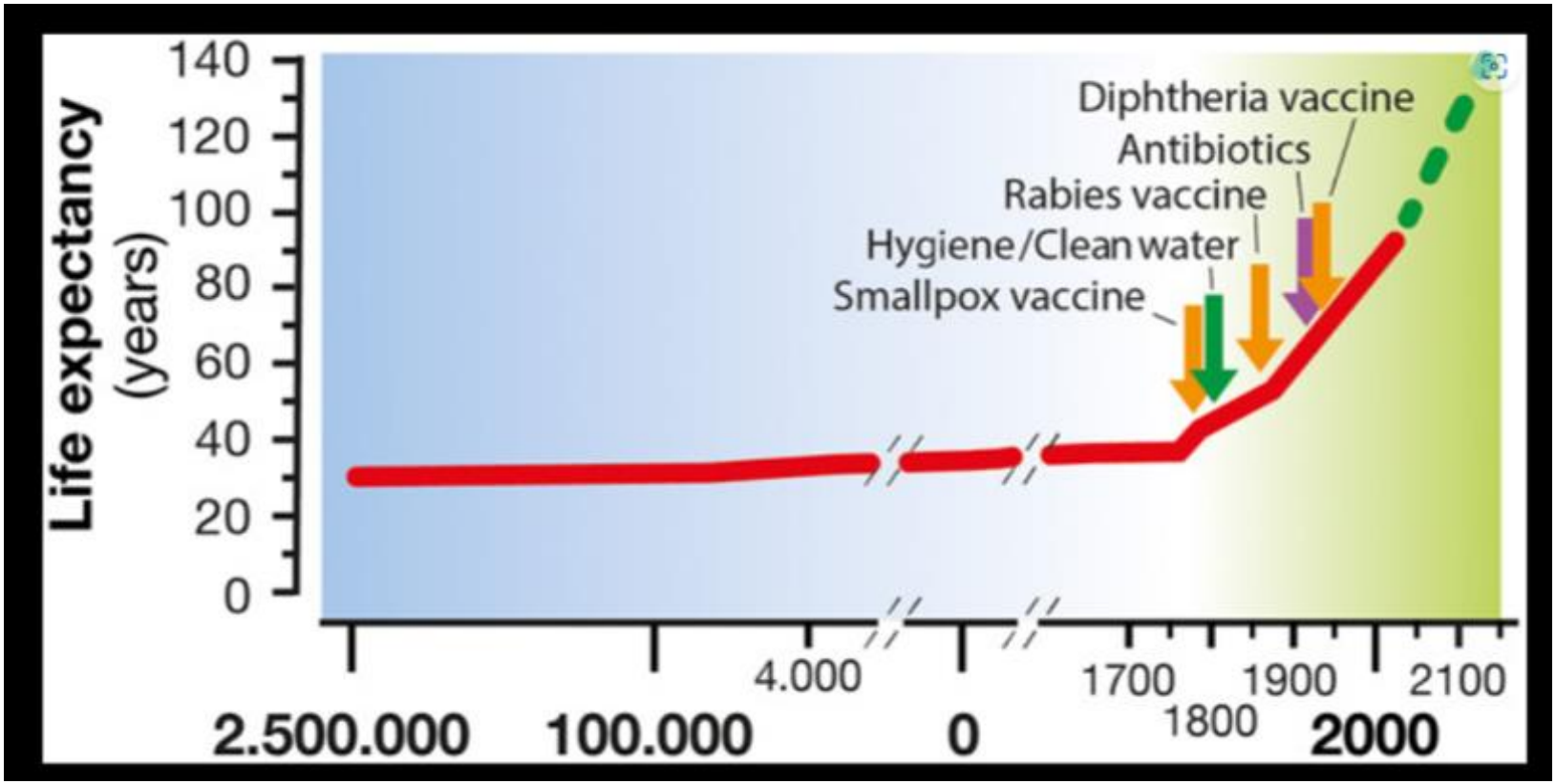
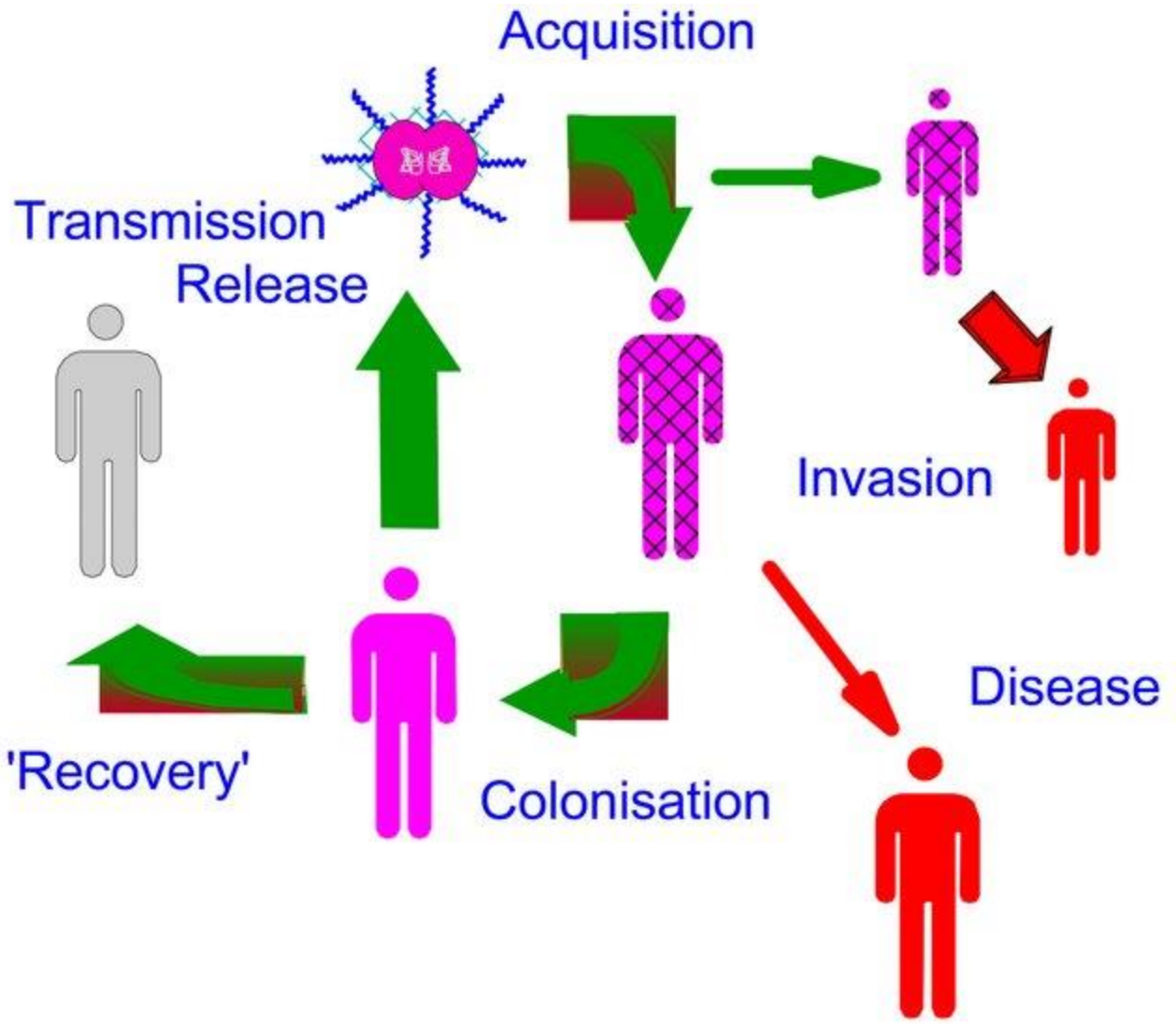
Vaccini contro le infezioni batteriche

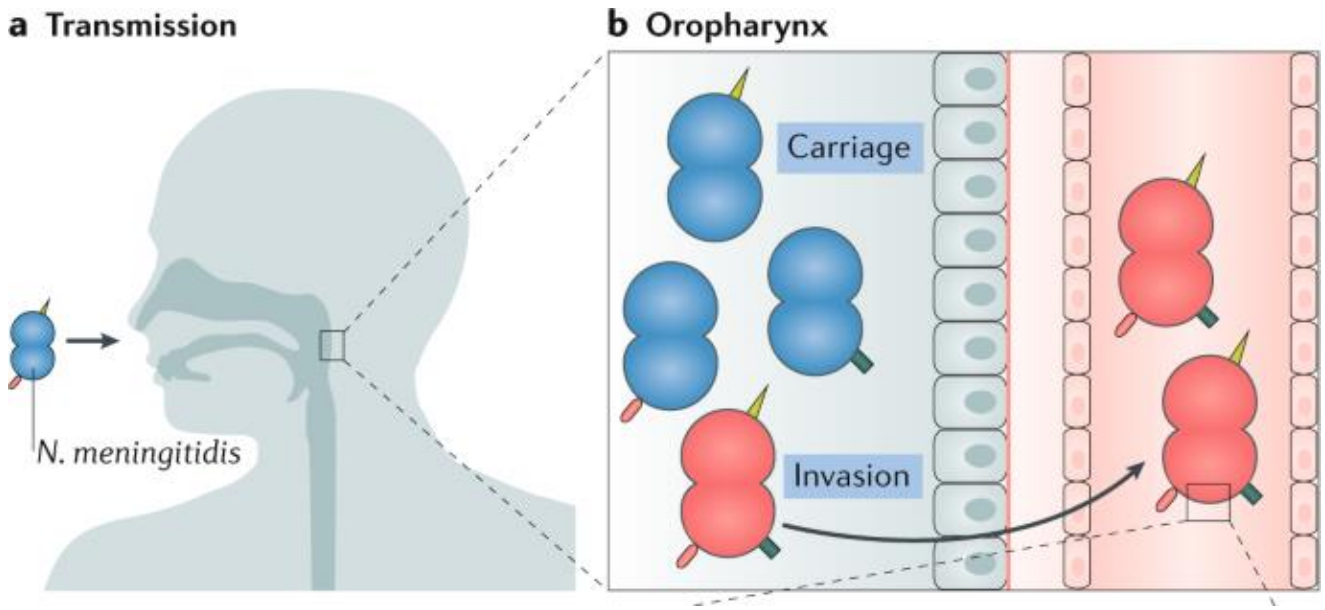


Rosini R, Nicchi S, Pizza M, Rappuoli R. Vaccines Against Antimicrobial Resistance. Front Immunol. 2020 Jun 3;11:1048. doi: 10.3389/fimmu.2020.01048.

Trasmissione di *Neisseria meningitidis* (Meningococcus, Men) nella popolazione



Meningococcal vaccines and herd immunity: Lessons learned from serogroup C conjugate vaccination programs.Trotter & Maiden 2009



Caugant DA, Brynildsrud OB. Neisseria meningitidis: using genomics to understand diversity, evolution and pathogenesis. Nat Rev Microbiol. 2020 Feb;18(2):84-96. doi: 10.1038/s41579-019-0282-6.

Vaccinare contro *Neisseria meningitidis* (Meningococcus, Men)

Vaccini polisaccaridici capsulari (CPS)

I primi tentativi di sviluppare vaccini furono effettuati dal 1900 al 1940, in risposta alle epidemie e all'aumento dei tassi di infezione verificatisi durante entrambe le guerre mondiali.

Questi vaccini erano preparati con batteri interi uccisi al calore

Si sono rivelati inefficaci a causa di dubbi sulla natura dell'immunità conferita e in particolare a causa dell'eccessiva risposta avversa del sistema immunitario, probabilmente dovuta alla presenza di grandi quantità di lipooligosaccaride (LOS).

Sophian 1912; Greenwood 1917; Gates 1918; Riding and Corkill 1932

La disponibilità di antibiotici divenne il trattamento più efficace della meningite batterica dalla seconda guerra mondiale in poi, tanto che la ricerca sul vaccino contro i meningococchi perse di interesse finché non fu osservata resistenza agli antibiotici, in particolare ai sulfonamidi.

(Miller, Siess and Feldman 1963)

Il ruolo della capsula polisaccaridica nella patogenesi

The *Neisseria meningitidis* Capsule Is Important for Intracellular Survival in Human Cells ▿ †
Infect Immun. 2007 Jul; 75(7): 3594–3603.

AMERICAN SOCIETY FOR MICROBIOLOGY | Infection and Immunity®

The *Neisseria meningitidis* Capsule Is Important for Intracellular Survival in Human Cells ▿ †

Maria Rita Spinosa, Cinzia Progida, [...], and Cecilia Bucci

Additional article information

Associated Data

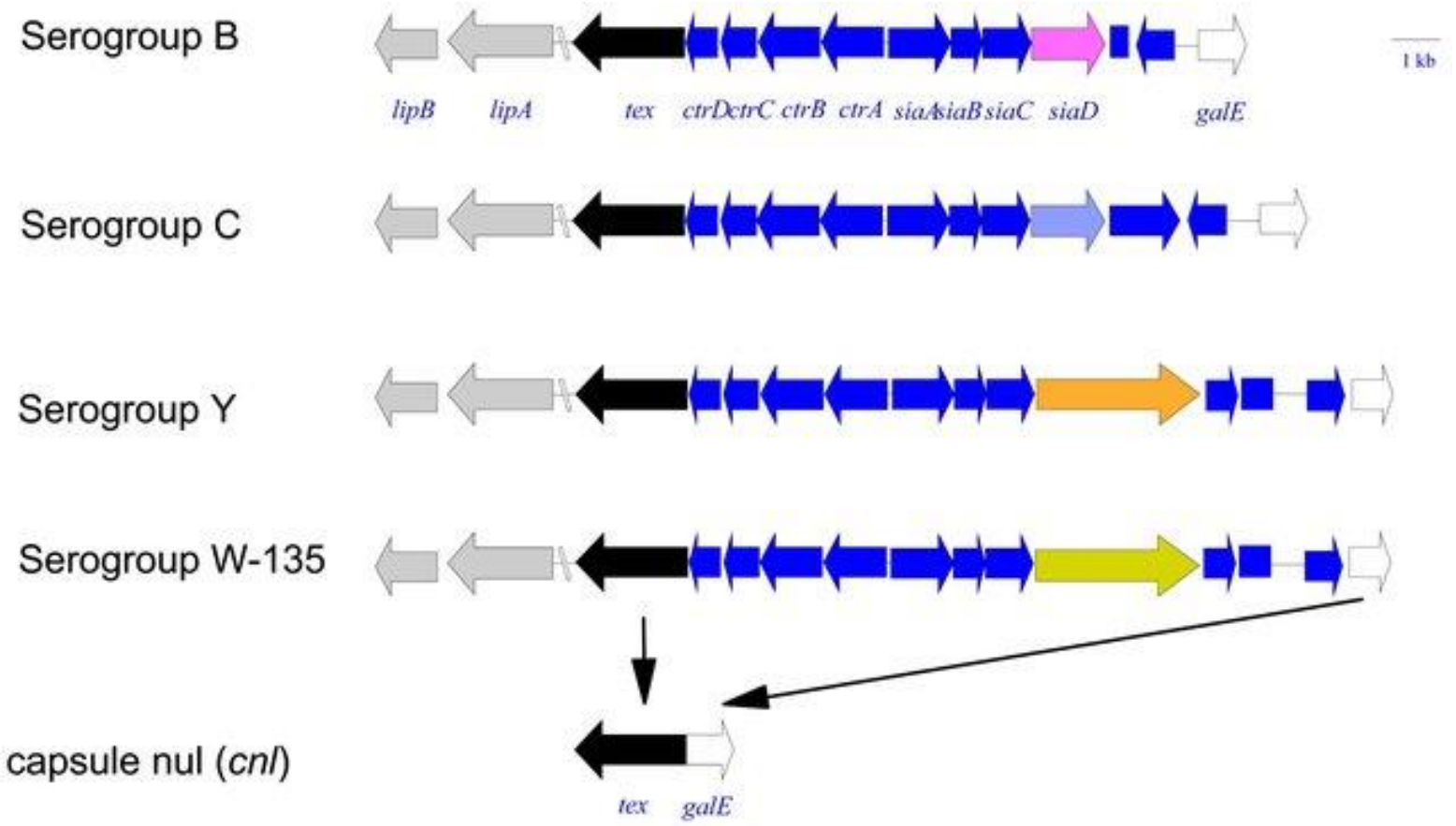
▸ Supplementary Materials

ABSTRACT

While much data exist in the literature about how *Neisseria meningitidis* adheres to and invades human cells, its behavior inside the host cell is largely unknown. One of the essential meningococcal attributes for pathogenesis is the polysaccharide capsule, which has been shown to be important for bacterial survival in extracellular fluids. To investigate the role of the meningococcal capsule in intracellular survival, we used B1940, a serogroup B strain, and its isogenic derivatives, which lack either the capsule or both the capsule and the lipooligosaccharide outer core, to infect human phagocytic and nonphagocytic cells and monitor invasion and intracellular growth. Our data indicate that the capsule, which negatively affects bacterial adhesion and, consequently, entry, is, in contrast, fundamental for the intracellular survival of this microorganism. The results of in vitro assays suggest that an increased resistance to cationic antimicrobial peptides (CAMPs), important components of the host innate defense system against microbial infections, is a possible

Gotschlich e colleghi pubblicarono nel 1969 un lavoro che descriveva lo sviluppo di un vaccino contro meningococco. Questi studi hanno descritto i fenomeni dell'immunità al meningococco legata all'età; il contatto con diversi ceppi di meningococchi nel corso della vita avvia, rafforza e amplia l'immunità naturale alla malattia meningococcica; che la suscettibilità alla malattia è correlata a bassi livelli di anticorpi sierici con attività battericida (SBA) contro l'agente patogeno.
L'unico preparato di CPS ad alto peso molecolare (>100 000 Da), prodotto mediante precipitazione con il detergente cationico Cetavlon (Gotschlich, Liu e Artenstein 1969), ha indotto in modo affidabile risposte anticorpali negli esseri umani.
Questi studi sono culminati nella sperimentazione del vaccino nei campi di addestramento militare di Fort Dix, nel New Jersey, usando un vaccino MenC CPS purificato.
Gli studi condotti su 28 245 reclute nel periodo 1969-1970 hanno mostrato un effetto protettivo del vaccino dell'89,5% contro la malattia MenC (Artenstein et al.1970; Gold e Artenstein 1971).
Questi studi hanno fornito le basi per l'utilizzo di tutti i successivi vaccini meningococcici basati su CPS e antigeni non capsulari.
I vaccini CPS semplici sono stati autorizzati sin dagli anni '70, come vaccini mono-, bi-, tri- e tetravalenti, in varie formulazioni contenenti MenA, MenC, MenW e MenY CPS.

