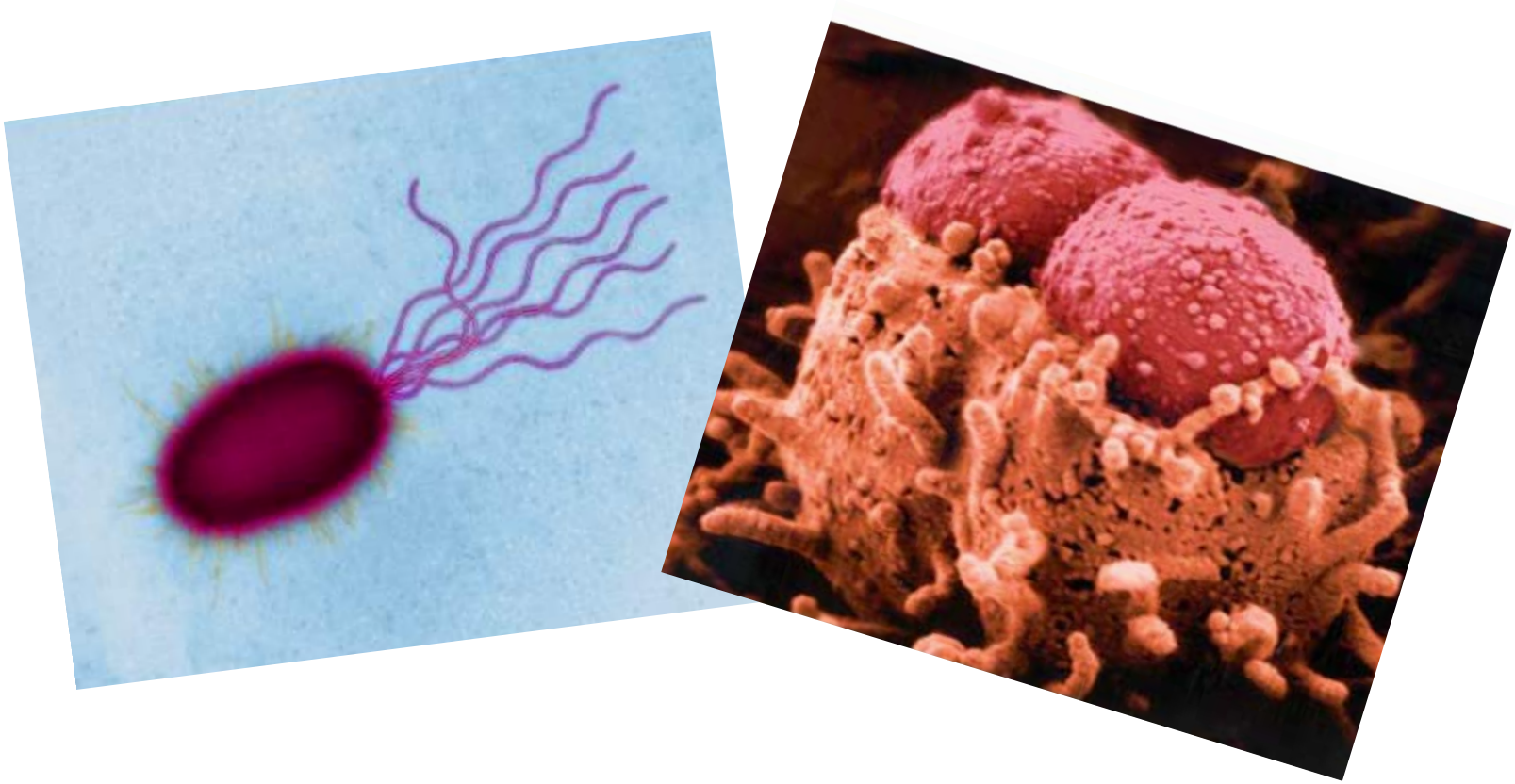
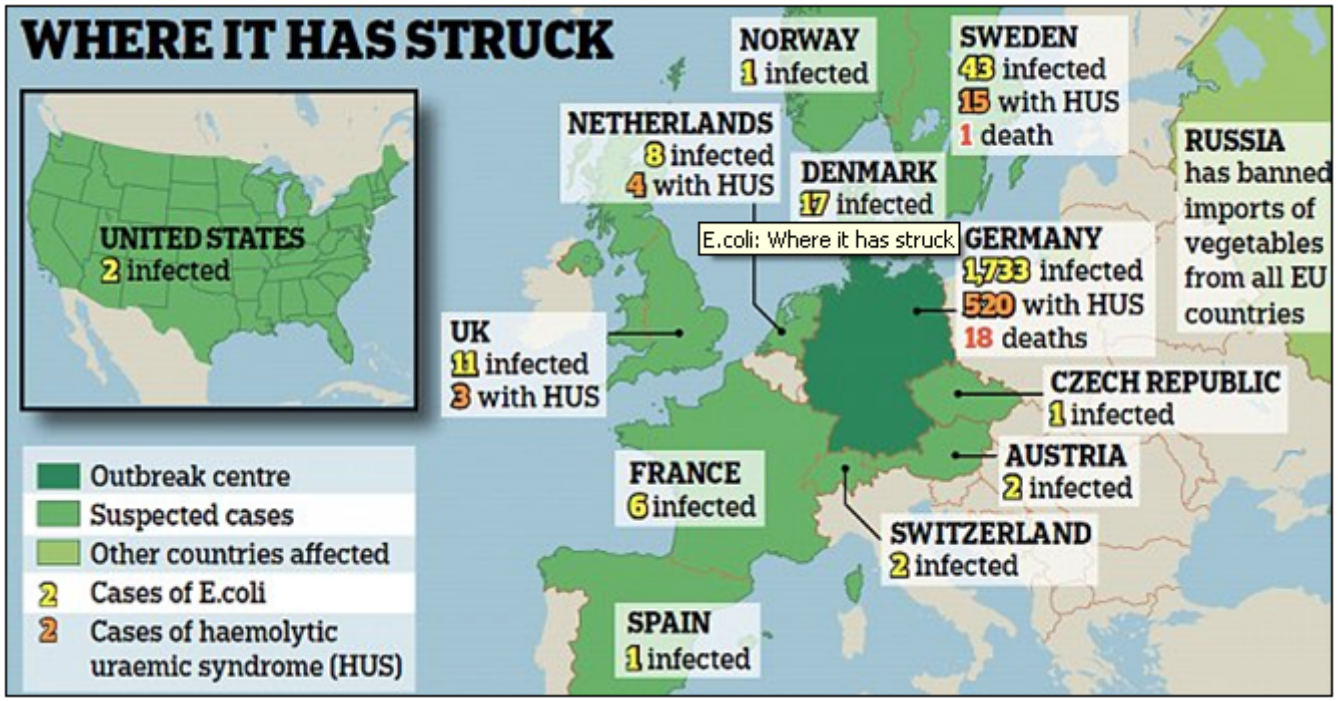
Biology of pathogenic *Escherichia coli*



Nathalie ROLHION
n.rolhion@imperial.ac.uk

Why a lecture on pathogenic *E. coli* ?

⇨ **recent outbreak in May-June 2011 : 3507 Europeans + 2 Americans contaminated**
39 deaths



Overview

INTRODUCTION

***Escherichia coli* :**

- member of the normal intestinal microflora of humans and others mammals

- model organism, laboratory workhorse

- important species in biotechnology :

-
-
-
-

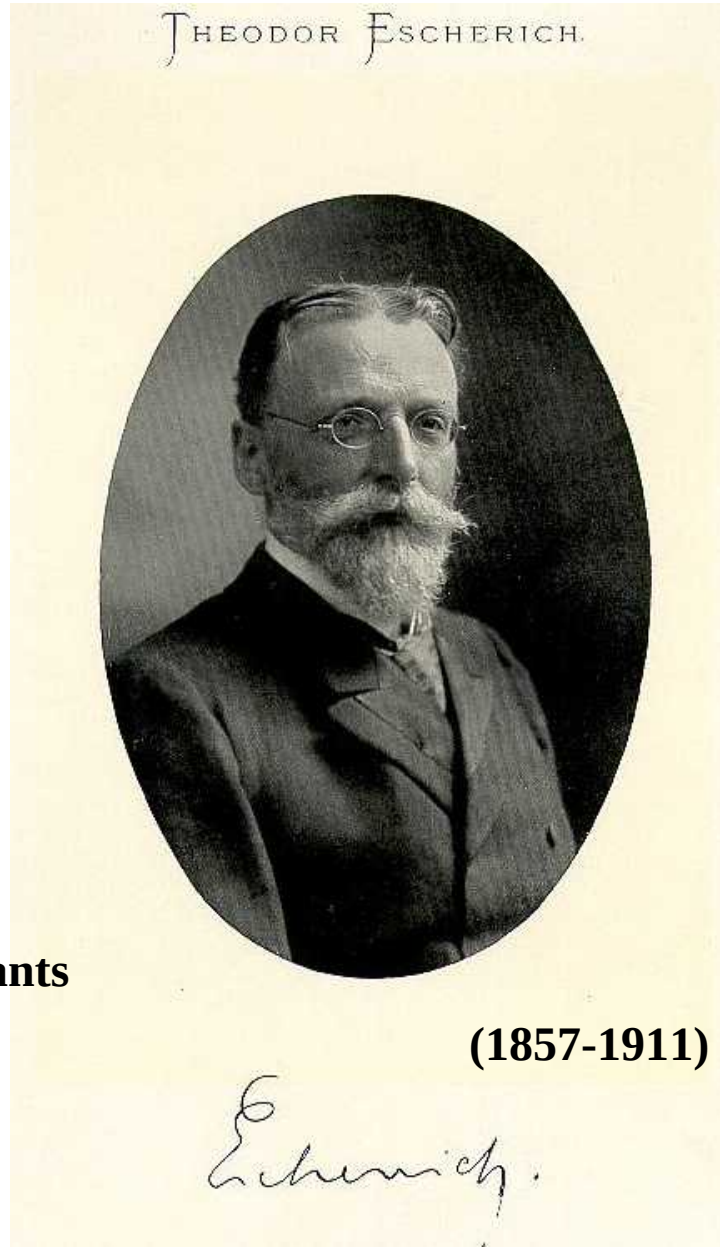
- highly versatile and frequently deadly pathogen

