Role, Circulation and Molecular Characterization of Integrons and ICEs in Clinical and Environmental *Vibrio*

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Mobile genetic elements are essential for the acquisition of new genetic information and genomic recombination. Among these, integrons and ICEs of the SXT/R391 family are involved primarily in antibiotic resistance diffusion and in other adaptive functions in *Vibrio*. Many studies indicate that mobile genetic elements are also important factors of genetic variability and suggest their role as potential vectors of cryptic functions. Resistance to drugs, molecular structure, geographic distribution and epidemiological relationship of these genetic elements circulating in *Vibrio spp* isolates from Africa and Vietnam (1985-2003) are described.

Keywords Integron; ICE; Vibrio

1. Mobile Genetic Elements and Genomic Plasticity

1.1 Integrons

Bacteria challenged and selected by natural compounds or commercial antibiotics are able to become resistant; drug resistance is not only acquired by spontaneous mutation but also by horizontal genetic transfer. The enrolment of exogenous genes is the most efficient way by which bacterial species can survive to environmental challenges, including exposure to antibacterial compounds.

Drug resistance is a major problem in treatment of infectious diseases and many studies are focused on elucidating resistance mechanisms and resistance spread. In the 1970s, multidrug resistance was in many cases associated with transmissible plasmids [1] and/or with transposons [2]. The importance of integrons in the acquisition of resistance genes was only recognized much later, in the late 1980s [3].

Integrons represent an important mechanism for the acquisition of antibiotic resistance genes in many bacteria [4], due to an integration system mediated by a specific integrase. These elements are not autonomously mobile but are able to capture, integrate and express exogenous gene cassettes. Integrons possess an integrase gene located in their conserved region, able to recombine these discrete units of circularized DNA, in a RecA-independent manner [5].

Integration occurs downstream of the resident Pc promoter at the *attI* site (primary recombination site), allowing the transcription of the captured genes. All integron-inserted cassettes share specific structural characteristics. They generally contain a single gene and an *attC* site or 59-base element (secondary recombination site), and are inserted within the two conserved regions, in the variable region [5, 6].

Integrons can be divided into mobile integrons and superintegrons. Mobile integrons are coupled with mobile DNA elements, such as insertion sequences (ISs), transposons and conjugative plasmids. All can be used by the integron as vehicles for intra- and inter-species transmission of genetic information, and are primarily involved in the spread of antibiotic-resistance genes.

So far, five classes of mobile integrons have been identified according to the sequence of their integrase gene. Here we mainly describe class 1 integrons, extensively found in clinical isolates, and containing most of the known antibiotic-resistance gene cassettes. Class 1 integrons are organised in two conserved regions (CS): 5'CS, containing *intI* gene, *attI* site and Pc; 3'CS, usually characterized by

qacEΔ1 and *sul1* resistance genes to quaternary ammonium compounds and sulfonamide, respectively [5, 6]. To date over 80 different gene cassettes from class 1 integrons have been described. They confer resistance to aminoglycosides, cloramphenicol, trimethoprim, streptothricin, rifampin, erythromycin, fosfomycin, lincomycin, quaternary-ammonium compounds, and to all known β-lactams [7, 8].

In the late 1990s, some studies investigating mobile integrons and a cluster of repeated DNA sequences on chromosome 2 of *V. cholerae* genome, led to the identification of a distinct type of integron [5]. This integron codes for a specific integrase related to mobile integron integrase, but is characterized by a large number of gene cassettes, and by its chromosomal location that is not associated with mobile elements. These elements were named superintegrons and are now known to be integral components of many γ-proteobacterial genomes [9]. They have been identified in the *Vibrionaceae*, in their close relatives the Xanthomonads, and in Pseudomonads.

1.2 Integrative Conjugative Elements (ICEs)

In 1996 Waldor *et al.* discovered in the chromosome of *V. cholerae* O139 MO10 from India a new integrative conjugative element, SXT^{MO10}[10]. It was named SXT, as acronyms for sulfamethoxazole and trimethoprim resistances. After its discovery deeply changed the understanding of resistance circulation in *V. cholerae* epidemics. Since that date SXT has been considered first as a conjugative transposon [10], then as a CONSTIN [11] and eventually as an ICE [12].