Rappresentazione schematica della regione CPS nei meningococchi che esprimono capsule contenenti acido sialico

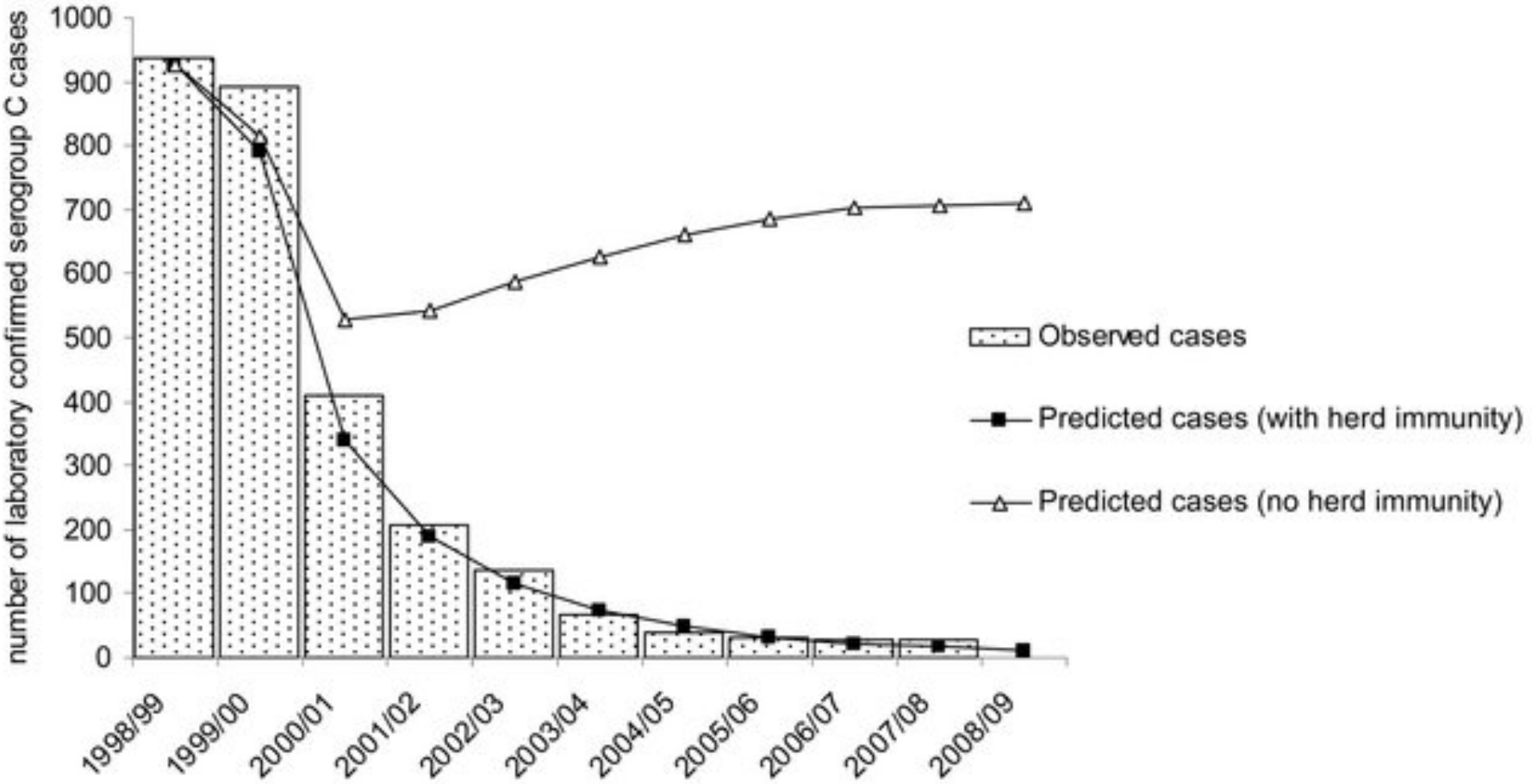


Meningococcal vaccines and herd immunity: Lessons learned from serogroup C conjugate vaccination programs. Trotter & Maiden 2009

Successivamente la ricerca è continuata mediante lo sviluppo e l'uso dei vaccini coniugati CPS (Gasparini e Panatto 2011).

La coniugazione di un CPS batterico con una proteina induce risposte anticorpali più forti grazie rispetto al corrispondente semplice vaccino basato solo su CPS

Proteine altamente immunogeniche, tra cui i tossoidi difterico e tetanico e la variante non tossica della tossina difterica CRM197, sono state coniugate a diversi Men CPS per produrre vaccini glicoconiugati. Il primo vaccino coniugato meningococcico ad essere utilizzato è stato un vaccino coniugato MenC (MCC) per neonati, bambini piccoli e adolescenti, introdotto nel programma di immunizzazione del Regno Unito nel 1999, in risposta alle epidemie di MenC ST-11 che si sono verificate negli adolescenti.



Meningococcal vaccines and herd immunity: Lessons learned from serogroup C conjugate vaccination programs.Trotter & Maiden 2009

L’esempio più notevole dell’impatto dei vaccini coniugati Men CPS è quello legato all’introduzione di un vaccino coniugato MenA, il MenAfriVac (Frasch, Preziosi e LaForce 2012; Tiffay et al.2015) nei paesi dell’Africa sahariana e sub-sahariana, che ha portato all’eliminazione della malattia sostenuta da ceppi di meningococco sierotipo MenA, il più diffuso in quei paesi (Djingarey et al.2015).

MenAfriVac ha ridotto con successo il carrier nasofaringeo e ha generato una protezione di gregge.

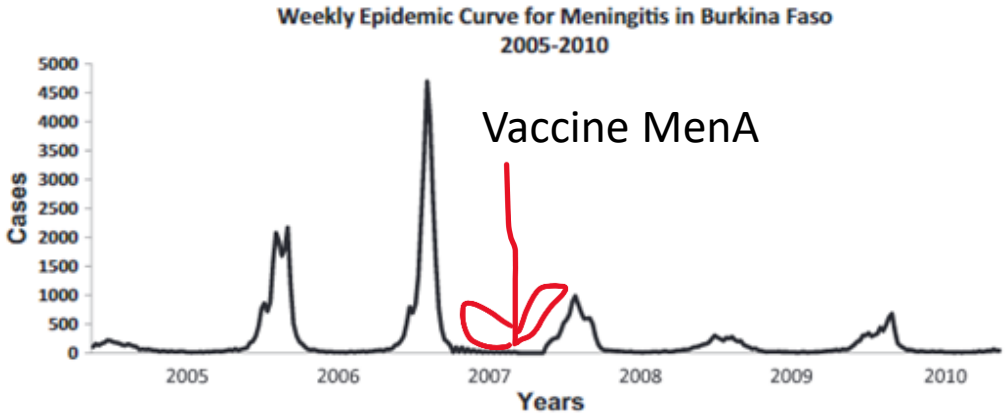


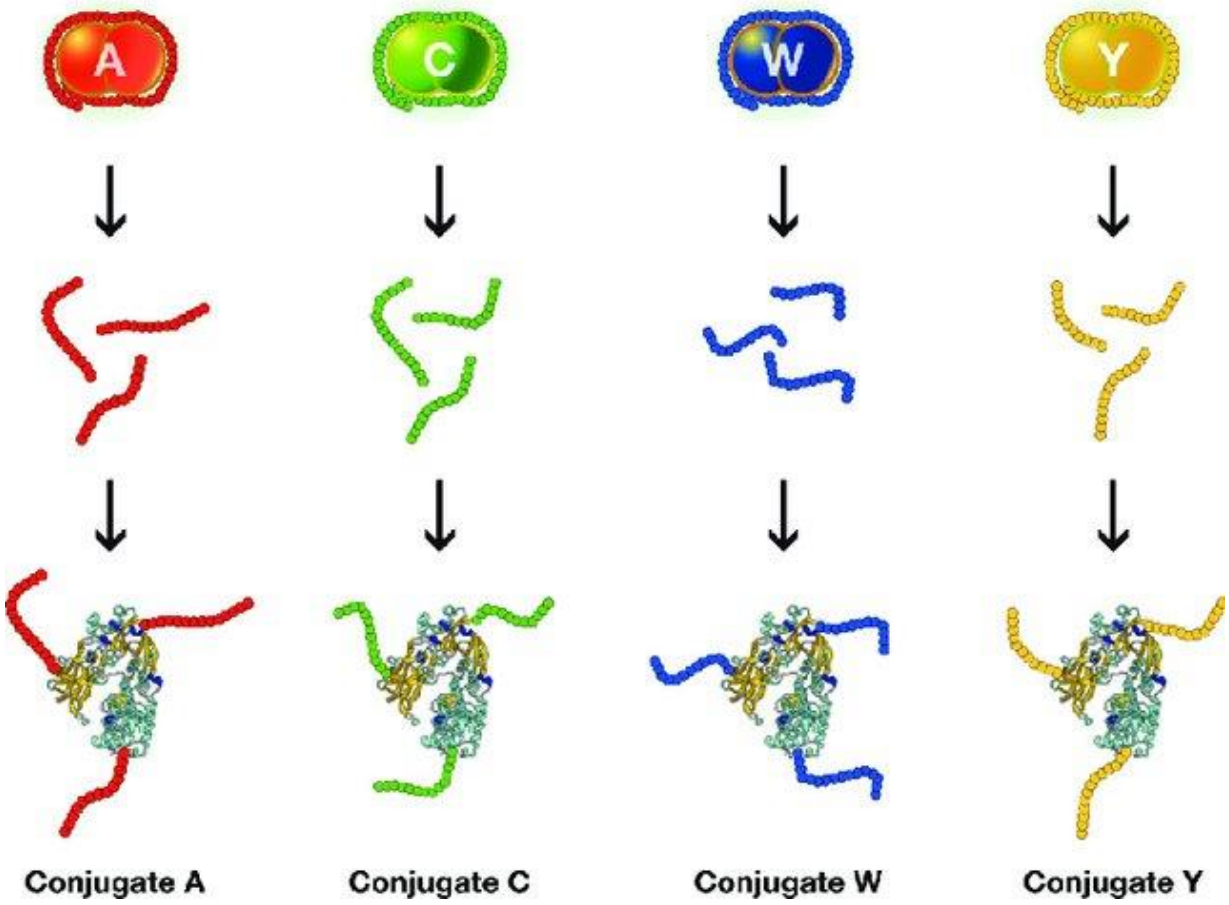
Fig. 1. Weekly reported meningitis cases – Burkina Faso 2005–2010.

.....Il ruolo degli adiuvanti

Cos’è un adiuvante e perché viene aggiunto a un vaccino?
Un adiuvante è un ingrediente utilizzato in alcuni vaccini che aiuta a creare una risposta immunitaria più forte nelle persone che ricevono il vaccino. In altre parole, gli adiuvanti aiutano i vaccini a funzionare meglio. Alcuni vaccini prodotti da germi uccisi contengono adiuvanti naturali e aiutano l’organismo a produrre una forte risposta immunitaria protettiva.

Adjuvant	Composition	Vaccines
Aluminum	One or more of the following: amorphous aluminum hydroxyphosphate sulfate (AAHS), aluminum hydroxide, aluminum phosphate, potassium aluminum sulfate (Alum)	Anthrax, DT, DTaP (Daptacel), DTaP (Infanrix), DTaP-HepB-IPV (Pediarix), DTaP-IPV (Kinrix), DTaP-IPV (Quadracel), DTaP –IPV/Hib (Pentacel), DTaP-IPV-Hib-HepB (VAXELIS), HepA (Havrix), HepA (Vaqta), HepB (Engerix-B), HepB (PREHEVBRIO), HepB (Recombivax), HepA/HepB (Twinrix), HIB (PedvaxHIB), HPV (Gardasil 9), Japanese encephalitis (Ixiaro), MenB (Bexsero, Trumenba), Pneumococcal (Prevnar 13, Prevnar 20, VAXNEUVANCE), Td (Tenivac), Td (Mass Biologics), Td (no trade name), Tdap (Adacel), Tdap (Boostrix), Tick-Borne Encephalitis (TICOVAC)
AS01B	Monophosphoryl lipid A (MPL) and QS-21, a natural compound extracted from the Chilean soapbark tree, combined in a liposomal formulation	Zoster vaccine (Shingrix)
AS04	Monophosphoryl lipid A (MPL) + aluminum salt	Human papillomavirus, or HPV (Cervarix)
CpG 1018	Cytosine phosphoguanine (CpG), a synthetic form of DNA that mimics bacterial and viral genetic material	HepB (Heplisav-B)
Matrix-M™	Saponins derived from the soapbark tree (*Quillaja saponaria* Molina)	COVID-19 vaccine (Novavax COVID-19 Vaccine, Adjuvanted)
MF59	Oil in water emulsion composed of squalene	Influenza (Fluad and Fluad Quadrivalent)
No adjuvant	–	Chickenpox, cholera, COVID-19 (includes mRNA Pfizer-BioNTech, mRNA Moderna and adenoviral Johnson & Johnson/Janssen), dengue, Ebola, Hib (ActHIB, HIBERIX), measles, mumps & rubella (MMR), meningococcal (Menactra, Menveo, MenQuadfi), polio (IPOL), rabies, rotavirus, seasonal influenza (except Fluad and Fluad quadrivalent), smallpox and monkeypox (ACAM2000, JYNNEOS), Typhoid, yellow fever, zoster live (Zostavax)

Successivamente sono stati sviluppati e autorizzati vaccini meningococcici coniugati CPS quadravalenti che includono MenA, MenC, MenW e MenY CPS (Dull e McIntosh 2012; Vella e Pace 2015). Recentemente, un vaccino coniugato MenACWY CPS è stato fortemente raccomandato per la vaccinazione degli adolescenti nel Regno Unito in risposta all'aumento dei casi di infezione causata da MenY e in particolare da un clone ipervirulento di MenW (Ladhani et al.2016a).