Non pathogenic *E. coli*

- **discovered in 1885**

- **1919 : *Escherichia coli***

- **1935 : a strain of *E. coli* was shown to be the cause of an outbreak of diarrhoea among infants**



(1857-1911)

Non pathogenic *E. coli*

- member of the normal intestinal microflora

- colonization of the GI tract of most warmed-blooded animals within hours or a few days after birth

- adhesion to mucus of the large intestine/colon

- benefit to their hosts : beneficial symbiotic relationship

Non pathogenic *E. coli*

- ***Enterobacteriaceae*** **family (the enteric bacteria)**

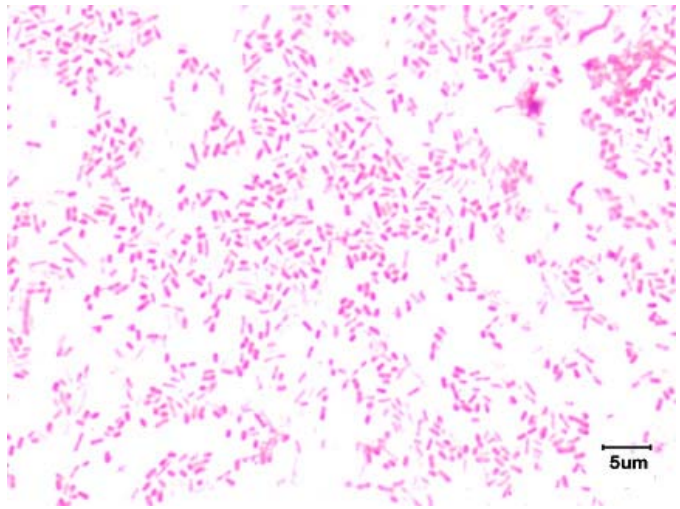
- **Gram negative bacterium**
- **facultative anaerobic**
- **shape :**
  long :
  diameter :

-optimal growth :

good adaptation to its characteristic habitats
response to environmental signals

- flagella, peritrichous arrangement

- 1997 : complete genome sequence



Non pathogenic *E. coli*

ARTICLE

The Complete Genome Sequence of *Escherichia coli* K-12

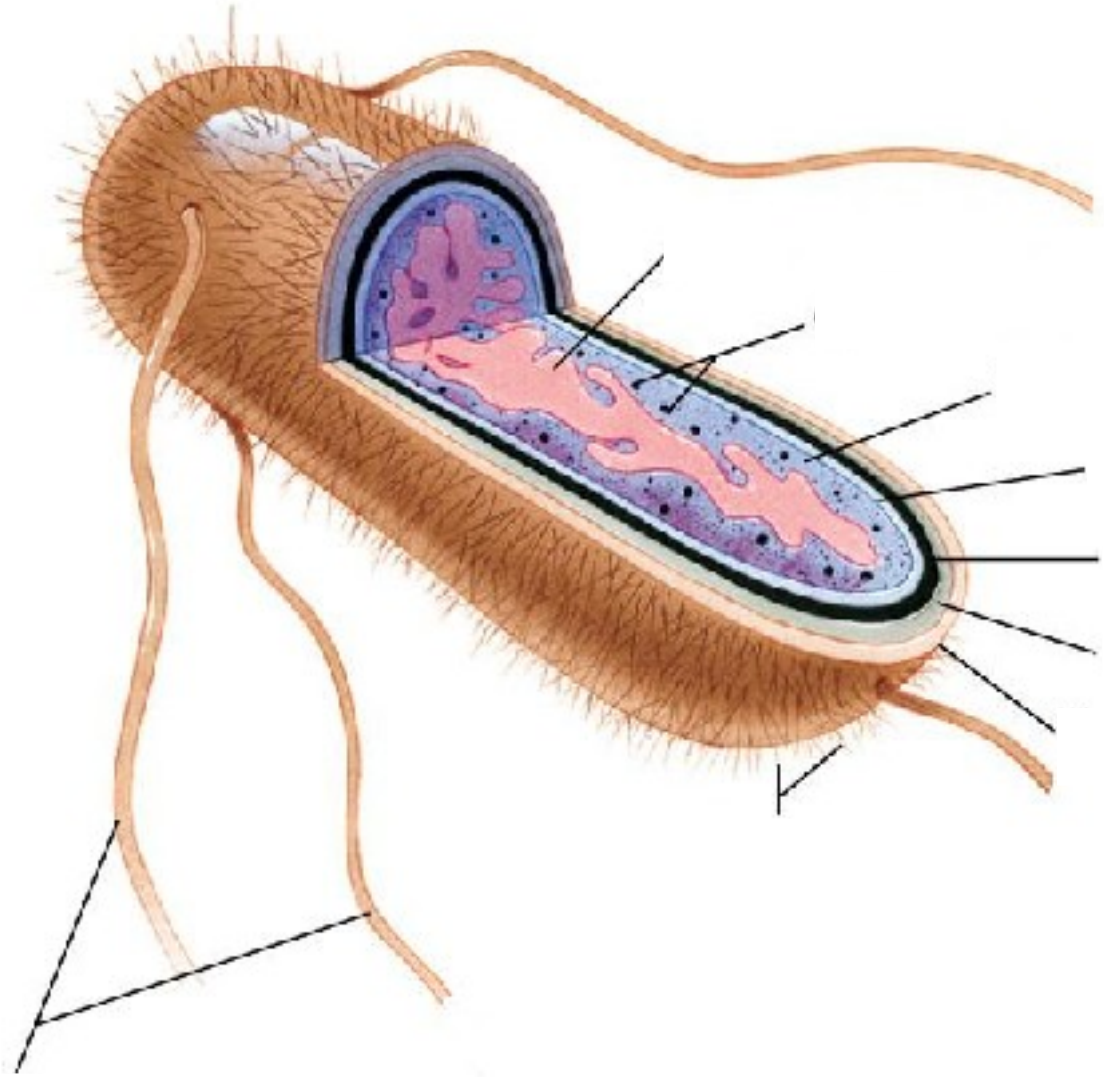
Frederick R. Blattner,* Guy Plunkett III,* Craig A. Bloch, Nicole T. Perna, Valerie Burland, Monica Riley, Julio Collado-Vides, Jeremy D. Glasner, Christopher K. Rode, George F. Mayhew, Jason Gregor, Nelson Wayne Davis, Heather A. Kirkpatrick, Michael A. Goeden, Debra J. Rose, Bob Mau, Ying Shao

The 4,639,221–base pair sequence of *Escherichia coli* K-12 is presented. Of 4288 protein-coding genes annotated, 38 percent have no attributed function. Comparison with five other sequenced microbes reveals ubiquitous as well as narrowly distributed gene families; many families of similar genes within *E. coli* are also evident. The largest family of paralogous proteins contains 80 ABC transporters. The genome as a whole is strikingly organized with respect to the local direction of replication; guanines, oligonucleotides possibly related to replication and recombination, and most genes are so oriented. The genome also contains insertion sequence (IS) elements, phage remnants, and many other patches of unusual composition indicating genome plasticity through horizontal transfer.

