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Do Antimicrobial Resistance and Virulence Share Same Genetic Elements

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Abstract Antimicrobial resistance (AMR) is a growing global health challenge with increased health care costs, mortality and morbidity. Over the years, microbes of clinical importance have coevolved with their hosts and have developed virulence mechanisms through mutations and horizontal gene transfer in order to compete and withstand hash environmental conditions including those posed by antimicrobial agents. Antimicrobial resistance and virulence mechanisms promote survival of microorganisms in their hosts. Several research studies have shown putative association between antimicrobial resistance and virulence but overlooked genetic link between the two. This review focuses on establishing whether antimicrobial resistance and virulence share the same genetic elements.

Keywords: *Antimicrobial resistance, virulence, AIEC, gene profile*

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1. Introduction

Emergence of antimicrobial resistance is one of the leading public health concerns and an important threat to effective treatment of infectious diseases. There is a growing concern of resistance in pathogenic bacteria including *Escherichia coli spp*., *Klebsiella spp*., *Mycobacteria spp*., *Streptococcus spp*., *Staphylococcus spp*., *Neisseria spp*., and *Shigella spp*., among others increasing health care costs, and morbidity [1]. Antimicrobial resistance occurs when microorganisms resist effective action of antimicrobial agents (drugs) to which they were previously susceptible. Pathogenic bacteria become resistant through various mechanisms including modification of the antimicrobial molecule, decreased permeability and efflux pumps, alteration of target sites, resistant biochemical pathways, and global cell adaptation facilitated by horizontal gene transfer and mutations [2,3].

The ability of pathogenic bacteria to cause infection is dependent on virulence factors. Virulence is defined as the ability of an organism to cause disease. Characteristics of virulence factors possessed by pathogenic bacteria include; ability to attach, invade, and colonize host specific tissues, production of toxins, enzymes, and compounds that cause damage to the host; usually encoded in plasmids [3]. Several studies have been carried out to characterise the molecular mechanisms of antibiotic resistance and virulence in most pathogenic bacteria. This review establishes whether antimicrobial resistance shares the same genetic elements with virulence factors, with a focus on Adherent invasive Escherichia coli pathotype (AIEC); *E. coli* strains implicated in the pathogenesis of Crohn's disease.

2. AIEC LF82 Sequenced Genome

Non diarrheagenic Adherent invasive *E. coli* strains, belonging to B2 clade have long been associated with Crohn's disease; a type of chronic inflammatory bowel disease [4,5,6]. AIEC strains are phenotypically characterized by their ability to adhere, invade intestinal epithelium, and replicate in macrophages without causing apoptosis, but stimulate increased production of tumour necrosis factor alpha (TNF-α) [5,7,8,9,10]. Despite the availability of phylogenetic information on *E. coli* pathotypes, the origin of AIEC pathotype is unclear. Genomic studies reveal that AIEC pathotype is closely related to Uropathogenic *E. coli* (UPEC); Extraintestinal pathogenic *E. coli*, and thus, proposed to have derived from the latter [11,12,13].

E. coli strain LF82 has been extensively studied and considered as the prototype strain for AIEC pathotype. The LF82 strain has a total size genome of approximately 4881,487 bp, that is, a circular chromosome whose size is 4,773,108 bp and a 108,379bp sized plasmid. In the study by Miquel *et al.*, annotation revealed that *E. coli* LF82 strain contains 4,376 coding sequences (CDSs) with 121 CDSs being on the plasmid (p1LF82) genome. Of the 121 CDSs, 97 were also identified in *Yersinia spp*., and *Salmonella enterica serova* plasmid (pHCM2 and pMT1) genomes only [14].

3. Antimicrobial Resistance in AIEC Strains

Generally, bacteria are known to have genome plasticity enabling them to cope with varying environmental changes. They have evolved mechanisms to withstand environmental threats including antimicrobial agents (Box1). In *E. coli* strains, resistance develops through two major strategies; (i)horizontal gene transfer facilitated by mobile genetic elements such as plasmids, insertion sequences, integrons, transposons, and integrative conjugative elements which actively support the acquisition and spread of resistance genes encoded in plasmid DNA or chromosomal DNA, and (ii)through mutations in genes [2,15]. Horizontal gene transfer is the most common driver of bacterial evolution achieved through three main stages; transformation (uptake of foreign genetic material), transduction (transfer of genetic material from one bacterium to another by way of bacteriophage), and conjugation (transfer of genetic material mediated by plasmids) [2,16]. On the other hand, mutational resistance occurs when bacteria develop mutations in genes that play a role in the activity of an antimicrobial agent.

