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On the Role of Genomic Islands in Bacterial Pathogenicity and Antimicrobial Resistance

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Abstract The problem of antimicrobials use failure is increasing everyday due to the transfer of virulence and antimicrobial resistance genes among different bacterial species where Genomic Islands (GEIs) have a major role. Virulence and antimicrobial resistance genes are encoded on GEIs where they can excise from a host chromosome, transfer and integrate into a new chromosome. Nonpathogenic strains become pathogenic by horizontal transfer of GEIs which in turn contributes in evolution of bacteria. GEIs was originated for symbiotic purposes then became pathogenic due changes in the environment and genes acquisition and they are useful in bacterial diagnosis as they are species-specific in some strains. The locus of enterocyte effacement (LEE) pathogenicity islands (PAIs) of Enteropathogenic *E.coli* (EPEC), *Staphylococcus* PAIs encoding toxic shock syndrome toxin (TSST), *Salmonella* genomic island 1(SGI1) of *S.* Typhimurium, the AbaR and Tn*6167* GEIs of *A. baumannii* and the cytotoxin-associated gene (*cag*) and *tfs4* GEIs of *H. pylori* are well known examples of GEIs. Target DNAs are edited by Clustered regularly interspaced short palindromic repeats (CRISPRs) and their associated (Cas) proteins causing bacterial evolution and pathogenicity. Genomic signature variation is utilized for detection of GEIs in host chromosome.
Keywords *Antimicrobials; Evolution; Horizontal; Islands; Pathogenic; Resistance*

1. Introduction

GEIs are large regions on the chromosome that have a base composition which is different from the whole genome, encode an integrase and are inserted at tRNA loci (Figure 1) [1]. GEIs may be pathogenicity and resistance islands when they encode disease-causing toxins and antibiotic resistance genes respectively. Also, it can be of other functions like carbon and nitrogen sources utilization [2-3].

Genomic content of the islands differs between different species and between the strains of the same species. All GEIs contain a recombination module which consists of an integrase, *attL* and *attR* (attachment sites), and in some cases excisionases or recombination directionality factor (RDFs) as shown in (Figure 1) [1].

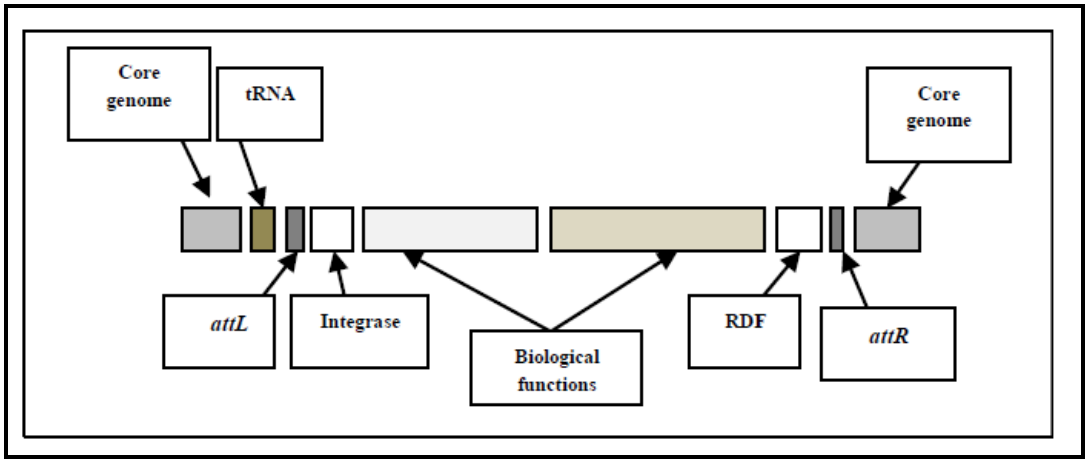

Figure 1: *Main Components of a Genomic Island*

2. Characteristics of GEIs

Bacterial genomes consist of the core gene pool which only contains chromosomal genes encoding basic cellular functions and the flexible gene pool which comprised of mobile genetic elements such as plasmids, transposons, phages, integrons, genomic islets (<10kb) and GEIs (>10kb) [4]. These genetic elements are transmitted between different bacterial genera and species. GEIs harbor plasmid and / or phage-derived sequences like transfer genes or integrases and insertion sequence (IS) elements. These DNA units are inserted into tRNA genes and may be unstable, due to flanking direct repeats which represent recognition sequences for enzymes involved in the excision of mobile genetic elements [5]. The repeats are homologous to the sequence of phage attachment sites and promote integration into and excision out of the bacterial genome [6]. They are created during mobile genetic element integration into the chromosome of the host, by site-specific recombination resulting in the duplication of the integration site [5]. Most integrases of the GEIs are inserted adjacent to tRNA loci [1].

Integrases are specific for tmRNA more than tRNA sites, as the *ssrA* gene encoding tmRNA was over represented more than six fold as site of integration compared to other tRNA loci. Also, selenocysteinyl-tRNA gene (tRNA-SelC) and the tRNA-Arg gene are preferred as integration sites on the contrary of tRNA-Met and tRNA-Ala genes [7-8]. In *E.coli*, the *asnT*, *aspV*, *LeuX*, *metV*, *pheV* and *thrW* genes were the most frequently targeted tRNA genes [9]. As determined by prophage identification method of the sequenced genomes, tRNA-Arg, -Leu, -Thr and -Ser were the most frequent sites of insertion and tmRNA was less prevalent as a target [10].