www.sciencemag.org • SCIENCE • VOL. 277 • 5 SEPTEMBER 1997

: 3.4 billion– base pair

Non pathogenic *E. coli*



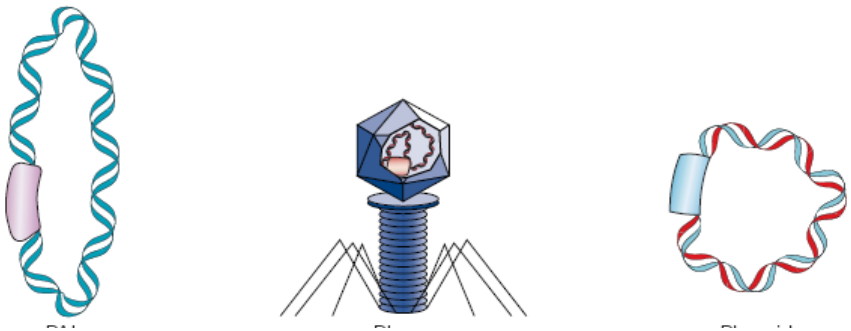
Pathogenic *E. coli* : common features

- acquisition of specific virulence attributes

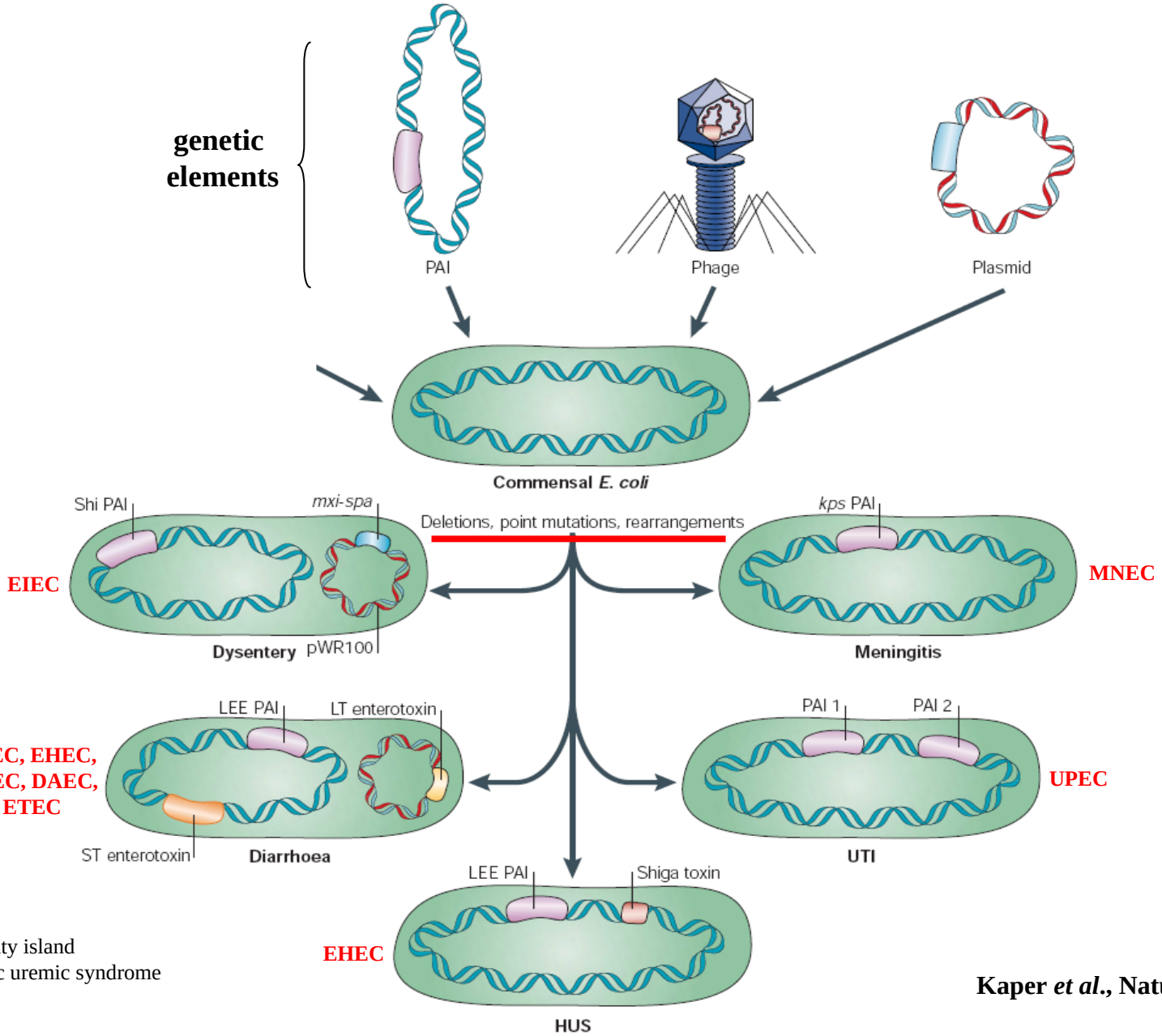
⇨ increased ability to adapt to new niches :

⇨ cause a broad spectrum of disease

- encoded on genetic elements



Pathogenic *E. coli* : common features



PAI : pathogenicity island
HUS : haemolytic uremic syndrome

Kaper *et al.*, Nature reviews, 2004

Pathogenic *E. coli* : common features

- 3 general clinical syndromes :

⇨ **enteric/diarrhoeal disease : EPEC**
EHEC
ETEC
EAEC
DAEC
EIEC

⇨ **urinary tract infections (UTIs) : UPEC**

⇨ **sepsis/meningitis : MNEC**

classification based on their unique virulence factors and identification only by these traits

- APEC
REPEC

Pathogenic *E. coli* : common features

Examples of the virulence determinants of pathogenic *E. coli*

Adhesins
CFAI/CFAII
Aggregative adherence fimbriae (AAFs)
type 1 fimbriae
Pap fimbriae
S fimbriae
intimin (non-fimbrial adhesin)
EPEC adherence factor

Invasins
for intracellular invasion and spread

Motility/chemotaxis
flagella

Toxins
Heat-labile toxin
Heat-stable toxin
Shiga toxin
cytotoxins
endotoxin (LPS)

Secreted proteins
type III effectors
autotransporters

Antiphagocytic surface properties
capsule (K antigens)
LPS

Defense against serum bactericidal reactions
LPS
K antigens

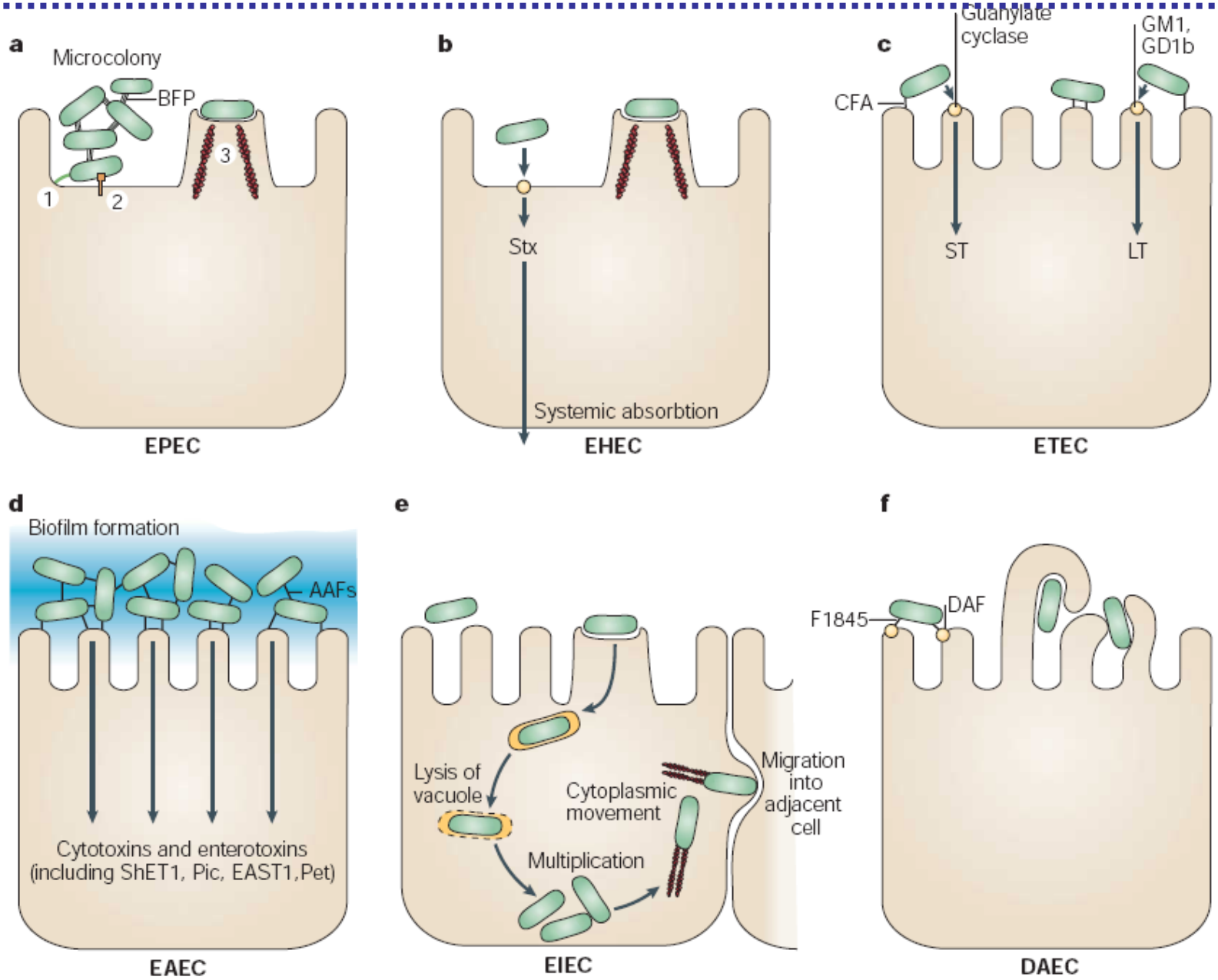
Defense against immune responses
capsules
LPS
antigenic variation

Others
genetic exchange by transduction and conjugation
drug resistance
siderophores and iron uptake systems

cf tables 1 and 2

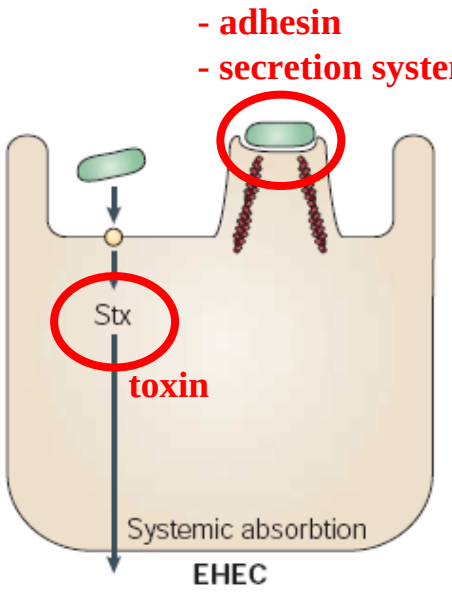
Adapted from Tokar K., Pathogenic *E. coli*.

