New and emerging SXT/R391 integrative conjugative elements as vehicles for stable mobile element transfer and spread of antibiotic resistance in both human and animals

Michael P Ryan[1, 2], Patricia Armshaw[1] and J Tony Pembroke[*1, 2]

[1]Molecular Biochemistry Laboratory, Department of Chemical Sciences, School of Natural Sciences and the [2]Bernal Institute, University of Limerick, Ireland

*Corresponding author: email: tony.pembroke@ul.ie Tel: 353 61 202491

The integrative conjugative elements, ICE's SXT and R391 are the prototypes of a group of gram negative integrative elements known as the SXT/R391 group. R391 was identified in a clinical isolate of *Providencia* in the late 1960's in South Africa, while SXT was initially isolated in 1992 in a clinical isolate of *Vibrio cholerae* O139 and variants have since been isolated in pandemic strains throughout the world. Subsequent sequencing of both elements demonstrated a high degree of structural similarity leading to the group being classified as the SXT/R391 group. The SXT/R391 ICE elements are characterised as integrating into a specific chromosomal site within gram *–ve* hosts, being extremely stable and promiscuous and possessing a number of element hotspots for integration of heterologous DNA including increasingly, antibiotic resistance determinants. This makes such ICE's highly adapted for antibiotic spread. New evidence emerging indicates that SXT/R391-like ICE's are increasingly being identified worldwide particularly in Asia not only from *Vibrio* species, where they have been found widely in human clinical isolates, but from other gram *-ve* associated infections of domestic animals and fish. Evidence of more such elements may emerge in the future as a new trapping vector *p*IceCap has been developed to capture them in a circular form, aiding characterisation. The types of the novel ICE's now emerging, their comparison with prototype elements and the antibiotic resistances associated with them are important given their promiscuous nature and stability.

Keywords: SXT/R391-like ICE's; stable mobile elements; antibiotic spread

1. Introduction

Integrative conjugative elements (ICE's) are a type of bacterial mobile genetic element that integrate into the host chromosome and are capable of conjugating widely [1-5]. ICE elements belonging to the SXT/R391 group are amongst the best characterized and studied of the enterobacterial elements with some 100 examples of different SXT/R391 ICE's being reported so far. This group were initially classified as IncJ plasmids [6] however inability to isolate them coupled to the observation that they were capable of *rec*A independent chromosomal integration led to their classification as conjugative transposons [7, 8] and later as ICE's. The prototype of this group, the element R391, was initially identified in *Providentia rettgeri* from a clinical isolate in South Africa [9]. Isolation of a circular transfer form of the ICER391 [10] led to the ability to sequenced the entire element [11] Sequencing showed it to be an integrating element with phage, plasmid and transposon characteristics with a type IV conjugative transfer system. Soon afterwards the SXT element, prevalent in epidemic strains of *Vibrio cholerae* and found in all pandemic strains of the O14 group, was shown upon sequencing to be highly homologous to R391 [12]. To unify the nomenclature elements related to R391 and SXT in terms of sequence and structure were termed ICEs and related elements were classified as SXT/R391 elements [13]. The ICE's SXT, R391, R392, R705, pMERPH and R997 elements were shown to integrate into the *prf*C gene (encoding an essential peptide release factor) of their enterobacterial host causing truncation of the *prf*C gene but restoring function by the element encoding a new "n" terminus for the hybrid encoded protein [14, 15]. This unusual integration into a unique 17bp sequence within the *prfC* gene is catalyzed by the ICE encoded integrase and leads to a single stable copy of the element within its host [15]. The 17bp integration site is conserved in a wide range of proteobacterial genera as shown by bioinformatics analysis [16] and hence SXT/R391 ICE's are capable of stable transfer to a wide variety of bacterial hosts. Several methods can be utilized to identify ICE elements including pulse field gel electrophoresis [17], PCR to identify the unique and well conserved integrase gene [18] and genomic sequencing [19].