GEIs have G+C content and codon usage which differ from the core genome. Some GEIs carry a set of virulence-related genes called PAIs. These sets of virulence genes might have been acquired as a block, so causing a dramatic change in the organism's behavior [3; 2]. Pathogenic potency and / or transmission to new recipient hosts increase by PAI acquisition. PAIs contain different virulence genes like toxin-encoded genes (e.g. α-hemolysin), iron-uptake systems (e.g. yersiniabactin), adhesions-encoded genes (e.g. P-related pilli, S-fimbriae), invasion systems, protein secretion systems (e.g. type Ш secretion systems, type IV secretion systems) and other effectors (Tia of enterotoxigenic *E.coli*). Virulence-related genes may be encoded by a single PAI type or different PAIs combination [6].

3. Integration and Excision of GEIs

GEIs for which self-mobility has reported, can excise from their chromosomal location and reintegrate into the new host's chromosome target site encoding the full capacity for horizontal self transfer to another cell as shown in (Figure 2). Integrative conjugative elements (ICEs) include GEIs that exhibit simultaneously all these features and self-transfer by conjugation [11], but GELs that do not self-transfer by conjugation but by phage packaging release and infection are not called ICEs. GEIs are often inserted in the 3¯- end of tRNA genes [12; 13; 8]. Site-specific phage-like recombinases called integrases are usually encoded by the GEI itself and catalyze GEI insertion [8]. The integrases are also involved in GEIs excision, with the help of an excisionase [14-16]. Integrase is encoded by an *int* gene which is often located at one extremity of the island and adjacent to the tRNA gene in the integrated GEI form. Following excision, a single copy of the recombination site (*attP*) is formed due to closing up of both GEIs ends [16-19]. Also, a single copy of the recombination site is again formed on the chromosome (*attB*) and excision is mostly non-replicative [14; 20]. GEI can reintegrate from the excised form back into the *attB* site of the same host or, after transfer to a new cell, in a suitable *attB* site in a new cell. Site-specific recombination between a 15- and 20-bp motif within the 3¯extremity of the tRNA gene leading to integration of GEI into the chromosome [12], [8]. Most excised GEIs don't replicate independently from the host's chromosome but rely instead on reintegration or horizontal transfer to a new host and reintegration to proliferate [20-21]. Staphylococcal pathogenicity islands (SaPIs) are phage-type GEIs, which have intimate relationships with certain temperate phages involving phage-induced excision, replication and packaging were also capable of self-replication [20; 22].

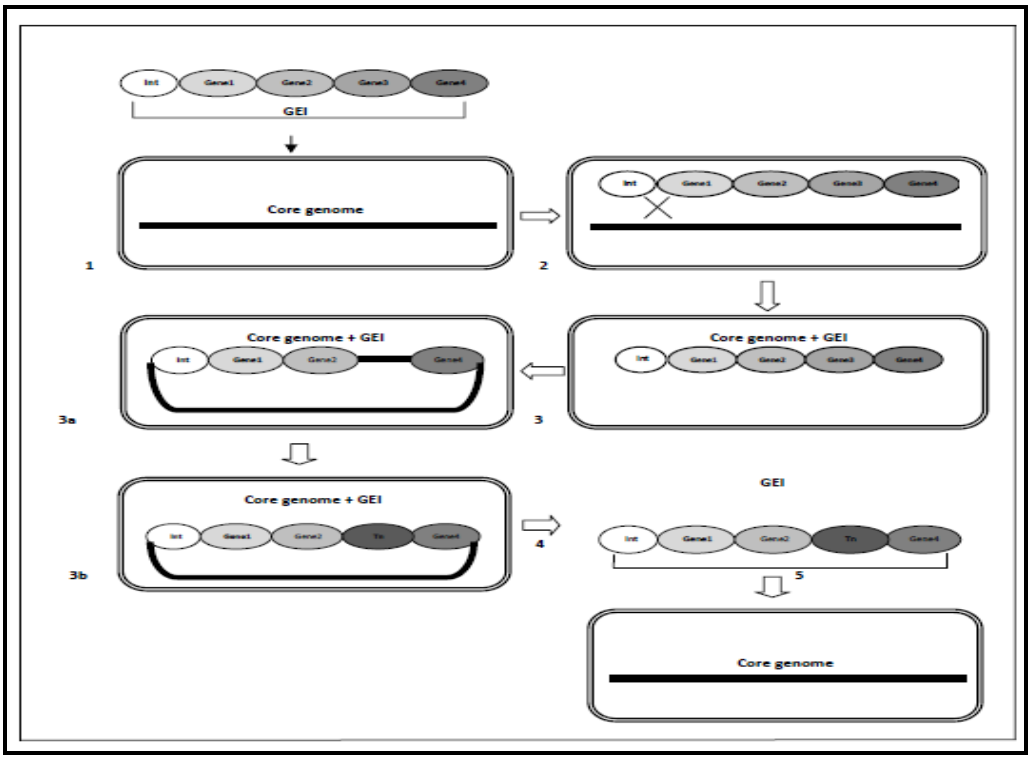

Figure 2: *Integration, Development and Excision of GEIs. 1. Acquisition by HGT; 2. Integration into the Host's Genome by Site-Specific Recombination; 3. Development of the GEI by Genetic Rearrangements, Loss (a) or Acquisition (b); 4. Excision from the Genome; 5. Transfer to a New Host*

