clams production area; SWR, seawater beach area of Rimini.

Results

On the whole, 19 *V. vulnificus* strains were newly isolated from 64 water samples of different sources: from Cesenatico (16 samples at each stations, SWC1, SWC2, BWC3, BWC4), and Rimini (3 seawater samples at the beach area SWR). The environmental conditions (mean values) at the water sampling stations at the time of sampling, are reported in Table 2. The samples from the beach areas of Cesenatico (SWC1, SWC2) were all negative for *V. vulnificus*, whereas 19% (3 out of 16) of the brackish waters samples SWC3 were positive, as well as 31% (5 out of 16) of the brackish

waters samples SWC4, and 33% (1 out of 3) of the seawater samples of the beach area of Rimini (SWR). The biochemical characteristics of all isolates utilized in the present study are omitted, but they resulted all attributable to the biotype 1, being indole positive.

Genotyping of isolates

All the 40 strains were positive for the species-specific genes *vvh*A and *hsp*, with variability for the other markers even in the same sample (734, 776, 798, 883, 915, 958, 1023): 55% (n=22) were of the environmental (E) genotype (*vcg*E, 16S rRNA type A, CPS2), 10% (n=4) of the clinical (C) genotype (*vcg*C, 16S rRNA type B, CPS1) and 35% (n=14) showed a mixed (M) geno-

type. Particularly, among the E strains, 5 resulted untypeable for CPS being negative for both CPS1 and CPS2 as the reference strain DSM 11507; among the M strains, 1 resulted positive for both CPS1 and CPS2, as the reference strains DSM 10143. The results of the PCR-based genotyping of the isolates are reported in Table 3.

Antimicrobial susceptibility testing

V. vulnificus reference strains and 75% (30 out of 40) of the isolates, including all of them of the C genotype, were sensitive to all the tested antibiotics. 25% (10 out of 40) of the isolates, all of them of the E or M genotype, were sensitive to CTX, AMP, IPM, MEM, C, CN, PRL, SXT, LEV and SAM, but showed intermediate sensitivity

to AK (4 strains of the M genotype and 2 strains of the E genotype), to CIP and CAZ or TE or KZ (4 strains of the E genotype), and the only one strain showing resistance to KZ was of the E genotype. FoFor the detail of the interpretative zone diameter (mm) see Table 4.

Discussion

V. vulnificus is a halophilic Gram-negative bacillus, commonly found worldwide in warm coastal waters with moderate salinity, sediments, and a variety of seafood including shrimp, fish, and bivalve shellfish (Jones and Oliver, 2009).

The water temperature during our study, ranging from 22.9 to 23.7 °C, was certainly favourable to *V. vulnificus*, because a temperature higher than 20°C is largely considered the most suitable for this organism

Table 3. Genotypes of the *Vibrio vulnificus* strains considered in the study and their source.

Sample N°/strain N°	Source	vvhA	hsp	vcgC	16S rRNA B	CPS1	vcgE	16S rRNA A	CPS2
628/7	RG	+	+	-	-	+	+	+	-
677/25	Cc	+	+	-	-	-	+	+	+
731/16	RG	+	+	-	-	-	+	+	+
731/17	RG	+	+	-	-	-	+	+	+
731/18	RG	+	+	-	-	-	+	+	+
734/4	RG	+	+	+	+	+	-	-	-
734/6	RG	+	+	-	-	-	+	+	+
734/8	RG	+	+	-	-	+	+	+	-
734/9	RG	+	+	-	-	+	+	+	-
734/14	RG	+	+	-	-	-	+	+	+
734/15	RG	+	+	-	-	-	+	+	+
734/16	RG	+	+	+	+	+	-	-	-
734/17	RG	+	+	+	+	+	-	-	-
734/18	RG	+	+	-	-	-	+	+	+
759/14	RG	+	+	-	-	-	+	+	+
776/6	RG	+	+	-	-	-	+	+	+
776/8	RG	+	+	-	-	-	+	+	+
776/10	RG	+	+	-	-	-	+	+	-
786/10	RG	+	+	+	+	+	-	-	-
798/10	SWR	+	+	+	+	+	-	+	-
798/12	SWR	+	+	+	+	+	-	+	-
798/14	SWR	+	+	+	+	+	+	+	-
798/15	SWR	+	+	+	+	+	+	+	-
880/3	BWC4	+	+	-	-	-	+	+	-
883/2	BWC3	+	+	-	-	-	+	+	-
883/3	BWC3	+	+	-	-	-	+	+	-
883/4	BWC3	+	+	-	-	-	+	+	-
883/5	BWC3	+	+	-	-	-	+	+	+
883/6	BWC3	+	+	-	-	+	+	+	-
915/2	BWC4	+	+	-	-	-	+	+	+
915/6	BWC4	+	+	-	-	+	+	+	-
915/18	BWC4	+	+	-	-	-	+	+	+
942/3	BWC4	+	+	-	-	+	-	+	-
945/2	BWC3	+	+	-	-	-	+	+	+
953/2	BWC3	+	+	-	-	-	+	+	-
958/4	BWC4	+	+	-	-	+	+	+	-
958/5	BWC4	+	+	-	-	-	+	+	+
958/6	BWC4	+	+	-	-	+	+	+	-
1023/4	RG	+	+	-	-	+	+	+	-
1023/5	RG	+	+	+	-	+	+	+	+
Reference strains									
DSM 11507		+	+	+	+	-	-	-	-
DSM 10143		+	+	-	-	+	+	+	+
NCTC 11066		+	+	-	-	-	+	+	+
NCTC 11067		+	+	+	+	-	-	+	+