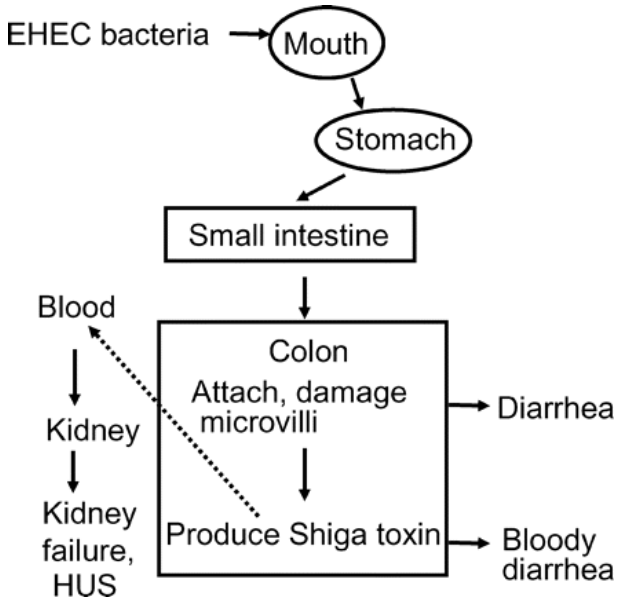
Gastrointestinal infections



Kaper *et al.*, Nature reviews, 2004

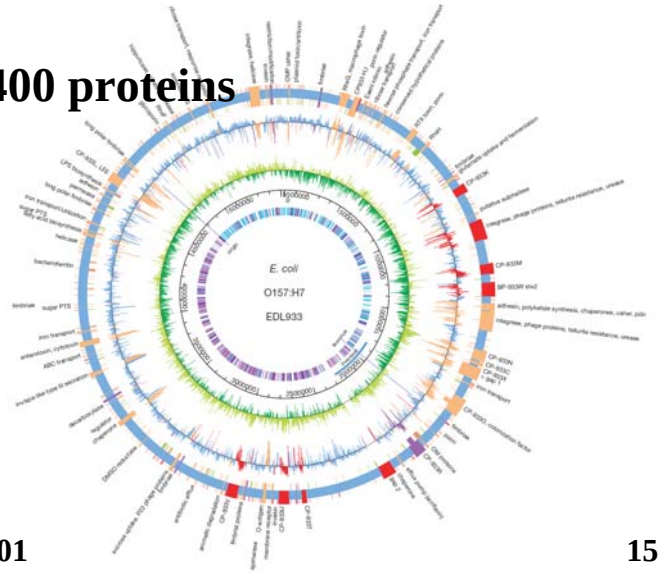
EHEC : enterohaemorrhagic *E. coli*





Gyles, J. Anim Sci., 2001

- in the popular press : Hamburger *E. coli*
  Hamburger disease
- O157:H7 serotype
- reservoir of EHEC : bovine intestinal tract
- infectious dose : 10-100 bacteria
- mild diarrhea,
  hemorrhagic colitis,
  hemolytic uremic syndrome (HUS, 5-10%)
- genome : 5.5 mbp, 5400 proteins



Perna *et al.*, Nature, 2001

EHEC : enterohaemorrhagic *E. coli*

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Q&A: E. coli O157

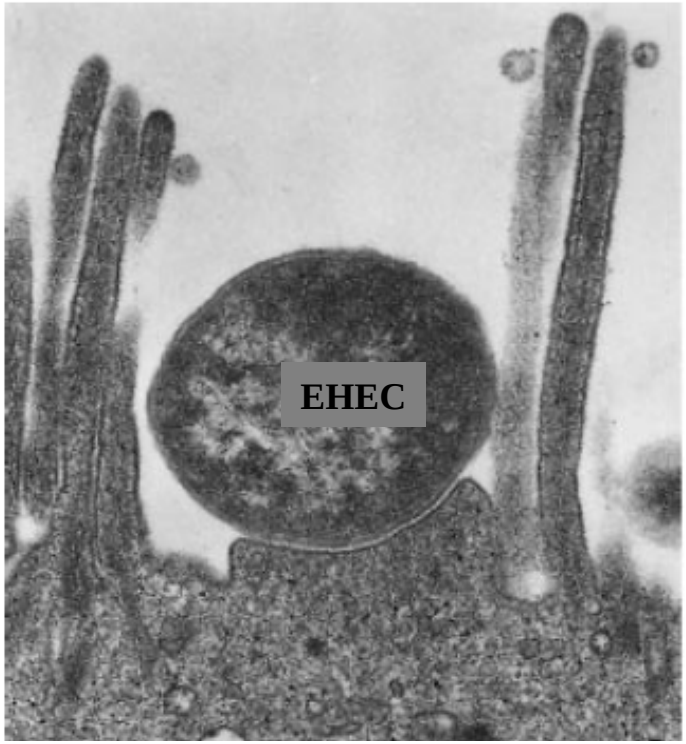
How e-coli spreads from livestock to humans

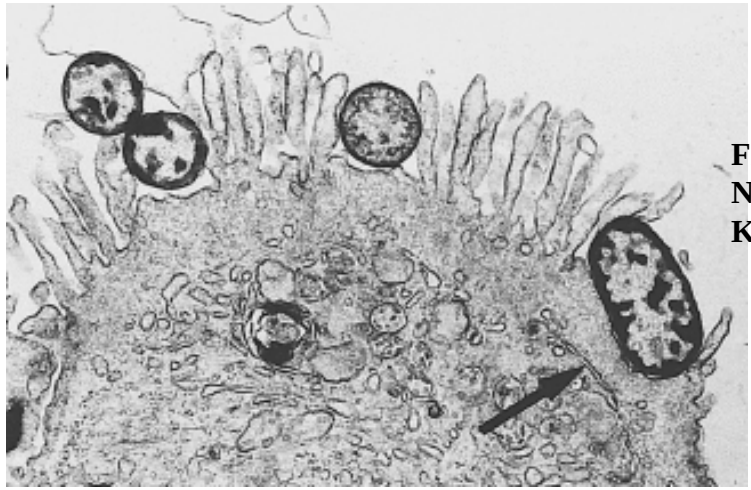
http://news.bbc.co.uk/1/hi/8254710.stm

EHEC infection

- attaching and effacing lesion : intimate adhesion, cytoskeletal changes

⇨ effacement of intestinal microvilli and pedestal-like structures





Frankel *et al.*, Mol mic., 1998
Nougayrede *et al.*, Cell mic., 2003
Kaper *et al.*, Nature reviews, 2004

PAI of EHEC

- 35-kbp PAI : **LEE** (Locus of Enterocyte Effacement)

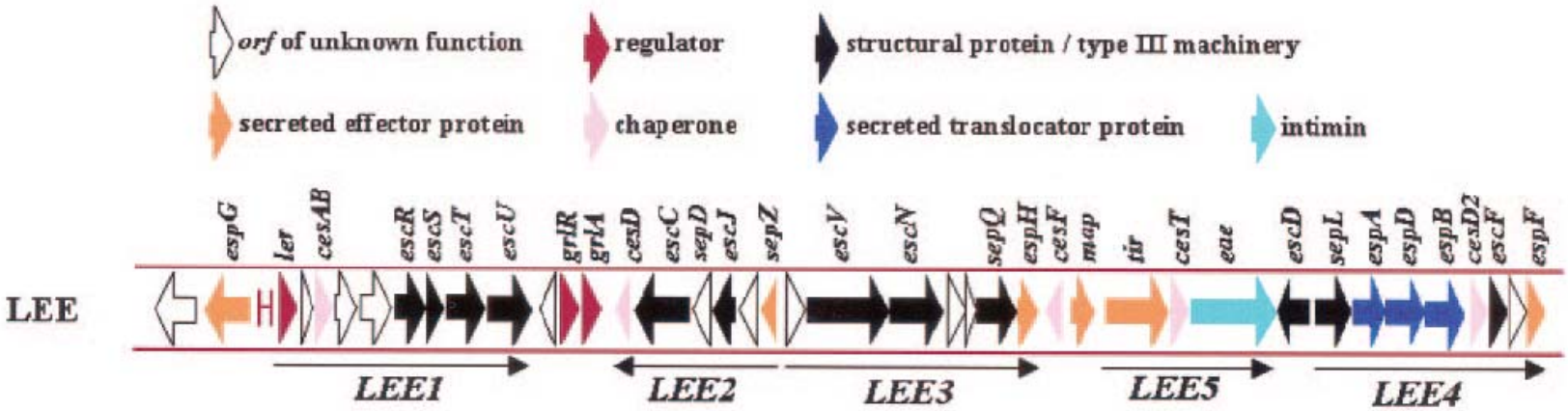
Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 1664–1668, February 1995
Microbiology