4. How do GEIs Move between Bacteria?

GEIs move between different bacterial species by horizontal gene transfer (HGT), transformation has been demonstrated in Gram-positive and Gram-negative, pathogenic and environmental bacteria. *H. influenza*, *N. gonorrhea*, *S. pneumonia*, *B. subtilis* and *Acinetobacter* spp. ADP1 are naturally transformable bacteria which take up free DNA from their surrounding environment [23-27].

After acquisition of the foreign DNA by natural transformation, part of it is degraded and the other part could be inserted into the host's genome, thus causing bacterial species evolution. Taking up species-specific DNA for transformation appears clearly in several bacterial species like *H. influenza* and *N. gonorrhea.* Efficiency of taking up species-specific DNA in *H. influenza* and *N. gonorrhea* requires the presence of a C.10. nucleotide - long DNA uptake sequence, which is found in the respective genomes at high frequency (C.1400 copies) [28; 23].

Secretion of chromosomal DNA in the surrounding environment in a noncontact-dependent manner by a novel type 4 secretion system (T4SS) in *N. gonorrhea* has been identified [29]. This T4SS is localized in the large, horizontally acquired gonococcal genetic island (GGT) present in the *N. gonorrhea* chromosome, so, by facilitating chromosomal DNA secretion, this GEI also encodes the mechanism of its own transfer [30-31]. Conjugation is the transfer of DNA from donor to recipient bacteria through a specialized apparatus which consists of a cell-envelope spanning translocation channel linked to a tube-like structure known as a pilus in Gram-negative bacteria or to the surface-associated adhesions in Gram-positive bacteria. A large and versatile family of T4SS-dependent transport systems contains conjugation systems [32-33]. T4SSs are encoded by multiple genes organized into a single operon. T4SSs can be classified into several types according to the organization of genetic determinants, shared homologues and evolutionary relationships [34]. T4SSs have a role in the horizontal transfer of a wide variety of GEIs in a broad spectrum of bacteria, including *Haemophilus* spp., *Pseudomonas* spp., and *S. enterica* serovar Typhi. [34-36]. Genes necessary for formation of a conjugative pilus and other proteins necessary for the island transfer are encoded by the T4SS of ICE Hin 1056 [35]. A better explanation of how GEIs can propagate and efficiently enable bacterial population to adapt to rapidly changing environments can be provided by the presence of a highly evolved and efficient conjugation system for mobilizing GEIs [37].

DNA transfer from one bacterium to another via bacterial viruses, bacteriophages is called transduction. As passengers in their genomes, many bacterial genes, including GEIs can be transferred by phages. SaPI family of *Staph. aureus* islands is a good example [38]. Certain resident temperate phages induce excision and replication of SaPI family and play a role in the packaging of SaPI family into a small phage-like particles [39; 20; 38], that are transmitted from donor to recipient cells at frequencies commensurate with the plaque-forming titer of the phage [40]. Interestingly, different Staphylococcal phages induce replication of SaPI bov2 (a member of the SaPI family of GEIs), which is encapsidated and transferred to a variety of recipient bacteria, including different Staphylococcal strains [38]. Yersinia high-pathogenicity island (HPI) of *Yersinia pseudotuberculosis* is also transferred by bacteriophages [15].

Host background has a strong influence on the GEIs transferability [36]. Also, transfer frequencies of ICEclc depend on the type of donor cell, even with closely-related *P. aeruginosa* strains [41]. GEIs have a characteristic gene pool which differs from the core DNA genome and encode unique genes which are not homologous to other genomes [42]. Ecological niches provide their residents with naked DNA segments in the form of chromosomal DNA fragments, ICEs, phages, plasmids and other mobile genetic elements [43].

Among all HGT mechanisms, phage transduction is the main force in genetic transfer between different species [44]. Bacterial host can be converted by bacteriophages encoding virulence factors

from a non-pathogenic strain to a virulent strain or a strain with increased virulence through 'lysogenic conversion'. Diverse repertoire of proteins such as type III secretion effectors, extracellular toxins, enzymes required for intracellular survival, adhesions for bacterial host attachment and factors involved in avoiding host immune defences are provided to the bacterial host by the genes harboured by bacteriophages [45]. As demonstrated, transduction is a central player in the formation of PAIs. *V. cholera* VPI-1 PAI is a good model supporting this concept as it can be transmitted among strains by the transducing vibriophage, CT-T1 [46]. General transduction also plays a role for transfer of SaPI1 and related islands in *Staph. aureus* [47].