Integrative and conjugative elements (ICE) are linear DNA sequences able to integrate into the bacterial genome and to transfer by conjugation. They constitute a large class of mobile genetic elements, nowadays identified in Gram + and Gram – bacteria, encoding many properties such as antibiotic and heavy metal resistance, complex degradation pathways, error-prone DNA repair system, recombination system and virulence factors [13].

SXT is a 99.5 kb ICE that mainly contributes to horizontal transmission and rearrangement of resistance genes in *V. cholerae*. This element is able to mobilize plasmids and chromosomal DNA from strain to strain [14] and shares several common features with R391. This integrating mobile element discovered in *Providencia rettgeri***,** was initially considered as a conjugative plasmid, belonging to incompatibility group IncJ [15]. Due to the impossibility of isolating an extrachromosomal replicative form of R391, the integration of this mobile element into the chromosome was assumed and confirmed [16], enrolling it in the class of ICEs.

The enzyme involved in integration/excision of both SXT and R391 is an integrase, able to catalyze the insertion of the element into the host chromosome at the 5' end of *prfC* gene, restoring the gene integrity and preserving its functionality [11, 17]. The presence of a circular replicative intermediate, formed through specific recombination of the left and right ends of the ICE, was established by PCR experiments [11].

All ICEs characterized by highly related int_{sxt} genes and ability to integrate into *prfC* are now grouped in the SXT/R391 family [18]. The genetic structure of SXT/R391 elements is comprised of a large conserved scaffold [19] containing three functional modules: a) maintenance, promoting integration and excision; b) dissemination, containing genes that specify the synthesis of the mating machinery, including *tra* genes associated to IS sequences; and c) regulation, including the genes and the mechanisms that regulate ICE transfer. Genes specific for each ICE of the SXT/R391 family are interspersed in the conserved sequence, and three Hotspots have been identified near the *tra* genes as targets for different insertions [19]. Figure 1a shows how the molecular analysis of these regions allows discrimination between SXT and R391.

The high variability of resistance patterns conferred by SXT related elements is well demonstrated.