The elements themselves can undergo stress inducible enhanced conjugative transfer, which may be an evolutionary adaptation which enhances survival of the SXT/R391 ICE's [18, 20, 21]. The basis of this enhanced transfer is due to a stress inducible excisionase encoded by the ICE's which enhances chromosomal excision providing elements for conjugative transfer and spread [22]. One of the key characteristics of SXT/R391 ICE elements is that they encode an unusual UV sensitizing function [18, 23]. Analysis of this genotype in the ICER391 via deletion analysis [24] has demonstrated that an inducible transfer gene termed *orf43* in the ICER391, upon overexpression [25, 26] allows low-level transfer of SXT/R391 ICE elements upon host damage. This has been termed a trap door mechanism and hypothesized to be a unique adaptive tool to allow ICE's escape and transfer even following extreme damage to the host

[27]. Thus inducible transfer to a wide range of protobacterial genera, integration in a stable manner, ability to recombine and integrate heterologous DNA with adaptive function such as antibiotic resistance determinants make such elements key drivers and maintainers of antibiotic resistance spread. Indeed in the SXT system it has been shown that these ICE's can also mobilise other genomic islands [28] and virulence plasmids [29] particularly in *Vibrio cholerae* strains

2. Lessons from comparative analysis of ICE elements

Comparative sequence analysis of the known SXT/R391 elements has revealed that they share extensive sequence homology, gene synteny and ability to integrate adaptive genes such as antibiotic resistance, restriction systems and metal resistance determinants [16, 30]. Comparison is compromised somewhat because each ICE on sequencing has been annotated separately and uniquely such that the gene nomenclature of SXT and R391 are different as are subsequent annotations as new ICE's have been discovered [11, 12, 19, 30]. The elements vary in size between 80kb to 110kb with core ICE genes showing extensive homology and more than 50 of the core genes showing extensive synteny [30]. The variability observed when comparing SXT/R391 ICE's is generally associated with a number of hotspots present within the ICE's [31]. Five hotspots for integration of DNA termed HS1-HS5, located within intergenic regions have been determined (Figure 1) based on sequence comparisons with known ICE elements, while variable regions have also been found to exist within the ICE SXT-R391 family, one (Variable region III) of which interrupts the *rum*B gene is seen in many ICEs such as SXT [30]. Comparative analysis has shown that the core genes encode functions for transfer and maintenance of the elements such as the IncA/C like conjugative transfer genes [30, 32], the *int* and *xis* genes, and control genes such as *orf90* and *orf91* (as per the R391 nomenclature) [11,16]. The incA/C transfer-like genes and *bet* and *exo* recombination genes and a small number of genes of unknown function are highly homologous (34-78%) and syntenous to genes associated with the *Yersinia pestis* IP275 plasmid *p*IP1202 however other genes such as *int*, *xis* and transcriptional activators (related to *orfs90/91*) are absent [30, 32] from this and may have a phage origin.

3. Antibiotic resistance spread through ICE elements

Recently based on use of DNA probes, genomic sequencing and comparative bioinformatic analysis there have been a large number of novel SXT/R391-like ICEs determined (Table 1). These elements have been identified in microbes of animal origin, as opposed to human clinical isolates, in chickens, pigs and in fish. The isolates follow the known ICE structure, as determined in previous characterized ICE's. The insertions are located into hotspots of preferred locations with many encoding antibiotic resistance determinants including resistance to SXT, tetracycline, streptomycin, penicillin's and cephalosporins. Data summarized in Table 1 suggests that the SXT/R391-like ICE's emerging from animal sources may be an important vehicle for the transfer of drug resistance.

Most antibiotic resistance genes in SXT/R391 elements are found within variable region III that disrupts the *rumB* gene (see Fig. 1, Table 1) possibly via carriage on a transposable element. This region is highly variable and can have between 9 to 17 genes. Antibiotic resistances found here include genes for resistance to ampicillin (amp) and cephalosporins (Bla_{HMS-1} and Bla_{CMY-2}) chloramphenicol (*floR*), streptomycin (*strBA*), sulfamethoxazole (*sulII*), erythromycin (Ery^r - *mphRK*, *mrx,),* tetracycline (*tetA*) and trimethoprim (*dhfR18*). Others are found in the Hotspots described above (Table 1).