5. GEIs and Bacterial Evolution

HGT facilitated by GEIs have a crucial role in bacterial species evolution. This is due to simple acquisition and loss of GEI-borne genes and possibility of GEIs transferring parts of a host's chromosomal DNA into the recipient bacteria, upon excision from the host genome [48]. Furthermore, secretion systems on many GEIs can be used not only for the transfer of GEIs and the GEI-encoded products, but also for the transfer of the host's chromosomal DNA. Secretion of chromosomal DNA in the gonococcus via the GGI-encoded T4SS is a good example [29]. GGI-encoded type T4SS secrete chromosomal DNA in the environment which is subsequently taken up by natural transformation, thus facilitating recombination that contributes to antigenic variation and antimicrobial resistance [29]. There is a significant impact on the host bacterium evolution as GEIs could undergo a recombination with the host's chromosome transferring important clinical or fitness traits encoded by GEI-borne genes. GEIs facilitate evolution by 'quantum leaps' as their acquisition or loss can rapidly and dramatically alter the life style of a bacterium whether they encode pathogenicity or biodegradation-related genes [49-52].

Successful treatment of infectious diseases is put in increasing doubt due to the emergence and dissemination of antibiotic resistance which is a serious threat to public health [53]. Methicillin-resistant *Staph. aureus* is a challenge with respect to treatment of *Staph. aureus* infections, as the methicilllin resistance is encoded by a GEI, which is termed Staphylococcal cassette chromosome mec (SCCmec). As a result of HGT, SCCmec variants are found with increasing frequency in coagulase-negative *Stapylococci* [54]. Certain SCCmec like type I SCCmec of *Staph. aureus* could be more than antibiotic resistance islands as they encode a plasmin-sensitive surface protein (PIs) which influences adherence and invasion of *Staph. aureus* [55]. The *Salmonella* GEI 1 (SGI1), which was first, discovered in *S. enterica* serovar Typhimurium phage type DT104, encoding resistance genes to ampicillin, cholramphenicol, streptomycin, sulfonamides and tetracycline. SGI1 also have 15 open reading frames (ORFs) which may function as potential virulence factors and have no homology to any known genes [56]. Also, SGI1 is also found in *S. enterica* serovar Typhimurium phage type DT120, in *S. enterica* serotype *agona* and even in *S. enterica* serotype *paratyphi B* [57].

6. Symbiosis and Pathogenesis

Both symbiosis and pathogenesis relationships have many similarities [58], no intentional pathogenic mechanism exists and pathogenicity is due to a combination of events, as the basic requirements (for pathogens and symbionts) involve overcoming the numerous physical, cellular, and molecular barriers presented by the host, typically this entails contacting and entering the host body, growth and replication using nutrients from host tissues, avoidance of host defences, persistence and replication and finally exiting and infecting new hosts. So, pathogenicity is not intentional at all [59], which is clear in the following species:

6.1. Neisseria

N. meningitidis inhabits human nasopharynx mucosa and is carried by 5-10% of healthy individuals [60]. Meningitis arises when the bacteria leaves the mucosa and invades the bloodstream [37]. Ninety-five percentages of genes were common to all pathogenic *Neisseria* spp. and nonpathogenic *N. lactamica* [61]. Surprisingly, 85 of the 127 suspected virulence genes in the pathogens were also found in the nonpathogens. The suspected virulence genes found in both pathogens and the nonpathogens fell into the categories of surface proteins, iron acquisition, pilus-associated and LPS biosynthesis/regulation. Change in the nature of *N. meningitidis* strains from commensal to pathogenic is due to a variety of mobile elements (such as GEIs) which causing variation of their surface proteins [60]. Without the specific combination of pathogenic potential genes, *N. lactamica* remains a harmless commensal although possession of the 'virulence gene' of the pathogen, as the similarity between the gene complements of the commensals and pathogens suggest that the virulence of the pathogenic *Neisseria* spp. may not lie within the genes they possess per se, but rather in 'a genetic personality' which is a result of the combination of these genes, sequence variations that alter the function of gene products, the possession of genes for which a virulence phenotype has not yet been identified, and/or in differences in the regulation of genes between the species [61].

6.2. Salmonella

Many of *Salmonella* virulence genes exist on PAI and approximately 82% of the virulence genes in *Salmonella* can also be found in a nonpathogenic lab *E .coli* strains which fall into the categories of transcriptional control, DNA repair, nutrient biosynthesis and enzymes [62]. Not surprisingly is that *Salmonella* and *E. coli* have similar genes as they both face similar stressors whether in a host or outside a host. The presence of these genes in nonpathogenic species suggests that they promote survival within nutritionally deprived and/or potentially lethal environments that microorganisms encounter inside and outside animal hosts [62]. The distinguishing genes between *Salmonella* and *E.coli* are located on PAIs in *Salmonella* and are responsible for host recognition/invasion and survival in macrophages [62]. PAIs in *Salmonella* may have been obtained at some point in the past from nonpathogenic bacteria (which had a symbiotic relationship with macrophages). Before the fall, an environment was not hazardous or harsh, so HGT did not probably occur and after the fall, bacteria must adapt and survive so HGT would be necessary, but the side effects of pathogenicity in humans, animals and plants may occur [37].