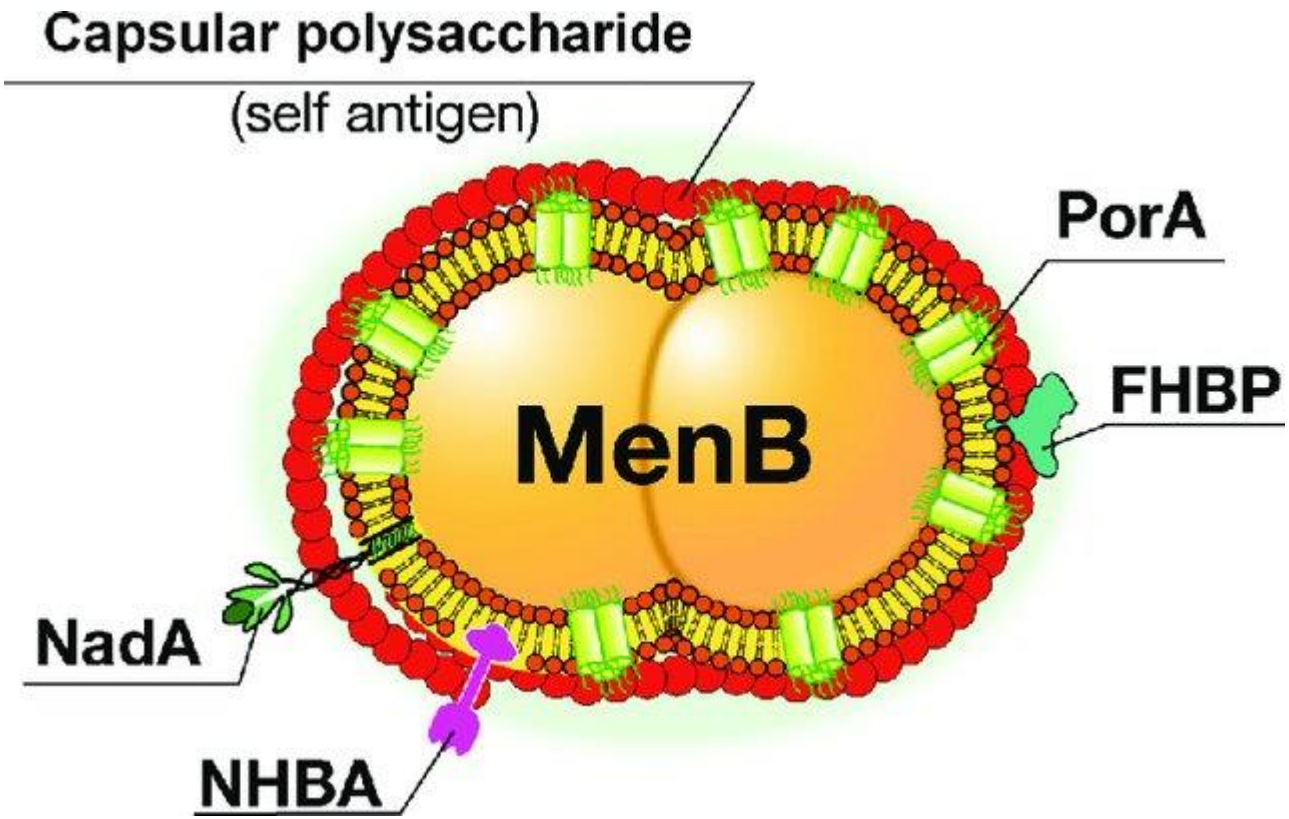


L’approccio vaccinale CPS non ha avuto successo per MenB

Il CPS MenB è stato testato su diverse centinaia di volontari, ma pochi individui hanno mostrato una risposta anticorpale protettiva (Wyle et al.1972).
È stato descritto che gli anticorpi contro MenB CPS hanno scarsa attività battericida in un modello di embrione di pollo con esposizione meningococcica (Frasch et al.1976).

La difficoltà a sviluppare vaccini MenB CPS si basa sull'osservazione che l'omopolimero del polisaccaride legato all'acido α (2-8) N-acetil-neuraminico è strutturalmente identico a una modifica della molecola di adesione delle cellule neurali dei mammiferi (Toikka et al.1998) e quindi, i vaccini possono indurre anticorpi autoimmuni che potenzialmente reagiscono in modo crociato con il tessuto celebrale fetale.

Da quando la strategia del vaccino CPS è stata ritenuta inutile per prevenire la malattia MenB, la ricerca si è concentrata intensamente sull’utilizzo di componenti non CPS come potenziali vaccini contro MenB.



The challenge of developing universal vaccines, August 2011

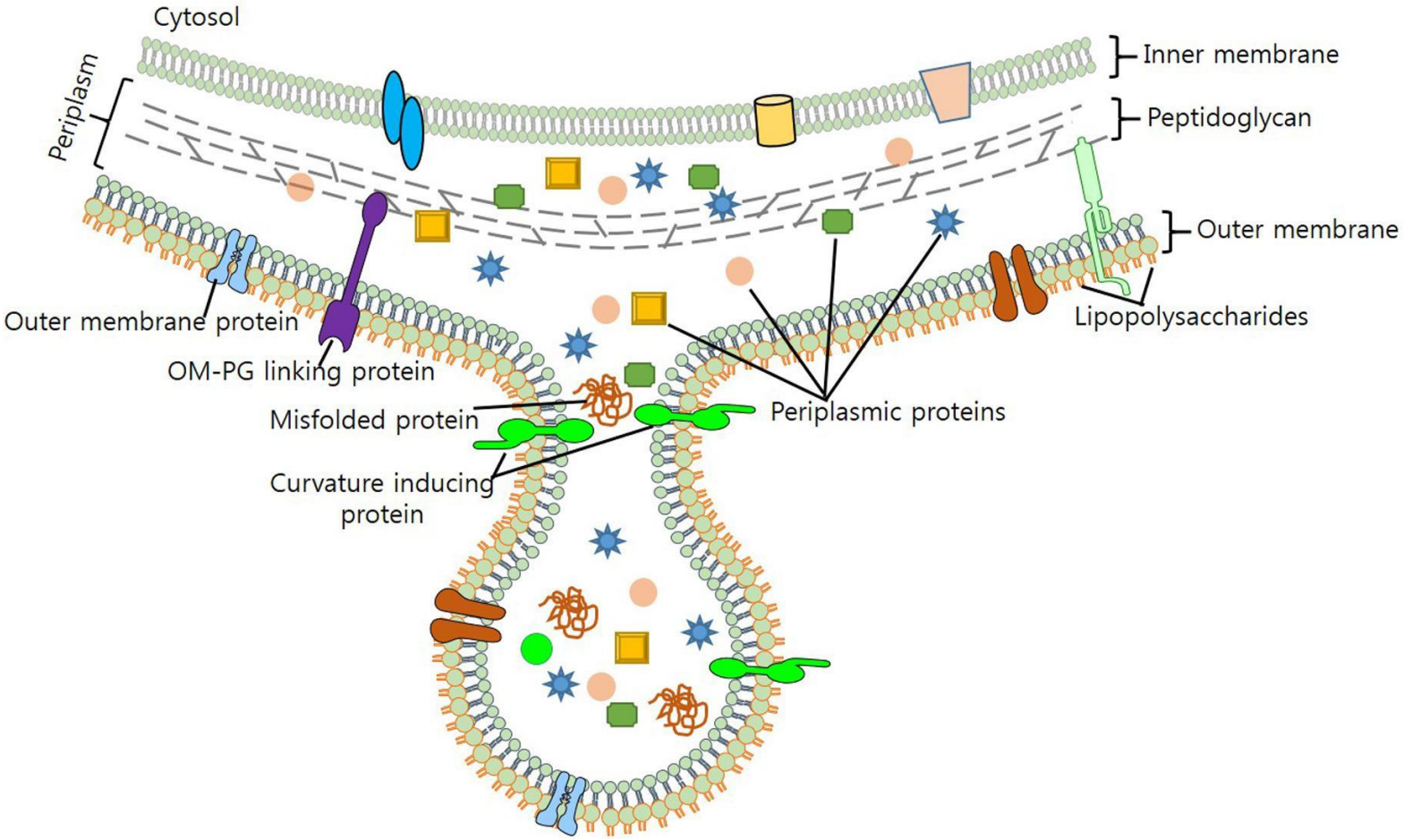
•F1000 Medicine Reports 3(1):16

Outer Membrane Vesicles (OMVs) dei batteri Gram-negativi

Le OMV dei batteri Gram-negativi sono delle particelle di membrana esterna di origine endocitica. Le OMV prodotte da diverse specie batteriche, e rilasciate nell'ambiente extracellulare sembrano svolgere un ruolo essenziale per la sopravvivenza del batterio.

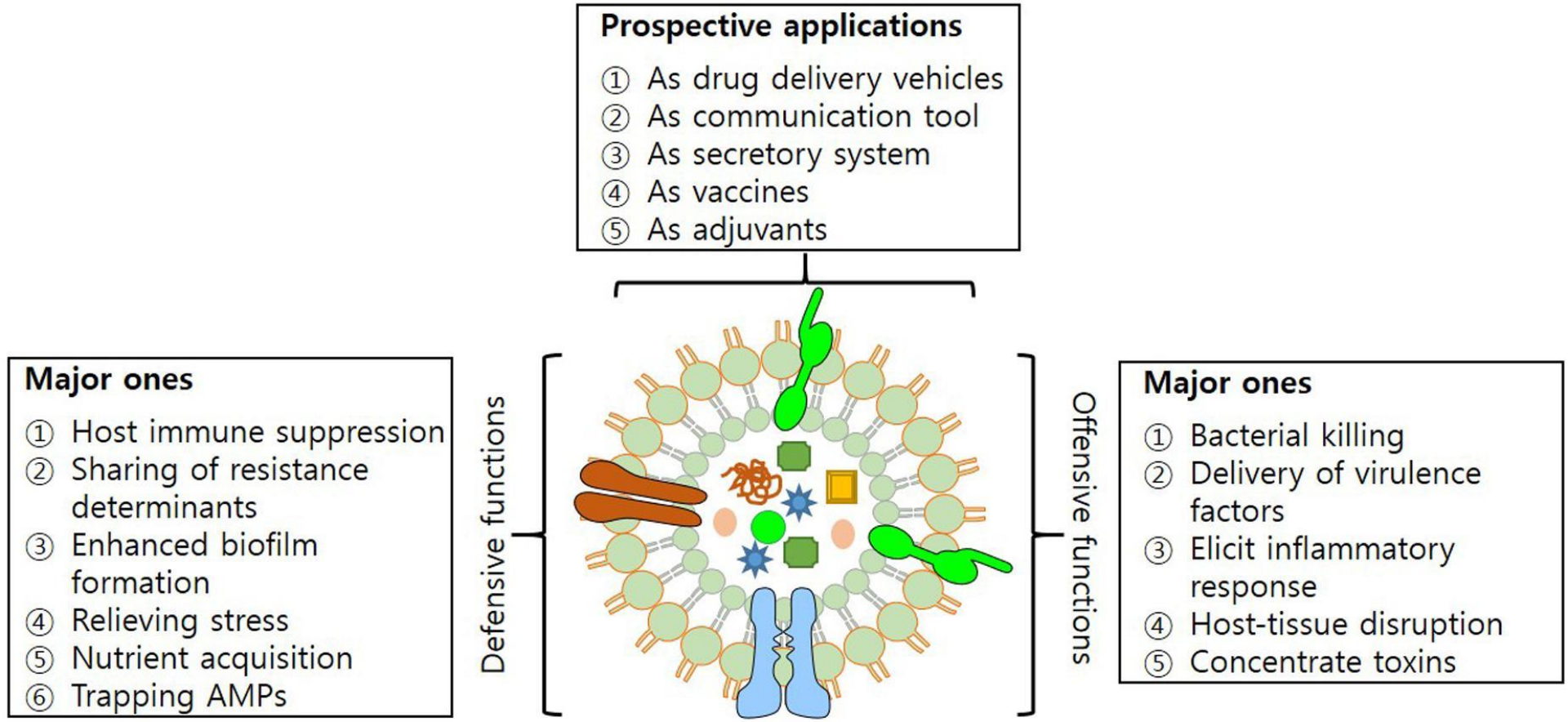
Contengono proteine bioattive, tossine e fattori di virulenza, le OMV svolgono un ruolo fondamentale nelle interazioni batteri-batteri e batteri-ospite. Le OMV aiutano i batteri ad adattarsi a diverse nicchie, a competere con altri batteri e, cosa più importante, a svolgere un ruolo cruciale nell’interazione ospite-patogeno.

Outer Membranes of Gram-negatives are characteristically shed from the surface as ‘vesicle blebs’ (OMV)



Front. Microbiol., 09 June 2017
Sec. Infectious Agents and Disease
https://doi.org/10.3389/fmicb.2017.01053

Figura che illustra i ruoli offensivi e difensivi delle OMV utilizzati nelle interazioni batteri-batteri e batteri-ospite; e le loro potenziali applicazioni.



Lo studio della produzione di OMV in condizioni di stress ha ampliato gli orizzonti sul loro potenziale utilizzo in campo vaccinale e ha anche aperto nuove frontiere nella delineazione del meccanismo molecolare coinvolto nella patogenesi delle malattie.

Svolgendo diverse funzioni biologiche e fisiopatologiche, le OMV rappresentano una grande promessa nella lotta alle malattie batteriche, soprattutto in alternativa agli antibiotici il cui calo di efficacia per la resistenza è in forte aumento.

Le OMV sono studiate come agente eziologico di una serie di malattie infettive e sfruttate nello sviluppo di strumenti diagnostici e come vaccini contro diverse specie patogene Gram-negativi

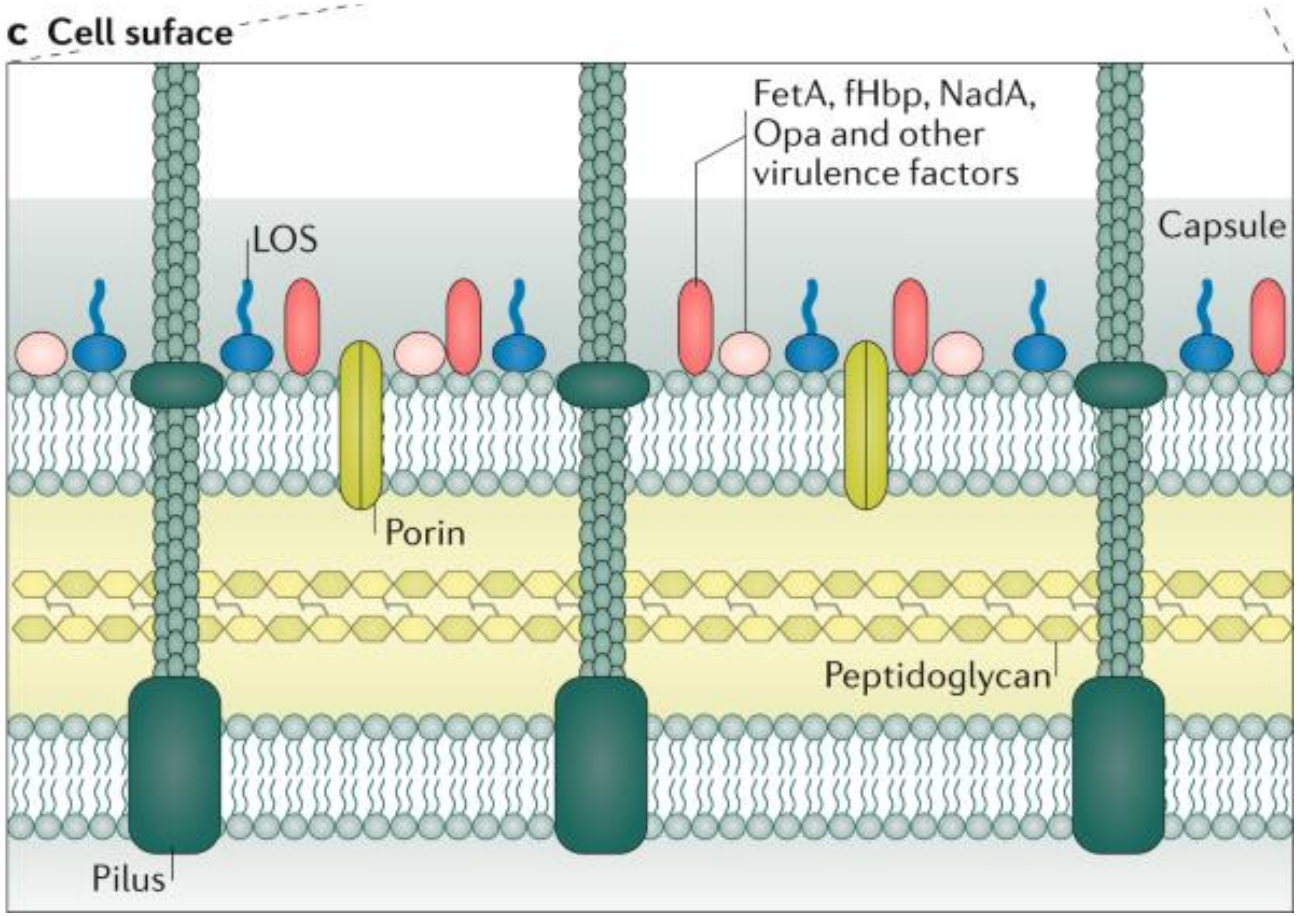
Per I vaccini definiamo tre tipi di OMV:

- Le OMV naturali non trattate "(n)OMV" nativi;
- Le OMV trattate con desossicolato di sodio (NaDOC) e utilizzate come vaccini nell'uomo sono definiti "vaccini OMV";
- Le OMV modificate mediante manipolazione genica (con o senza trattamento NaDOC) sono definite come "(m)OMV" modificati

L'ipotesi di utilizzare le OMV come vaccini ha portato allo sviluppo di due vaccini OMV: VA-MENGOCOC-BC™, sviluppato presso il Finlay Institute di Cuba, e MenBvac™ presso il Norwegian Institute of Public Health (Holst et al.2013). I vaccini sono stati sviluppati dai ceppi clonali omologhi di MenB e utilizzati per controllare con successo le epidemie in corso in entrambi i paesi.
Per produrre questi vaccini OMV vengono utilizzati vari metodi (Frasch et al.2001; Holst et al.2009), inclusa la crescita in condizioni di ferro (Banerjee-Bhatnagar e Frasch 1990) o condizioni limitanti lo zinco (Stork et al.2010) per indurre l'espressione di proteine regolate, ma l'estrazione con NaDOC (0,5%) è il passo comune e chiave per ridurre il contenuto di LOS.