Table 1 Summary of a selection of novel SXT/R391-ICEs determined recently and emerging from animal sources showing the pattern of antibiotic resistance and the insertion location within the core ICE genome.

ICE Type	Resistances	ICE Insert Location	Reference
ICE*Pvu*CHN2213	*flo*R, *str*B, *str*A, *sul*2	III	33
ICE*Pmi*CHN1586	*dhf*R, *flo*R, *str*B, *str*A, *sul*2	III	33
ICE*Pmi*CHN904	*flo*R, *str*B, *str*A, *sul*2, *tet*AR, Bla_{HMS-1}	III, HS4	33
ICE*Pmi*CHN3300	*dhf*R, *flo*R, *str*B, *str*A, *sul*2, *dhf*R	III, HS3	33
ICE*Pmi*CHN3335	*flo*R, *tet*R/A *str*B, *str*A, *sul*2	III	33
ICE*Pmi*CHN2407	$Bla_{HMS-1,}$ *tet*AR	III, HS4	33
ICE*Pmi*CHN2410	Bla_{HMS-1}	III	33
ICE*Pmi*CHN2416	Bla_{HMS-1}	III	33
ICE*Pmi*CHN901	*dhf*R, *flo*R, *str*B *str*A *sul*2, Bla_{HMS-1}	III	33

ICE*Pmi*CHN902	*dhf*R, *flo*R, *str*B *str*A *sul*2, Bla_{HMS-1}	III	33
ICE*Pmi*CHN903	*dhf*R, *flo*R, *str*B *str*A *sul*2, Bla_{HMS-1}	III	33
ICE*Pmi*CHN905	*flo*R, *str*B. *str*A. *sul*2, $Bla_{HMS-1,}$ *tet*AR	III, HS4	33
ICE*Pmi*CHN3237	Bla_{HMS-1}	III	33
ICE*Val*AO56-1	*strBA*, *sul*2	HS5	34
ICE*Val*HN396	hydroperoxide^{R}	HS3	34
ICE*Vsc*Spa1	RM	HS5	35
ICE*Pmi*Fra 1	Bla_{CMY-2} (AmpC)	III, V	36
ICE*Pmi*Jpn1	Bla_{CMY-2} (AmpC)	III, V	37
ICE*Mfu*Ind1a	RM, czcA	HS5, HS2	38
ICE*Mpr*CHN1	Multidrug	HS1	38
ICE*Pmi*Chn1	*floR, tetG, StrAB, sul2*	III	39
ICE*Vch*Chn6	Amp^{r}, Sul, Str (Hg Cd Pb)	HS3, HS4	40
ICE*Vpa*Chn1	Str^{r}	III, HS3	40
ICE*Vpa*Chn2	Amp^{r}	III	40
ICE*Vch*Chn0143	Sm^{r}, Su^{r}, Tm^{r}, Tc^{r}	III	41
ICE*Vch*Chn0956	Sm^{r}, Su^{r}, Tm^{r}	III	41
ICE*Vch*Chn1605	Sm^{r}, Su^{r}, Tm^{r}	III	41
ICE*Vch*Chn1627	Sm^{r}, Su^{r}, Tm^{r}, Tc^{r}	III	41
ICE*Vch*Chn1909	Sm^{r}, Su^{r}, Tm^{r}	III	41
ICE*Vch*Chn1944	Sm^{r}, Su^{r}, Tm^{r}	III	41
ICE*Vch*Chn2255	Sm^{r}, Su^{r}, Tm^{r}, Tc^{r}	III	41
ICE*Vch*Chn2605	Sm^{r}, Su^{r}, Tm^{r},	III	41
ICE*Vch*Chn4210	Sm^{r}, Su^{r}, Tm^{r}, Tc^{r}	III	41
ICE*Vch*Chn57	Sm^{r}, Su^{r}, Tm^{r}, Tc^{r}	III	41
ICE*Vch*ChnAHV1003	Ery^{r}, Sm^{r}, Su^{r}, Tm^{r},	III	41
ICE*Vsp*Por3	Tc^{r}	III	42

