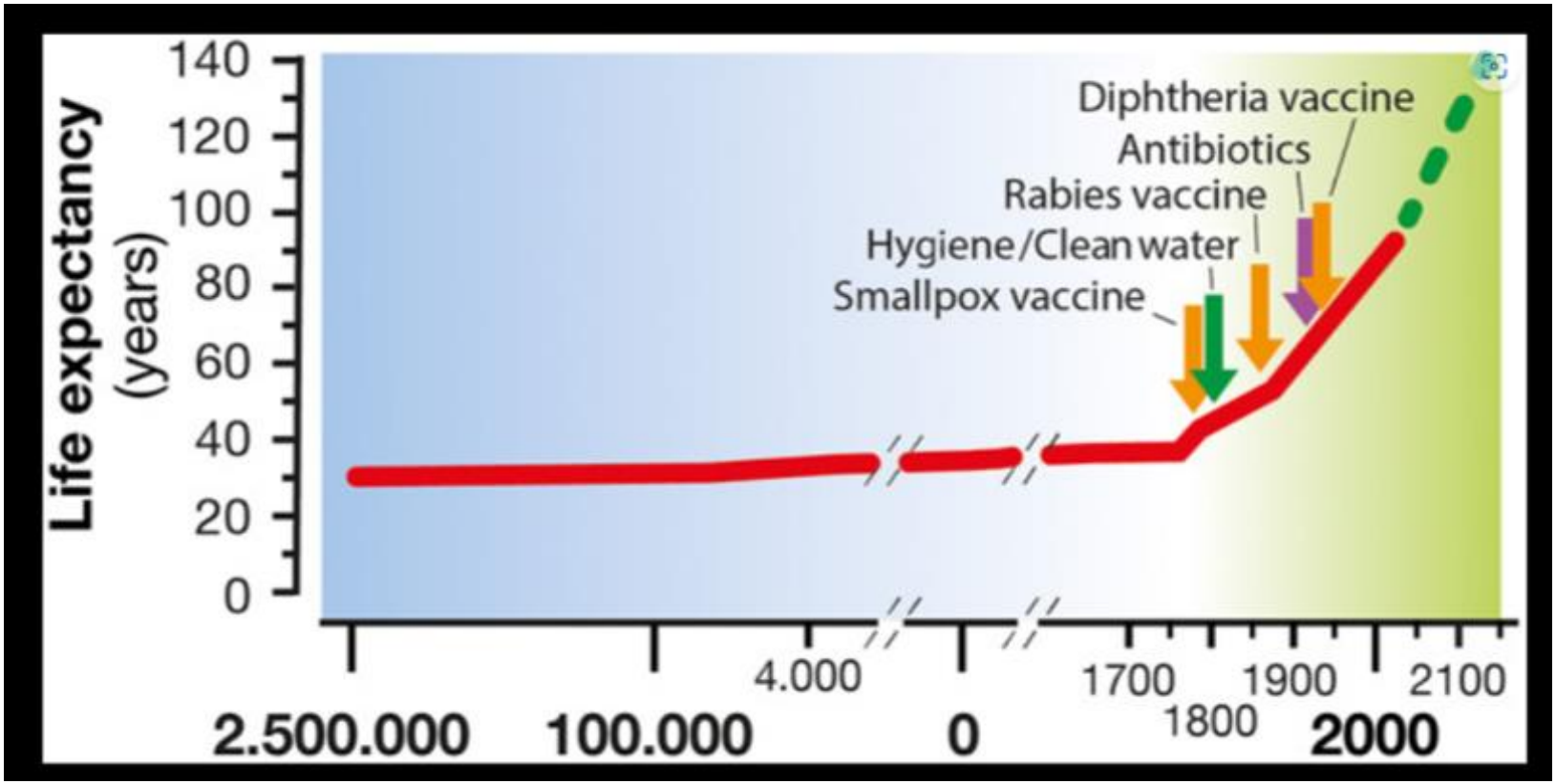