Data la complessità dei vaccini OMV, la domanda da porsi è: quali antigeni dirigono le risposte immunitarie protettive?

Gli studi della sperimentazione vaccinale norvegese utilizzando il western blot hanno dimostrato che gli anticorpi contro la proteina PorA e la proteina Opc specifiche del sierotipo forniscono il contributo più importanti all'attività battericida



Caugant DA, Brynildsrud OB. Neisseria meningitidis: using genomics to understand diversity, evolution and pathogenesis. Nat Rev Microbiol. 2020 Feb;18(2):84-96. doi: 10.1038/s41579-019-0282-6.

Esempi di strategie per identificare potenziali antigeni del vaccino meningococcico

Method	Protocol	Reference
Reverse vaccinology	See next slides	
Pan-genome analysis	Multistep comparative analysis of entire *Neisseria* genomes identifies potential pan-neisserial vaccine candidates	Pajon *et al.* (2009)
Proteomics/immunoproteomics	Human sera from colonised individuals, convalescents and OMV vaccinees examined for specific reactivity with meningococcal proteins, using 2D gel electrophoresis and western blotting. Correlation of protein detection with increased SBA identifies candidate vaccine antigens	Mendum *et al.* (2009); Williams *et al.* (2009, 2014)
	SCAPE (2D free method for proteome analysis) and bioinformatics identifies candidate OMP	Gil *et al.* (2009)
	2D gel-based platform integrating surface and immunoproteomics identifies novel potential immunogens and validates others (e.g. PorA, MIP, fHbp)	Tsolakos *et al.* (2014)
	Surface-display screening/reporter fusion/phage-display-based systems to characterise still undefined meningococcal secretome	Gagic *et al.* (2016)
Targeting meningococcal factors required for pathogenesis	Mouse model of meningococcal systemic infection used to identify candidate vaccine antigens that protect mice from lethal challenge	Sun *et al.* (2005)
Expression library immunisation	Screening of genetic libraries selected on the basis of induction of murine SBA identifies protective pools of defined antigens	Yero *et al.* (2007)
Transcriptional profiling	Transcriptomes of meningococci grown in blood identify upregulated proteins as putative vaccine candidates	Echenique-Rivera *et al.* (2011); Hedman *et al.* (2012)
	Transcriptomes of meningococci exposed to human sera and after interactions with epithelial and endothelial cells	Kurz *et al.* (2003)

Reverse vaccinology

Vaccino contro MenB basato su proteine

Il sequenziamento dell’intero genoma ha aperto la strada a nuovi approcci per lo sviluppo di vaccini meningococcici fornendo informazioni sul proteoma completo. Dal proteoma si possono selezionare i candidati proteici al vaccino ed identificare i presunti antigeni protettivi.

Questo nuovo approccio in silico è stato chiamato "vaccinologia inversa" (RV). Si basa sul genoma e sul proteoma ed è stato descritto per la prima volta da Rino Rappuoli e colleghi nel 2000 (Rappuoli 2001) per la scoperta di potenziali antigeni nel ceppo MenB MC58 e ha portato alla sviluppo del vaccino Novartis Bexsero™/4CMenB.

10 Mar 2000

Complete genome sequencing of Neisseria meningitidis serogroup B

10 Mar 2000
Vol 287
1809-1815

Science. 2000 Mar 10;287(5459):1809-15. doi: 10.1126/science.287.5459.1809.

Complete genome sequence of Neisseria meningitidis serogroup B strain MC58

H Tettelin [1], N J Saunders, J Heidelberg, A C Jeffries, K E Nelson, J A Eisen, K A Ketchum, D W Hood, J F Peden, R J Dodson, W C Nelson, M L Gwinn, R DeBoy, J D Peterson, E K Hickey, D H Haft, S L Salzberg, O White, R D Fleischmann, B A Dougherty, T Mason, A Ciecko, D S Parksey, E Blair, H Cittone, E B Clark, M D Cotton, T R Utterback, H Khouri, H Qin, J Vamathevan, J Gill, V Scarlato, V Masignani, M Pizza, G Grandi, L Sun, H O Smith, C M Fraser, E R Moxon, R Rappuoli, J C Venter

Affiliations + expand

PMID: 10710307 DOI: 10.1126/science.287.5459.1809

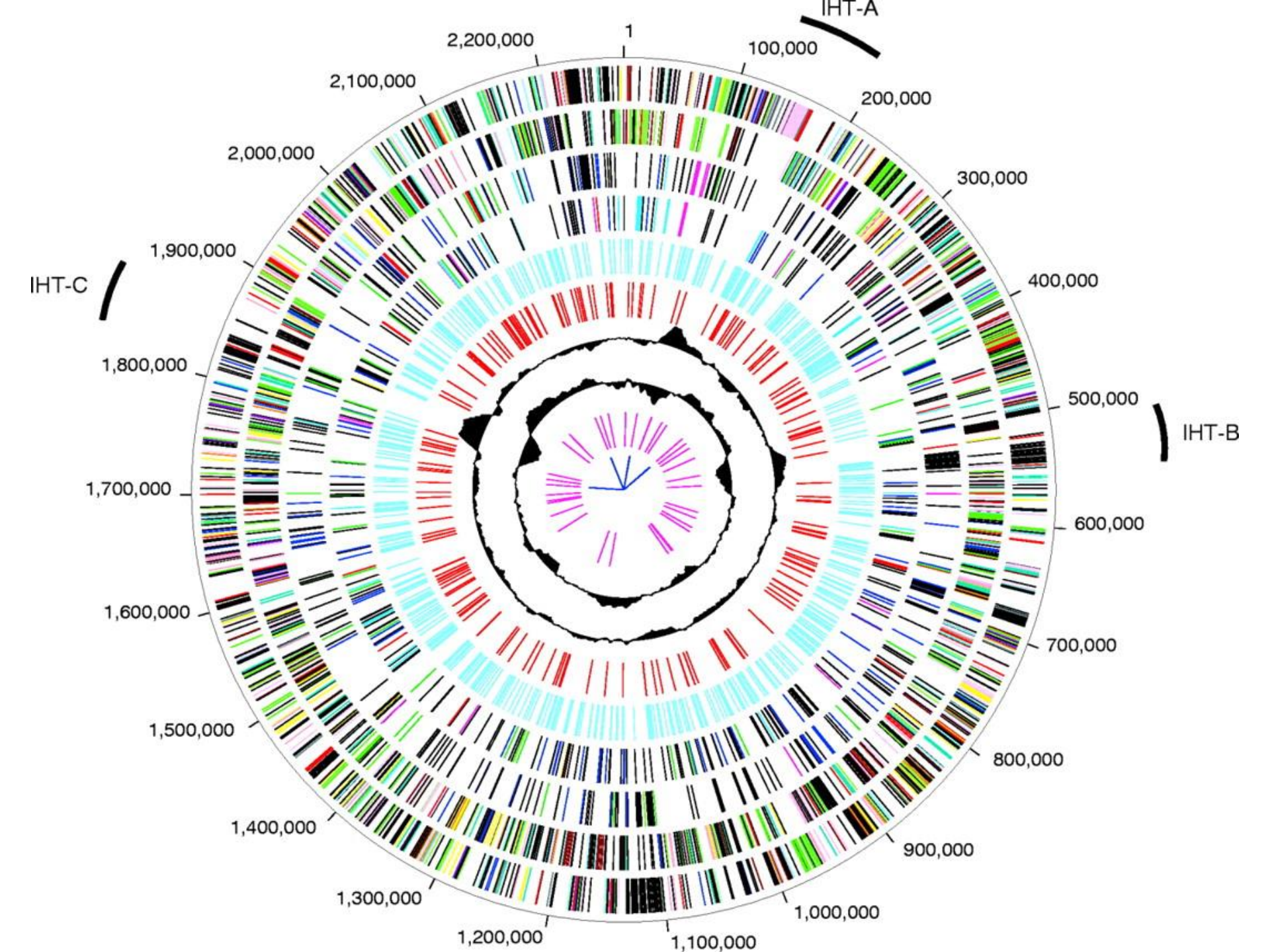
Abstract

The 2,272,351-base pair genome of Neisseria meningitidis strain MC58 (serogroup B), a causative agent of meningitis and septicemia, contains 2158 predicted coding regions, 1158 (53.7%) of which were assigned a biological role. Three major islands of horizontal DNA transfer were identified; two of these contain genes encoding proteins involved in pathogenicity, and the third island contains coding sequences only for hypothetical proteins. Insights into the commensal and virulence behavior of N. meningitidis can be gleaned from the genome, in which sequences for structural proteins of the pilus unique to serogroup B capsular polysaccharide synthesis can

The 2,272,351–base pair genome of *Neisseria meningitidis* strain MC58 (serogroup B), a causative agent of meningitis and septicemia, contains 2158 predicted coding regions, 1158 (53.7%) of which were assigned a biological role.

Three major islands of horizontal DNA transfer were identified; two of these contain genes encoding proteins involved in pathogenicity, and the third island contains coding sequences only for hypothetical proteins. Insights into the commensal and virulence behavior of *N. meningitidis* can be gleaned from the genome, in which sequences for structural proteins of the pilus are clustered and several coding regions unique to serogroup B capsular polysaccharide synthesis can be identified.

Finally, *N. meningitidis* contains more genes that undergo phase variation than any pathogen studied to date, a mechanism that controls their expression and contributes to the evasion of the host immune system.


IHT-A
IHT-B
IHT-C
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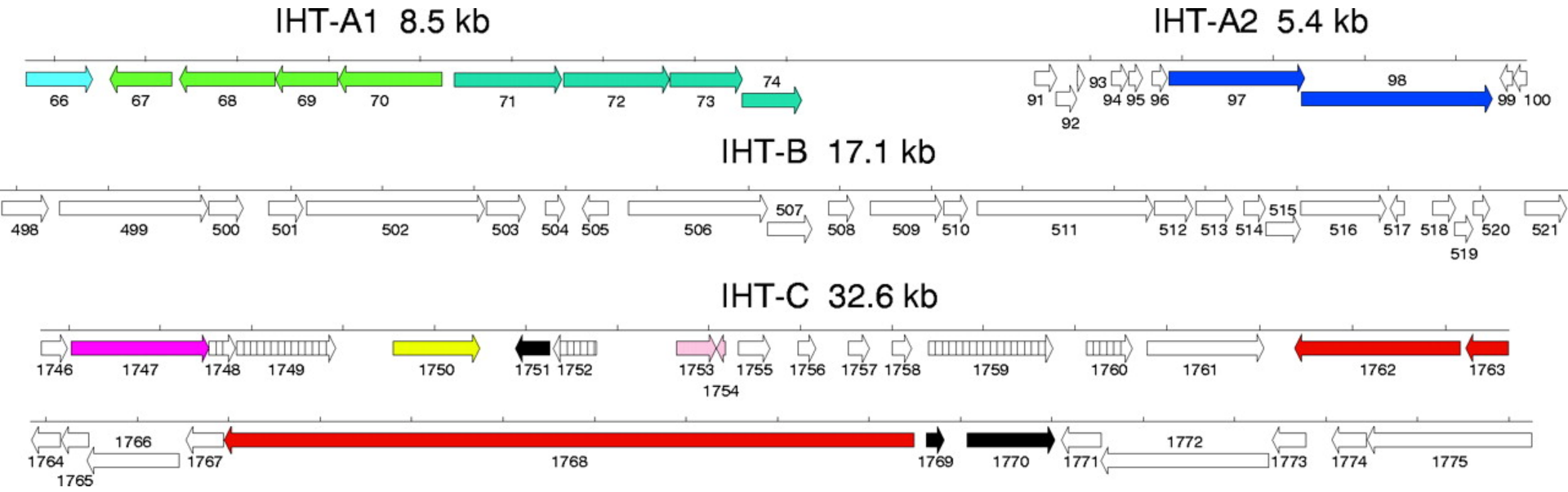
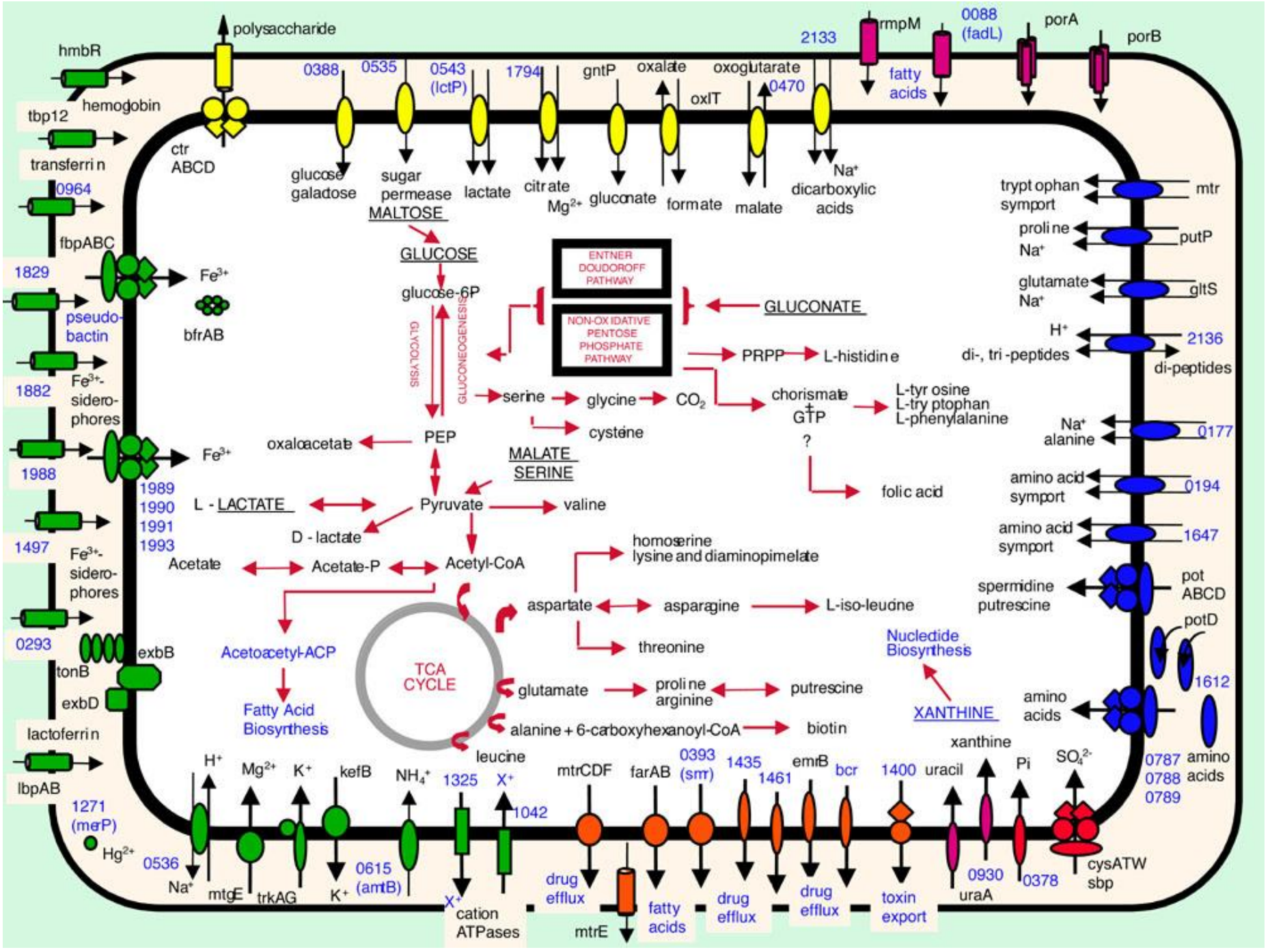


Figure 3 Structure of the putative islands of horizontally tranferred DNA (IHTs) in the N. meningitidis strain MC58 genome. Empty boxes are hypothetical proteins and striped boxes are conserved hypothetical proteins.

IHT-A1: NMB0066, adenine rRNA methylase ErmC; NMB0067 to NMB0070, capsule biosynthesis proteins SiaD, SiaC,SiaB, and SynX; NMB0071 to NMB0074, capsule export proteins CtrA, CtrB, CtrC, and CtrD.

IHT-A2: NMB0097 and NMB0098, disrupted secreted protein and ABC transporter.