4. A novel transfer mechanism associated with SXT/R391-like ICE's

The ability of SXT/R391-like ICE's to transfer widely amongst enterobacterial hosts, integrate, become stably maintained in the host genome and contain integrative hotspots for the acquisition of resistance determinants makes their behavior different from plasmid counterparts. In total 19 ICE genes can be classified as being directly involved in ICESXT/R391 conjugative transfer, either in preparation of the ICE DNA for transfer or in construction of the type IV conjugative machinery. These genes are *mob*l, *tra*I/D/J, *tra*L/E/K/B/V/A, *dsb*C, *tra*C, *trh*F, *tra*W/U/N and *tra*F/H/G and also required is the origin of transfer *ori*T [30]. Recently it has been shown that in addition to this system, a novel 'trap door' mechanism exists which can result in ICE transfer as a last ditch resort [27] in cases where the host is damaged. Under damage conditions (such as UV or chemical damage), members of the SXT/R391 group can induce a regulatory loop which is controlled by the ICE repressor *orf*96. Upon cleavage it results in transcription of *orf* 90 and *orf* 91 which are themselves transcriptional activators [20, 43]. These activate transcription of *orf*4 encoding an ICE excisionase which results in excision of the element to form a circular transfer intermediate [10, 22]. Activation of *orf* 90 and 91 also results in upregulation of *orf*43, a *tra*L homolog, whose overexpression results in lysis of the host cell [24 - 27]. This lysis event allows escape of the circular intermediate making it available for uptake via transformation. This trap door mechanism is an added survival mechanism which has consequences for ICE survival and spread over and above traditional conjugative transfer mechanisms. This lysis explains an unusual feature of SXT/R391 elements which showed lower damage survival following treatments such as UV irradiation [23].

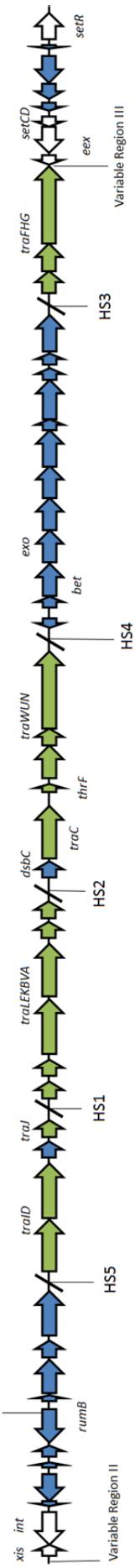

Figure 1 Genetic organization of the ICER391 [11] showing the hotspots for insertion HS1-HS5 that occur in the majority of SXT/R391 ICES. In addition there are a number of variable insertion sites which occur in some SXT/R391 like ICE's. These have been termed Variable regions I-IV. Recombination events occur at high frequency into these regions resulting in the acquisition of genes of known or indeed unknown function. These regions also acquire the majority of the antibiotic resistance determinants observed in many of the newer ICE-like elements.

It now appears that this decreased survival may in fact be an adaptive lysis mechanism to allow element escape under adverse conditions.

Such an array of mechanisms, stable integration into a conserved 17 bp integration site in a large selection of hosts catalyzed by a conserved integrase, ability to pick up adaptive functions easily in selective hotspots, possession of a type IV conjugative transfer system, ability to mobilise pathogenicity and genomic islands and finally an adaptive fail safe mechanism of escape when the host is damaged makes the SXT/R391 group of ICE's an important player in dissemination of antibiotic resistant determinants in human and animal systems.