A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens

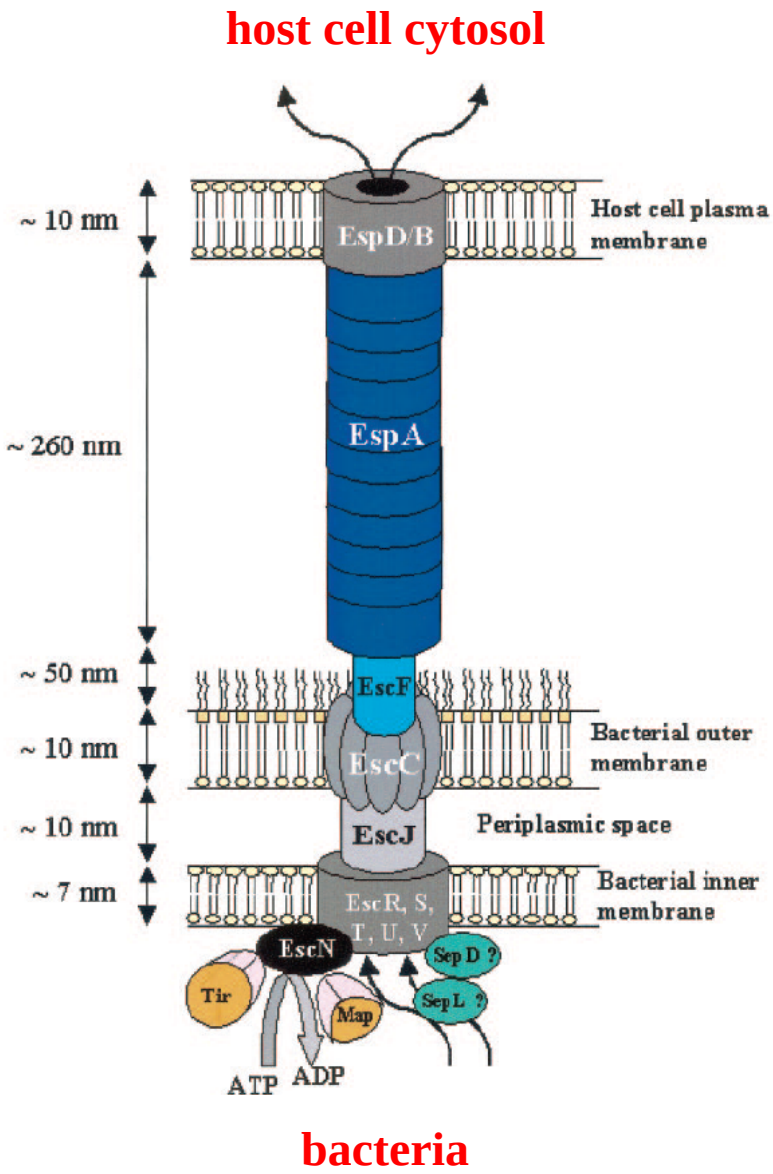
(bacterial pathogenesis/epithelial cells/attaching and effacing lesions)

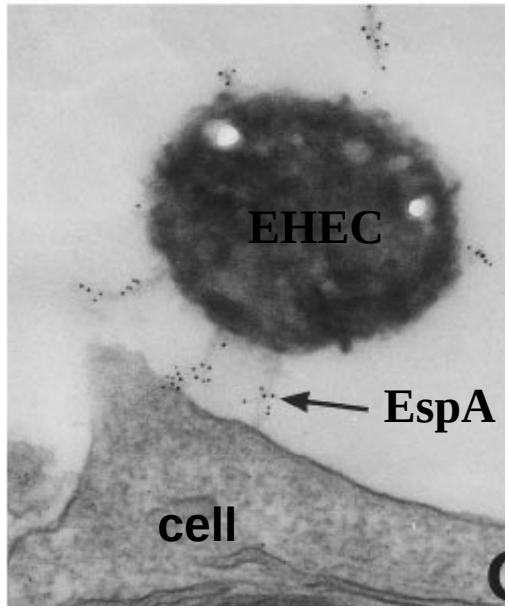
TIMOTHY K. MCDANIEL*†, KAREN G. JARVIS*, MICHAEL S. DONNENBERG†‡§, AND JAMES B. KAPER*†¶

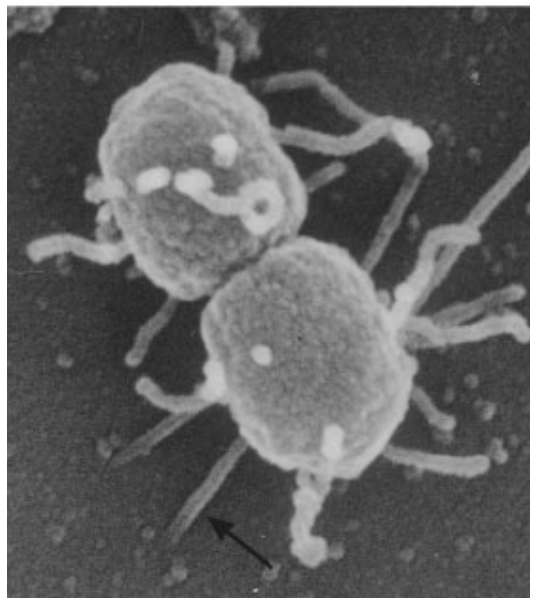
⇨ **type III secretion system and effector proteins**



Type III secretion system of EHEC







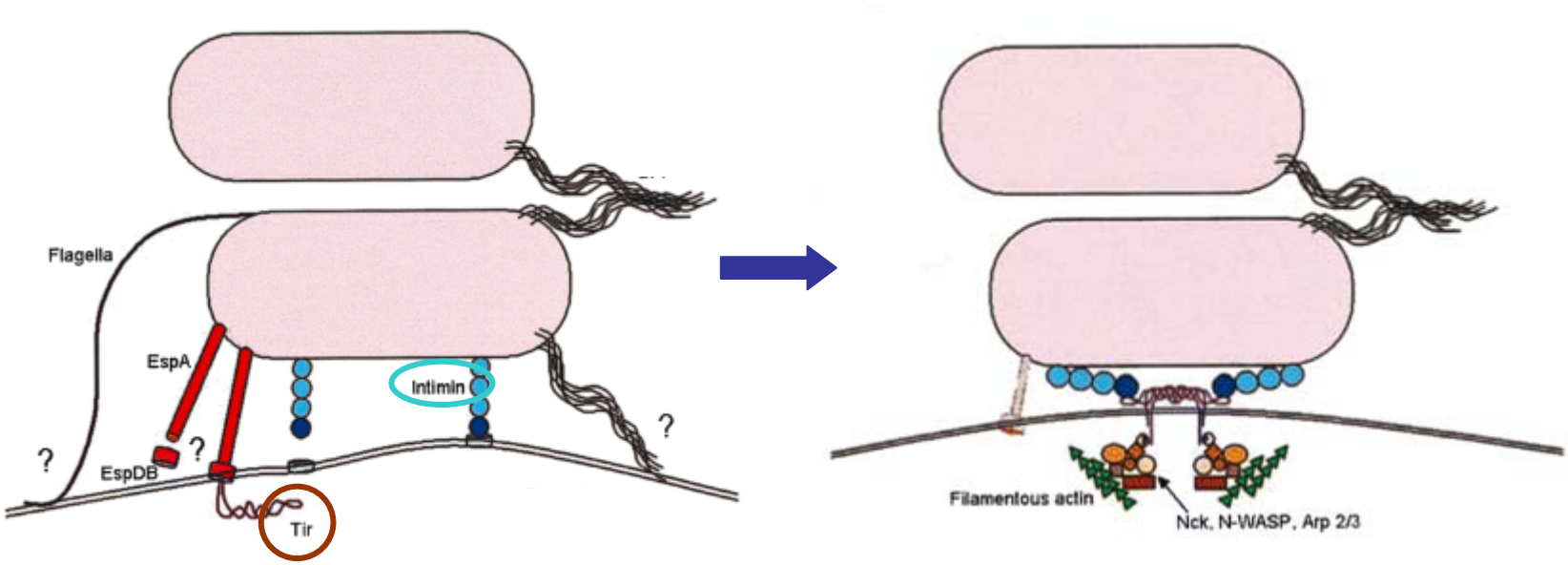
Garmendia *et al.*, Infect and Immun, 2005

