GenEpi-BioTrain Virtual training 23

Virulence Profiling of *E. coli* Pathotypes

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Intended Learning Objectives

Specific objectives of this session:

1. Understand the challenges in typing of *E. coli* and *Shigella* in the laboratory
2. Understand the biological diversity within the *E. coli* pathotypes (incl. *Shigella*) and the significance for their virulence.
3. Learn about the challenges in quality control of whole-genome sequencing (WGS) *E. coli* data.
4. Analyse and interpret the different sub-typing methods, including MLST, cgMLST, and serotyping of *E. coli*.
5. Apply virulence profiling and typing using simple bioinformatics tools and suggest the resulting pathotype
6. Learn about real-life challenges in tracing emerging clones of pathogenic *E. coli*.

Outline

This session consists of the following elements

Day 1 – March 3

1. Introduction to *E. coli* pathotypes incl. *Shigella*
2. Introduction to typing tools and virulence factor databases
3. Intro to an exercise on *E. coli* subtyping and virulence profiling

Day 2 – March 10

4. Discuss results of typing and virulence profiling
5. Learn about outbreaks with specific *E. coli* pathotypes

Agenda

Agenda	Speakers	Hours
ECDC introduction and rules of conduct		09:00 – 09:10
***1. E. coli* virulence and pathotypes** ○ *E. coli* diagnostic in the laboratory – incl. notifiable results ○ *E. coli* pathotypes; virulence and genetic markers	Dr Cecilia Jernberg (ECDC) Dr Jette Kjeldgaard (DTU)	09:10 – 09:30 09:30 - 10:20
Break		10:20 – 10:30
***1. E. coli* genomics and quality control (QC)** ○ What to be aware of, when working with a diverse organism as *E. coli* ○ Presentation of QualiBact – species-specific QC parameters	João Cardoso, bioinformatician (DTU)	10:30 – 11:00
3. Taxonomic analysis and typing: ○ Concept, application, WGS-based tools	Dr Jette Kjeldgaard	11:00 – 11:20
Break		11:20 - 11:30
3. Virulence gene databases ○ Available databases; specificity, inputs, and outputs ○ Applications of databases	João Cardoso, bioinformatician	11:30 – 11:50
3. Interpretation of virulence gene results ○ Virulence profiling and pathotypes	Dr Jette Kjeldgaard	11:50 – 12:10
3. Introduction to the exercise.	Dr Jette Kjeldgaard	12:10 – 12:20
Q&A and conclusion	Dr Jette Kjeldgaard and João Cardoso	12:20 – 12:30

Intended Learning Objectives of this session

Introduction to *E. coli* pathotypes incl. *Shigella*

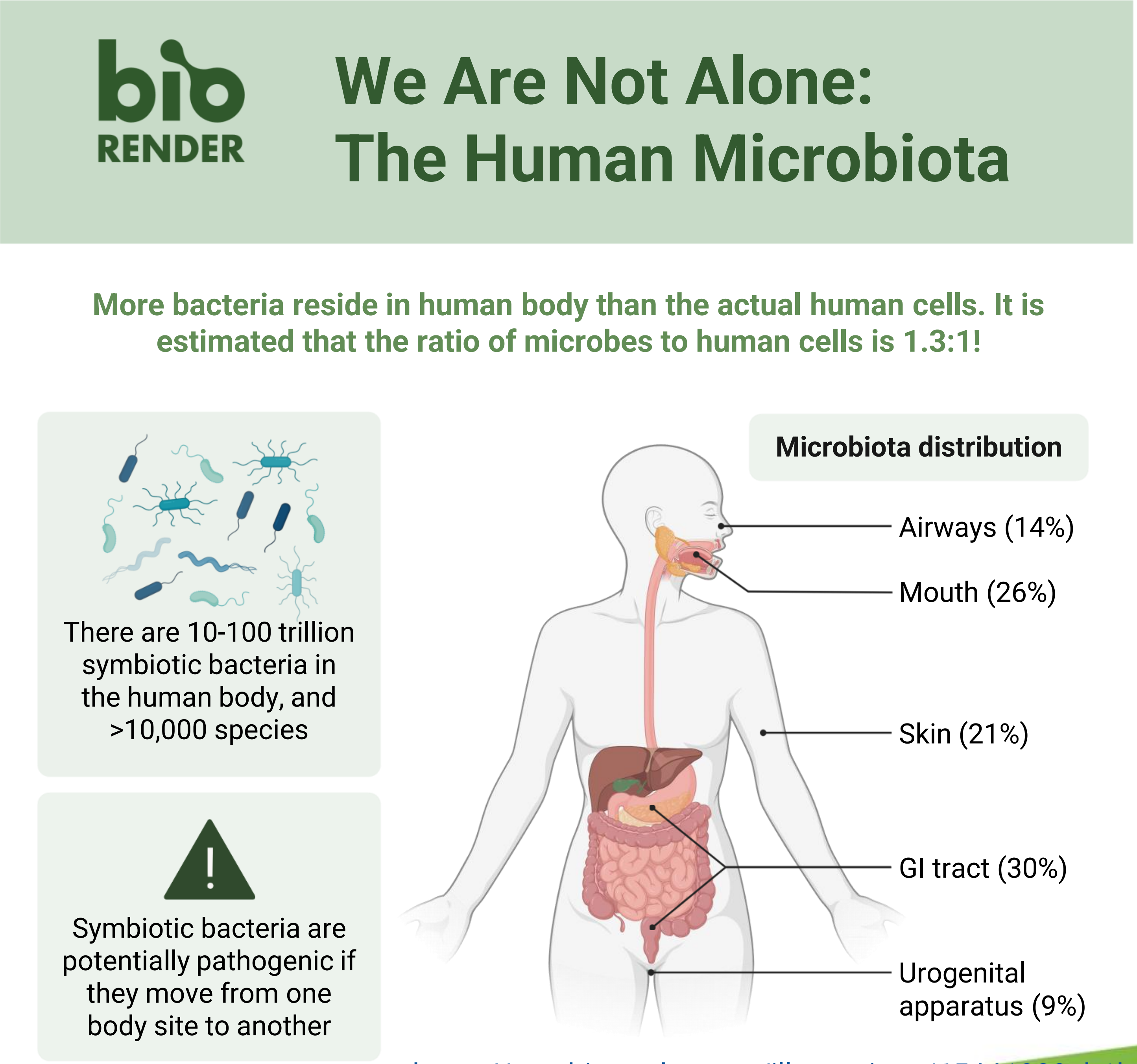
- *E. coli* diagnostic in the laboratory – incl. notifiable results (Cecilia Jernberg, ECDC)
- Which types of infections are caused by *E. coli,* and why?
- What do ‘virulence genes’ cover, and how can we use them to understand the differences in pathogenicity of E. coli?