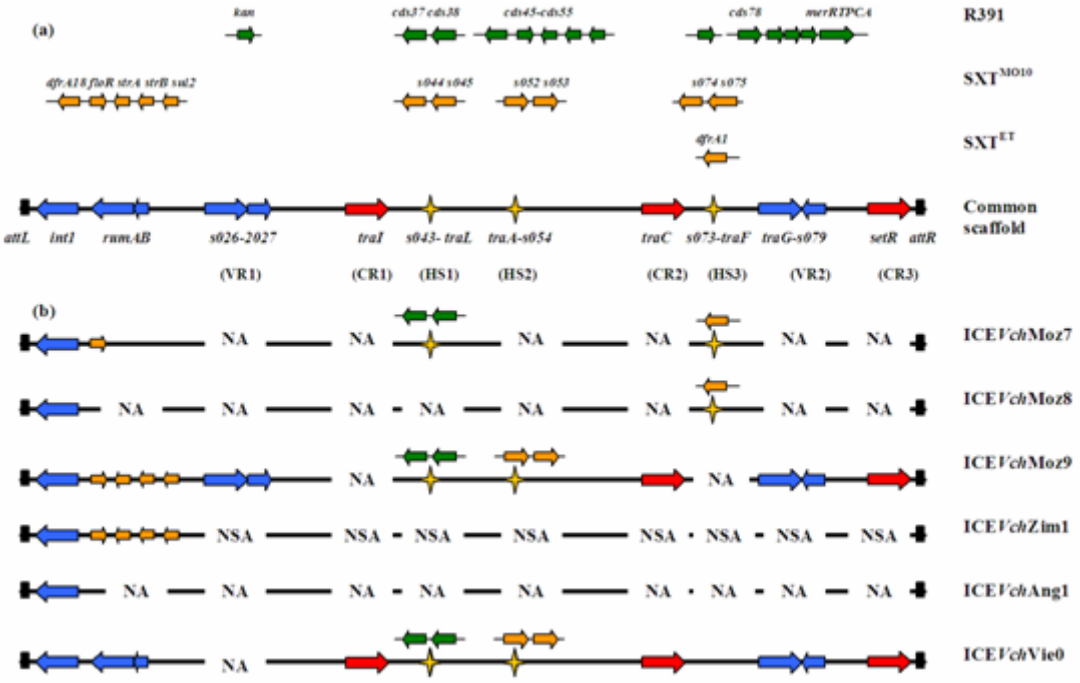

Fig. 1 Molecular structure of ICEs of SXT/R391 family and characterization of six ICEs. (a) Linear representation (not to scale) of the common scaffold of ICEs of SXT/R391 family. The black line represents the conserved genes in SXT and R391. Specific insertion for SXT (orange) and R391 (green) are depicted above the common scaffold. PCR amplification of eleven regions was performed to characterize the ICEs. Identification: presence of int_{SXT} and integration into *prfC* chromosomal gene. Characterization: three conserved common regions *traI*, *traC* and *setR* (red). Distinction between SXT and R391 molecular profiles: *rumAB*, two variable regions (VR1, VR2) and three Hotspots (HS1, HS2, HS3; yellow stars). For PCR procedure details about this characterization see Bani *et al.* [20]. (b) Schematic representation of the six ICEs of SXT/R391 family, belonging to isolates from different countries described in our work. ICE*Vch*Zim1 in representative also for ICE*Vch*Swa1. ICE*Vch*Ang1 is representative also for ICE*Vch*Ang2 and ICE*Vpa*Ang1. The *rumAB* operon of ICE*Vch*Vie0 is interrupted by a relic cluster containing *tnp*, *tnpA* and *s021*. NA: Not Amplified. NSA (Not Submitted to Amplification)

Resistance genes to chloramphenicol (*floR*), streptomycin (*strA* and *strB*), sulfamethoxazole (*sul2*), trimethoprim (*dfrA18* or *dfrA1*) and tetracycline (*tetA* and *tetR*) characterize SXT with different arrangements. In SXT^{MO10} found in *V. cholerae* O139, resistance genes are embedded in a transposon-like element, the antibiotic resistance cluster, that interrupts the *rumAB* operon. Interestingly *rumAB* operon is associated with UV protection in IncJ R element group [17]. SXT^{ET} (ICE*Vch*Ind1), isolated in clinical *V. cholerae* O1 [21], is closely related but not identical to SXT^{MO10}; it shows a shorter resistance cluster in *rumAB* operon, lacking *dfrA18*, but containing *dfrA1* located close to *traF* transfer gene, as represented in Fig. 1a. SXT^{LAOS} (ICE*Vch*Lao1), a new SXT/R391-like element isolated in Laos [22], is characterized by *tet*A resistance gene and by the absence of trimethoprim resistance genes. A derivative of this element, ICE*Vch*Vie1, bearing also *tetR* gene was isolated in Vietnam [23]. We can infer that this resistance profile plasticity found in SXT/R391 family allows the host strains to better face the antibiotic challenge.

Recently, new variants of SXT-related ICEs were described in *V. fluvialis* clinical isolates (ICE*Vfl*Ind1), in *V. cholerae* environmental isolates (ICE*Vch*Mex1) and in *Photobacterium damselae* subsp. *piscicida* (ICE*Pda*Spa1) [24-26] reinforcing the hypothesis that SXT/R391 ICEs are widely distributed among different *Vibrio* species and environmental γ-proteobacteria and represent a large class of polymorphic genetic elements.

Another interesting ICE feature is represented by elements devoid of resistant genes, described in different *V. cholerae* isolates [20, 21, 26, 27]. Their circulation and maintenance provide an evidence of the possible presence of cryptic genetic information encoding for favourable factors, beside drug resistance.

Some ICEs bear genes for antimicrobial compounds biosynthesis (Tn5376, *L. lactis*), for DNA repair (Tn5252, *S. pneumoniae*), for nitrogen fixation and symbiotic growth with plant roots (*M. loti*) [28]. The hypothesis that ICEs participate in the mobilization of virulence factors is highly supported by recent data. ICE*Ec*1 in *E. coli*, carries the pathogenicity island HPI, already described in *Y. pestis*, coding for an iron uptake system involved in the expression of virulence genes [29].