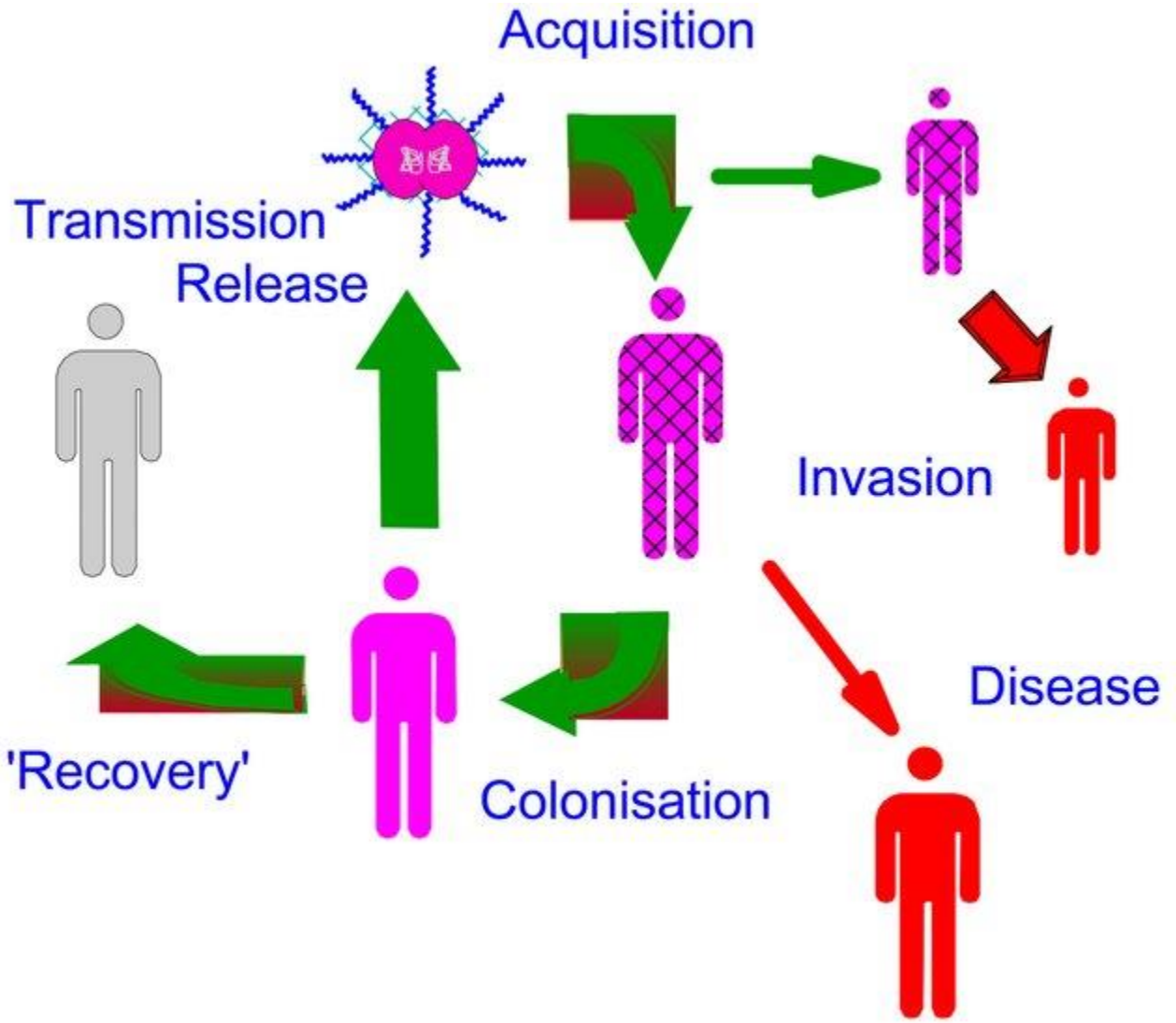
Vaccine against bacterial infections