AIEC strains are naturally resistant to most commonly used antimicrobial agents. Study by Dogan *et al.*, identified AIEC strains resistant to antimicrobials including tetracycline, trimethoprim/sulfamethoxazole, ampicillin, clarithromycin among others with varied resistance determinant genes; *tetA*, *tetB*, *tetC bla-TEM*, *sulI*, *dhfrI*, *catI* [17]. Whereas the AIEC reference strain LF82 is naturally resistant to ampicillin with unclear determinant [7,18].

Box 1. Resistance Mechanisms

Over the years, bacteria have evolved resistance mechanisms that enable them to withstand antimicrobial agents, rendering them ineffective. These include:

- **Efflux pumps:** complex machineries that force out compounds toxic to the bacteria cells such as antimicrobial agents out of the cell, resulting in resistance. Genes encoding these pumps are usually in either plasmid or chromosomal DNA. This mechanism confers resistance to antimicrobial agents including β-lactams, quinolones, and tetracyclines among others. A good example is Tet efflux systems that pump out tetracycline drugs out of *E. coli* cytoplasm [2,19].
- **Permeability and porins:** most antimicrobial agents have intracellular targets that allow them to exert their effect. Passage to these targets is achieved through porins, which are diffusion protein channels such as *OmpF*, *OmpC*, and *PhoE* found in *E. coli* strains. However, bacteria reduce permeability by shifting the type of porins expressed, reducing expression levels and impairing the porin functionality. Penicillins are usually affected by this mechanism [2,20].
- **Resistant biochemical pathway:** bacteria are able to develop new target sites that are not inhibited by antimicrobial agents but perform similar biochemical functions as the original targets. A classic example of drug enabled resistant through this mechanism is that conferred against Sulfonamides [2].
- **Alteration of target site:** point mutations in genes that encode antimicrobial agent target sites and alteration of binding sites by bacterial enzymes decrease the affinity of antimicrobial agents to target sites. Examples include macrolide resistance attained through methylation of ribosome by methylase, and fluoroquinolone resistance achieved through point mutations in genes (*gyrA-gyrB* and *parC-parE*) encoding subunits of the enzyme's DNA gyrase, and topoisomerase IV [2].
- **Inactivation of antibiotics:** some resistant bacteria produce modifying enzymes; β-lactamases such as methylase, acetylase, and phosphorylase encoded in either plasmid or chromosomal DNA and inactivates antimicrobial agents by inducing chemical changes to the drug. An example is TEM-1, a β-lactamase known to hydrolyse ampicillin in some E. coli strains [19]. Other than β-lactams, aminoglycosides are also affected by this mechanism.

4. Virulence Factors in AIEC Strains

The pathogenesis of AIEC in Crohn's disease is linked to genes involved in the processes of adhesion and invasion of intestinal epithelial cells, motility, iron intake, capsule and biofilm formation including *pic*, *auf*, *ibeRAT*, *pdu*, *ipfA*, *gipA*, *ompA*, *ompC*, and *yfgl* all from distinct virulence groups (Table1, key table). Adhesive and invasive interactions of AIEC strains with the target host cell; carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6-receptor) is facilitated by virulence factors including flagella, outer membrane proteins (*ompA*, *ompC)*, type 1 fimbriae [11,21].

Flagella is a cell organelle known to mediate motility activities in most bacteria. In AIEC strains, flagella are covered with flagellin a tip protein encoded by the *fliC* gene which facilitates adhesion and is linked to biofilm formation. Flagellin coupled with outer membrane protein (*omp*) activity serves as bacterial adhesion and activates its specific ligand Toll-like receptor 5 (TLR-5) which mediates signalling and production of TNF-α by microphages, thereby, inducing inflammation. Bacterial appendages designated as type 1 fimbriae, located on the tip of the fimbriae function by binding to CEACAM6 receptors and promote adhesion. Type 1 fimbria with subunits *fimA*, *fimF*, *fimG*, *fimH* and long polar fimbria (encoded by *lpf* gene) facilitate attachments to Peyer's patches. However, *fimH* variants caused by mutations in the gene are associated with enhanced adhesion and antimicrobial resistance [10,21,22].