ICEs should not then be considered just a mean of resistance transmission among clinical isolates, but as a potential vector for genetic information, widely distributed among bacterial strains of different origin.

2. Occurrence of Class 1 Integron Resistance Cassettes in Clinical Vibrio Isolates

Mobile genetic elements able to transfer multiple drug resistance among *V. cholerae* strains have been described in numerous African cholera epidemics since the early eighties and are considered a major public health problem [1]. Only recently have investigators studied the characteristics of class 1 integrons, both chromosomally and plasmid located, to explain variability in the spread of multiple drug resistance in African epidemics [30, 31].

So far class 1 integrons appear, by means of their cassettes content, to confer resistance to amikacin/tobramycin, β-lactams, erythromycin, gentamicin, kanamycin, aminoglycosides and trimethoprim to *V. cholerae* [22, 31-34]. To our knowledge, no resistance integrons were found in *Vibrio spp* other than *V. cholerae*, with the exception of the cases of *Vibrio fluvialis*, containing a class 1 integron characterized by an *aac(3)-Id* cassette, coding for resistance to aminoglycosides [24], and *V. parahaemolyticus* here described [27].

Horn of Africa was the port of entry of the seventh cholera pandemic in the seventies into Africa. We had the chance to examine two representative *V. cholerae* O1 clinical strains, isolated in Somalia in 1985 [35], showing different profiles of multiple drug resistance: CZM7, representative of the early stage of the cholera epidemic, prevailing in North West, and CZM36, the prevalent late epidemic strain in Mogadishu. As drug resistance is often associated with genetic mobile elements, we preliminarily submitted the strains to conjugation experiments, and followed resistance transfer to recipient strains. Both were characterized by large multiresistant conjugative plasmids of the same incompatibility group incC. In-F and In-B primers, respectively located in the 5' and 3' conserved sequences (CS) regions, were used to identify by PCR experiments class 1 integron-bearing strains among *Vibrio* isolates under study. Thus, it was shown that *V. cholerae* isolate CZM7 contained two different class 1 integrons containing *aadA1* or *dfrA15* cassettes, coding for spectinomycin and trimethoprim resistance, respectively. Since both were able to be transferred into recipient strains by bacterial conjugation procedures, it was possible to assess their location on the resident plasmid, confirmed by resistance transfer and class 1 integron PCR detection in the exconjugants. Conversely, the CZM36 plasmid was devoid of resistance integrons but characterized by tetracycline resistance; its emergence was supposedly selected by tetracycline treatment of cholera cases, leading to the exclusion of CZM7 plasmid of the same incompatibility group. This is an interesting model of integron circulation (emergence or loss) mediated by conjugative plasmids.

An additional example of plasmid located integrons is illustrated by our studies of cholera epidemics in the nineties in Angola [27], a country epidemiologically linked to the Great Lakes region of cholera epidemics.

The cholera epidemic in Angola started in 1987 and multiple resistances to drugs became soon an important public health problem [36]. The ubiquitous presence of the conjugative p3iANG plasmid, sizing about 80 kb, from Cabinda enclave in the north to Cuando Cubango province in the south, was the

main cause of resistance spread among epidemic *V. cholerae* O1 in Angola during the period 1991-1996 [27].

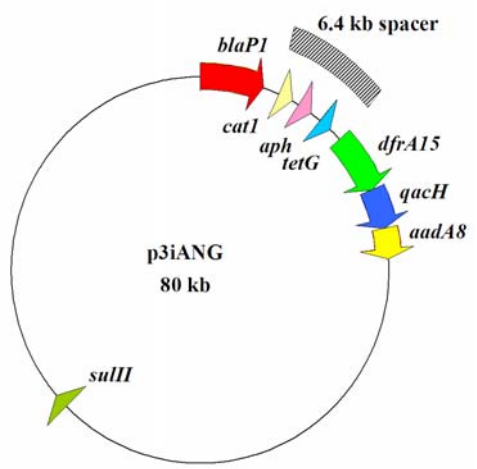

Fig. 2 p3iANG plasmid map (approximate sizes, not to scale). The three class 1 integrons are represented with solid arrows containing respectively *blaP1*, *dfrA15* and *qacH-aadA8* (joined in tandem) cassettes. The spacer, spanning 6.4 kb, is indicated by a striped rectangle, aside the plasmid. Triangles represent the four resistance cassettes: *cat1*, *aph* and *tetG* inside the spacer; *sulII* outside the integron region. Relative order and locations are roughly approximated. Further evidences indicate the presence of *tetR* too.