5. Trapping and characterisation of new ICE's

As identifying novel integrative SXT/R391-like ICE's can be difficult a new vector has been developed to aid their recovery and identification. pIceCap is a trapping vector [30] based on a modified F-plasmid pXX704, which lacks transfer genes. This vector is similar to the use of an F'prime vector containing the SXT/R391 chromosomal integration site used previously to monitor chromosomal integration of SXT/R391 ICEs [8]. This pIceCap vector contains the attachment site *att*B for SXT/R391 ICES allowing ICE catalyzed integration. This vector is maintained in an *E.coli* host harbouring a deletion in the *prf*C gene (where SXT/R391 ICEs integrate), thus forcing integration into the pIceCap vector [30]. To trap novel ICE elements conjugation between potential ICE harbouring strains and this *E.coli* harbouring the pIceCap forces integration into the trapping vector. The vector can then be isolated and sequenced, once size increase due to the integrating ICE has been verified. This should aid in recovery of more such elements in the future for ease of characterisation.

References

[1] Pembroke JT, McGrath B., MacMahon C. The role of conjugative transposons in the Enterobacteriaceae. Cellular and Molecular Life Sciences. 2002; 59: 2055-64.

[2] Burrus V, Waldor MK. Shaping bacterial genomes with integrative and conjugative elements. Research in Microbiology. 2004; 155: 376-86.

[3] Ryan MP, Pembroke Pembroke JT, Adley CA. Novel Tn*4371*-ICE like element in *Ralstonia pickettii* and Genome mining for comparative elements. *BMC Microbiology* 2009; 9: 242.

[4] Brochet M, Couve E, Glaser P, Guedon G, Payot S. Integrative conjugative elements and related elements are major contributors to the genome diversity of *Streptococcus agalactiae*. Journal of Bacteriology. 2008; 190: 6913-17.

[5] Van Houdt R, Toussaint A, Ryan MP, Pembroke JT, Mergeay M, Adley CA. The Tn*4371* ICE family of bacterial mobile genetic elements. In: Roberts AP, Mullany P, editors. Mobile Genetic Elements. Landes Bioscience; 2012; p179-200.

[6] Hedges RW. R-factors from *Proteus mirabilis* and *Proteus vulgaris*. Journal of General Microbiology. 1975; 87: 301-31.

[7] Murphy DB, Pembroke JT. Transfer of the IncJ plasmid R391 to recombination deficient *E. coli*: Evidence that R391 behaves as a conjugal transposon. FEMS Microbiology Letters. 1995; 134: 153-58.

[8] Murphy DB, Pembroke JT. Monitoring of chromosomal insertions of the IncJ group elements R391 and R997 into the chromosome of *Escherichia coli* K12. FEMS Microbiology Letters. 1999; 174: 355-61.

[9] Coetzee J, Datta N, Hedges RW. R factors from *Proteus rettgeri*. Journal of General Microbiology. 1972; 72: 543-52.

[10] Pembroke JT, Murphy DB. Isolation and Analysis of a circular form of the IncJ conjugative transposon-like elements R391 and R997: implications for IncJ incompatibility. FEMS Microbiology Letters. 2000; 187: 133-38.

[11] Böltner, D, MacMahon C, Pembroke JT, Strike P, Osborn AM. R391 is an 89 kb conjugative genomic island comprising elements related to plasmids, phages, and transposable elements. Journal of Bacteriology. 2002; 184: 5158-169.

[12] Beaber JW, Hochhut B, Waldor MK. Genomic and functional analyses of SXT, an integrating antibiotic resistance gene transfer element derived from *Vibrio cholerae*. Journal of Bacteriology. 2002; 184: 4259-269.

[13] Burrus V, Marrero J, Waldor MK. The current ICE age: biology and evolution of SXT-related integrating conjugative elements. Plasmid. 2006; 55: 173-83.

[14] Burrus V, Waldor MK. Control of SXT integration and excision. Journal of Bacteriology. 2003; 185: 5045-54.

[15] McGrath BM, Pembroke JT**.** Detailed analysis of the insertion site of the mobile elements R997, pMERPH, R392, R705 and R391 in *E. coli* K12. FEMS Microbiology Letters*.* 2004*;* 237: 19-26.