7. GEIs and Bacterial Diagnosis

Determination of the presence and typing of a certain PAI could be used as a marker for certain pathogens or classes of pathogens, as the presence of certain PAI is specific for certain bacterial pathogens [6]. PAI I_{536} and PAI III_{536} of the uropathogenic *E. coli* (UPEC) strain 536 are demonstrated as strain-specific PAI. LEE which is responsible for effacement of microvilli on enterocyte, intimate attachment, actin accumulation and pedestal formation, this type of PAI is characteristic of a defined pathogenic effect. Also, the LEE is present in EPEC and enterohemorrhagic *E. coli* (EHEC), but also in *C. rodentrum* and *H. alvei* [63]. The presence of the O122 PAI in an *E. coli* strain indicates the carrier strain to be a member of attaching and effacing lesions causing *E. coli* strain (AEEC), as this PAI is characterized for AEEC, because it was not found in other *E. coli* groups [64].

Also, for shiga toxin-producing *E. coli* (STEC) of serotype O26:H11/H^-, high pathogenicity island (HPI) can be used as a highly specific marker and for O128:H2/H^- STEC strains, can be used as a good indicator. The presence of HPI is restricted to *Yersinia* and *E. coli* [65]. In *Shigella* and in *Salmonella* strains belonging to group I which are pathogenic for humans and warm-blooded animals, HPI was never detected [66]. Although, in the closely-related *S. enterica* subspp. *arizonae* (group IIIa), *S.*

enterica subspp. *diarizonae* (group IIIb) and *S. enterica* subspp. *indica* (group VI), HPI could be detected [67]. HPI presence in a certain *Salmonella* strain, determined for example by PCR, is a strong indicator for this *Salmonella* strain not belonging to the pathogenic *Salmonella* group I, so, HPI can function as a marker in differential *Salmonella* diagnostics [6].

8. Regulation of GEIs and Adaptive Behavior

Tetracycline determinants in Bacteroides are studied for GEI regulation behavior. Induction of two regulatory genes, *rteA* and *rteB*, both of which stimulate transcription of a third factor *rteC,* which influences excision of the ICEs CTnDOT and CTnERL from Bacteroides and this stimulates conjugative transfer of the elements from Bacteroides up to 10000-fold when cells are grown in the presence of tetracycline [68]. Excision of 108-kb GEI called PAPI-1, formation of an intermediate circular form and reintegration into either of the two $tRNA^{cys}$ genes in *P. aeruginosa* (PA4541 and PA0976) facilitates the transfer of PAP1-1 GEI which was discovered in *P. aeruginosa* strain PA14 [19]. The Soj protein is related to the ParA family of proteins, which are responsible for correct segregation of low-copy plasmids during cell division, Soj protein is expressed by the circular form of PAPI-1 GEI at early stationary phase, and it can protect the circular form of PAPI-1 either directly from degradation or indirectly by promoting the integration of the circular form back into the chromosome. So, a *soj* gene encoded by PAPI-1 itself was required for the maintenance of the element; both in integrated and in circular form [19].

A novel recombination directionality factor (RDF) called Rdfs, which is encoded by the gene msi109 also stimulates the excision of the ICEMISymR7A symbiosis island of *M. loti* strain R7A. A putative relaxases, RlxS is also required for transfer of the ICEMISymR7A. The genes *rdfS* and *rlxS* are part of the same cluster of which two other genes are homologous to the conjugative protein TraF. Also, the excised form of ICEMISymR7A was more abundant in stationary phase than exponential phase of *M. loti*, similarly to the clc element [16].

Factors including the degree of homology between the transferred DNA and the bacterial host, the metabolic compatibility adaptations to their abiotic environment, gene expression systems, gene transfer mechanisms, the mismatch repair and restriction endonuclease systems control the frequency of successful DNA exchange between bacteria belonging to different genera.

GEIs depend on site-specific reintegration into an appropriate chromosomal target site, in the absence of which the element is unlikely to be maintained. However, once integrated, a GEI will be automatically maintained by chromosomal replication. Excision of GEI facilitates its loss which is a strong advantage for growth of cells without GEIs, as occurs with the UPEC *E.coli* isolate 536, which contains five PAIs and in chronic infection, some of them are deleted.

There is a unidirectional cross-talk between integrases of different PAIs which means that the integrase of one of the PAIs could actually mediate the excision of another PAI [69]. GEI expression is globally influenced by the host in which it resides. Thus, GEIs constitute part of global regulating networks and genes on particular GEI can be regulated by regulators present on the same GEI, by regulators encoded by the host bacterium or by regulators harboured by other GEIs. Also, genes on the bacterial chromosome can be regulated by GEI-borne regulators [53]. Paradigmal regulatory networks involving intensive cross-talk between GEI-borne regulator and gene components of the host genome include *Salmonella* pathogenicity islands (SP1-1 and SP1-2) of *S. enterica* and Vibrio pathogenicity island (VPI) of *V. cholera*. Regulators frequently contributing to regulation of GEIs comprise the two-component response regulator family, AraC family, alternative sigma factors and histone-like proteins [4; 70].