Microbiota

Normal microbiota
- commensals
- Symbiotics =
  - Opportunists

Pathogens
- Primary pathogens
- Opportunistic pathogens

Difference?
- Virulence factors



We Are Not Alone: The Human Microbiota

More bacteria reside in human body than the actual human cells. It is estimated that the ratio of microbes to human cells is 1.3:1!

There are 10-100 trillion symbiotic bacteria in the human body, and >10,000 species

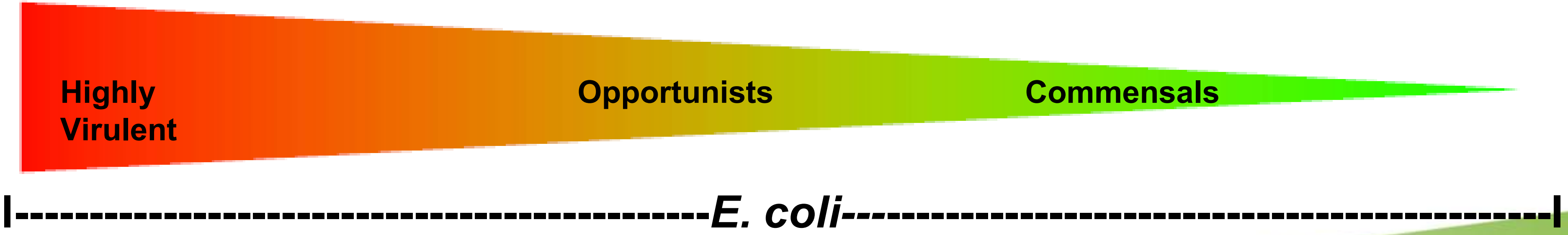
Symbiotic bacteria are potentially pathogenic if they move from one body site to another

https://app.biorender.com/illustrations/65441839cb1beca3631c4457

Pathogenicity and virulence

- Pathogenicity:
  - the ability of a microbe to cause disease in a host
- Virulence:
  - the relative capacity of a microbe to cause disease/symptoms in a host
  - degree of pathogenicity, the severity of disease
- Virulence factor:
  - a constituent of the pathogen that damages the host (e.g. a toxin) or is essential for viability in the host (e.g. capsule or iron-uptake system)