IHT-C: NMB1747, tspB protein; NMB1750, PivNM-2; NMB1751, NMB1769, and NMB1770, transposases; NMB1753 and NMB1754, bacteriophage-related proteins; NMB1762, NMB1763, and NMB1768, toxin/toxin-related homologs.


polysaccharide
hmbR
hemoglobin
tbp12
transferrin
0964
fbpABC
1829
pseudo-bactin
1882
Fe3+-sidero-phores
1988
1497
Fe3+-sidero-phores
0293
exbB
tonB
exbD
lactoferrin
lbpAB
1271 (merP)
Hg2+
ctr ABCD
Fe3+
bfrAB
Fe3+
1989
1990
1991
1993
0388
0535
0543 (lctP)
1794
gntP
oxalate
oxoglutarate
oxlT
0470
2133
rmpM
fatty acids
0088 (fadL)
porA
porB
glucose galactose
sugar permease
lactate
citrate Mg2+
gluconate
formate
malate
Na+ dicarboxylic acids
MALTOSE
GLUCOSE
glucose-6P
ENTNER DOUDOROFF PATHWAY
NON-OXIDATIVE PENTOSE PHOSPHATE PATHWAY
GLUCONATE
GLYCOLYSIS
GLUCONEOGENESIS
PRPP
L-histidine
serine
glycine
CO2
cysteine
chorismate
GTP
L-tyrosine
L-tryptophan
L-phenylalanine
?
folic acid
PEP
oxaloacetate
MALATE
SERINE
Pyruvate
L - LACTATE
D - lactate
valine
Acetate
Acetate-P
Acetyl-CoA
homoserine
lysine and diaminopimelate
aspartate
asparagine
L-iso-leucine
threonine
Acetoacetyl-ACP
Fatty Acid Biosynthesis
TCA CYCLE
glutamate
proline
arginine
putrescine
alanine + 6-carboxyhexanoyl-CoA
biotin
leucine
Nucleotide Biosynthesis
XANTHINE
trypt ophan symport
mtr
proline Na+
putP
glutamate Na+
gltS
H+
2136
di-, tri-peptides
di-peptides
Na+ alanine
0177
amino acid symport
0194
amino acid symport
1647
spermidine putrescine
pot ABCD
potD
1612
amino acids
amino acids
0787
0788
0789
xanthine
uracil
Pi
SO4 2-
cysATW sbp
0930
uraA
0378
H+
Mg2+
K+
kefB
NH4+
1325
X+
1042
mtrCDF
farAB
0393 (smr)
1435
1461
emrB
bcr
1400
0536
Na+
mtgE
trkAG
K+
0615 (amtB)
X+
cation ATPases
drug efflux
mtrE
fatty acids
drug efflux
drug efflux
toxin export

REPORTS

Identification of Vaccine Candidates Against Serogroup B Meningococcus by Whole-Genome Sequencing

10 Mar 2000
vol 287
1816-1820

MARIAGRAZIA PIZZA, VINCENZO SCARLATO, VEGA MASIGNANI, MARZIA MONICA GIULIANI, BEATRICE ARICÒ, MAURIZIO COMANDUCCI, GARY T. JENNINGS, LUCIA BALDI, ERIKA BARTOLINI, BARBARA CAPECCHI, CESIRA L. GALEOTTI, ENRICO LUZZI, ROBERTO MANETTI, ELISA MARCHETTI, MARIROSA MORA, SANDRA NUTI, GIULIO RATTI, LAURA SANTINI, SILVANA SAVINO, MARIA SCARSELLI, ELISA STORNI, PEIJUN ZUO, MICHAEL BROEKER, ERIKA HUNDT, BERNARD KNAPP, ERIC BLAIR, TANYA MASON, HERVÉ TETTELIN, DEREK W. HOOD, ALEX C. JEFFRIES, NIGEL J. SAUNDERS, DAN M. GRANOFF, J. CRAIG VENTER, E. RICHARD MOXON, GUIDO GRANDI, AND RINO RAPPUOLI fewer Authors Info & Affiliations

SCIENCE · 10 Mar 2000 · Vol 287, Issue 5459 · pp. 1816-1820 · DOI: 10.1126/science.287.5459.1816

To identify potential vaccine candidates, we determined the genome sequence of the virulent strain MC58 [see (11)]. While the sequencing project was in progress, unassembled DNA fragments were analyzed to identify open reading frames (ORFs) that potentially encoded novel surface-exposed or exported proteins (12).

We identified 570 such ORFs and, by means of the polymerase chain reaction (PCR), we amplified and cloned the DNA sequences of these hypothetical genes in *Escherichia coli* to express each polypeptide as either His-tagged or glutathione S-transferase (GST) fusion proteins (13). We obtained successful expression with 350 ORFs (61%).

A total of 350 candidate antigens were expressed in *Escherichia coli*, purified, and used to immunize mice.

The sera allowed the identification of proteins that

-are surface exposed,

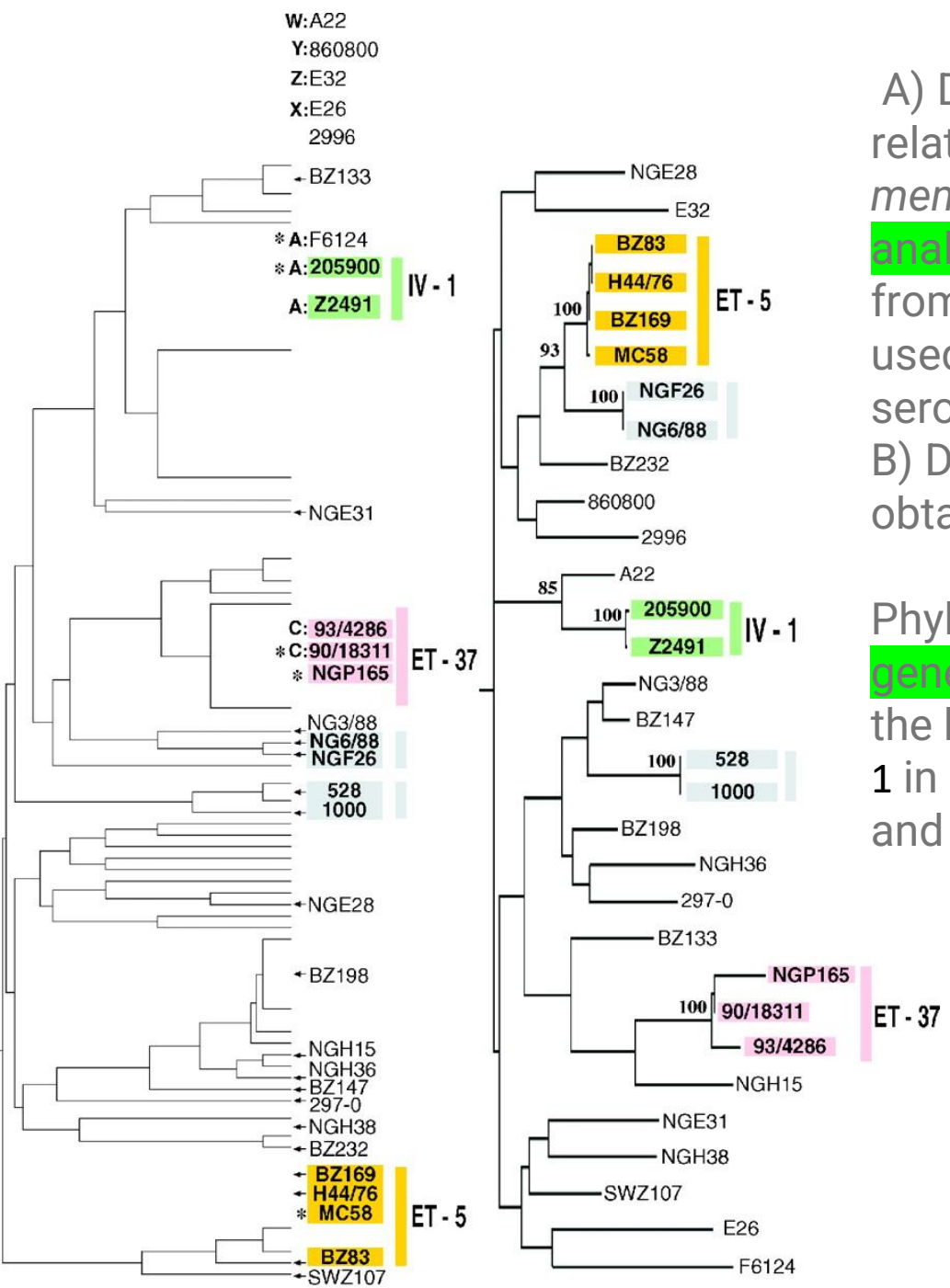
-are conserved in sequence across a range of strains,

-induce a bactericidal antibody response, a property known to correlate with vaccine efficacy in humans.

we used a collection of strains isolated worldwide and over many years to investigate whether the new candidate molecules were conserved and accessible to antibodies.

Our aim was to select strains representative of the diversity found in natural populations of MenB.

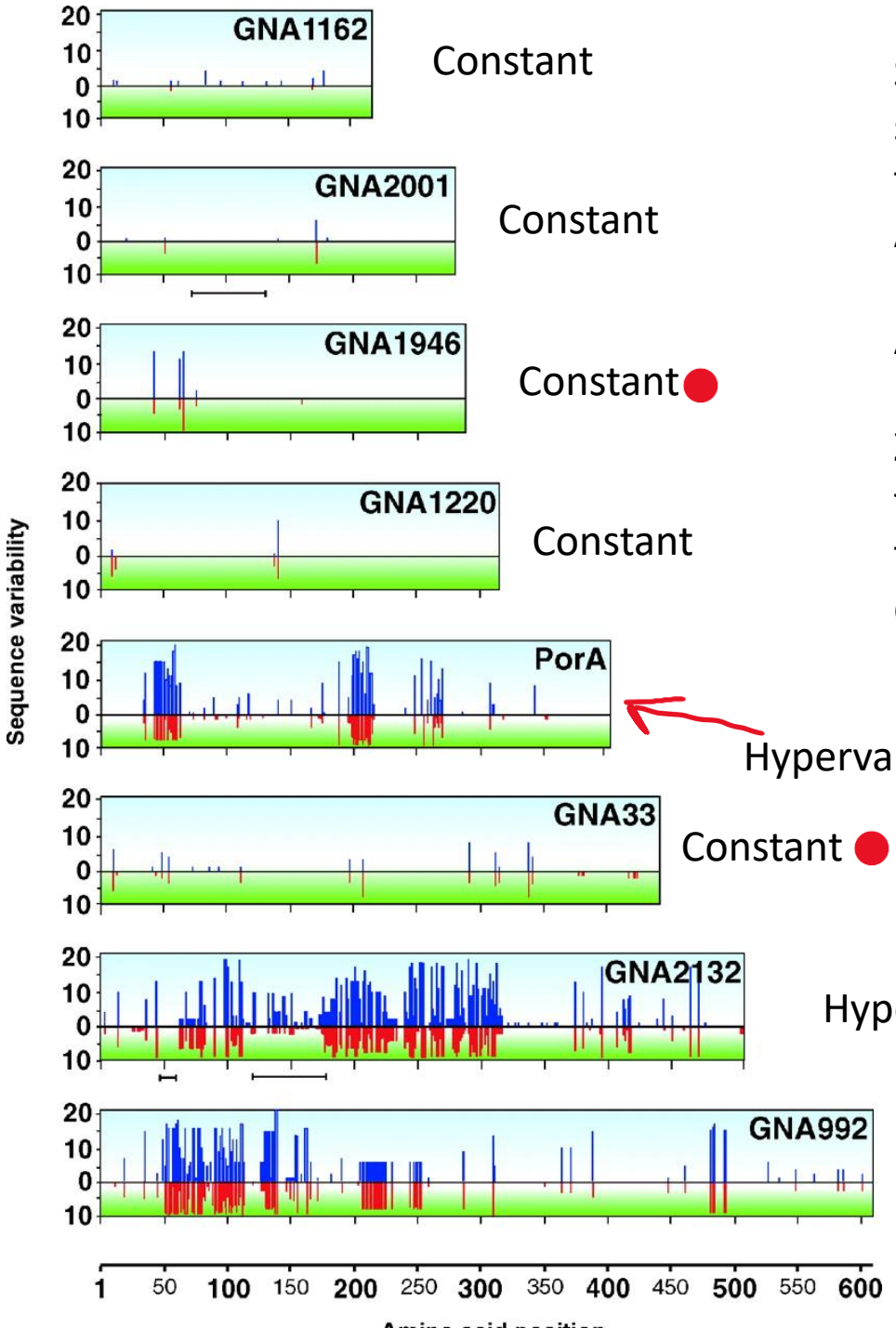
We used a phylogenetic tree from 107 strains constructed by multilocus enzyme electrophoresis (MLEE) and validated by multilocus sequence typing (MLST) to select 22 representative, disease-associated MenB strains



A) Dendrogram showing genetic relationship among 107 *N. meningitidis* strains based on MLST analysis of six gene fragments [adapted from Maiden *et al.* The dendrogram was used to select strains representative of serogroup B meningococcus population B) Dendrogram of *N. meningitidis* strains obtained from the conserved genes.

Phylogenetic analysis based on the new genes provided a dendrogram that clusters the hypervirulent strains ET-5, ET-37, and IV-1 in agreement with the results of MLEE and MLST reported in (A).

Antigen (length, amino acids)	Remarks/similarities*	FACS	ELISA	Serum bactericidal activity (SBA)
GNA33 (441)	Lipoprotein/similar to*E. coli* membranebound lytic transglycosylase A (MltA) of *E. coli* and of *Synechocystis* sp. (22)	++++†	13,000	1/16,000‡
GNA992 (591)	Outer membrane protein/similar to Hsf and Hia of*Haemophilus influenzae* and FhaB of *Bordetella pertussis* (26)	+++	2,750	1/256
GNA1162 (215)	Lipoprotein/no significant similarities	++	1,270	1/4
GNA1220 (315)	Membrane protein/contains a stomatin-like domain	+++	1,000	1/256
GNA1946 (287)	Lipoprotein/similar to HlpA of *H. influenzae*, belongs to the NlpA family of lipoproteins (27)	+++	13,100	1/32
GNA2001 (251)	Outer membrane protein/similar to P60 invasion-associated extracellular proteins (28)	++	500	1/512
GNA2132 (488)	Lipoprotein/low similarity to transferrin binding proteins	++	1,700	1/16,000
GST§	—	–	<50	<1/4
OMV§	Mixture of proteins containing mainly PorA	++++	260,000	1/32,000



Constant

Constant

Constant

Constant

Hypervariable (PorA)

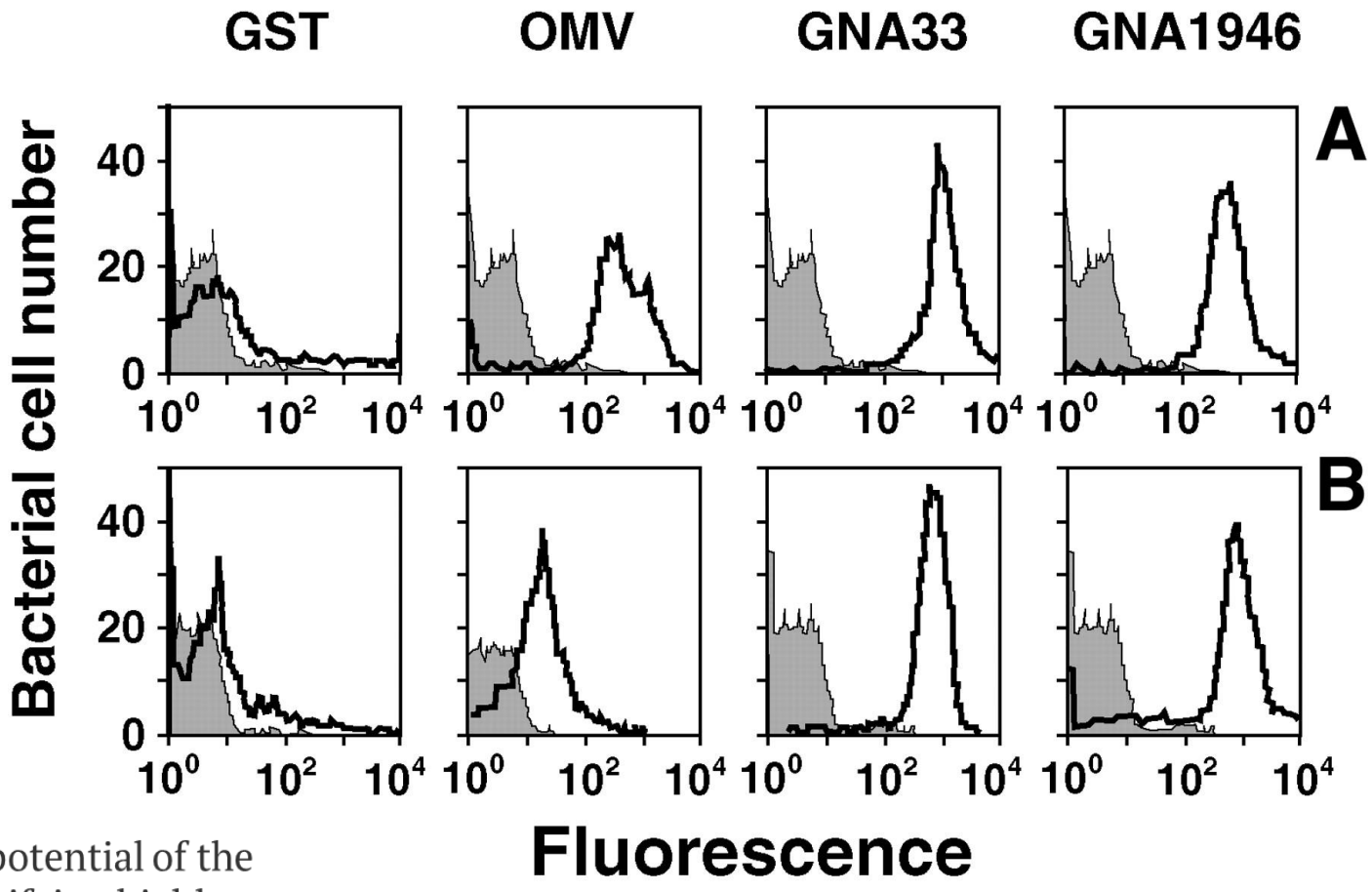
Constant

Hypervariable

Hypervariable

Schematic representation of amino acid sequence variability within N. meningitidis of the seven antigens and of PorA.
Abscissa, amino acid position; ordinate, number of strains analyzed.
Amino acid differences within the nine N. meningitidis strains from serogroups A, C, Y, X, Z, and W135 are indicated by red lines below the 0. Height of blue and red lines represents the number of strains with amino acid changes. Variable regions appear as blue and red peaks.