Histone-like nucleoid structuring protein (H-NS) is a pleiotropic regulator that modulates gene expression of Gram-negative bacteria in response to environmental stimuli, such as temperature and osmolarity [71], it represents the bacterial functional equivalent of histone that plays a role in local supercoiling of DNA [72]. H-NS can selectively silence horizontally-acquired genes by targeting sequences with proportionally high AT content, as the expression of more than 400 genes was shown to be upregulated in the *Salmonella*-hns mutant, out of which more than ninety percentages were acquired by HGT [72]. The GC content of most of the H-NS repressed genes was lower than the average GC content of the host Salmonella genome. Conserved sequence motifs that represent the high affinity DNA-binding sites for H-NS have been identified [73], these motifs found in AT-rich regions of DNA whether in operons or in PAIs genes [73]. Targeting sequences with different GC content by H-NS represent an elegant and efficient mechanism of regulation of newly acquired GEIs [53].

9. How do GEIs Contribute to Virulence?

The ability of a bacterial strain to infect a host, persist, proliferate and transfer to a new host in a specific niche is known as bacterial pathogen fitness. A simultaneous acquisition of many genes by HGT that allow the bacteria to rapidly gain complex virulence functions and to exploit new environmental niches increasing bacterial fitness [50]. Specific contribution of PAIs to virulence of pathogens can be demonstrated by some examples including the *cag* PAI of *H. pylori*, the TSST PAI of *Staphylococcus*, the LEE PAI of *E. coli* and related species and the AbaR4 island of *A. baumannii*.

10. The *cag* and *tfs4* GEIs of *H. pylori*

H. pylori colonizes over half of the world's population as a human gastric pathogen. Most *H. pylori*-infected people will exhibit some degree of gastritis, approximately 10% of infected people will develop peptic ulcer disease and approximately 1% will develop gastric cancer. *Cag* PAI is one of the defined *H. pylori* strains virulence factors. Peptic ulcer-causing *H. pylori* strains possess the *cag* PAIs, which is absent from strains isolated from patients with uncomplicated gastritis [74]. The *cag* PAI may become lost during colonization of animals, as demonstrated from association between *cag* PAI-negative *H. pylori* strains and strains that are mouse-adapted, less virulent and can better colonize mice [75]. The *cag* PAI is a 37-40 kb chromosomal region that was acquired by horizontal transfer and inserted at the distal end of the glutamate racemase gene (*glr*) which is essential for bacterial cell wall synthesis. *Cag* has a distinct G+C content, and is flanked by DRs of 31 bp that probably function as sites for recombination and deletion of the locus. Twenty seven ORFs and an additional element which is not present in all of the *cag* positive strains were predicted (Figure 3).

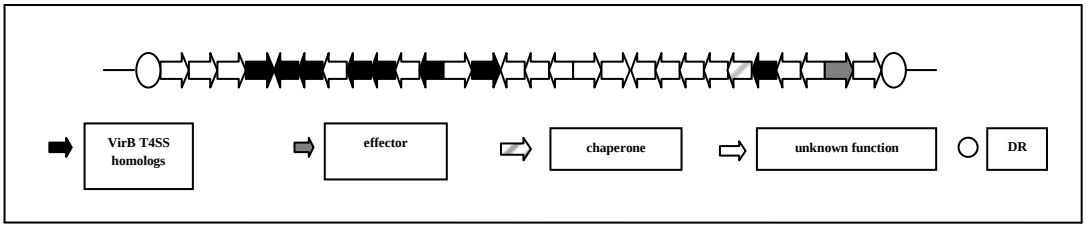

Figure 3: *cag PAI of H. pylori*

Cag PAI genes encode a functional T4SS and CagA which is the effector protein of *H. pylori* T4SS, and eight of T4SS are homologues to components of the *A. tumefaciens* virB operon prototype T4SS [76-77]. T4SS transport proteins through a complex channel structure directly through the inner and outer bacterial membranes into the cytoplasm of the host cell [78-79]. CagA has multiple effects on host signal transduction pathways, the cytoskeleton and cell junctions as it interacts with a large number of host proteins [80]. CagA becomes phosphorylated by Scr kinases after translocation into host cells and is recruited to the plasma membrane where it interacts with a number of host proteins.

Particular pathways are activated by this interaction and leads to actin polymerization, cell elongation, pedestal formation and proliferation of gastric epithelial cells. *Cag* PAI induces expression of proinflammmatory cytokines, such as interlukin-8 (IL-8), which contributes to *H. pylori*-induced inflammation in the stomach [75; 81]. Another *cag* protein, namely CagF which interacts with CagA and might function as a chaperone-like protein for CagA [82]. A subset of 17 genes in the *cag* PAI is required for the translocation of CagA and a subset of 14 genes is required for the stimulation of IL-8 synthesis in host cells. Most of the *cag* PAI genes are required for the formation of a functional T4SS which translocates the bacterial effector protein CagA into host cells and induces the synthesis and secretion of chemokines, such as IL-8 [43].

The complement of factors involved in mobilization of the *H. pylori* disease- associated *tfs4* GEI have been investigated, *tfs4* encodes a VirD2-like relaxase with distinctive DNA binding and nicking activity and Tfs4 VirD2 likely initiates mobilization of *tfs4* by specific interaction at a chromosomal transfer origin sequence, so the pathogenic potential of *H. pylori* strains may be increased by Tfs4 VirD2-mediated mobilization of *tfs4* [83].