Interestingly p3iANG plasmid circulated also in environmental *V. cholerae* O1, isolated from Bengo River, water supply to Luanda population and possible reservoir of *Vibrio* epidemic strains.

Conjugation experiments and PCR testing demonstrated that it contained an array of clustered integrons. Three different integrons were identified and characterized by the following cassettes: the *dfrA15* dihydrofolate reductase gene for trimethoprim resistance, the *blaP1* β-lactamase gene and the *qacH* gene for quaternary ammonium compound resistance. The latter was followed by an *aadA8* 3'-adenyl transferase gene, for aminoglycoside resistance. This resistance cluster spans about 19 kb of the p3iANG plasmid, as shown in Fig. 2: the *blaP1* integron is located on the left, and the tail to head *dfrA15* and *qacH-aadA8* integron tandem on the right. They are separated by a 6.2 kb spacer, containing *cat1*, *aph* and *tetG*, resistance genes to cloramphenicol, kanamycin and tetracycline, respectively. Unpublished evidences of the presence of a transposase gene inside the spacer, suggest that this assemble may be an autonomous mobile element itself, including resistance factors found in other bacterial strains. The two integron cassettes, *blaP1* and *dfrA15*, show a complete sequence identity to the cassettes previously described in *V. cholerae* O1 clinical isolates in Thailand [34]; the *qacH* cassette was described in *E. coli,* carried by the plasmid located class 1 integron In53 [37]. The high variability of plasmid located integrons in epidemic *V. cholerae* O1, is also highlighted by the presence of a large conjugative resistance plasmid with class 1 integron borne gene cassettes (encoding resistance to trimethoprim, *dfrA12* and aminoglycosides, *aadA1*) found in 1996 in Guinea-Bissau [31]

Resistance factors encoded by integrons can also be chromosomally located and represent a widespread feature of *Vibrio*. Along with plasmid located integrons in *V. cholerae*, in Angola we found *V. parahaemolyticus* isolates containing the *dfrA15* class 1 integron. Although these strains contained a conjugative resistance plasmid, many evidences demonstrated that this integron was not associated with it, but was chromosomally located. We have no evidence to hypothesize identity or common origin with the analogous integron found on the *V. cholerae* O1 p3iANG plasmid; however, the presence of *dfrA15* class 1 integron, may indicate a strong pressure on *Vibrio* strains to select resistance to cotrimoxazole, widely used in drug therapy. Although superintegrons have already been described in many *Vibrio spp.* including *parahaemolyticus* [38], so far this is the only report of the presence of class 1 integrons in *V. parahaemolyticus* isolates [27].

The presence of chromosomally located resistance integrons was demonstrated to be a very common feature in *V. cholerae* O1 multiresistant epidemic strains, at least in Africa [35]. In a large study of representative *V. cholerae* O1 clinical strains from Mozambique, Rwanda, Swaziland and Zimbabwe, from 1994 to 1998, the presence of *dfrA15* and *aadA1* integron cassettes in all the isolates was revealed by PCR [35]. The *dfrA15* trimethoprim resistance cassette is associated with sulfamethoxazole resistance coded by integron 3'CS and its circulation may be once again related to the wide usage of cotrimoxazole.

The *aadA1* aminoglycoside resistance cassette, was present in almost all isolates and it was also described in Guinea Bissau [31]. Some authors reported the presence of *aadA2* in *V. cholerae* O1 isolated in South Africa from migrant workers from Mozambique [30], and we above described the presence of *aadA8* in Angola. The high incidence of *aadA* cassettes, the most frequently found resistance gene in the variable region of mobile integrons, may be related to the environmental origin of aminoglycosides, produced by naturally resistant microorganisms. During its environmental life cycle, *Vibrio* could be in contact with aminoglycoside producing bacteria, and thus be selected for the acquisition of this resistance.