- Not all bacteria are pathogenic

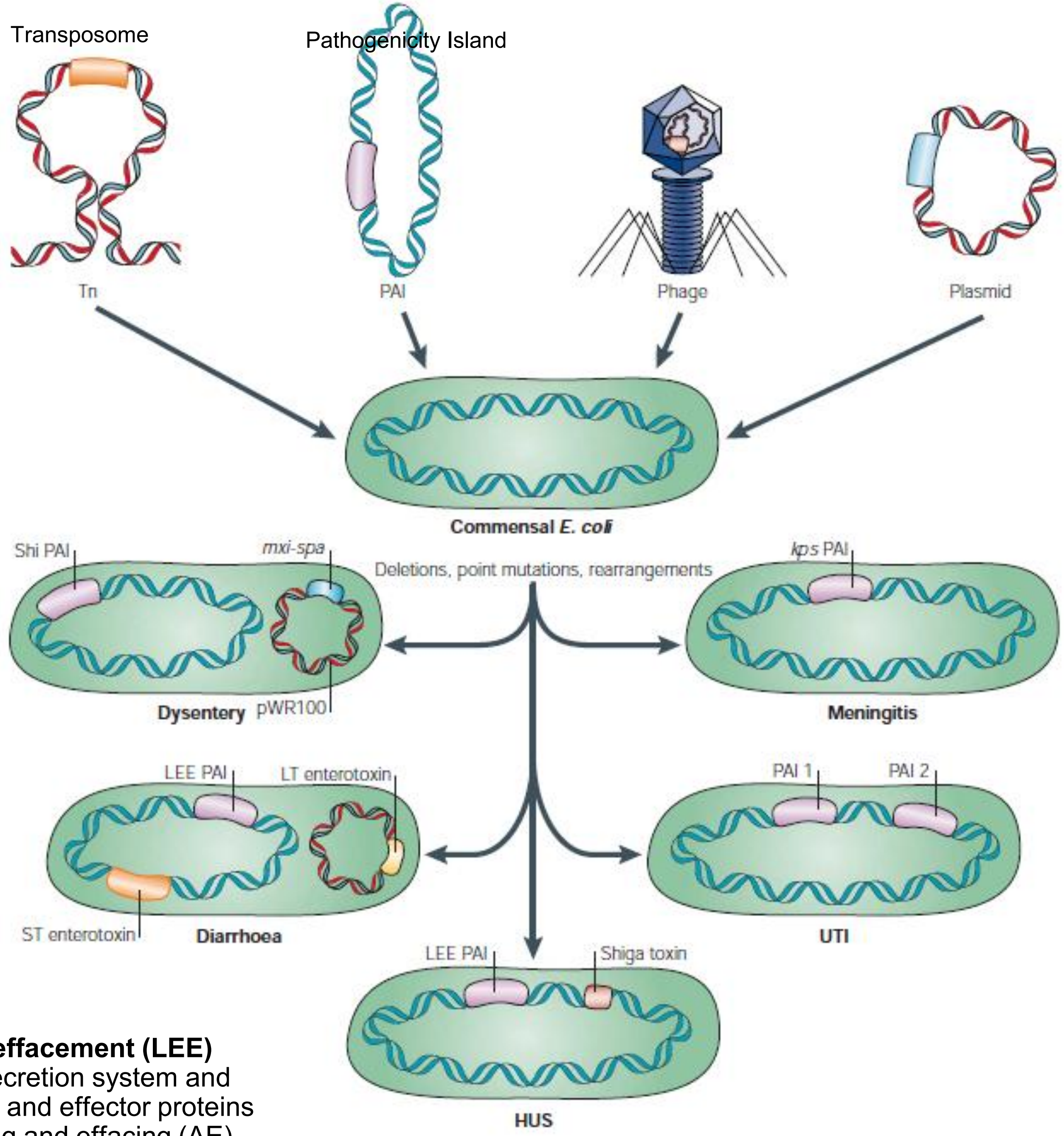


Highly Virulent **Opportunists** **Commensals**

|--*E. coli*---|

Escherichia coli infections

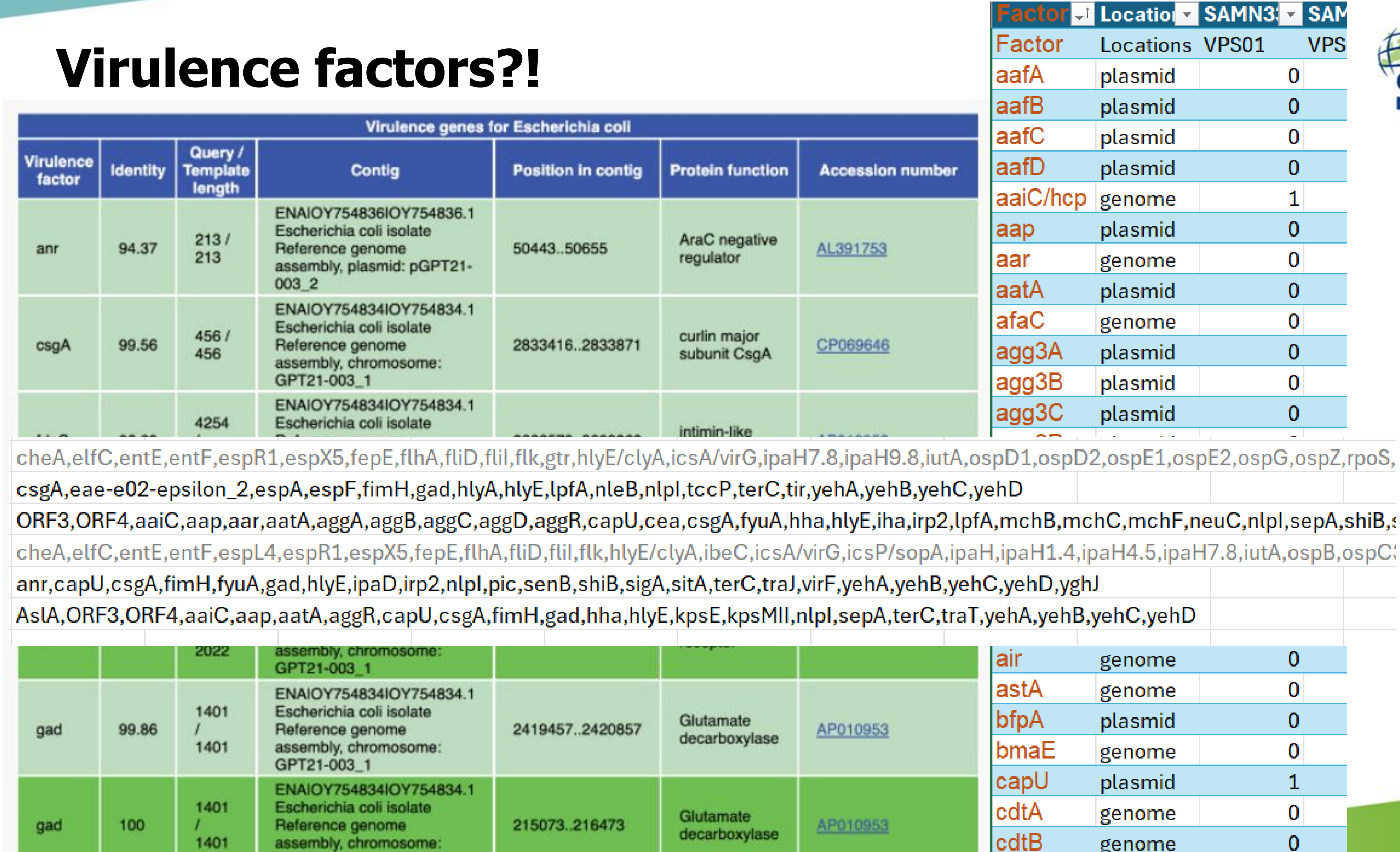
… all depending on the virulence factors



The **locus of enterocyte effacement (LEE)** encodes the Type III secretion system and associated chaperones and effector proteins responsible for attaching and effacing (AE) lesions in the large intestine

https://doi.org/10.1038/nrmicro818

Virulence factors?!