Frankel *et al*, Mol mic., 1998

Type III secretion system of EHEC

***eae* gene : intimin protein, bacterial outer membrane**

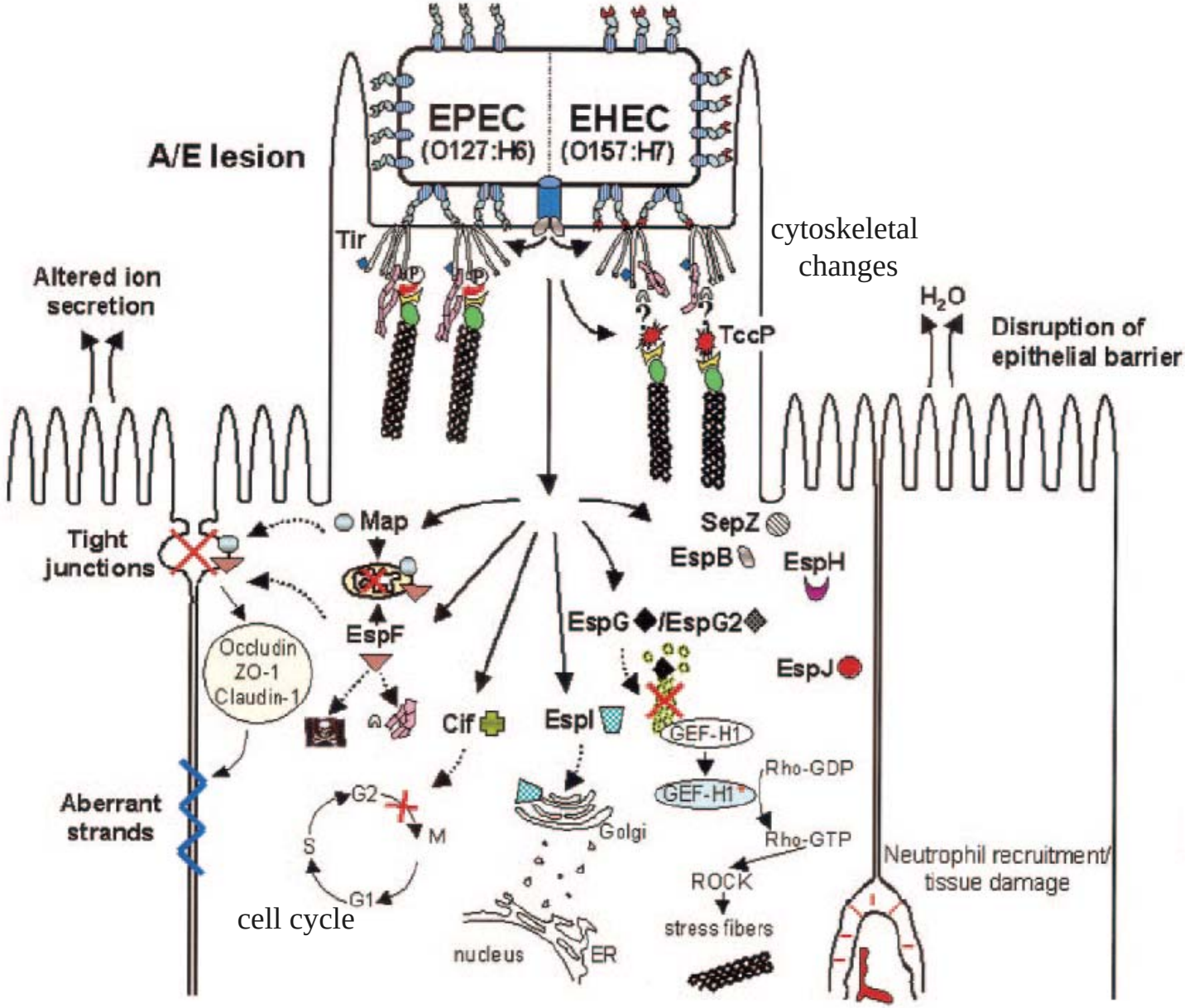
***tir* gene : Tir protein, secreted, intimin receptor, anchored in the host cell membrane**



intimate adhesion

Nougayrede *et al.*, Cell mic., 2003

Type III secretion system of EHEC



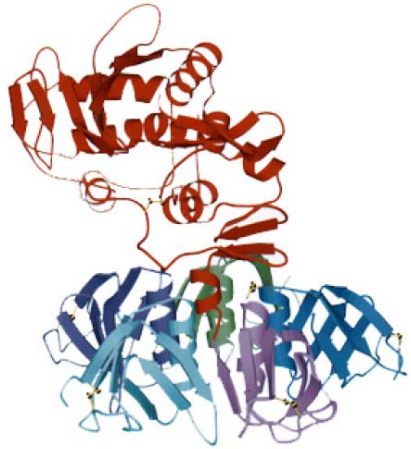
Adapted from Garmendia *et al.*, Infect Immun, 2005

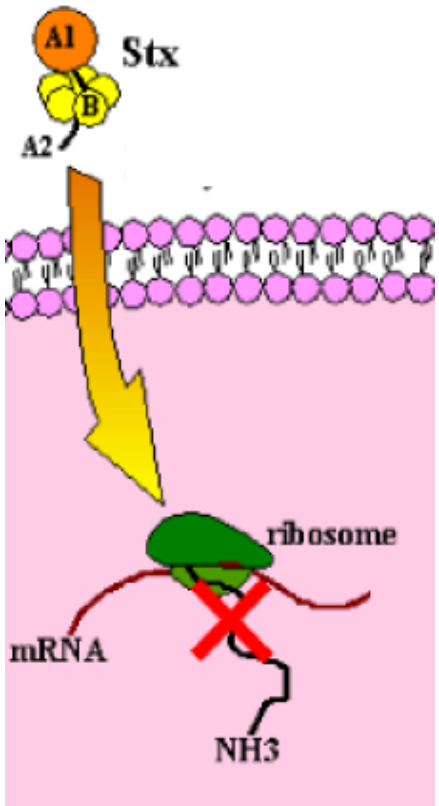
Toxins of EHEC

- most characteristic virulence factors : shiga-toxins

- encoded by phages

- AB_5 toxin





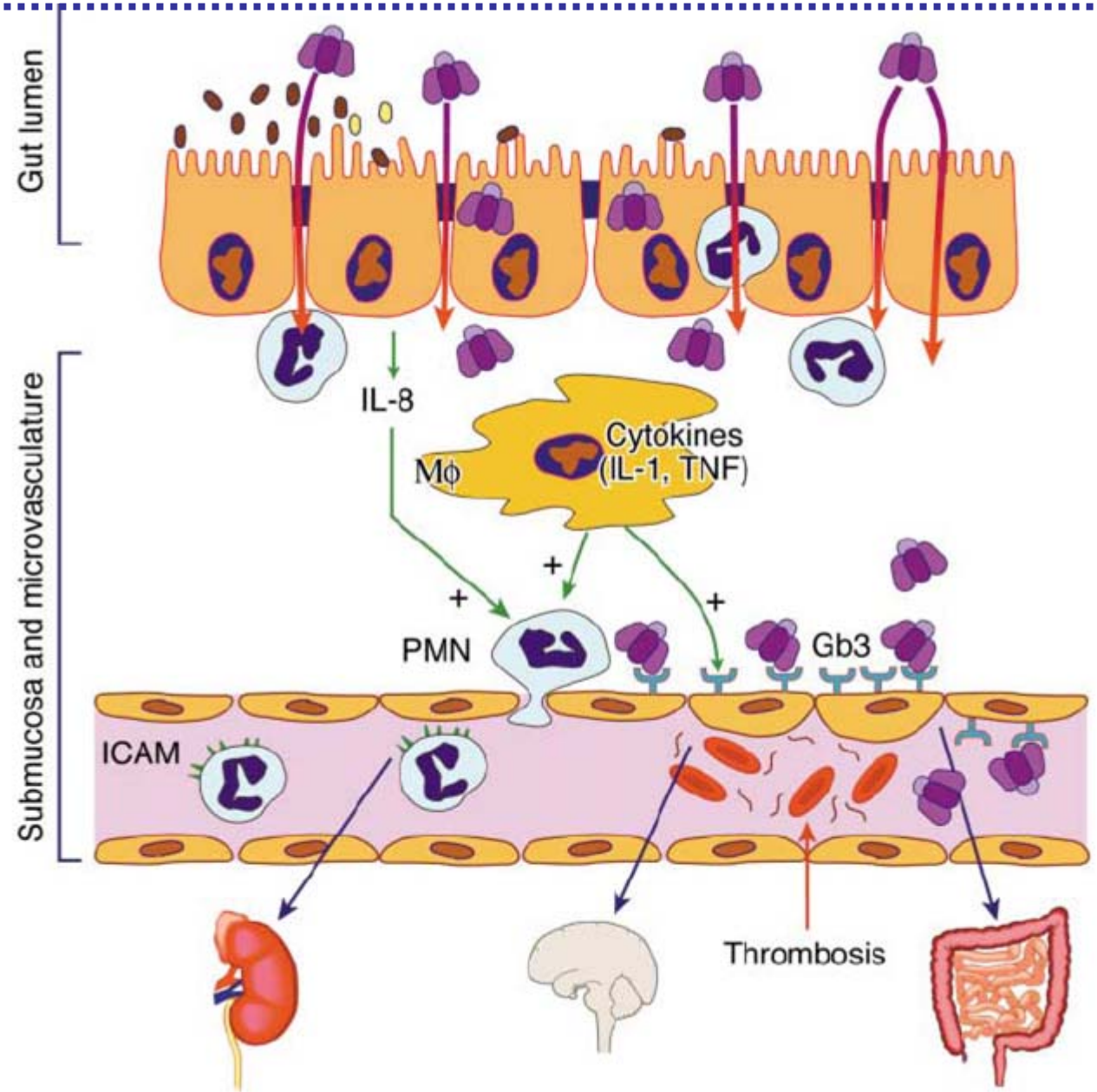
B subunits : binding to specific glycolipids on the host cell
specifically globotriaosylceramide (Gb3)