FACS analysis showing binding of polyclonal OMV, GNA33, and GNA1946 antisera to the ethanol-treated homologous 2996 (A) and heterologous BZ232 (B) strains. Gray profiles show binding of preimmune sera; white profiles show binding of immune sera. Negative controls include sera of mice immunized with GST.



In addition to proving the potential of the genomic approach, by identifying highly conserved proteins that induce bactericidal antibodies, we have provided candidates that will be the basis for clinical development of a vaccine against an important pathogen. This vaccine is likely to elicit cross protection not only against group B*N. meningitidis* but also against other serogroups and species of pathogenic *Neisseria*.

Table 2. Presence of genes in *Neisseria*.

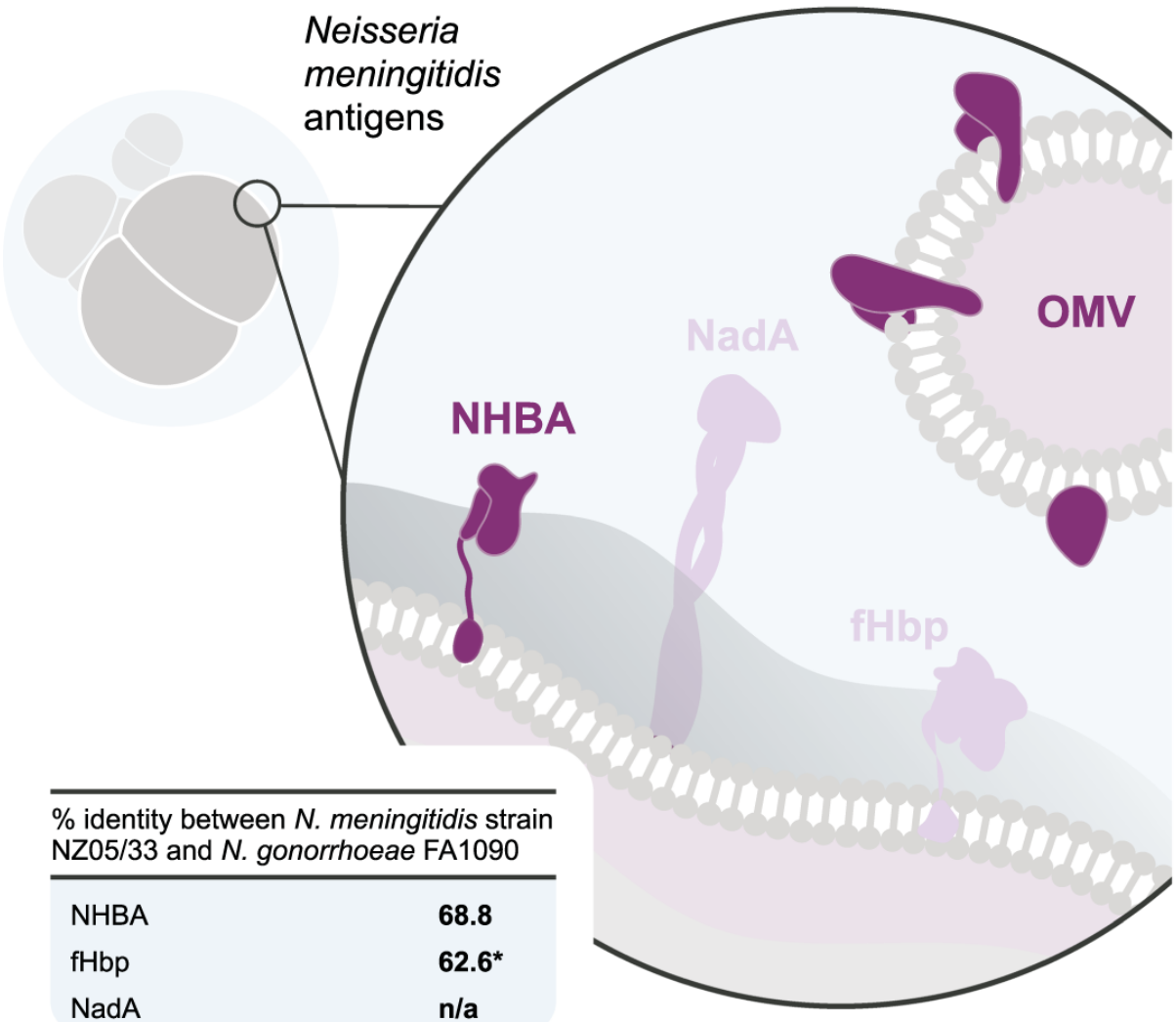
Gene	*N. meningitidis* B (22 strains)	*N. meningitidis* A,C,Y,X,Z,W135 (9 strains)	*N. lactamica* (1 strain)	*N. cinerea* (1 strain)	*N. gonorrhoeae* (3 strains)
*gna*33	+	+	+	+	+
*gna*992	+*	+	+/−†	+/−	−
*gna*1162	+	+	+	+	+
*gna*1220	+‡	+	+/−	+/−	+
*gna*1946	+	+	+	+/−	+
*gna*2001	+	+	+	+/−	+
*gna*2132	+	+	+	−	+
porA	+	+	+	−	+

*In strains NG6/88 and NGF26 the start codon is 222 bases downstream from the starting codon in the other strains. †+/− indicates a negative PCR but positive Southern blotting. ‡In strain BZ133 a deletion of 31 nucleotides causes a frameshift in this gene.

Table 1. **Historical milestones tracking the impact of new technologies on vaccine discovery and design**

Milestone years	Approach to discover and design vaccines	Technologies and description	Comments and references
1796	Classical vaccinology	**Growing microorganisms:** Growth of microorganisms allows making killed and live-attenuated vaccines or to discover antigens used for subunit vaccines.	1796: Jenner starts growing cowpox in cows (Willis, 1997; Baxby, 1999), marking the beginning of vaccinology.
1995			1995: Venter publishes the first sequencing of the entire genome from a bacterium (Fleischmann et al., 1995).
2000	Reverse vaccinology	**Genomics, high-throughput protein expression, and animal models:** Vaccine antigens are discovered using the genomic information without the need for growing microorganisms. Antigens selected in silico are expressed and screened in animal models.	2000: The first vaccine candidates based on antigens discovered by genomics are reported (Pizza et al., 2000).
2012			2012: The first genome-based vaccine receives regulatory approval (European Medicines Agency, 2012).
2002			2002: Burton proposes to use human mAbs to design new vaccines (Burton, 2002).
2008	Reverse vaccinology 2.0	**Genomics, high-throughput protein expression, animal models, human monoclonals, B cell repertoire deep sequencing, proteomics, and structure-based antigen design:** Genomics is used not only for antigen discovery, but also for antigen expression, conservation, and for epidemiology. Human monoclonals are used to identify protective antigens/epitopes. Structural characterization of the Ab–antigen complex is used to instruct antigen design.	2008: Dormitzer, Ulmer, and Rappuoli propose the term "structural vaccinology" to identify the emerging structure-based antigen design (Dormitzer et al., 2008).
2013			2013: Graham and Kwong first report that RSV pre-fusion F antigen successfully derived from structure-based design is protective in the animal model (McLellan et al., 2013a).

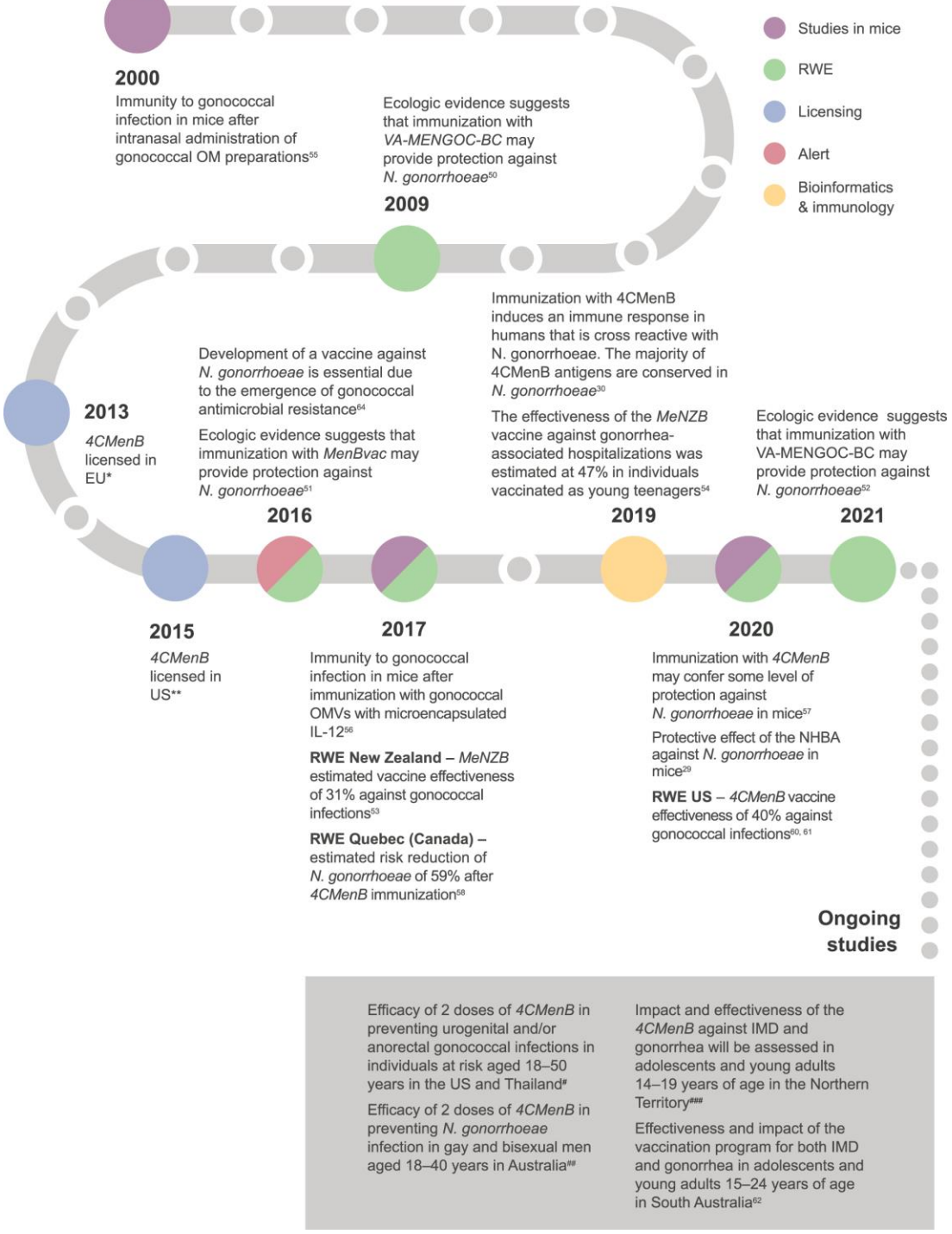
Ruiz García, Y., Sohn, WY., Seib, K.L. *et al.* Looking beyond meningococcal B with the 4CMenB vaccine: the *Neisseria* effect. *npj Vaccines* **6**, 130 (2021). https://doi.org/10.1038/s41541-021-00388-3

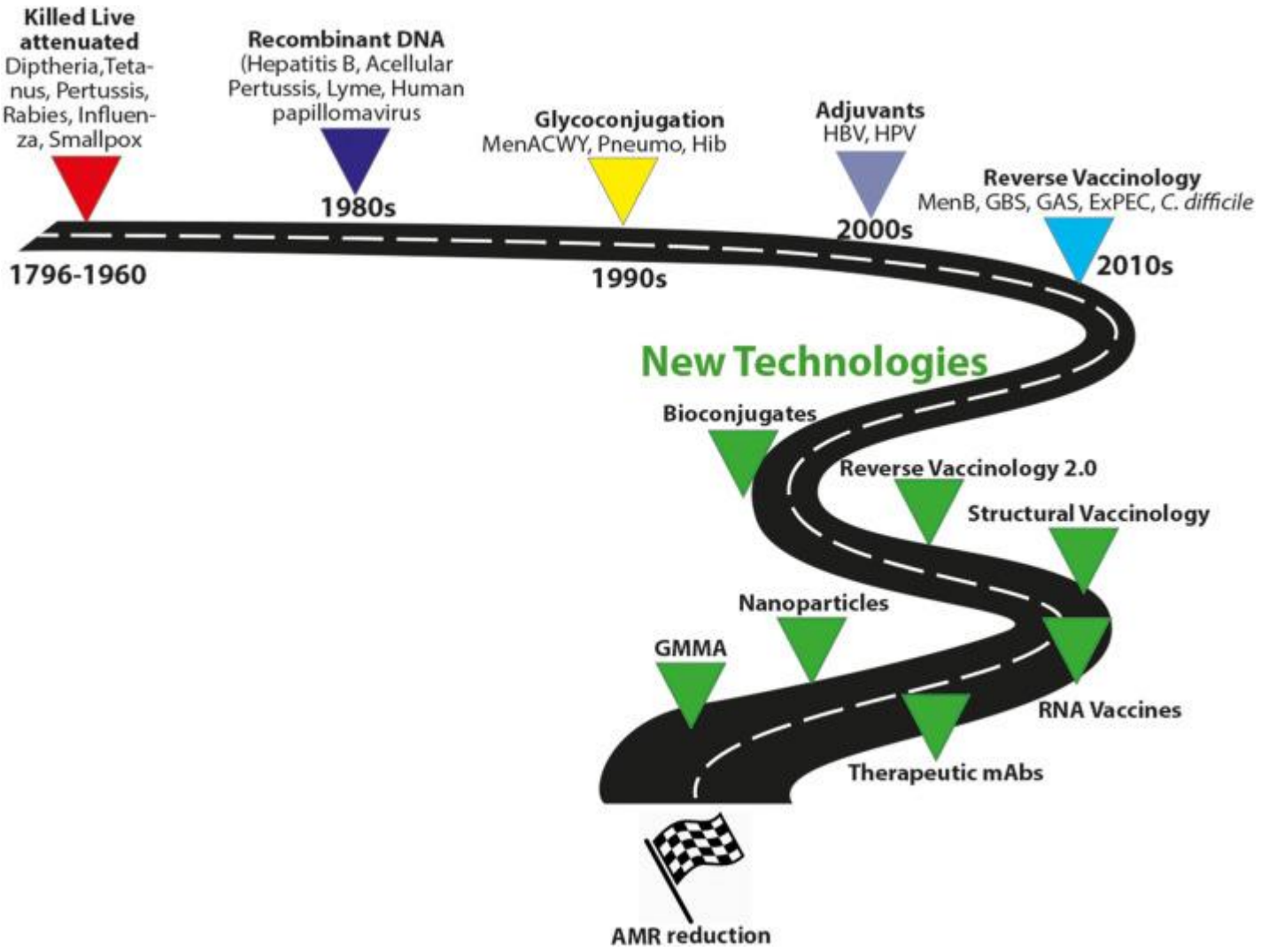


% identity between *N. meningitidis* strain NZ05/33 and *N. gonorrhoeae* FA1090	
NHBA	**68.8**
fHbp	**62.6***
NadA	**n/a**