Virulence genes for Escherichia coli

Virulence factor	Identity	Query / Template length	Contig	Position in contig	Protein function	Accession number
anr	94.37	213 / 213	ENAIOY754836IOY754836.1 Escherichia coli isolate Reference genome assembly, plasmid: pGPT21-003_2	50443..50655	AraC negative regulator	AL391753
csgA	99.56	456 / 456	ENAIOY754834IOY754834.1 Escherichia coli isolate Reference genome assembly, chromosome: GPT21-003_1	2833416..2833871	curlin major subunit CsgA	CP069646
		4254	ENAIOY754834IOY754834.1 Escherichia coli isolate		intimin-like	
		2022	assembly, chromosome: GPT21-003_1			
gad	99.86	1401 / 1401	ENAIOY754834IOY754834.1 Escherichia coli isolate Reference genome assembly, chromosome: GPT21-003_1	2419457..2420857	Glutamate decarboxylase	AP010953
gad	100	1401 / 1401	ENAIOY754834IOY754834.1 Escherichia coli isolate Reference genome assembly, chromosome:	215073..216473	Glutamate decarboxylase	AP010953

Factor	Locatio	SAMN3	SAM
Factor	Locations	VPS01	VPS
aafA	plasmid	0	
aafB	plasmid	0	
aafC	plasmid	0	
aafD	plasmid	0	
aaiC/hcp	genome	1	
aap	plasmid	0	
aar	genome	0	
aatA	plasmid	0	
afaC	genome	0	
agg3A	plasmid	0	
agg3B	plasmid	0	
agg3C	plasmid	0	
air	genome	0	
astA	genome	0	
bfpA	plasmid	0	
bmaE	genome	0	
capU	plasmid	1	
cdtA	genome	0	
cdtB	genome	0	

cheA,elfC,entE,entF,espR1,espX5,fepE,flhA,fliD,flil,flk,gtr,hlyE/clyA,icsA/virG,ipaH7.8,ipaH9.8,iutA,ospD1,ospD2,ospE1,ospE2,ospG,ospZ,rpoS,

csgA,eae-e02-epsilon_2,espA,espF,fimH,gad,hlyA,hlyE,lpfA,nleB,nlpl,tccP,terC,tir,yehA,yehB,yehC,yehD

ORF3,ORF4,aaiC,aap,aar,aatA,aggA,aggB,aggC,aggD,aggR,capU,cea,csgA,fyuA,hha,hlyE,iha,irp2,lpfA,mchB,mchC,mchF,neuC,nlpl,sepA,shiB,

cheA,elfC,entE,entF,espL4,espR1,espX5,fepE,flhA,fliD,flil,flk,hlyE/clyA,ibeC,icsA/virG,icsP/sopA,ipaH,ipaH1.4,ipaH4.5,ipaH7.8,iutA,ospB,ospC

anr,capU,csgA,fimH,fyuA,gad,hlyE,ipaD,irp2,nlpl,pic,senB,shiB,sigA,sitA,terC,traJ,virF,yehA,yehB,yehC,yehD,yghJ

AslA,ORF3,ORF4,aaiC,aap,aatA,aggR,capU,csgA,fimH,gad,hha,hlyE,kpsE,kpsMII,nlpl,sepA,terC,traT,yehA,yehB,yehC,yehD

Escherichia coli groups

Two major groups;

- Diarrhoeagenic *E. coli* (DEC)

ETEC - enterotoxigenic *E. coli*
EPEC – enteropathogenic *E. coli*
EHEC (Incl. STEC) - enterohaemorrhagic *E. coli*
EIEC – enteroinvasive *E. coli* – Incl. *Shigella*
EAEC - enteroaggregative *E. coli*
DAEC – diffusely adhering *E. coli*

- Extraintestinal *E. coli* (ExPEC)
  - Uropathogenic (UPEC)
    - Urinary tract infections (UTI’s, sepsis)
  - Neonatal meningitidis (NMEC) *E. coli*

Do not edit
How to change the design

Have you been working with subtyping of E. coli before, incl. serotyping, biotyping and pathotyping?

ⓘ The Slido app must be installed on every computer you’re presenting from

slido

Diarrhoeagenic *E. coli* pathotypes

ETEC - enterotoxigenic *E. coli*

- causing travelers' diarrhea (watery, can be severe)

EPEC – enteropathogenic *E. coli*

- mild to severe (watery) diarrhea, vomiting (in children <2 y; LMIC countries)

EHEC (Incl. **STEC**) - enterohaemorrhagic *E. coli*/Shiga toxin producing *E. coli*

- bloody diarrhea and HUS (kidney failure)
- intense inflammatory response

EIEC – enteroinvasive *E. coli* – Incl. *Shigella*

- Enterocolitis or mild form of bacillary dysentery, similar to that caused by *Shigella* spp
- abdominal cramps, diarrhea (blood and mucus), vomiting, fever

EAEC/EAggEC - enteroaggregative *E. coli*

- persistent diarrhea in children <2 y

DAEC – diffusely adhering *E. coli*

- mild to moderate diarrhea in children >2 y, adolescents

Additionally…
AIEC -Adherent-invasive *E. coli*
EAggSTEC – enteroaggregative shiga toxin producing *E. coli*
….

Virulence factors and infection

How to establish a microbial infection?