[16] Armshaw P Pembroke JT. Integrative Conjugative Elements (ICE's) of the SXT/R391 group as vehicles for acquisition of antibiotic resistance determinants, stable maintenance and transfer to a wide range of enterobacterial pathogens. In Méndez-Vilas A, Editor. Microbial pathogens and strategies for combating them: science, technology and education. Formatex. 2013, p 439-44

[17] Pembroke JT, McGrath BM. Pulsed Field Gel Electrophoresis to rapidly detect the presence of IncJ conjugative transposon-like elements. Letters in Applied Microbiology. 2005; 41: 258-61.

[18] McGrath BM, O'Halloran JA, Piterina A, Pembroke JT. Molecular probes to detect IncJ elements: a family of integrating, antibiotic resistance mobile genetic elements. Journal of Microbiological Methods. 2006; 66: 32-42

[19] Pembroke JT, Piterina AV. A novel ICE in the genome of *Shewanella putrefaciens* W3-18-1: Comparison with the SXT/R391 ICE-like elements. FEMS Microbiology Letters. 2006; 264 (1) 80-88.

[20] Beaber JW, Hochhut B, Waldor MK. SOS response promotes horizontal dissemination of antibiotic resistance genes. Nature. 2004; 427: 72–74.

[21] Armshaw P Pembroke JT. UV Stress responsive genes associated with enterobacterial integrative conjugative elements of the ICE SXT/R391 group. In de Brujin F. Editor. Stress and environmental regulation of gene expression and adaption in bacteria. Wiley Publishers. 2016, p 519- 31.
[22] O'Halloran JA, McGrath BM, Pembroke JT. The *orf*4 gene of the enterobacterial ICE, R391, encodes a novel UV inducible recombinational directionality factor, Jef, involved in excision and transfer of the ICE. FEMS Microbiology Letters. 2007; 272: 99-105.
[23] Pembroke JT, Stevens E. The effect of plasmid R391 and other IncJ plasmids on the survival of *Escherichia coli* after UV irradiation. Journal of General Microbiology. 1984; 130: 1839-844.
[24] Armshaw P, Pembroke JT. Generation and analysis of an ICE R391 deletion library identifies genes involved in the element encoded UV-inducible cell-sensitising function. FEMS Microbiological Letters. 2013; 342; 45-53.
[25] Armshaw P, Pembroke JT. Control of expression of the ICE R391 encoded UV-inducible cell-sensitising function. BMC Microbiology. 2013; 13 195-202.
[26] Armshaw P, Pembroke JT. Examination of the cell sensitising conjugative transfer gene of ICE R391, orf43 suggests a secondary role in ICE transfer enhancement to recipient cells. FEMS Microbiological Letters. 2015; 362(4) 1-7.
[27] Ryan MP, Armshaw P, Pembroke JT. SXT/R391 ICE elements encode a novel 'trap-door' strategy for mobile element escape. Frontiers in Microbiology. 2016; 7:829
[28] Daccord A, Ceccarelli D, Burrus V. Integrating conjugative elements of the SXT/R391 family trigger the excision and drive the mobilization of a new class of *Vibrio* genomic islands. Molecular Microbiology. 2010; 78: 576-88.
[29] Osorio CR, Marrero J, Wozniak RAF, Lemos ML, Burrus V, Waldor MK. Genomic and functional analysis of ICE*Pda*Spa1, a fish-pathogen-derived SXT-related integrating conjugative element that can mobilize a virulence plasmid. *J Bacteriol* 2008; 190: 3353-361.
[30] Wozniak RAF, Fouts DE, Spagnoletti M, Colombo MM, Ceccarelli D, Garriss G, Dery C, Burrus V, Waldor MK. Comparative ICE Genomics: insights into the Evolution of the SXT/R391 Family of ICEs. PLoS Genetics. 2009; 5: 13.