OMV proteins	% identity between *N. meningitidis* strain NZ05/33 and *N. gonorrhoeae* FA1090
FbpA	**99.1**
MafA adhesin	**98.8**
Antioxidation AhpC TSA family glutaredoxin	**98.5**
FkpA	**97.8**
TonB-dependent receptor (NMB0964)	**96.9**
MtrE	**96.4**
Hypothetical protein	**96.3**
TonB-dependent receptor (NMB1497)	**96.1**
OMP85	**95.0**
FrpB	**94.3**
Putative lipoprotein NMB1126/1164	**94.2**
OMP P1	**94.0**
Tbp1	**93.7**
NspA	**93.7**
RmpM	**93.4**
PilQ	**91.4**
LptD	**89.8**
LysM peptidoglycan -binding domain containing protein	**88.7**
PorB	**67.3**
OpcA	**43.8**
PorA	**n/a**
LbpA	**n/a**

Ruiz García, Y., Sohn, WY., Seib, K.L. *et al.* Looking beyond meningococcal B with the 4CMenB vaccine: the *Neisseria* effect. *npj Vaccines* **6**, 130 (2021). https://doi.org/10.1038/s41541-021-00388-3





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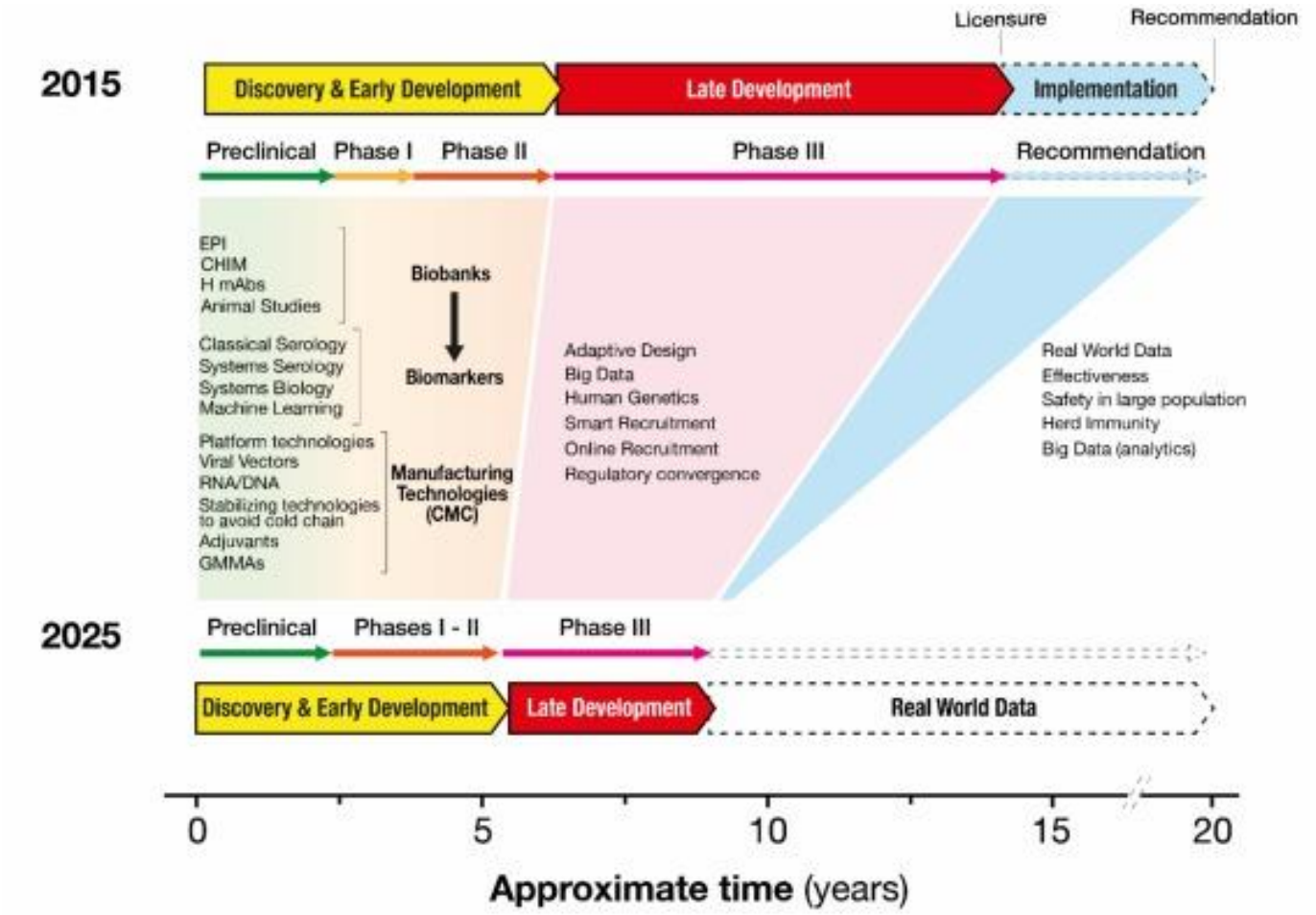

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frontiers in Microbiology

ORIGINAL RESEARCH
published: 24 June 2021
doi: 10.3389/fmicb.2021.665858

Multi-Omics Approach Reveals the Potential Core Vaccine Targets for the Emerging Foodborne Pathogen *Campylobacter jejuni*

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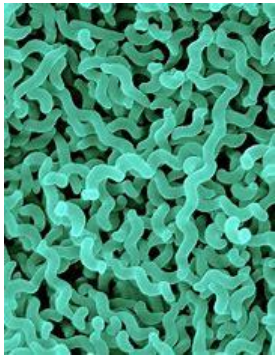
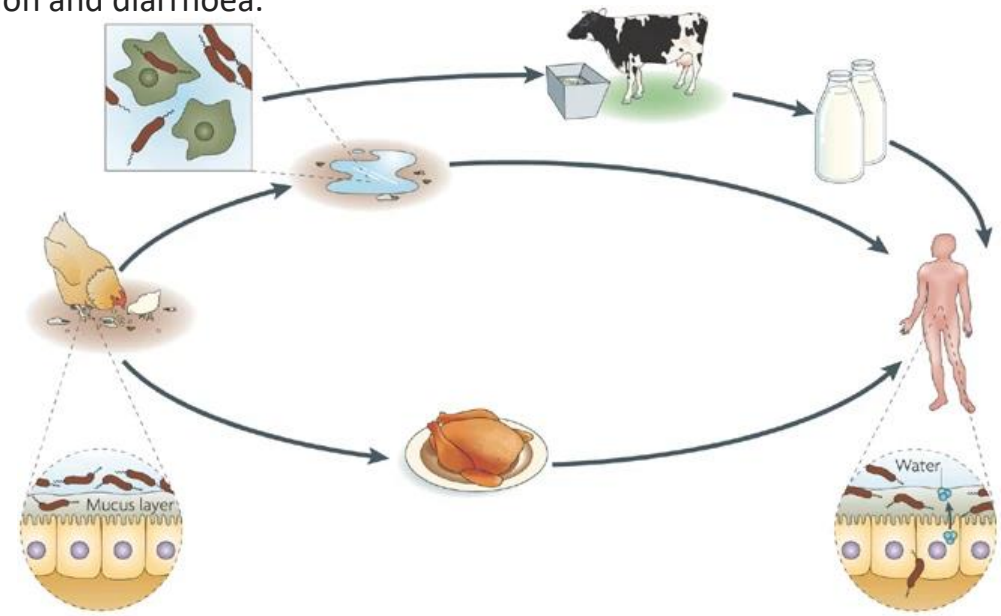


Thermotolerant campylobacters are the most frequent cause of bacterial infection of the lower intestine worldwide.
C. jejuni belongs to the epsilon class of proteobacteria, in the order *Campylobacteriales*; this order includes two other genera, *Helicobacter* and *Wolinella*. Like *C. jejuni*, members of these genera have small genomes (1.6–2.0 megabases)

Figure 1: The sources and outcomes of *Campylobacter jejuni* infection.
Several environmental reservoirs can lead to human infection by *C. jejuni*. It colonizes the chicken gastrointestinal tract in high numbers, primarily in the mucosal layer, and is passed between chicks within a flock through the faecal–oral route. *C. jejuni* can enter the water supply, where it can associate with protozoans, such as freshwater amoebae, and possibly form biofilms. *C. jejuni* can infect humans directly through the drinking water or through the consumption of contaminated animal products, such as unpasteurized milk or meat, particularly poultry. In humans, *C. jejuni* can invade the intestinal epithelial layer, resulting in inflammation and diarrhoea.



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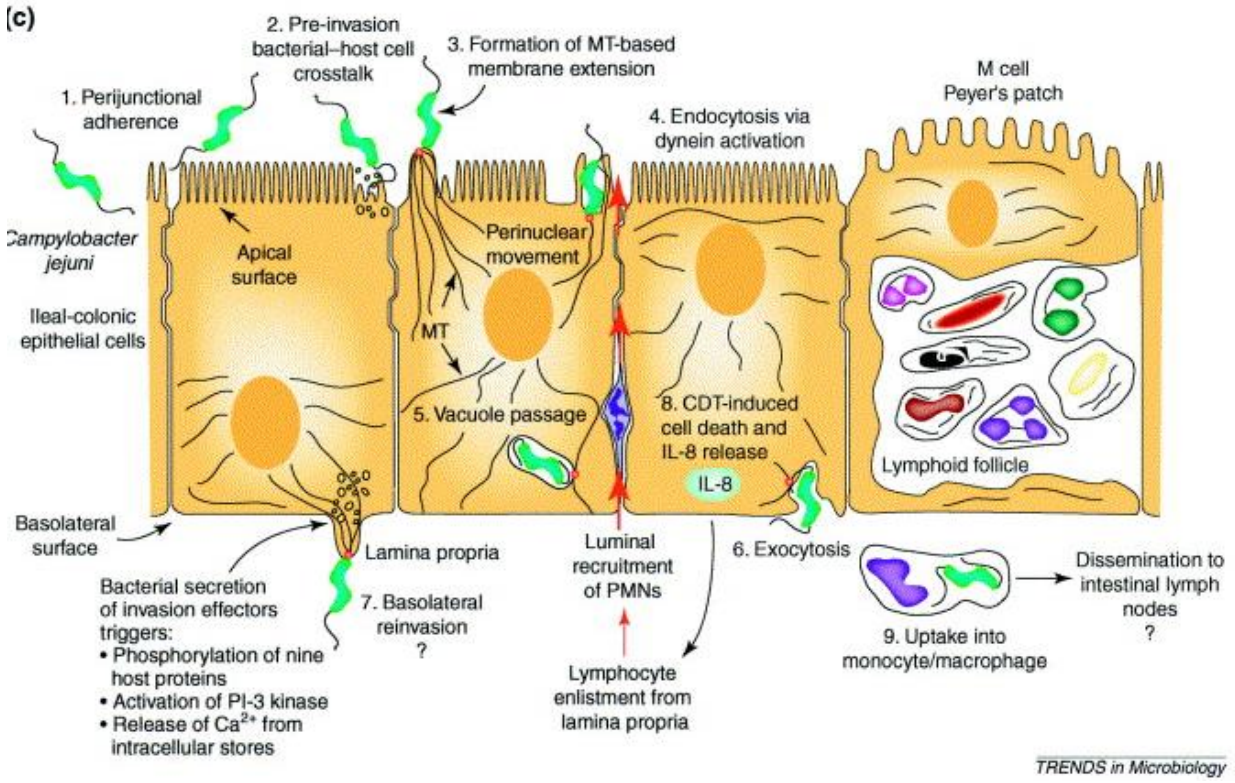
The determination of the complete genome sequence of several *C. jejuni* strains and plasmids has heralded the beginning of a new era of *C. jejuni* research. These projects have revealed the potential mechanisms by which *C. jejuni* associates with the host; for example, the complete sequencing of **pVir**, a plasmid that is found in some isolates of *C. jejuni*, has led to the identification of a **type IV secretion system** that has been demonstrated to have a role **in cell invasion and pathogenicity** in ferrets.

The publication of the genome sequence has also enabled the development of multiple genetic and genomic tools for use in *C. jejuni*, including microarrays, transposons for efficient random mutagenesis, signature-tagged mutagenesis, new reporter constructs and vectors for constructing in-frame deletion mutants and chromosomal point mutations.

Campylobacter jejuni pathogenesis

The mechanism of pathogenesis comprises four main stages: **adhesion to intestinal cells, colonization of the digestive tract, invasion of targeted cells, and toxin production**.



Genetic variation and natural transformation.

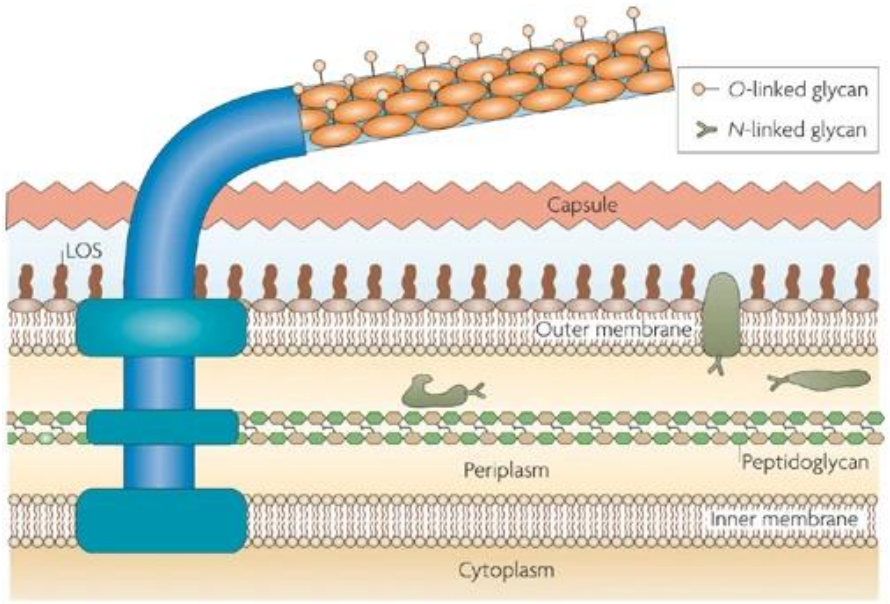
C. jejuni displays extensive genetic variation, which has arisen from intragenomic mechanisms as well as genetic exchange between strains. Sequencing the genome of *C. jejuni* has revealed:

the presence of hypervariable sequences that consist of homopolymeric tracts

lack of clear homologues of many *E. coli* DNA-repair genes.

Most of the hypervariable sequences are in regions that encode proteins that are involved in the biosynthesis or modification of surface-accessible carbohydrate structures, such as the capsule, lipooligosaccharide (LOS) and flagellum.

The flagellin is modified by *O*-linked glycosylation. This modification is required for flagellar assembly and is, therefore, important for motility, virulence and epithelial cell adherence and invasion. The *N*-linked-glycosylation system modifies some periplasmic and outer-membrane proteins.



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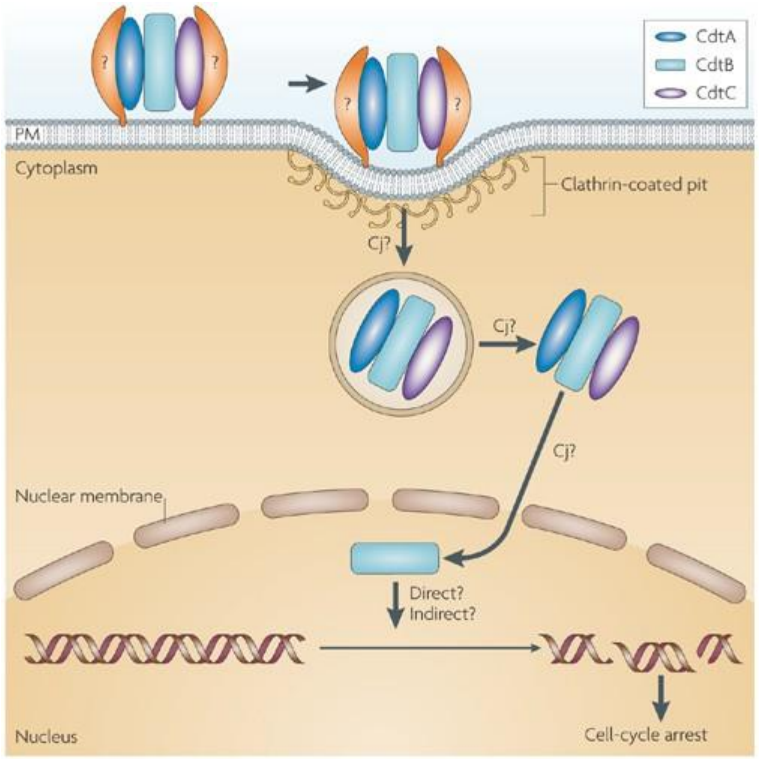
Cytolethal distending toxin.