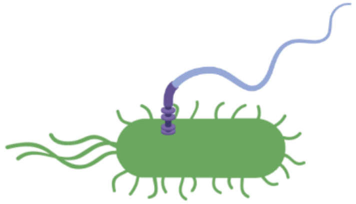
Multiple important factors:

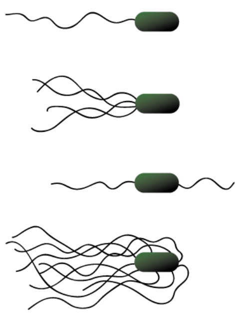
- Adhesion and motility
- Colonization/biofilm formation
- Avoidance of host defence mechanisms
- Nutritional factors
- Invasion
- Toxin or other aggressin production
- Secretion or delivery systems

Adhesion and motility (Flagella, fimbriae and pili)

Filamentous surface structures

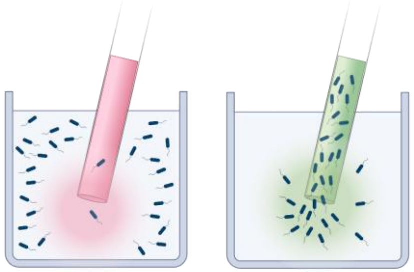
- Help *E. coli* interact with its environment, host tissues, and other bacteria
- Differ in structure, size, composition, and function





Flagella:

- Surface structure; long, thin filament(s); H-antigen
- Used for motility
- Situated in the outer membrane with a hook and a ‘motor’
- Enable bacteria to move toward nutrients or away from harmful substances (chemotaxis)



Common genes involved in flagella

Overview of some of the genes involved in expression of flagella:

Function	**Key Genes**	**Notes**
Master regulator	*flhDC*	Activates transcription of most flagellar operons
Basal body / hook	*fliE, fliF, fliG, fliM, fliN, flgB, flgC, flgD, flgE (hook), flgF, flgG, flgH, flgI, flgJ*	Core rotary motor + hook
Filament	***fliC****	Flagellin (main filament protein)
Motor proteins	*motA, motB*	Proton-driven rotary motor
Export apparatus	*flhA, flhB, fliP, fliQ, fliR*	Secretion system for exporting flagellar components
Regulation	*fliA (σ28), flgM*	*fliA* = flagellar sigma factor; *flgM* = anti-sigma factor

E. coli* H-antigen (flagellar) serotyping primarily relies on identifying variations in the ***fliC* gene.

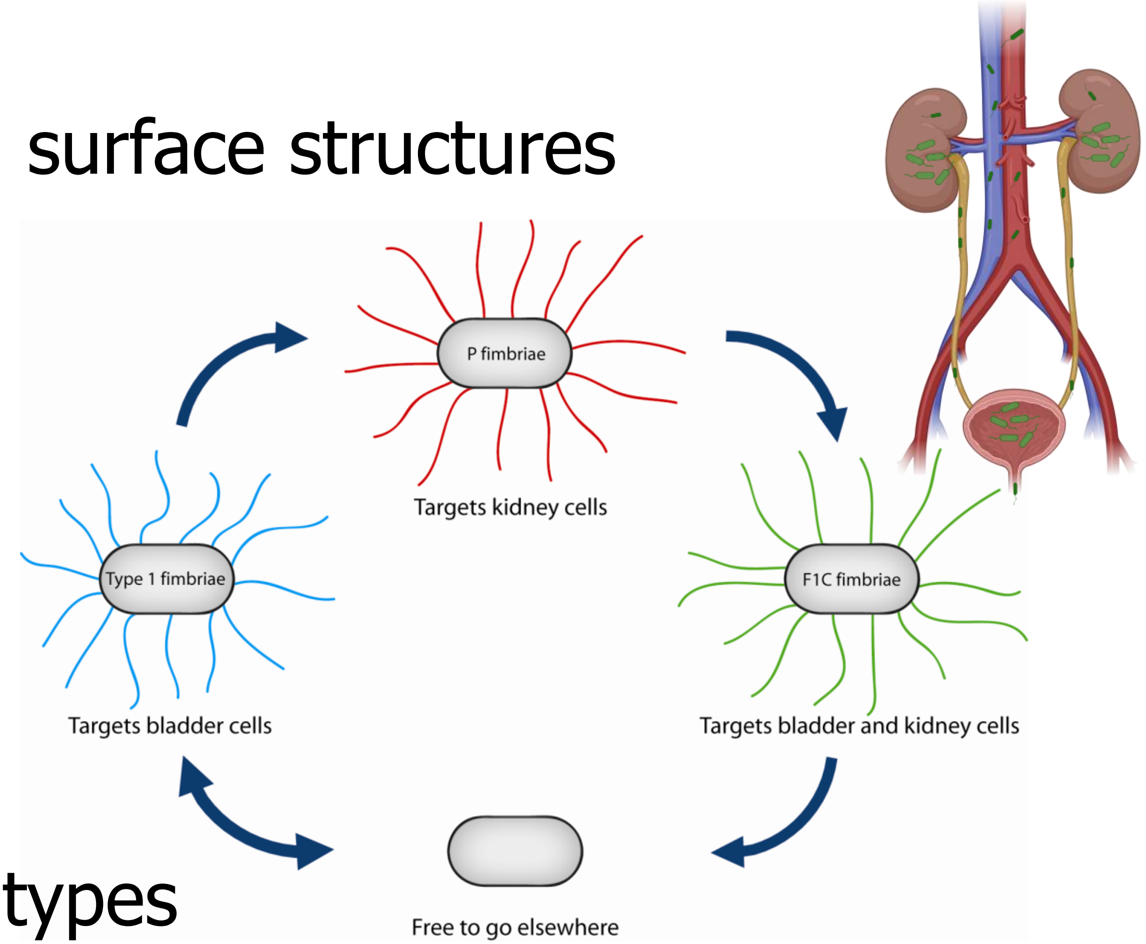
Other alternate flagellar genes like ***flkA***, ***flmA***, and ***fllA*** are used when *fliC* is not expressed.