A subunit :

Gb3 receptor : renal epithelial tissues, CNS neurons and endothelium

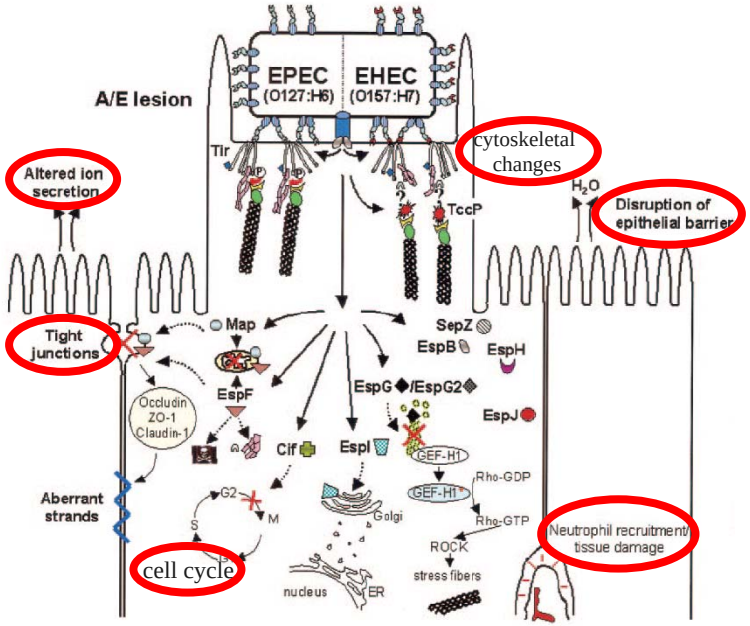
Schmitt *et al.*, Emerg Infect Dis., 1999

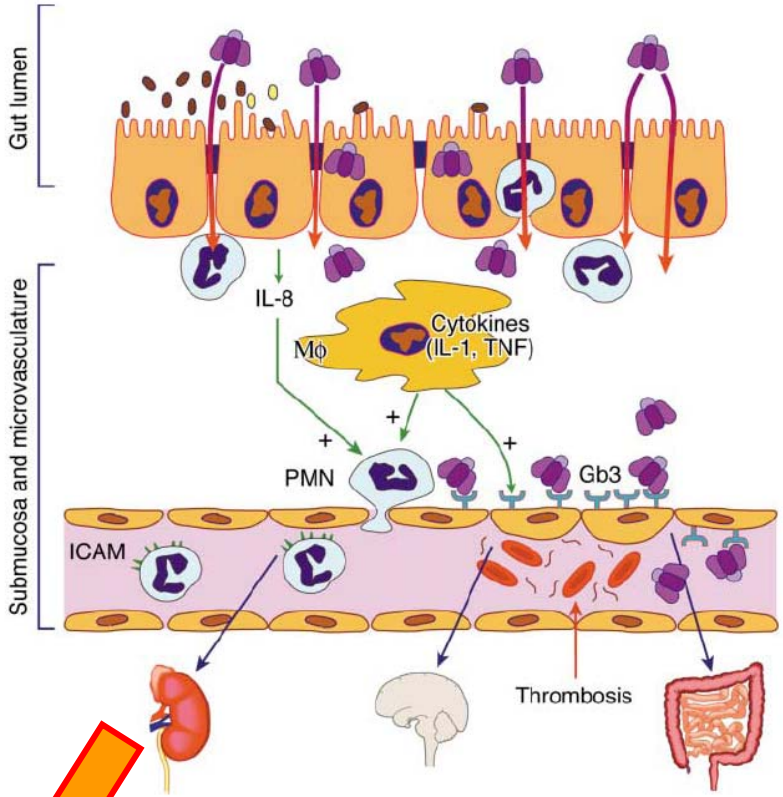
Toxins of EHEC



O'loughlin and Robins-Browne, Microbes and infection, 2001

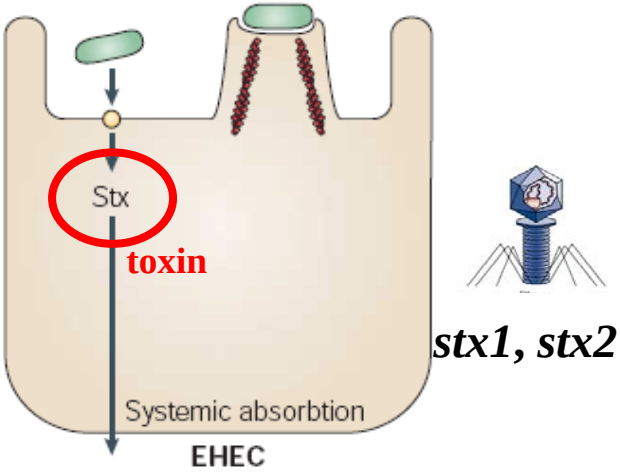
EHEC pathogenesis



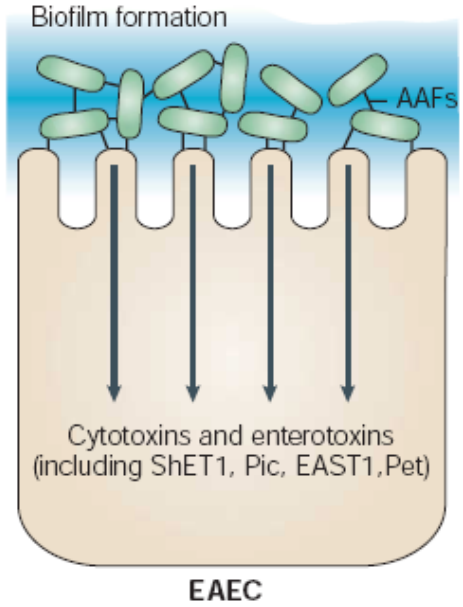


bloody diarrhea
thrombosis
kidney failure, HUS

EAHEC : Enteroaggregative haemorrhagic *E. coli*

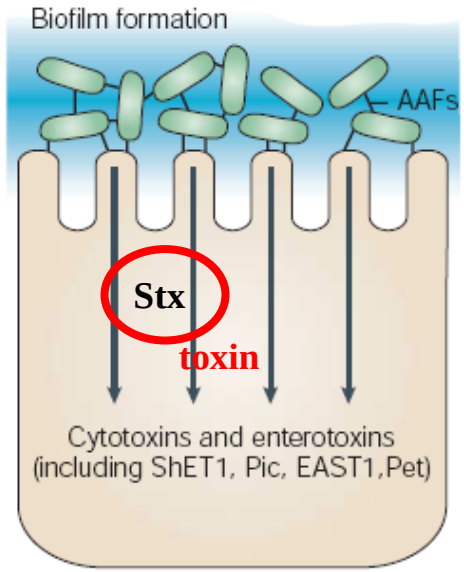


stx1, stx2



Enteroaggregative haemorrhagic *E. coli* =

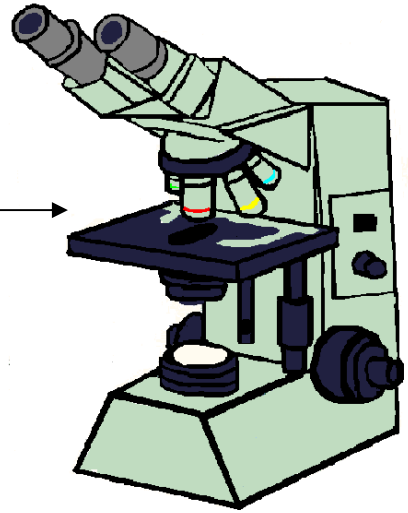
haemolytic uremic syndrome



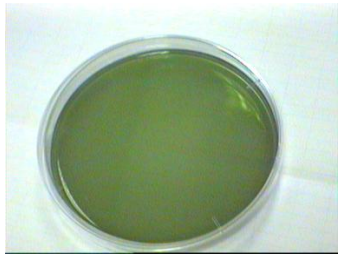
Laboratory diagnosis

sample

→ **Gram stain** →



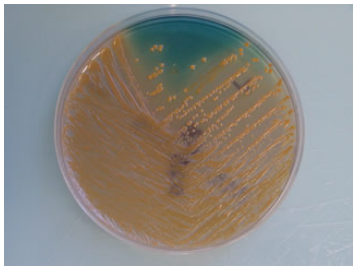
☞ ***Gram negative***



antibiotic choice ⇐

antibiogramme

isolation and growth on selective medium



→

→ **PCR**

API20E : identification ☞ ***E. coli***

☞ ***enteric bacteria***

Antibiotic therapy…

- **antibiotics : amoxicillin, semi-synthetic penicillins, many cephalosporins, carbapenems, aztreonam, trimethoprim-sulfamethoxazole, ciprofloxacin, nitrofurantoin….**