We already mentioned the chance of resistance integron loss, depending on the level of antibiotic selective pressure. An example of this phenomenon is well described by the history of Vietnamese cholera epidemics.

It was reported that *V. cholerae* O1 epidemic strains from 1979 until 1990, although resistant to aminoglycosides, did not show such resistance mediated by integron cassettes. The *aadA1* class 1 integron cassette emerged in 1994 [33]. Ehara *et al.* kept tracking the evolution of aminoglycosides resistance circulation and its association with mobile genetic elements, and found a trend leading to susceptibility: resistance was related to *aadA1* class 1 integron cassette in 1995, coded by ICE*Vch*Vie1 (see next section for more details about ICEs in Vietnam) in 2000 and absent in 2002 [23].

We analyzed Vietnamese *V. cholerae* O1 isolates of 2003. Our strains, although showing a limited resistance pattern if compared with other epidemics [1, 30, 32] were resistant to aminoglycosides [20]. Therefore, our finding concerning the re-emergence of aminoglycosides resistance in 2003, inverts in some measure the general trend of drug resistance loss registered in the last decade.

We investigated the presence of resistant, conjugative or cryptic plasmids in the isolates, however no evidence of their presence was found, and no class 1 integrons were identified. It is not surprising that low resistant strains were devoid of mobile genetic elements, since these are the major means of drug resistance acquisition in *V. cholerae*.

3. Circulation of ICEs of the SXT/R391 Family in Vibrio Strains

After the discovery of R391 element in South Africa in the sixties, due to the interest generated by the emergence of SXT^{MO10} in India in *V. cholerae* [10], a correlated SXT element in *V. cholerae* O1 was discovered 30 years later in South Africa [30]. We considered this finding very interesting and decided to look for the presence of ICEs in *V. cholerae* O1 isolates from Mozambique, Swaziland and Zimbabwe in 1997-1998. These countries bordering South Africa and belonging to the same epidemic area, Austral Africa, were subjected to recurrent cholera epidemics in the eighties and in the nineties.

In Mozambique [35], preliminary analysis of several isolates by PCR experiments revealed the presence of genetic elements possessing int_{SXT} gene, able to conjugate and to integrate into *prfC*: conditions for their belonging to SXT/R391 family [18].

To better characterize the molecular profile of these ICEs, the strains were submitted to additional PCR amplification for detection of a few important regions as described in Fig. 1a. Thanks to this procedure, we detected the absence of the conserved region *traI* in all the ICEs. Among those tested, we discriminated three different ICEs belonging to the SXT/R391 family, according to their molecular profiles (unpublished results). Figure 1b shows 1) ICE*Vch*Moz7 positive only for *floR* and *dfrA1* resistance genes, characterized by a peculiar molecular profile, including an inserted region in Hotspot 1 belonging to R391, 2) ICE*Vch*Moz8 containing *dfrA1* only, and a specific molecular rearrangement, and 3) ICE*Vch*Moz9, positive for *floR*, *strA*, *strB* , *sul2* and *dfrA1*, (the same resistance pattern of ICE*Vch*Ind1, used as reference strain) and including an inserted region in Hotspot 1 belonging to R391.

The hybrid SXT and R391 rearrangement is not surprising. Since the two elements do not exclude each other they can be harboured by the same cell and have the chance of recombining parental regions [13]. It should be highlighted that this high molecular variability in the ICE population, probably due to self-arrangement by recombination events, confers different resistance features to *V. cholerae* O1 strains, isolated in the same area and period.

We also found that SXT related ICEs were circulating in Zimbabwe and in Swaziland [35]. No direct comparison may be done with those found in Mozambique and in South Africa, since no molecular characterization was reported. These findings are strong evidence of a wide circulation of ICEs of SXT/R391 family in Austral Africa, unlike in other epidemic areas and periods, such as in Somalia and Rwanda, where ICE presence was not found in the strains examined [35].