Fimbriae and pili

Fimbriae and pili are hair-like protein surface structures

They play a crucial role in:

- Adherence to host cells
- Colonization of specific tissues
- Biofilm formation
- Host specificity
- Virulence in different *E. coli* pathotypes



Fimbriae in urinary tract infections

Common genes in fimbriae/pili expression

Pathotype	Key fimbriae / Pili	Major gene clusters	Role in pathogenesis
ETEC	CFA/I, CFA/II, CFA/IV; animal fimbriae (F4/K88, F5/K99, F6/987P, F18)	*cfaA–D, cs1–cs3, fae, fan, fas, fed*	Adherence to small intestine; essential for colonization and delivery of LT/ST enterotoxins
EPEC	Bundle-forming pilus (BFP)	*bfpA–P (*EPEC adherence factor plasmid)	Localized adherence (LA), microcolony formation; early step in A/E lesion development
EHEC/ STEC	Long polar fimbriae (LPF), curli, other adhesins (Iha, Saa)	*lpfA–D, csgA–G, iha, saa*	Colonization of large intestine, biofilm formation, support of A/E lesions (with *eae*/*tir*)
UPEC	Type 1 fimbriae, P fimbriae, S fimbriae, Dr adhesins	*fimA–H, papA–K, sfa, dra/afa*	Adherence to bladder (type 1 fimbriae) and kidney epithelium (P fimbriae), central in cystitis and pyelonephritis
EAEC	Aggregative adherence fimbriae (AAF/I–V)	*aggA–D, aafA–D, agg3, agg4, agg5*	Aggregative (“stacked-brick”) adherence, mucus induction, persistent diarrhea
DAEC	Dr adhesins	*afa/dra, daa*	Diffuse adherence on epithelial cells; associated with UTIs and some pediatric diarrhea
NMEC	Type 1 fimbriae, S fimbriae	*fimA–H, sfa*	Invasion of brain microvascular endothelial cells; contributes to meningitis
ALL	YHD/Yeh fimbriael cluster	*yehA-D*	The yeh cluster is widely distributed in *E. coli*

Avoidance of host defence mechanisms

- Survival through stomach acid
  - Glutamate decarboxylase enzymes *gad*-genes (*gadA-C* + *E*)
  - Arginine decarboxylase (*adiA+C*)
  - Lysine decarboxylase (*cadA*+B)

- Increased serum survival
  - Mainly in ExPEC: *iss*-gene

- Outer membrane antigens, LPS (O-type – somatic)

- Capsule formation (K-antigen)
  - Extracellular, polysaccharide layer (biofilm formation)
  - Cover antigens, prevents recognition
  - Protect against phagocytosis and antibiotics
  - especially important in ExPEC (invasive strains)

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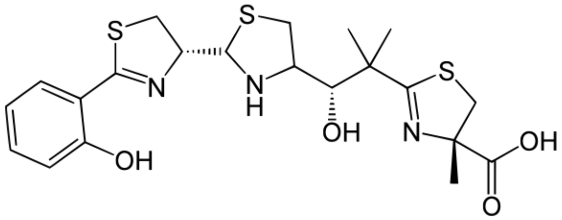
Common capsule and antigen genes in *E. coli*

Capsules are often strain specific and not a defining virulence feature

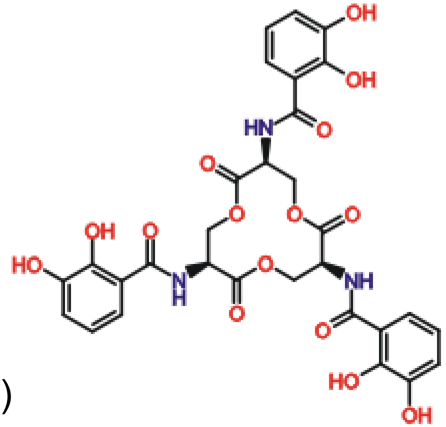
Capsule Group	Main Gene Clusters	Capsule Type Examples	Notes
Group 1	*cpsA–C*, *wzx*, *wzy*	O-antigen	
Group 2	*kpsDMTE*, *kpsF, kpsS, kpsU* + variable region	K1, K5, K12, K100	Most common in pathogenic strains
Group 3	*kpsDMTE*, *kpsCSU*	K10, K54	Often plasmid-encoded
Group 4	*gfcA–D*, *wza*, *wzb*, *wzc*	O-antigen	Found in commensals (EHEC)

Nutritional factors - iron

- Iron is essential for all microbes – and man = competition for iron is fierce
- Inside a host, iron is scarce, poorly soluble or tightly bound by host proteins
- Mechanisms used for iron uptake:
  - Siderophores – iron binding complexes
  - Direct iron (ferrous) uptake
  - Haeme uptake
  - Haemolysin - enzymatic degradation
  - ….

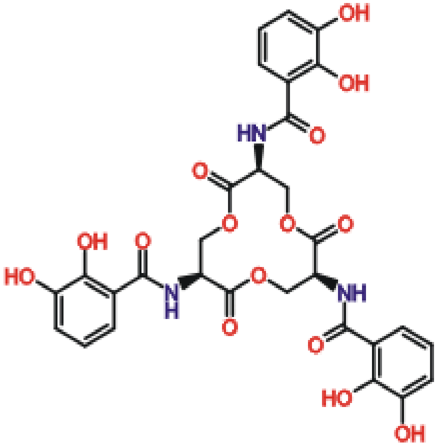

Yersiniabactin of *E. coli*


Haemolysis (Atlas.sund.ku.dk)


enterobactin of *E. coli*

Nutritional factors - Genes for Iron acquisition

- Siderophores (UPEC, EHEC)
  - Specific membrane-located receptor proteins used to transport iron-siderophores into the cell
    - Enterobactin synthesis *entA-F*
      - uses *tonB–exbB–exbD* energy system, *febA-D/G for* transport and *fes* for release
    - Salmochelin (*iro*-genes); Aerobactin (*iuc*-genes, *iutA*);
    - Yersiniabactin (*ybt / irp / fyu* –genes)
- Non-sideophore systems
  - Iron ion transport
    - *feoA/B/C*; *fecA-E/I/R; fur*
- Haemolysin
    - *hlyA* (UPEC + others)
    - *hlyE, clyA* (EIEC/Shigella + others)
    - *ehxA* (EHEC/STEC)