RG, *Ruditapes philippinarum* from Goro; Cc, *Carretta carretta*, blood; BWC3, brackish water of Cesenatico (channel flowing into the sea); BWC4, Cesenatico inland branch; SWR, seawater of the beach area of Rimini.

Table 4. *Vibrio vulnificus* strains showing intermediate sensitivity or resistance to the tested antibiotics.

Strain	Source	CTX	CIP	CAZ	AMP	IPM	MEM	AK	TE	C	CN	PRL	SXT	KZ	LEV	SAM
734/15	RG	S	S	S	S	S	S	S	I (18)	S	S	S	S	S	S	S
776/6	RG	S	S	S	S	S	S	S	S	S	S	S	S	I (17)	S	S
776/8	RG	S	S	S	S	S	S	I (16)	S	S	S	S	S	S	S	S
798/10	SWR	S	S	S	S	S	S	I (16)	S	S	S	S	S	S	S	S
915/2	BWC4	S	I (20)	I (16)	S	S	S	S	S	S	S	S	S	S	S	S
945/2	BWC3	S	S	S	S	S	S	I (16)	S	S	S	S	S	S	S	S
953/2	BWC3	S	S	S	S	S	S	S	S	S	S	S	S	R (12)	S	S
958/4	BWC4	S	S	S	S	S	S	I (16)	S	S	S	S	S	S	S	S
1023/4	RG	S	S	S	S	S	S	I (16)	S	S	S	S	S	S	S	S
1023/5	RG	S	S	S	S	S	S	I (16)	S	S	S	S	S	S	S	S

BWC3, brackish water of Cesenatico (channel flowing into the sea); BWC4, Cesenatico inland branch; SWR, seawater beach area of Rimini; RG, *Ruditapes philippinarum* from Goro; Cc, *Carretta carretta*, blood; AMP, ampicillin; SAM, ampicillin-sulbactam; PRL, piperacillin; KZ, cefazolin; CTX, cefotaxime; CAZ, ceftazidime; IPM, imipenem; MEM, meropenem; AK, amikacin; CN, gentamicin; TE, tetracycline; CIP, ciprofloxacin; LEV, levofloxacin; SXT, trimethoprim-sulfamethoxazole; C, chloramphenicol; S, sensitivity; I, intermediate sensitivity; R, resistance. Zone diameter is expressed as mm.

(Cañigral *et al*., 2010). On the contrary, the salinity, ranging from 28,8 to 39.5 ppt, was undoubtedly higher than indicated as suitable by many authors (between 15 to 25 ppt), and stressed in the comprehensive review of Horseman and Surani (2011), suggesting that temperature and salinity may be not the only environmental factors influencing *V. vulnificus* growth and survival as speculated by DePaola *et al*. (2006).