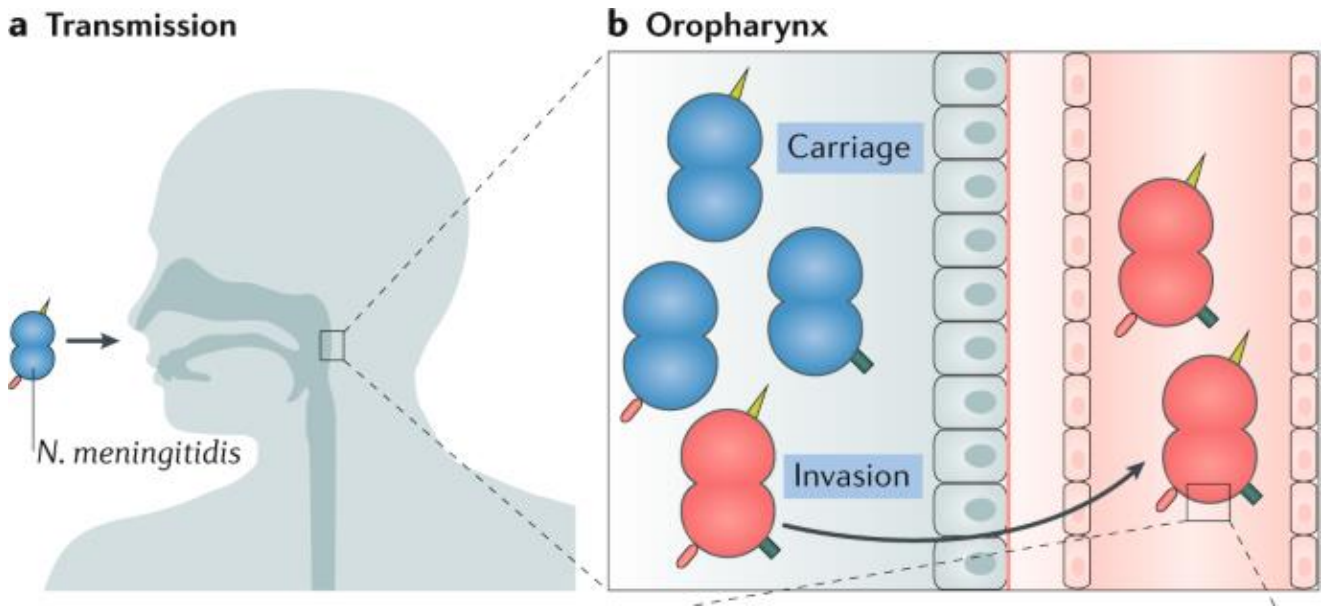


Rosini R, Nicchi S, Pizza M, Rappuoli R. Vaccines Against Antimicrobial Resistance. Front Immunol. 2020 Jun 3;11:1048. doi: 10.3389/fimmu.2020.01048.

Transmission of *Neisseria meningitidis* (Meningococcus, Men) in the population



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.

Neisseria meningitidis (Meningococcus, Men) vaccination

Capsule polysaccharide (CPS) vaccines

The earliest attempts to develop Men vaccines were made from 1900 to 1940, in response to epidemic disease and increased infection rates occurring during both World Wars. These vaccines were heat-killed whole bacteria preparations, which ultimately proved unsuccessful due to poorly controlled clinical studies, questions over the nature of the immunity conferred and notably because of excess reactogenicity, in hindsight likely due to the presence of large amounts of lipooligosaccharide (LOS).