11. The LEE PAI

The LEE was initially described in an EPEC strain and enables it to attach to host intestinal epithelium and efface brush border microvilli causing infant diarrhea [63]. It is a 35 kb PAI which contains genes necessary for this phenotype [84]. The LEE contains 41 ORFs and is originated as five polycistronic operons (LEE1-LEE5). G+C content of the LEE (38%) is strikingly lower than that of the rest of the chromosome (50.8%). SelC tRNA gene is the chromosomal integration site of LEE in the EPEC reference strain E2348/69, but other sites are found in different EPEC strains. The LEE PAI have different modules including: (i) a type 3 secretion system (T3SS) that is essential for formation of the attaching and effacing histopathology in EPEC and is used as a molecular syringe to translocate effector proteins into host cells, (ii) EspA, EspD and EspB; the secreted translocator proteins which are required for translocating effectors into host cells, (iii) the adhesin (intimin, EAE) which mediates intimate attachment to Tir on the host cell cytoplasmic membrane and (iv) the secreted effector proteins EspF, EspG, EspZ, EspH and Tir, the intimin receptor, chaperoned by CesT. Direct interaction of Tir with the bacterial outer membrane protein intimin, as well as the host cytoskeleton has been demonstrated as translocation of effector molecules by T3SS into the host cells results in changes of the host cell cytoskeleton arrangement leading to actin-rich pedestals formation in which the Tir effector is located at their tip [85-86].

Different regulators encoded within the PAI, on a plasmid, and on the core genome are controlling LEE genes. These regulators include the global-regulator of LEE-activator (GrLA), global-regulator of LEE-repressor (GrLR), LEE-encoded regulator (Ler), the plasmid-encoded regulator (Per), the DNA-binding protein H-NS and the integration host factor (IHF) [87-88].

12. AbaR-type and Tn*6167* resistance islands of *A. baumannii*

A. baumannii is responsible for serious infections and nosocomial outbreaks and finding appropriate therapies is a great challenge due to the increasing resistance to multiple antimicrobials [89]. An 86-kb genomic region was identified in an epidemic multidrug resistant (MDR) *A. baumannii* with a 26% mortality rate. The region contains 88 predicted ORFs, of which 82 were assigned functions based on amino acid similarities with other proteins that were primarily from *Pseudomonas*, *Salmonella* and *Escherichia* spp. G+C content of this DNA region (52.8%) varies from the G+C content of *A. baumannii* chromosome (38.8%), which suggests that they were acquired by genetic transfer, 45 from the 52 antibiotic resistance genes were located within this region, which indicates that this area is a hotspot for resistance genes integration, similar to *Salmonella* spp. and *E. coli* genes [70]. *StrA* and

strB which encode streptomycin resistance and *cmlA* which encodes chloramphenicol resistance that had not previously been found in *Acinetobacter* spp. were identified.

The structural variations of AbaR-type resistance islands in MDR *A. baumannii* have been demonstrated. AbaR1 is integrated into the ATPase gene (comM gene) and contains a large cluster of antimicrobial and heavy metal resistance genes. In the same region of comM in *A. baumannii* isolates, there is significant diversity among the AbaR-type resistance islands. Also, AbaR transposons have been found in *A. baumannii* since the 1970s [90-96].

The GC2 *A. baumannii* isolate includes a 37 kb genomic resistance island, Tn*6167*, in the comM gene, which carries Tn*6022*Δ1 at one end interrupted by a novel insertion sequence ISAba17. The *sul2* (sulphonamide resistance) and *strA*-*strB* (streptomycin resistance) genes and *tet* (B) tetracycline resistance determinant are at the other end in the configuration ISAba1-*sul2*-CR2Δ-*tetA*(B)-*tetR*(B)-CR2-*strB*-*strA*. Also, transposon Tn*2006* carrying $bla_{oxa\text{-}23}$ was found in an 11 kb region located between Tn*6022*Δ1 and the other resistance genes. The 17.6 kb Tn6166 from the GC2 *A. baumannii* reference strain can be derived from Tn*6167* via a single deletion arising adjacent to Tn*6022*Δ1 and causing loss of a large central segment [97].

13. *Staphylococci* and *Salmonella* PAIs

Staph. aureus is a common pathogen causing a range of acute and pyogenic infections. Toxins of *Staph. aureus* including hemolysins, staphylococcal exotoxins (Set) and superantigens (SAgs) are major virulence factors. Certain *Staph. aureus* strains possess secreted virulence factors known as TSST which function as superantigens toxins [98]. High fever, rash, vomiting, diarrhea and renal and hepatic dysfunction are the TSST consequences. Staphylococcal pathogenicity islands (SaPIs) were the first clearly defined PAI characterized in Gram-positive bacteria, SaPI is a series of discerte 15-20 kb chromosomal elements that are mobilized at high frequencies by certain staphylococcal phages. The chromosomal *tst* gene, encoding TSST-1 is located on SaPI [99]. SaPI1 is the prototype of the SaPI family. SaPI1 is 15.2kb long, carries a *tst* gene, flanked by 17bp DR sequences, and is inserted in an *attC* site close to the *tyrB* gene.