We should mention here ICE*Vch*Vie0, in relation to the correlation between ICEs and drug resistance [20]. This ICE was described in a *V. cholerae* O1 strain, isolated in Vietnam in 1990, and was reported not to contain any of the typical SXT resistance genes. This finding was consistent with its *rumAB* region arrangement, containing a gene relic matching SXT^{S} and devoid of the SXT^{MO10} resistance gene cluster [21], as in Fig. 1b.

ICEs devoid or having lost resistance genes had already been detected sporadically among strains isolated in Vietnam in 2000 and in Laos in 1998-2000 [23]. These ICEs seem to be more widespread than supposed and may constitute a wide cryptic class of mobile genetic elements bearing undiscovered adaptive functions [25].

This idea is reinforced by the finding of undefined ICEs in Angola among 1991 and 1993, devoid of any resistance factor [27]. We found a wide circulation of ICEs in several clinical *V. cholerae* O1 and *V. parahaemolyticus*, in environmental *V. cholerae* non O1, and *Providencia spp*. This group of ICEs is very peculiar: they are positive only for *int* gene and do not seem to integrate in *prfC* site, thus their classification was not easy. However, according to V. Burrus (personal communication) they resulted positive for *traG*, a gene involved in the SXT/R391 exclusion system [39], and were then included in SXT/R391 family [18]. We suspect that these three ICEs might infact be the same, but circulating in different bacterial species.

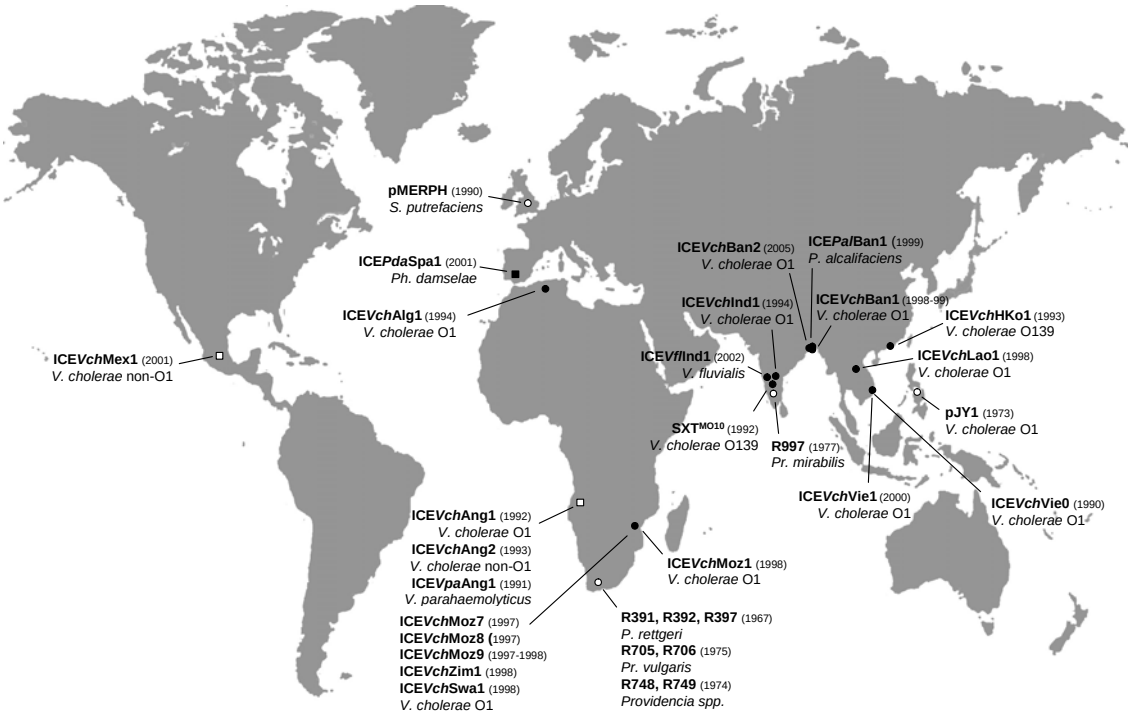

Fig. 3 World distribution of ICEs of SXT/R391 family, including the ICEs described in this study. Figure adapted from Burrus *et al.* [18].