[31] Beaber JW, Burrus V, Hochhut B, Waldor MK. Comparison of SXT and R391, two conjugative integrating elements: definition of a genetic backbone for the mobilization of resistance determinants. Cellular and Molecular Life Sciences. 2002; 59: 2065-070.
[32] Fricke WF, Welch TJ, McDermott PF, Mammel MK, LeClerc JE, White, DG, Cebula TA, Ravel J. Comparative genomics of the IncA/C multidrug resistance plasmid family. Journal of Bacteriology. 2009; 191: 4750-757.
[33] Li X, Du Y, DU P, Dai H, Fang Y, Z, Li N, LV N, Zhu B, Kan B, Wang D. SXT/R391 integrative and conjugative elements in *Proteus* species reveal abundant genetic diversity and multidrug resistance. Scientific Reports. 2016; 6: 37372.
[34] Luo P, He X, Wang Y, Liu Q, Hu C. Comparative genomic analysis of six new-found integrative conjugative elements (ICEs) in *Vibrio alginolyticus*. BMC Microbiology. 2016; 16:79.
[35] Rodríguez-Blanco A, Lemos ML, Osorio CR .Integrating conjugative elements as vectors of antibiotic, mercury, and quaternary ammonium compound resistance in marine aquaculture environments. Antimicrobial Agents and Chemotherapy. 2012; 56: 2619-26.
[36] Aberkane S, Compain F, Decré D, Dupont C, Laurens C, Vittecoq M, Pantel A, Solassol J, Carrière C, Renaud F, Brieu N, Lavigne JP, Bouzinbi N, Ouédraogo AS, Jean-Pierre H, Godreuil S. High Prevalence of SXT/R391-Related Integrative and Conjugative Elements carrying bla$_{CMY-2}$ in *Proteus mirabilis* isolates from Gulls in the south of France. Antimicrobial Agents and Chemotherapy. 2015; 60: 1148-52
[37] Harada S, Ishii Y, Saga T, Tateda K, Yamaguchi K. Chromosomally encoded bla*CMY-2* located on a novel SXT/R391-related integrating conjugative element in a *Proteus mirabilis* clinical isolate. Antimicrobial Agents and Chemotherapy. 2010; 54: 3545-50
[38] Badhai J, Das SK. Characterization of Three Novel SXT/R391 Integrating Conjugative Elements ICE*Mfu*Ind1a and ICE*Mfu*Ind1b, and ICE*Mpr*Chn1 Identified in the Genomes of *Marinomonas fungiae* JCM 18476T and *Marinomonas profundimaris* Strain D104. Frontiers in Microbiology 2016; 7: 1896.
[39] Lei CW, Zhang AY, Wang HN, Liu BH, Yang LQ, Yang YQ. Characterization of SXT/R391 integrative and conjugative elements in *Proteus mirabilis* isolates from food-producing animals in China. Antimicrobial Agents and Chemotherapy. 2016; 60: 1935–1938.
[40] Song Y, Yu P, Li B, Pan Y, Zhang X, Cong J, Zhao Y, Wang H, Chen L. The mosaic accessory gene structures of the SXT/R391-like integrative and conjugative elements derived from *Vibrio* spp. isolated from aquatic products and environment in the Yangtze River estuary, China. BMC Microbiology. 2013; 13: 214.
[41] Wang R, Yu D, Yue J, Ban K. Variations in SXT elements in epidemic *Vibrio cholerae* O1 El Tor strains in China. Scientific Reports. 2016; 6: 22733.
[42] Balado M, Lemos ML, Osorio CR. Integrating conjugative elements of the SXT/R391 family from fish-isolated *Vibrios* encode restriction–modification systems that confer resistance to bacteriophages. FEMS Microbiology Ecology. 2013; 83: 457-67.
[43] McGrath BM, O'Halloran JA, Pembroke JT. Pre-exposure to UV irradiation increases the transfer frequency of the IncJ conjugative transposon-like elements R391, R392, R705, R706, R997 and pMERPH and is recA+ dependent. FEMS Microbiology Letters. 2005; 243: 461-65.