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

The availability of antibiotics became significant for successfully treating bacterial meningitis from WWII onwards, such that meningococcal vaccine research languished until antibiotic resistance, in particular to sulphonamides, was observed in meningococci (Miller, Siess and Feldman 1963)

The role of the polysaccharide capsule for pathogenesis

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

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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

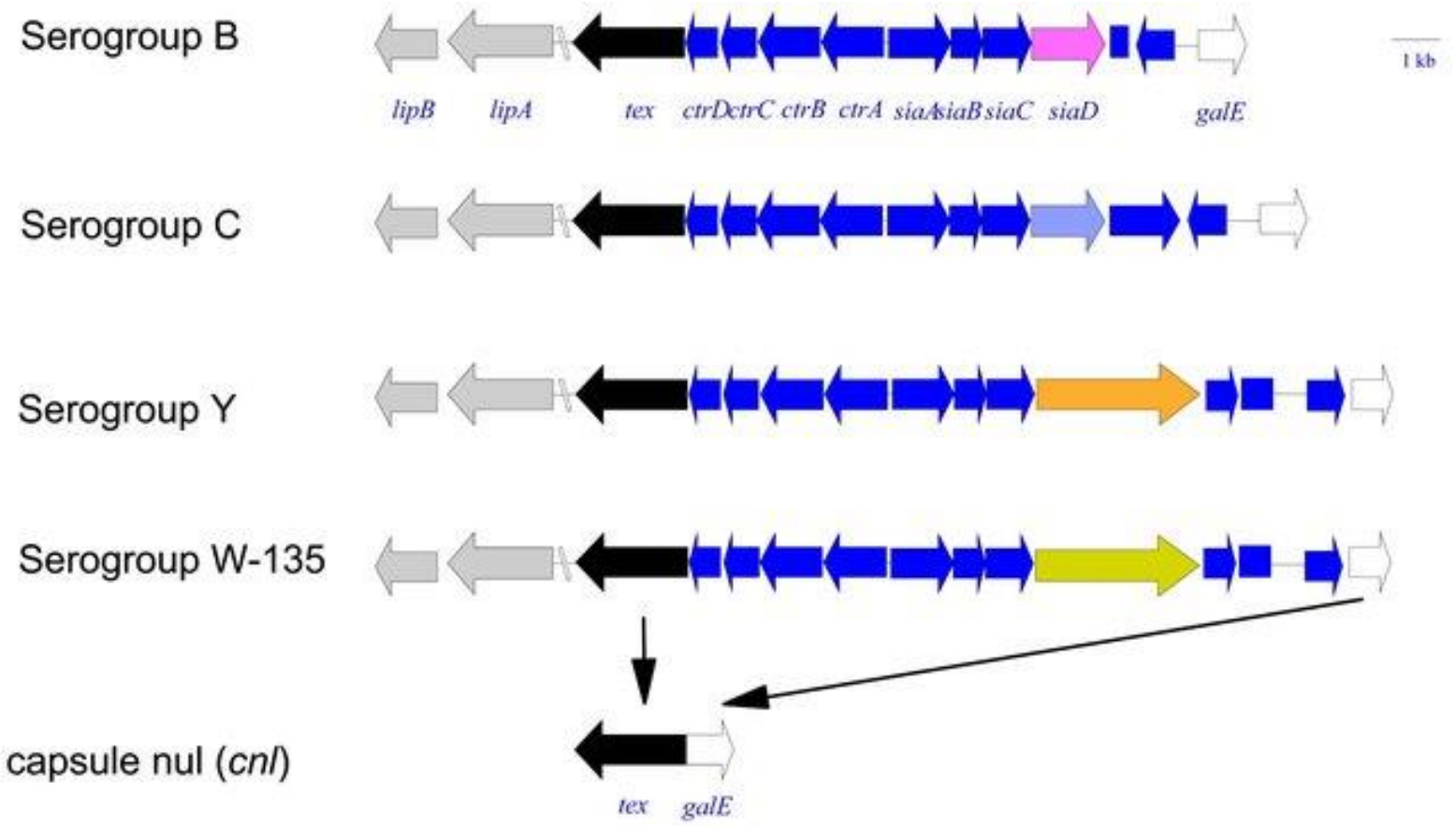
ABSTRACT

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 and colleagues published in 1969 Men vaccine development. These cardinal studies described the phenomena of age-related immunity to the meningococcus; that intermittent carriage of different strains of meningococci throughout life initiates, reinforces and broadens natural immunity to meningococcal disease; that susceptibility to disease correlates with low levels of serum antibodies with bactericidal activity (SBA) to the pathogen.
The only high-molecular weight CPS (>100 000 Da), produced by precipitation with the cationic detergent Cetavlon (Gotschlich, Liu and Artenstein 1969), reliably induced antibody responses in humans.
These studies culminated in the classic vaccination trial of a purified MenC CPS at the military training camps at Fort Dix, New Jersey.