From all these evidences, it appears that SXT-like elements are ubiquitous in very different geographic areas (see Fig. 3), especially in Austral Africa and that they are very variable. They are not a

specific feature of *V. cholerae* but a genetic element belonging to the *Vibrio* genus and other bacterial genera.

4. Fitness Impact of Integrons and ICEs in Vibrio

We should remind that horizontal transmission of exogenous DNA is almost ubiquitous in all bacterial genera and is the main strategy to evolve, adapt and survive to different environmental challenges, including antimicrobial drug exposure. The knowledge of how new bacterial pathovars emerge is of great scientific interest, and *Vibrio* offers a natural system to study the coevolution of bacteria and genetic elements involved in genome plasticity [5].

The discovery of mobile integrons contributed greatly to the understanding of resistance acquisition and organization in bacterial strains, after the description of plasmids, phages and transposons. Integrons confer to bacteria an efficient system of genetic capture, able to face both natural antibiotics in the environment and drug treatment in therapy. Gene cassette recruitment may also provide bacteria with new metabolic functions, conferring potential evolutional advantages, beside the simple antibiotic resistance.

Mobile integrons and superintegrons have identical structural organizations, and the antiquity of superintegrons suggests that they could be ancestral to mobile integrons, and could have evolved through the entrapment of *intI* genes and *attI* sites by mobile elements, such as transposons [5]. The high variability of gene cassettes associated with mobile integrons and the discovery of superintegrons, extend the importance of these elements, which potentially give bacteria new enzymatic functions and adaptive advantages [5], contributing to bacterial genome plasticity.

Also, ICEs were initially identified because of their association with an antibiotic resistance phenotype but then emerged as featuring a wide spectrum of additional acquired functions. The integration mechanism into a specific chromosomal site, shared by most ICEs, is a common characteristic with pathogenic genomic islands. Genomic island setting goes through gene capture and phage and transposon assembly, thus it might be evolutionary related to ICE. This might be a starting step toward the final evolution of a new genomic island. Therefore this has reinforced the idea that ICEs constitute a class of mobile elements more common and more composite than imagined, able to confer advantages through genome plasticity.

We have here described the high flexibility of the genetic structure of ICEs, not only in their resistant determinants and clusters, but also in their organization of transfer and regulative functional modules. The mechanism of insertion and the polymorphism of resistance genes, suggest that they might have been acquired later in the scaffold of the element, and then be allowed to be transferred. Also these resistance determinants can be easily lost when not required. We have found ICE*Vch*Vie0, that has lost all typical SXT resistance genes and also ICEs from Angola that apparently do not posses any. We have also found in aquatic environmental *Vibrio*, isolated in Mozambique, several ICEs of the SXT/R391 family that have an intact *rumAB* region, efficiently encoding for UV protective proteins (Taviani *et al.*, manuscript in preparation). Conserving ICEs devoid of resistance genes, but coding for other functions, may reflect a selective advantage in the environment where the strains are exposed more to UV radiation than to antibiotics, as is the case for clinical strains. Investigation of the origin and evolution of ICEs might be more fruitful approaching the microflora of the aquatic environment, where *Vibrio* is a natural component and clinical *Vibrio* strains also circulate.

In our opinion, the most suitable hypothesis for SXT origin in *V. cholerae*, might be environmental more than clinical. Furthermore, the critical spreading of SXT was reported in 1992, with the emergence of *V. cholerae* O139 in India [10], but the wide circulation of polymorphic ICEs in Africa, in different bacterial species since the sixties, and SXT–like elements in the nineties, is consistent with the idea of the autonomous emergence and spreading (if not the origin) of SXT/R391 family in Africa[18]. This picture is intriguing but is still a hypothesis that needs further molecular evidences that will clarify the African ICE phylogeny and their correlation with Indian ICEs.

In conclusion, it should be remarked that integrons and ICEs share the function of assembling new genetic information horizontally transmittable through different bacterial genera. This ability is

represented by the presence of an integrase, a powerful enzyme mean of genetic variability. This observation indicates the impact of these elements in the arrangement of emerging, environmental and pathogenic bacterial species and the importance of studying these elements.

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