Other virulence factors include vacuolating autotransporter toxin (*vat*-AIEC); an enzyme which facilitates penetration of AIEC strains into the cells by diminishing epithelial mucus viscosity encoded by *vat* gene [21]. Further, *ibeA* proteins play a role in invasion and survival of the microbes in phagolysosomes of host macrophages [14]. A unique gene; *pic* gene has been identified in AIEC strains and characterized as critical in invasiveness of the pathotype. AIEC strains possess

secretion systems; type VI secretion system (T6SS) associated with attenuation of host immunity, and type II secretion system (T2SS) associated with transportation of virulent determinants; hydrolytic enzymes, toxic compounds and adhesin subunits to extracellular spaces, unlike other intestinal *E. coli* pathotypes possessing type III secretion systems. These secretion systems have been observed in genomic islands (GI) associated with pathogenicity and antimicrobial resistance [11,23]. AIEC strains also possess iron uptake systems *chuA, Irp2* which promotes their survival inside macrophages [24].

Table 1. Box 2. Key table: Virulence gene profile [7,14]

Gene(s)	Description LF82
fimH	Type 1 fimH adhesin
csgA	Curli
yadC	Uncharacterized fimbrial-like protein
ydeQ	Uncharacterized fimbrial-like protein
yehA	Predicted Yeh fimbrial-like adhesin
yfcP	Hypothetical fimbrial-like protein
ppdD	Putative major pilin subunit
auf	Putative fimbrial-like protein
Ygi	Yqi fimbriae
lpfA	fimbrial-like protein
gipA	Peyer's patch-specific factor
ibeA	Invasion protein IbeA
pdu	coenzyme B12-dependent 1,2-propanediol catabolism
ratA	RatA-like protein
fepC	Ferric enterobactin transport ATP-binding protein fepC
chuA	Outer membrane hemin receptor chuA
fhuA	iron compound receptor (ferrichrome iron receptor)
sitA-D	Salmonella iron/manganese transport
iss	Serum survival
sepA	Extracellular serine protease
yfgL	Outer membrane Vesicle formation
ompC	Outer membrane porin protein C
ompA	Outer membrane porin protein A
nlpI	Lipoprotein NlpI
pic	Invasiveness
vat	Vacuolating autotransporter toxin

5. Link Between Antimicrobial Resistance and Virulence Genes

Advances in molecular characterisation of genomes have paved way for comprehensive analysis of pathogenicity in quest by researchers to establish the connection between resistance and virulence genes. Several genomic studies have shown mutations in target genes with critical biological functions giving rise to resistance and reduced virulence, while others have shown resistance and increased virulence. A population-based case-study by Zhang *et al.,* showed the prevalence of resistance in pathogenic *E. coli* isolates higher, than in commensal isolates [25]. Whereas a study on mutant *Pseudomonas aeruginosa* with mutations in *oprD* gene encoding carbapenem entry channel and transposon insertion, revealed increased virulence activity in the host and enhanced bacterial fitness [26,27].

Complete genome sequencing of AIEC strains such as LF82 and NRG857 have revealed the presence of 35 genomic islands (GI) with some encoding virulence genes, resistance genes, and uncharacterised genes which may provide deeper understanding if fully characterised in future studies [22]. GI-3 encodes virulence factors; *vat* gene for vacuolating autotransporter toxin, *insA* and *insB* genes for transposase, and type VI secretion system, GI-23 *emrY* and *emrK* genes for two multidrug resistance proteins, GI-36 tetracycline resistance gene (*tet*), GI-37 encompassing class 1 integron coding resistance determinants *mphA* (macrolides), *dfrAD* (sulfamethoxazole/trimethoprim), and *aaDA5* (aminoglycosides) [11,23].

Recent studies involving variants of *fimH* allele which encode type I fimbriae *fimH* adhesin (virulence factor) have linked pathogenicity and antimicrobial resistance. Independent studies by Benerjee *et al.*, and Price *et., al* suggested that subclone H30-R (fimH-30-R; *fimH* variant) in *E. coli* ST131(AIEC strain) led to the development of fluoroquinolone resistance. whereas subclone H30-Rx (fimH-30Rx; *fimH* variant) encoding virulent mobile genetic element led to simultaneous development of fluoroquinolone resistance and CTX-M- 15 extended spectrum beta-lactamase (ESBL) production in *E. coli* ST131 [22,28,29]. This suggests a connection that antimicrobial resistance and virulence genes may be co-selected and mobilized together in plasmids and/ or chromosomes.