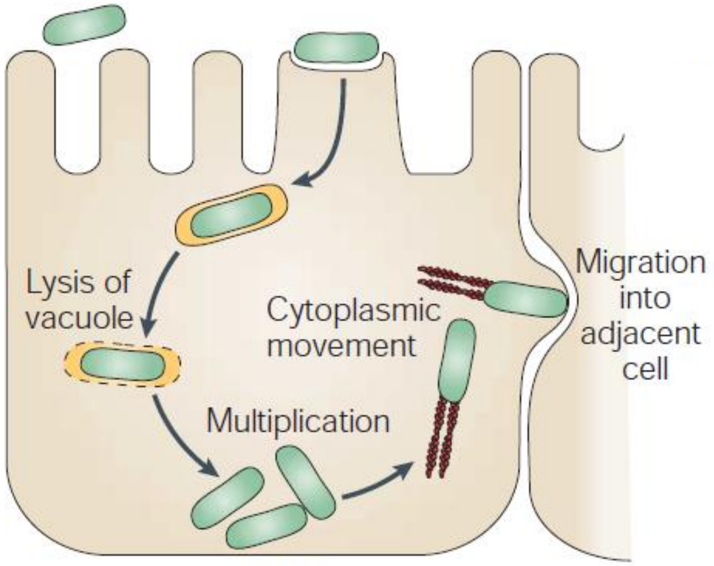
enterobactin of *E. coli*

Invasion/infection – EIEC/*Shigella*

EIEC/Shigella key invasion genes (on the pINV plasmid):

- Mediates active invasion of epithelial cells, allows intracellular replication and spread

- *ipaA, ipaB, ipaC, ipaD* – Invasion plasmid antigens
- ***ipaH*** gene for a Type III-secreted (T3SS) effector protein
- *mxi* genes – T3SS components
- *spa* genes – T3SS secretion apparatus
- ***virF,*** *virB* – transcriptional regulators
- *icsA, virG* – actin-based motility; intracellular spread



EIEC

Endocytic uptake of bacteria and disruption of endocytic vacuoles (phagocytosis)

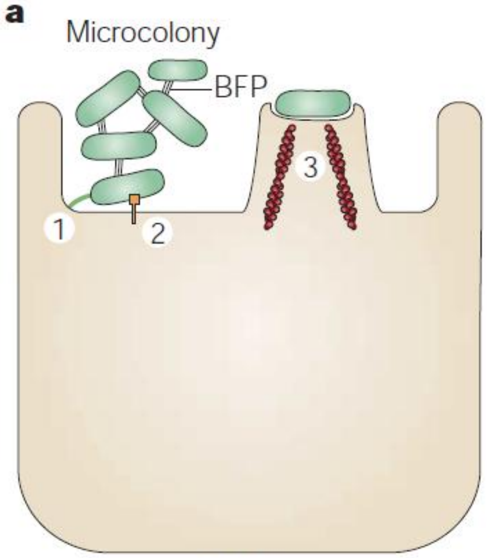
doi.org/10.1038/nrmicro818

Invasion/infection - EPEC

EPEC does not invade deeply like EIEC but performs limited effacement-associated invasion.

Destroy the normal microvillar structure, inducing the characteristic attaching and effacing (A/E) lesion

- EPEC attach via pili (*bfp*), insert intimin-receptor *tir* (1)
- *tir* is responsible for anchoring to the host cell via intimin *eae* and Type 3 secretion system (T3SS) (2)
- Arrangement of host cell cytoskeleton (3), > active ion secretion, > increased permeability



EPEC

doi.org/10.1038/nrmicro818

Invasion/infection - EPEC

- Key factors are on the Locus of Enterocyte Effacement (LEE) pathogenicity island (35-kb PAI)
    - ***eae*** – Intimin (attaching-effacing adhesion)
    - *tir* – Translocated intimin receptor (inserted into host membrane)
    - *esc/sep* genes – T3SS structural proteins
    - *espF, espG, espH, espZ, espB, espD* – effector proteins
    - *espC* - enterotoxin
    - *map* – mitochondrial-associated protein
- *nleA-C* genes – non-LEE encoded effectors
- Regulation of *espC*– *ler*
    - and in EPEC *per* is regulating *ler*

Invasion/infection – EHEC/STEC

- EHEC shares some LEE genes with EPEC, + produces Shiga toxins
- Key factors on the LEE pathogenicity island
  - ***eae*** – Intimin (attaching-effacing adhesion)
  - *tir* – Translocated intimin receptor (inserted into host membrane)
  - *esc/sep* genes – T3SS structural proteins
  - *espF, espG, espH, espZ, espB, espD* – effector proteins
  - Shiga toxins; ***stx1, stx2* – *several variants***

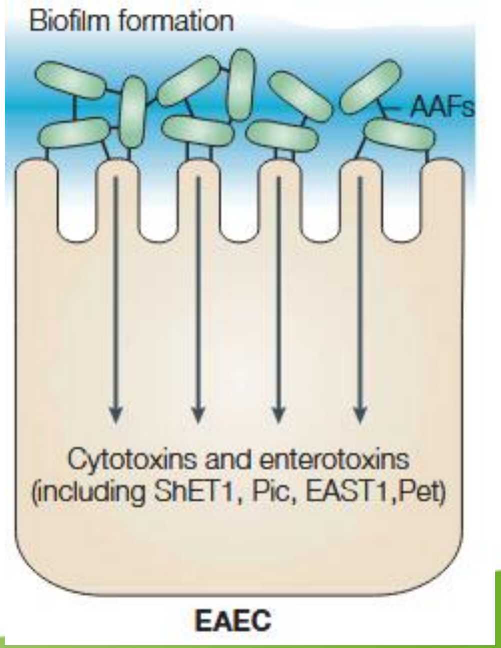
  - *nle* genes – non-LEE encoded effectors
  - *saa* (in some LEE-negative STEC) – autoagglutinating adhesin
  - *toxB* – adherence and epithelial interaction
  - ***ehxA* -** enterohaemolysin

Invasion/infection - EAEC

EAEC are not very invasive, but causes persistent mucosal colonisation, biofilm and inflammation.