- **antibiotic resistance : growing problem**

- **try to define or to find news therapeutic targets or news strategies :**

  ⇨ **targets : structural components of type III secretion system**

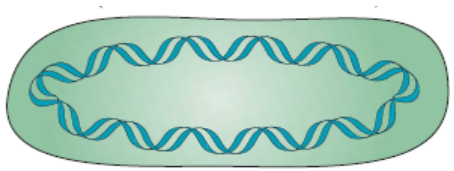
  ⇨ **strategies : block of bacterial adhesion (antibodies, receptor analogue)**

  block of host cell manipulation (inhibition of type III secretion system)

  vaccine development (toxins, siderophores…)

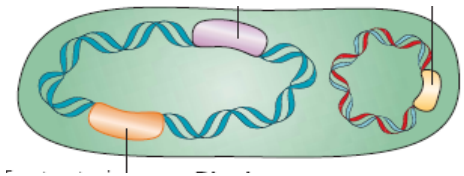
CONCLUSION

commensal *E. coli*



no toxins
no secretion systems/effectors
no adhesion factors
no invasion factors
no iron-transport system
no plasmids
smaller genome

pathogenic *E. coli*



toxins
secretion of effectors
adhesion factors = adhesins
invasion factors = invasins
iron-transport system
presence of plasmids
bigger genome (phages, plasmids, PAI…)

⇨ **adaptation**
⇨ **host cell manipulation**
⇨ **disease**

References- selected reading

** Tokar, K. Pathogenic *E. coli*. Online Textbook of bacteriology. University of Wisconsin-Madison, Department of Bacteriology. http://textbookofbacteriology.net/e.coli.html

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** Kaper J.B., Nataro J.P. and Mobley H.L. Pathogenic *Escherichia coli*. Nature Reviews Microbiology, 2004, 2, 123-140.

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** O'Loughlin EV, Robins-Browne RM. Effect of Shiga toxin and Shiga-like toxins on eukaryotic cells. Microbes Infect. 2001 May;3(6):493-507.

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** Dobrindt U, Hacker J. Targeting virulence traits: potential strategies to combat extraintestinal pathogenic *E. coli* infections. Curr Opin Microbiol. 2008 Oct;11(5):409-13.

http://www.youtube.com/watch?v=gnNFNI9_pT0

Supplementary documents

Kaper *et al.*, Nature reviews, 2004

Table 1 | ***E. coli* virulence factors: colonization and fitness factors**

Factor	Pathotype	Activity/effect
IcsA (VirG)	EIEC	Nucleation of actin filaments
Intimin	EPEC, EHEC	Adhesin, induces T_H1 response; 10 variants described
Dr adhesins	DAEC, UPEC	Adhesin, binds to decay-accelerating factor (DAF), activates PI-3-kinase, induces MICA; >10 Dr adhesins described
P (Pap) fimbriae	UPEC	Adhesin; induces cytokine expression
CFAs	ETEC	Adhesin, >20 different factors designated CFA, CS or PCF
Type-1 fimbriae	All	UPEC adhesin; binds to uroplakin
F1C fimbriae	UPEC	Adhesin
S fimbriae	UPEC, MNEC	Adhesin
Bundle-forming pilus (BFP)	EPEC	Type IV pilus
Aggregative adherence fimbriae	EAEC	Adhesin; >4 subtypes
Paa	EPEC, EHEC	Adhesin
ToxB	EHEC	Adhesin
Efa-1/LifA	EHEC	Adhesin
Long polar fimbriae (LPF)	EHEC, EPEC	Adhesin
Saa	EHEC	Adhesin
OmpA	MNEC, EHEC	Adhesin
Curli	Various	Adhesin; binds to fibronectin
IbeA, B, C	MNEC	Promotes invasion
AslA	MNEC	Promotes invasion
Dispersin	EAEC	Promotes colonization; aids mucous penetration
K antigen capsules	MNEC	Antiphagocytic; >80 K types
Aerobactin	EIEC	Iron acquisition, siderophore
Yersiniabactin	Various	Iron acquisition, siderophore
IreA	UPEC	Iron acquisition, siderophore receptor
IroN	UPEC	Iron acquisition, siderophore receptor
Chu (Shu)	EIEC, UPEC, MNEC	Iron acquisition, haem transport
Flagellin	All	Motility; induces cytokine expression through TLR5; >50 flagella (H) serotypes
Lipopolysaccharide	All	Induces cytokine expression through TLR4; >180 O types

CFA, colonization factor antigen; CS, coli surface antigen; MICA, MHC class I chain-related gene A; PCF, putative colonization factor; PI-3-kinase, phosphatidylinositol 3-kinase; TLR, Toll-like receptor.

Kaper *et al.*, Nature reviews, 2004

Table 2 | ***E. coli* virulence factors: toxins and effectors**

Factor	Pathotype	Toxin class	Target	Activity/Effect
Heat-labile enterotoxin (LT)	ETEC	AB subunit, type II effector	G_s	ADP ribosylates and activates adenylate cyclase resulting in ion secretion
Shiga toxin (Stx)	EHEC	AB subunit	rRNA	Depurinates rRNA, inhibiting protein synthesis; induces apoptosis
Cytolethal distending toxin (CDT)	Various	ABC subunit	DNA	DNaseI activity, blocks mitosis in G2/M phase
Shigella enterotoxin 1 (ShET1)	EAEC, EIEC*	AB subunit	–	Ion secretion
Urease	EHEC	ABC subunit	Urea	Cleaves urea to NH_3 and CO_2
EspC	EPEC	Autotransporter	?	Serine protease; ion secretion
EspP	EHEC	Autotransporter	?	Serine protease; cleaves coagulation factor V
Haemoglobin-binding protease (Tsh)	ExPEC, APEC	Autotransporter	Haem	Degrades haemoglobin to release haem/iron
Pet	EAEC	Autotransporter	Spectrin	Serine protease; ion secretion; cytotoxicity
Pic	UPEC, EAEC, EIEC*	Autotransporter	?	Protease, mucinase
Sat	UPEC	Autotransporter	?	Vacuolation
SepA	EIEC*	Autotransporter	?	Serine protease
SigA	EIEC*	Autotransporter	?	Ion secretion
Cycle-inhibiting factor (Cif)	EPEC, EHEC	Type III effector	?	Blocks mitosis in G2/M phase; results in inactivation of Cdk1
EspF	EPEC, EHEC	Type III effector	?	Opens tight junctions, induces apoptosis
EspH	EPEC, EHEC	Type III effector	?	Modulates filopodia and pedestal formation
Map	EPEC, EHEC	Type III effector	Mitochondria	Disrupts mitochondrial membrane potential
Tir	EPEC, EHEC	Type III effector	Nck	Nucleation of cytoskeletal proteins, loss of microvilli, GAP-like activity

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IpaA	EIEC	Type III effector	Vinculin	Actin depolymerization
IpaB	EIEC	Type III effector	Caspase 1	Apoptosis, IL-1 release; membrane insertion
IpaC	EIEC	Type III effector	Actin	Actin polymerization, activation of Cdc42 and Rac
IpaH	EIEC	Type III effector	Nucleus	Modulates inflammation (?)
IpgD	EIEC	Type III effector	PtdIns $(4,5)P_2$	Inositol 4-phosphatase, membrane blebbing
VirA	EIEC	Type III effector	Tubulin	Microtubule destabilization, membrane ruffling
StcE	EHEC	Type II effector	C1-esterase inhibitor (C1-INH)	Cleaves C1-INH, disrupts complement cascade
HlyA	UPEC	RTX toxins	Erythrocytes, Leukocytes	Cell lysis
Ehx	EHEC	RTX toxins	Erythrocytes, Leukocytes	Cell lysis
Cytotoxic necrotizing factors (CNF-1,-2)	MNEC, UPEC, NTEC		RhoA, Cdc42, Rac	Altered cytoskeleton, necrosis
LifA/Efa	EPEC, EHEC		Lymphocytes	Inhibits lymphocyte activation, adhesion
Shigella enterotoxin 2 (ShET2)	EIEC, ETEC		?	Ion secretion
Heat-stable enterotoxin a (STa)	ETEC	Heat-stable enterotoxins	Guanylate cyclase	Activates guanylate cyclase resulting in ion secretion
Heat-stable enterotoxin b (STb)	ETEC	Heat-stable enterotoxins	?	Increase intracellular calcium resulting in ion secretion
EAST	Various	Heat-stable enterotoxins	Guanylate cyclase	Activates guanylate cyclase resulting in ion secretion

*These factors have been characterized in *Shigella* species, but their presence in EIEC has not yet been established. EAST, enteroaggregative E.coli ST; GAP, GTPase-activating protein; IL, interleukin; PtdIns$(4,5)P_2$, phosphatidylinositol-4,5-bisphosphate.