Salmonella pathogenicity island 1 (SPI1) is the best characterized SPIs in the *S.* Typhimurium, it is approximately 40 kb in size, flanked by *fhlA* and *mutS* and has an overall GC content of 42%. Type III secretion system, its regulators and its secreted effectors are encoded by SPI1 [100]. The ability of SPI1-encoded secretion system to deliver effector proteins into the host cell cytoplasm control the virulence phenotype associated with SPI1. By delivery of these effectors, SPI1 enables *S.* Typhimurium to penetrate the intestinal epithelium, as the SPI1 mutants are attenuated for virulence orally, but not systemically in mice [101-102], as the role of SPI1 may be limited to gut colonization and other virulence determinants later acquired allowing establishment of systemic diseases [103]. The IncA/C helper plasmids are not only involved in the horizontal spreading of SGI1 gene cluster originally found in *S*. Typhimurium, but may also contribute to its evolution [104].

14. CRISPRs and Their Associated Cas Proteins

CRISPRs/ Cas system represents a defense mechanism against bacteriophages and plasmids, which acquire short spacer sequences from foreign genetic elements and insert these into their CRISPR arrays, creating past invaders memory. Short non-coding RNAs guide cas proteins to cleave invading complementary nucleic acid as shown in Figure (4). Growth inhibition and cellular filamentation result from chromosomal targeting via targeting of bacterial host's own genome. The toxic phenotype is abolished by mutation in the CRISPR repeats, the protospacer target, and protospacer-adjacent motif (PAM) beside the target and the cas operon. Chromosomal targeting results in deletion or remodeling of entire pre-existing PAIs leads to genomic alterations. Genetic engineering can use these

characteristics to delete targets of bacterial chromosome causing evolution and pathogenicity of bacteria [105].

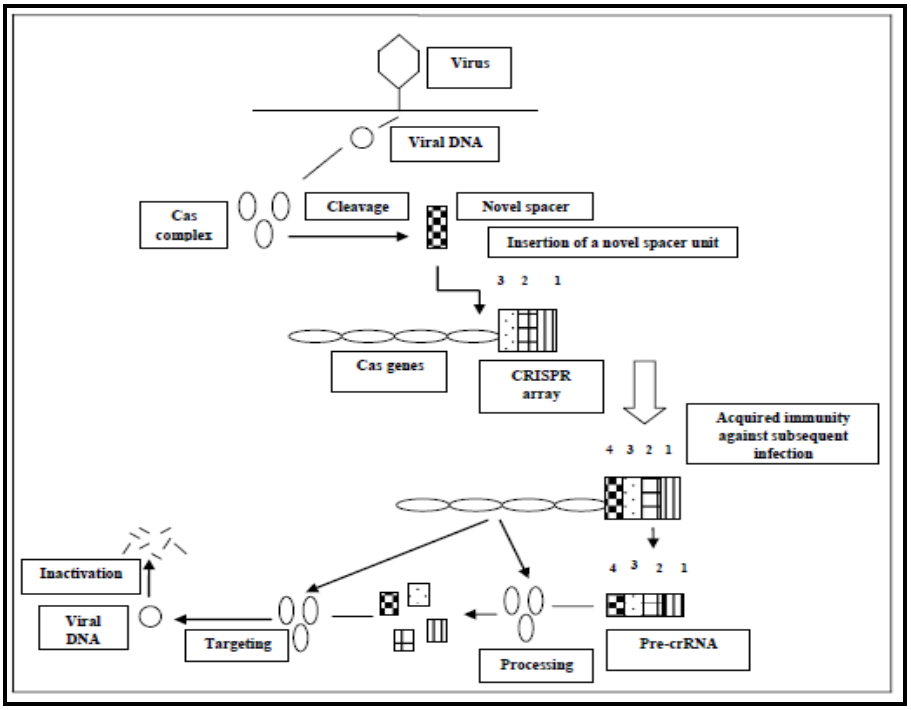

Figure 4: *CRISPR/Cas Mechanism of Action. After Insertion of Viral DNA a Cas Complex Recognizes Foreign DNA and Inserts a Novel Spacer Unit at the CRISPR Array, The CRISPR Spacer Array is transcribed into a Pre-crRNA that is processed into Mature crRNA, which are subsequently used as a Guide by a Cas Complex to Inactivate the Corresponding Invading Nucleic Acid*

15. Detection of PAIs in Diverse Bacterial Genomes

Microbial genomes are substantially homogenous in G+C content and genome signature [106], but the GEIs composition differs; albeit not strongly, from that of the overall genome. The gene segment are said to be GEIs putatively derived by lateral gene transfer when a genome segment deviates sufficiently from the average genome composition in G+C, and/or genome signature, and/or codon usage, and/or amino acid frequencies and/or contains a cluster of putative alien genes. GEI character of the segment is emphasized if it is identified using all the last five criteria [107].

16. Conclusion

GEIs are necessary for adaptation of bacteria to new environmental changes and are considered to be one of the main HGT tools between different bacterial species leading to dissemination of antimicrobial resistance and establishment of new pathogenic variants. CRISPR/Cas system is promising machinery for immunization against viral infections of bacteria and foreign DNA fragments. This system can be used for engineered genomic alterations contributing in bacterial evolution and pathogenicity.

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