The virulence is centered on adhesion, mucus interaction, inflammation and secretion of toxins (entero- and cytotoxins.)

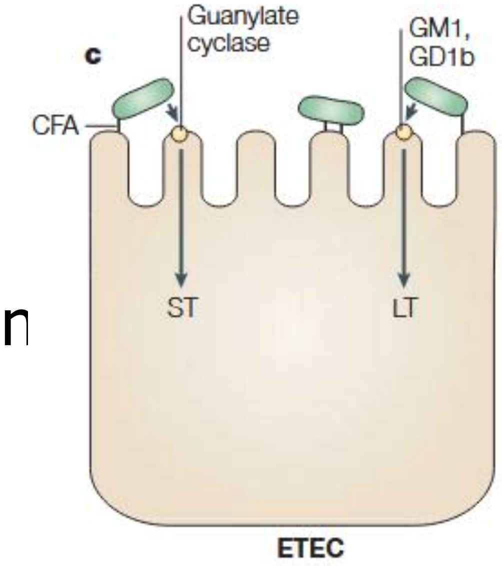
- Aggregative fimbriae - *aggA, aafA, agg3A, agg4A, agg5A*
- Dispersin – facilitating adhesion –*aap/aat, capU*
- Enterotoxin: Enteroaggregative *E. coli* ST (EAST1/*astA*)
- Autotransporter toxins –*pet, pic*
- AggR regulon; *aaiA–aaiV*-genes
- Haemolysin E - *hlyE*



Invasion/infection - ETEC

ETEC shows only limited invasion, relying on toxins (ST, LT)

Possible contributing genes:
tia - Toxigenic invasion locus A
tibA – autotransporter associated with adhesion/invasion
eatA – mucin-degrading protease facilitating epithelial interaction



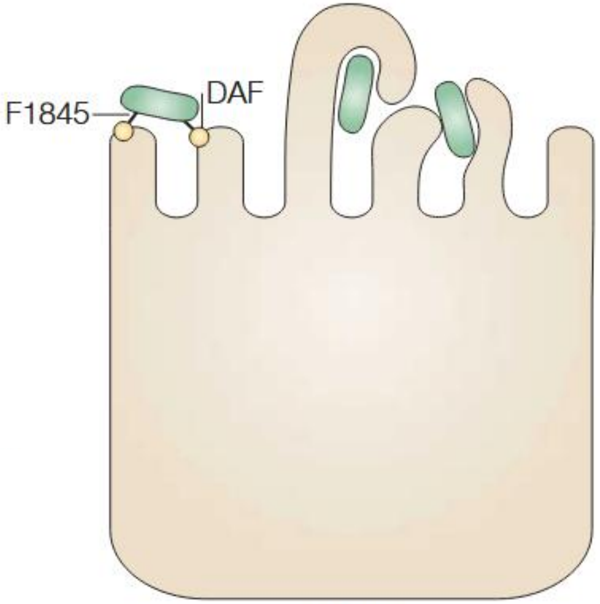
Toxins:
Heat-labile toxin: LT – *eltA, eltB*
Heat-stable toxins: STh and STp – *estA* (STa), *estB* (STb) - (*estIa* in VF)

Invasion/infection - DAEC

Presents a diffuse pattern of adherence > elongated microvilli > long finger-like cellular projections, wrapping the bacteria

Possible contributing genes:

- Dr fimbria: *afa/dra, daaE*
- F1845 is common



DAEC

Invasion/infection - NMEC

Neonatal Meningitis *E. coli* have special ability to survive in macrophages and serum, and to cross the intestinal - and blood–brain barrier

Key specific virulence genes:

- *ibeA, ibeB, ibeC* – Invasion of brain endothelium
- *ompA* – mediates binding to brain endothelial receptors
- *cnf1* – cytoskeletal manipulation
- *kps* genes, *neuC* (K1 capsule) – aids serum resistance and intracellular survival
- *fimH* – adherence to epithelial cells
- *vat* - vacuolating autotransporter toxin

Overview of common pathotype markers

E. coli pathotype	Key virulence markers	Virulence function
EPEC	*eae*, *bfpA*, EAF	*eae* = intimin; *bfpA* + EAF plasmid
ETEC	*elt* (LT), *est* (ST), CF genes (*cfa*, *cs1*–*cs7*)	LT and ST enterotoxins; CF = colonization factors
EIEC	*ipaH*, *ial*, *virF*	*ipaH* is multicopy → high sensitivity; similar to *Shigella*
EHEC/STEC	*stx1*, *stx2*, *eae*, *ehxA*, *lpf*	STEC = *stx*+; EHEC = *stx* + *eae*; *ehxA* = enterohemolysin, long polar fimbriae
EAEC	*aggR*, *aatA/aap*, *aaiC*	*aggR* = master regulator; EAEC is genetically diverse
DAEC	*daa*, *afa/dra* genes	Adhesins associated with diffuse adherence
AIEC	No single marker; sometimes *lpfA*, *fimH* variants used	Requires phenotypic confirmation (adhesion + macrophage survival)

References

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Virulence profiling

- Not an easy task with out good, curated resources or tools
- Typical markers will give a good indication
- Consider which genes and plasmid are important, not only toxins but also genes for adhesion/invasion
- Consider also regulatory genes
- Remember there is a big genetic overlap between some pathotypes
- We will try it in the exercise

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