Notwithstanding *Vibrio* spp. abundance has been largely considered unrelated to pollution or faecal waste (Cañigral *et al*., 2010), a significant correlation between the occurrence of *V. vulnificus* and the occurrence of faecal indicators was found in the Danish marine environments (Høi *et al*., 1998), moreover *V. vulnificus* was recovered from effluents of wastewater treatment plants, suggesting that nutrient enriched and faecal contaminated water may represent a favourable environment (Igbinosa *et al*., 2009; Cañigral *et al*., 2010). Accordingly, in the present study the higher percentage of positivity for *V. vulnificus* (33%) was found in recreational seawater heavily influenced by wastewater (SWR), followed by brackish water partially influenced by wastewater (the channel flowing into the sea, SWC3 19%, and its inland branch, SWC4 31%), whereas all samples from recreational seawater not directly influenced by wastewater (SWC1, SWC2) were all negative for *V. vulnificus*. Moreover, the previously reported

multi-year study on bivalve shellfish (*Ruditapes philippinarum*) harvested in the area of Goro, indicated that only 11,5% of the samples resulted positive for *V. vulnificus* (Serratore *et al*., 2016).

Combining these observation, it can be assumed that, in the area of interest, the conditions determined by a sizeable amount of nutrients and wastewater may enhance the prevalence of *V. vulnificus* in a water body more than the filter feeding activity in bivalve shellfish harvested in a B zone (Goro), considered moderately fecalized and classified according to Regulation EC 854/2004 (*E. coli* exceeding 230 MPN/100g, and ≤ 4.600 MPN/100g).

The genotyping of *V. vulnificus* isolates utilized in the present study showed a higher percentage of strains with the E genotype (55%) with respect to the C genotype (10%), but also the M genotype was represented with a high percentage (35%), confirming that the recovery of strains with the M genotype is not uncommon, as reported elsewhere (Nilsson *et al*., 2003; Chatzidaki-Livanis *et al*., 2006).

The susceptibility of *V. vulnificus* to antibiotics has been studied applying different protocols, and the results are often in disagreement, ranging from sensitivity to the majority of antibiotics (Han *et al*., 2007) to a widespread antibiotic intermediate sensitivity or resistance (Ottaviani *et al*., 2001; Baker-Austin *et al*., 2009; Shaw *et al*., 2014). Obviously, it is possible that strains

of different geographical origin may present different susceptibility to antibiotics, but the differences amongst the utilized protocols may also be at the origin of such a variety of results. In the present study the disk diffusion method on Mueller-Hinton agar, applied according to the Clinical Laboratory Standards Institute (CLSI, 2006), showed that 75% of the strains were sensitive to all the tested antibiotics. The strains showing intermediate sensitivity or even resistance (25%) were all of the E (n=6) and M (n=4) genotype, and aminoglycosides showed the worst performance, according to the study of Bier *et al*. (2015), but unlike this study, non-susceptibility to both quinolones (CIP) and cephems (CAZ) was also observed in one strain.

A recent extensive review on the antibiotic resistance profile of *V. vulnificus* in the United States and other countries including Italy, Brazil, Philippines, Malaysia, Thailand, China, India, Iran, South Africa and Australia, indicate that both environmental and clinical isolates show similar antibiotic resistance profiles (Elmahdi *et al*., 2015). Our data are not in agreement considering that 27% of the E genotype isolates (6 out of 22) and 29% of M genotype strains (4 out of 14) showed intermediate sensitivity to AK, or CIP and CAZ, or TE, or KZ, or resistance to KZ, whereas the strains of the C genotype were 100% sensitive to all the tested antibiotics, as the reference strains.

Conclusions

Notwithstanding *V. vulnificus* infection is considered one of the few foodborne illnesses with an increasing incidence worldwide (Bross *et al*., 2007), and the mortality rate may increase to 100% in patients with septicemia if treatment is delayed by 72 h (Bross *et al*., 2007), the awareness of antimicrobial resistance of *V. vulnificus* is not as well documented as for other foodborne bacterial pathogens (Elmahdi *et al*., 2015).

The present study evidenced the intraspecific variability of genotypes of *V. vulnificus* isolates even in the same sample, and confirmed that aminoglycosides show the worst performance against *V. vulnificus* as previously reported (Bier *et al*., 2015), but at odds with previously reported studies (Elmahdi *et al*., 2015) a different antimicrobial patterns among the different genotypes was also evidenced. Moreover our study documents non-susceptibility to ceftazidime (CAZ), ciprofloxacin (CIP), and tetracycline (TE), conversely indicated among the recommended antibiotics for the treatment of *V. vulnificus* infections (Elmahdi *et al*., 2015).

It can be concluded that more extensive studies are needed to characterize the antimicrobial susceptibility of the environmental strains of *V. vulnificus* with respect to their geographical origin, and to individuate the appropriate first-line antimicrobials, enabling clinicians to apply a timely and adequate medical therapy in case of human infection.

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