6. Identification of Genomic Signatures of Resistance and Virulence Genes

Genomics a fast-growing field involving mapping, sequencing, analysis and comparison of genomes for Eukaryotes and Prokaryotes has been used to identify and reveal the existence of resistance and virulence genes [19]. It has resulted in the rise of whole genome sequencing and bioinformatics approaches permitting collection of large datasets for clinically important isolates as well as deeper understanding of complex molecular mechanisms of resistance and virulence [30,31]. DNA sequencing technologies used in genomic studies include DNA amplification- polymerase chain reaction (PCR), Sanger dideoxy method, shotgun sequencing, life sciences pyrosequencing methods; Illumina, and single molecule real time sequencing (SMRT) among others (Box 3). After sequencing, draft genomes are assembled in order, removing all overlaps using software such as Velvet and SPAdes packages. Genomes are thereafter annotated (bottleneck in genomics) to identify genes and other functional regions before analysis and comparison with data contained on online databases such as MicrobesOnline and NCBI.

BLAST an online tool [30,31,32]. In a comparative genomics study by Barrios-villa *et al.,* on AIEC pathotypes, genomic DNA was sequenced using Illumina technology Trueseq, assembled using Velvet and SPAdes software, and annotated by RAST service and PROKKA utility. An online tool IslandViewer4 was used to identify genomic islands. The results produced were comparable with the LF82 reference strain on NCBI BLAST. In another genomics study by Miquel *et al.,* shotgun and capillary

sanger sequencing strategies were used to prepare genomic DNA. Assembly was facilitated by Phred/Phrap/Consed software, annotation by MicroScope system, and comparative analysis realized by TBLASTN program. Results produced had discrepancies and the author associated this with TBLASTN comparative analysis.

Table 2. Box 3. Tools and services used in genomics [19,32]

Subject	*Technology-Tool-Service*
Sequencing	PCR* Illumina technology Sanger dideoxy RNA-seq
Assembly	Velvet software SPAde software Phred/phrap/Consed software
Annotation	PROKKA* utility RAST *service MicroScope system SEED* service
Comparative Analysis	NCBI BLAST* TBLASTN* MicrobesOnline EMBL-EBI*

* See glossary

7. Conclusion

Antimicrobial resistance and virulence share a common purpose of ensuring survival of bacteria in adverse environment conditions. Antimicrobial resistance enables bacteria to overcome the action of antimicrobial drugs and survive in competitive environments, whereas virulence mechanisms enable bacteria to overcome host defence mechanisms and establish new niche. Most of their determinants encoded in chromosomal or plasmid DNA have been acquired or transmitted between species through horizontal gene transfer of mobile genetic elements, and through mutations. Similarities in resistance and virulence mechanisms revealed in various studies suggest a connection between the two processes based on genetic background which is usually overlooked and raises important questions (Box 4). With the advancement in molecular techniques and DNA sequencing technologies, there is need to consider further studies on genomic islands identified in various pathogenic bacteria species including AIEC strains, and complete characterization of uncharacterized genes including patho-adaptive mutations identified. Considering annotation of genomes as the bottleneck in genomics, comprehensive analysis of identified genes, and single nucleotide mutations should be carefully performed as it may be beneficial for future studies; providing solutions to major clinical problems.

Outstanding Questions

- Can genome sequencing and comprehensive analysis be the ultimate solution to the fight against antimicrobial resistance?
- Can the link between all antimicrobial resistance mechanisms for all drug classes and virulence genes be established?
- Are there genes coding both resistance and virulence?

Are there external factors influencing the putative connection between antimicrobial resistance and virulence genes?

8. Glossary

Abbreviations and terminologies:

EMBL-EBI: European Molecular Biology Laboratory Bioinformatics Institute; provides comprehensive genomic and molecular biology database information

NCBI BLAST: National Centre for Biotechnology Information; serves as sequence database search tool for genomic and molecular biology

PCR: Polymerase Chain Reaction; used to sequence and amplify nucleic acids (DNA&RNA)

PROKKA: Software tool for rapid annotation of prokaryotic genomes

RAST: Rapid Annotation Using Subsystem Technology; used to annotate assembled genomes

SEED: Database for bioinformatics research with subsystem approach used for annotation and allows comparative analysis

SMRT: Single Molecule Real Time Sequencing; model that identifies conserved proteins

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