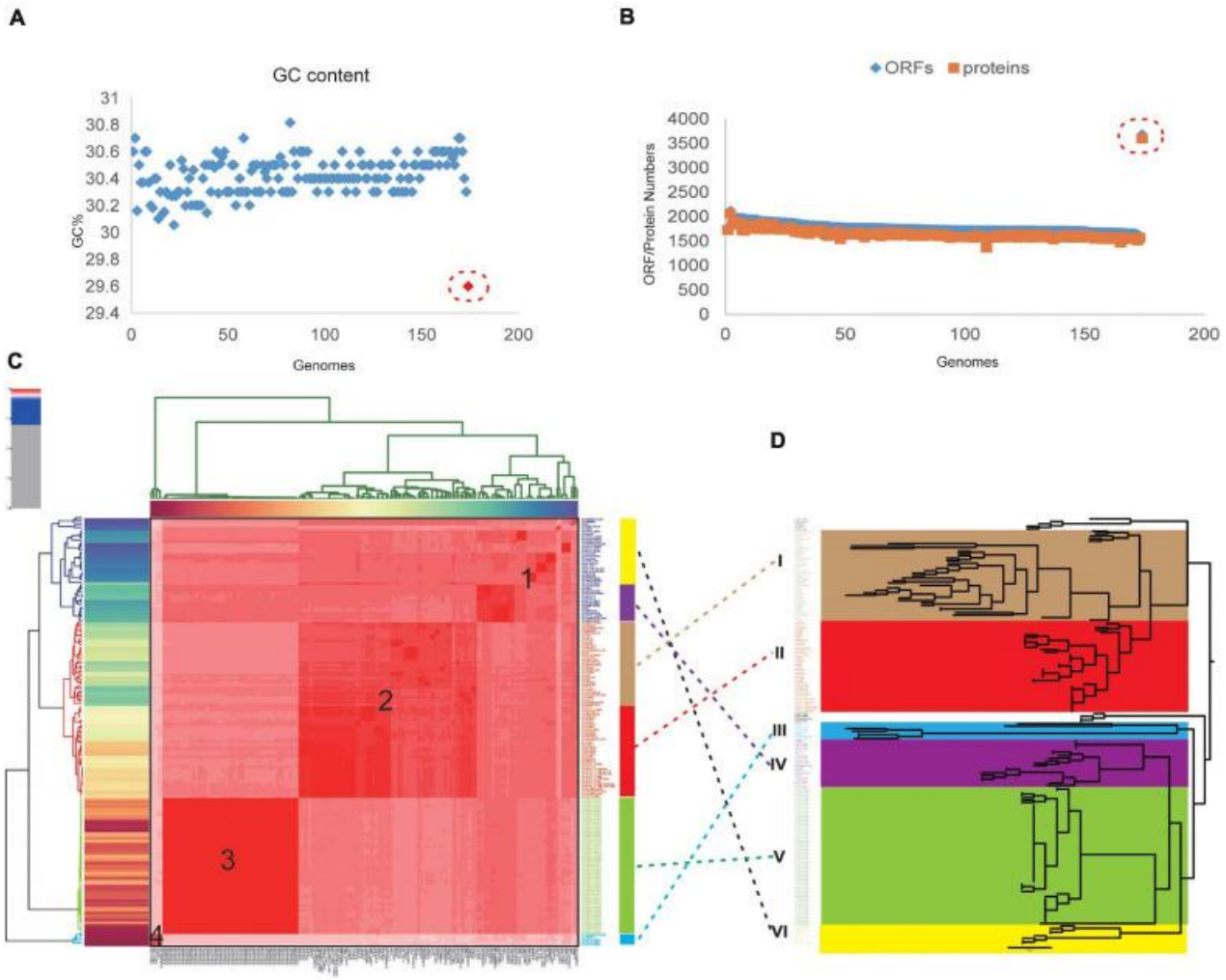
C. jejuni produces cytolethal distending toxin (CDT), which is also produced by a diverse group of other bacterial species. The toxin causes arrest at the G_1/S or G_2/M transition of the cell cycle, depending on the cell type. The active holotoxin is a tripartite complex of CdtA, CdtB and CdtC, although one study has indicated that CdtB and CdtC combined have some cytotoxicity without CdtA[1]



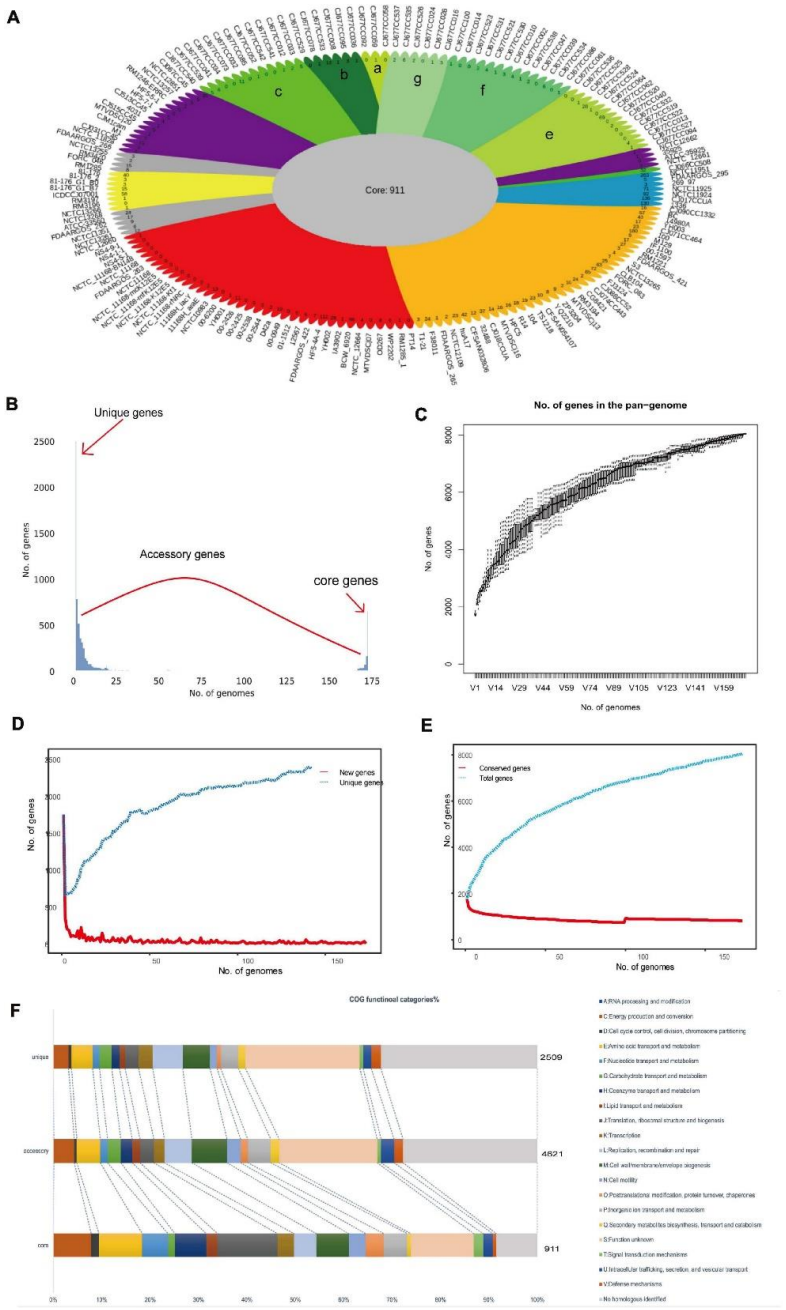
Campylobacter jejuni genomics

A total of 174 complete genome sequences of *C. jejuni* strains collected from different geographic locations and isolation sources were preliminarily analyzed. To be consistent with the genomic data, all of the sequences were annotated using the software Prokka. The correct taxonomy classification is essential for obtaining high-quality pangenomes (Wu et al., 2020).

In order to determine the taxonomic status and obtain a high-quality pangenome of *C. jejuni*, the ANI values were firstly calculated to estimate the genetic relatedness among the strains. ANI has become one of the main genome options for DNA–DNA hybridization for taxonomic purposes. The previously suggested species threshold of 95% ANI can represent the same species. We found that the ANI value of the C. jejuni strain 414 is about 91%, which is obviously different from the other 173 strains and may be an incorrect classification.



Using the whole-genome and core genome alignment concatenation approach, phylogenetic trees for the set of 173 genomes were constructed, the core genome tree could be divided into six main clades, in which nine strains were diverged independently of the other members.



Pangenome shape of *Campylobacter jejuni*.

(A) Pangenome flower plot showing the core genome and the different unique genes for each strain. *Different colors* represent the subgroups in the pangenome tree (the *colors* correspond to the different clades in the core genome tree).

(B) Gene accumulation curves for the pangenome. **(C)** Histogram of the prevalence of the different gene families in the pangenome. A total of 8,041 non-redundant gene families identified in 173 genomes are based on their frequency distribution.

(F) Distributions of the Clusters of Orthologous Genes (COG) categories in the core, accessory, and unique genes without homologs were marked in *gray*.

Characterization of the core virulence factors (VFs).
(A) Distributions of the virulence type categories of 145 VF proteins. The top three types are motility and export apparatus (*black*), colonization and immune evasion (*light blue*), and iron uptake (*brown*). **(B)** Distributions of the Clusters of Orthologous Genes (COG) types of core VFs, with the largest COGs mainly distributed in cell motility (*N*) and cell component (wall, membrane, and envelop) biogenesis (*M*), which accounted for more than 35% of the 145 core VFs. **(C)** Simulated location distributions of some core VFs based on Gene Ontology (GO) functional analysis that are mainly involved in motility, biosynthesis, metabolism, and transportation.

Core VF Estimation for Essentiality and Non-host Homologs

Essential genes are composed of the minimum set of genes required to support cell life and have greater therapeutic potentiality.

The identification of essential genes is a key step in designing therapeutic targets for bacterial infections.

Among the 145 core VFs, 94 (~65%) were predicted as essential genes. These genes are mainly involved in biological processes like ATP binding, DNA binding, and transferase and permease activities.

Afterward, the essential core VFs were aligned with the human proteome to confirm whether there is any similarity between them.

74 proteins showed hits below the threshold value and were considered as non-host homologous proteins. **These non-host homologous proteins can be preferably used for *C. jejuni* vaccine development to avoid autoimmune response or recombination and integration events in humans.**

Besides, proteins located in the periplasmic region, in outer membranes, and extracellularly are considered as effective vaccine candidates.

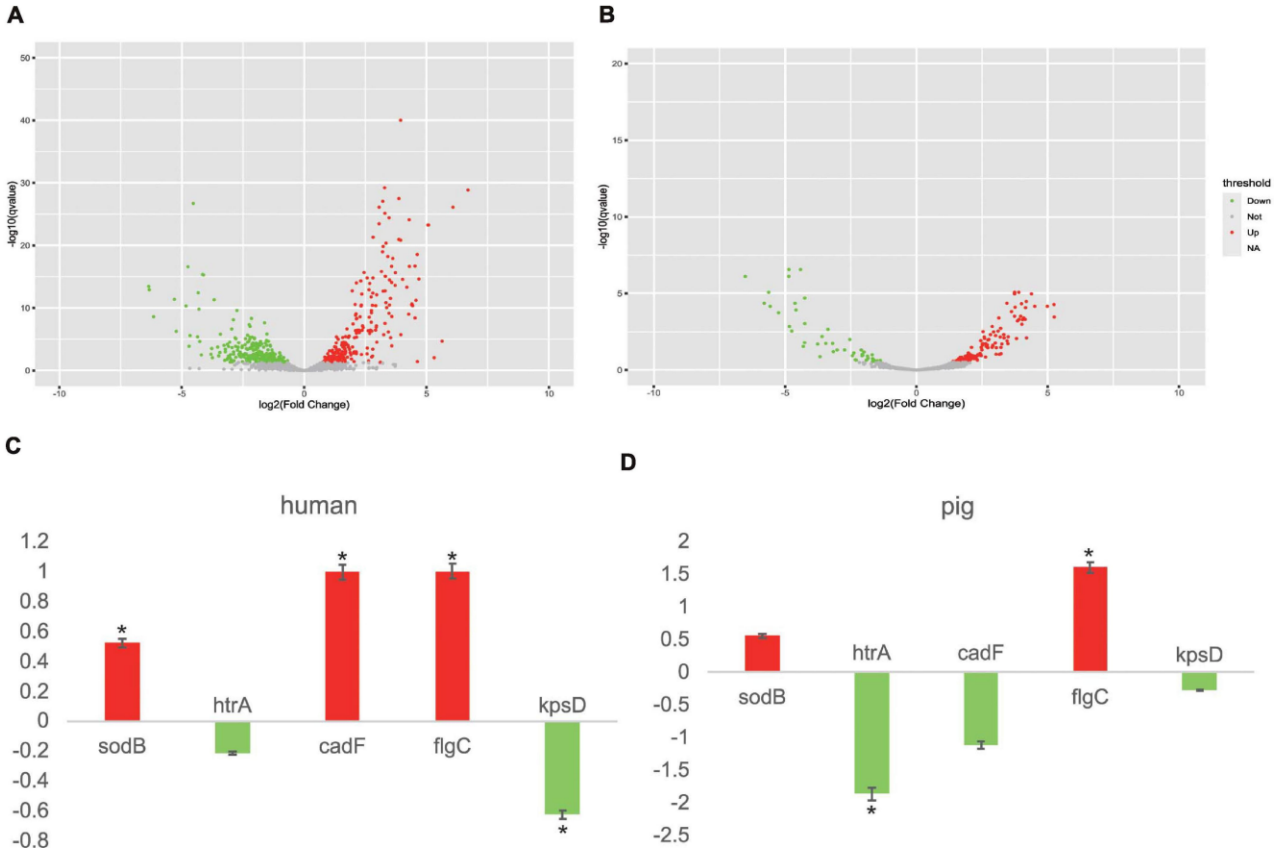
The core VF proteins for subcellular location revealed that 47 proteins were cytoplasmic, 19 were located in the cytoplasmic membrane, one was in the outer membrane, three were unknown, and four were periplasmic

It is known that outer membrane vesicles (OMVs) are a molecular complex consisting of lipopolysaccharides (LPS), outer membrane proteins, periplasmic proteins, lipids, and even cytoplasmic proteins, which are important vehicles for the simultaneous delivery of many effector molecules to host cells. Exposed proteins are often attractive targets for vaccine design, but sometimes not all proteins must be exposed to the surface, including some periplasmic proteins present in OMV preparations, which may also elicit an immunogenic response. Due to the role of OMVs in intestinal adhesion and invasion, and in regulating the dynamic interaction between host and pathogens, OMVs have become potential vaccine targets for a variety of intestinal pathogens. Therefore, the bacterial cell surface and secreted proteins, usually located in the extracellular, periplasmic, and outer membranes, could be more effective as vaccine candidates or diagnostic targets

Protein name	Location	PsortB score	TMHMM prediction	Molecular weight (kDa)	VaxiJen score	VaxiJen prediction
SodB	Periplasmic	9.44	Outside	24.81	0.5003	Probable antigen
FlgC	Periplasmic	9.44	Outside	18.30	0.4831	Probable antigen
HtrA	Periplasmic	9.76	Outside	51.01	0.5379	Probable antigen
KpsD	Periplasmic	9.44	Outside	60.84	0.4261	Probable antigen
CadF	Outer membrane	10	Outside	36.00	0.8043	Probable antigen

***Campylobacter jejuni* transcriptome analysis in human INT 407 and Caco-2 cells and the pig intestinal loop.**

the expression levels of 126 genes, including the 25 core VFs (which include *sodB*, *cadF*, and *flgC*) were increased and the expression levels of 148 genes (including 13 core VFs) were decreased under human immune stress.



For the five selected proteins, nearly all of them had an apparent differential to the stress in human and pig.
As well, the results in the pig ligated intestinal loop model showed that the expression levels of 33 core VFs, including *flgC*, have been increased and those of 23 core VFs, including *htrA*, have been decreased.

The oxidative stress response genes and the iron acquisition genes, including the potential vaccine targets *htrA*, *sodB*, and other core VFs such as *chuA*, *chuB*, and *chuD*, were expected to be decreased due to the intestinal mucus in the intestinal loop of pig.

This study found that the increased core VFs were mainly associated with the motility- and flagellar-related genes in both human and pigs and that the decreased core VFs were mainly related to iron transport system proteins.

These results indicate that the flagellar genes are important VFs, which are essential for *C. jejuni* motility and the secretion of virulence proteins. The differences in the gene expressions could be caused by the different transcriptional responses by different hosts or the need for a certain reaction time after infection.

The candidate proteins found in this study may be efficient vaccine targets both in human and other animals. With the development of more animal models, these core VFs can provide abundant gene resources, which may be beneficial to the study of the virulence mechanisms of *C. jejuni*