The large scale field trials in 28 245 recruits that followed in 1969–1970 showed a vaccine-protective effect of 89.5% against MenC disease (Artenstein *et al.*1970; Gold and Artenstein 1971).
Significantly, these studies have provided the basis for using SBA as the 'serological correlate of protection' for all subsequent meningococcal vaccines based on CPS and non-capsular antigens.
Plain CPS vaccines have been licensed since the 1970s, as mono-, bi-, tri- and tetravalent vaccines, in various formulations containing MenA, MenC, MenW and MenY CPS.

Diagrammatic representation of the CPS region in meningococci that express sialic acid containing capsules.



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

Perhaps the most outstanding example of the impact of Men CPS conjugate vaccines on disease is the introduction of an affordable MenA conjugate vaccine MenAfriVac (Frasch, Preziosi and LaForce 2012; Tiffay et al.2015) to **Saharan and Sub-Saharan countries of Africa, which has led to virtual elimination of MenA disease in those countries with immunisation programmes** (Djingarey et al.2015).

MenAfriVac successfully reduces nasopharyngeal carriage and generates herd protection.

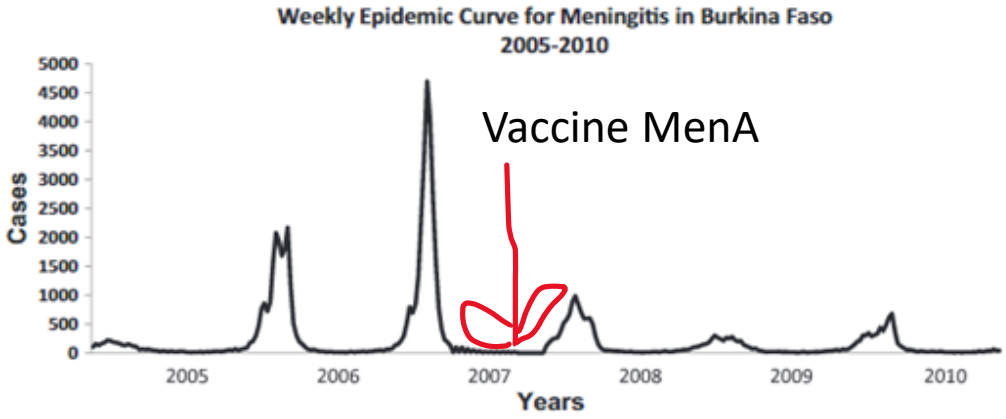
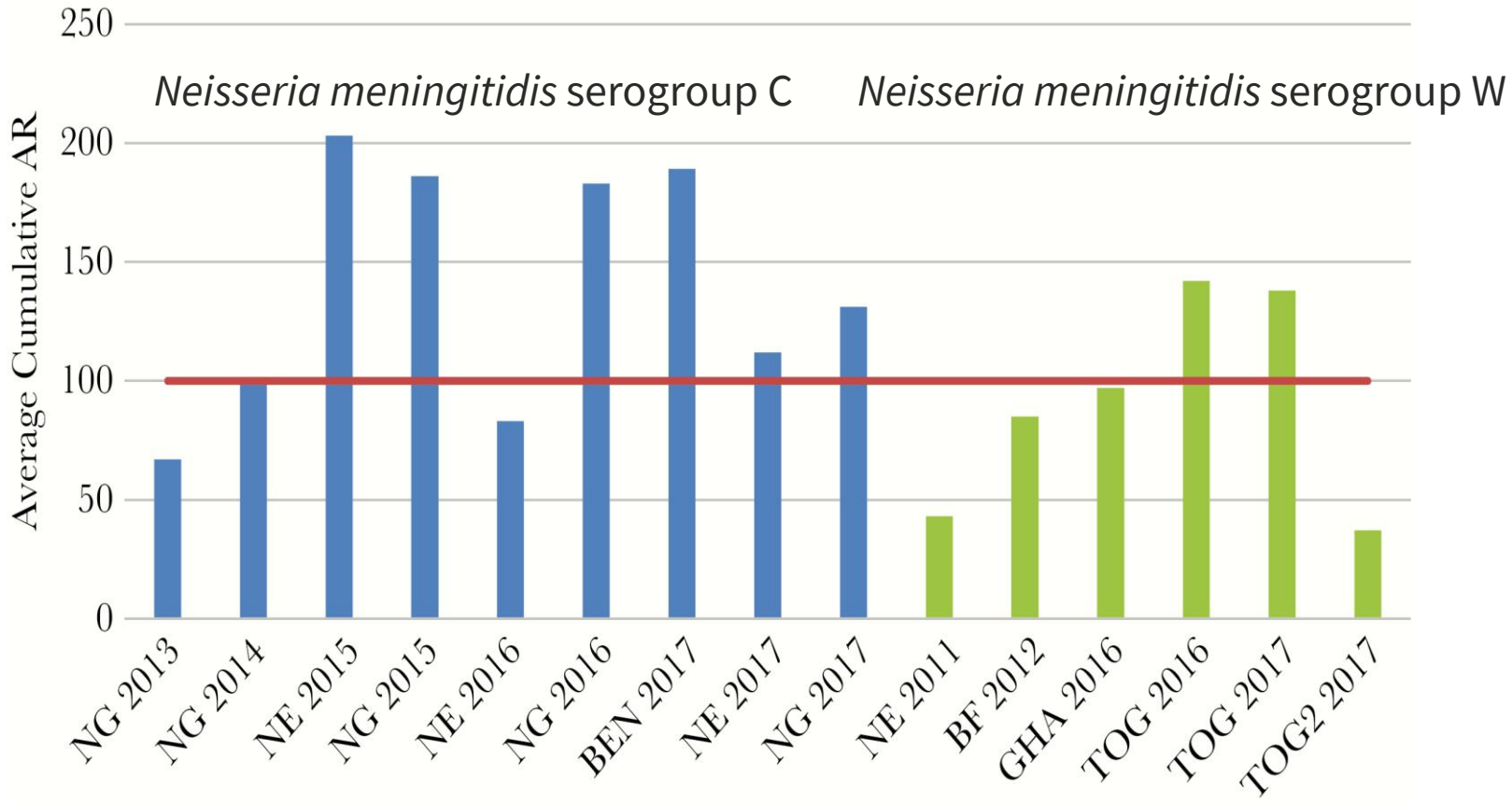


Fig. 1. Weekly reported meningitis cases – Burkina Faso 2005–2010.

Figure 1. Cumulative attack rates per outbreak (district average), stratified by serogroup, excluding special situations outbreaks in Cameroon and Ethiopia. *Neisseria meningitidis* serogroup C outbreaks are shown in blue and *N. meningitidis* W outbreaks in green. Red line indicates epidemic criterion. Abbreviations: AR, attack rate; BEN, Benin; BF, Burkina Faso; GHA, Ghana; NE, Niger; NG, Nigeria; TOG, Togo.



J Infect Dis, Volume 220, Issue Supplement_4, 1 December 2019, Pages S225–S232, https://doi.org/10.1093/infdis/jiz355

Plain CPS vaccines have been licensed since the 1970s, as mono-, bi-, tri- and tetravalent vaccines, in various formulations containing MenA, MenC, MenW and MenY CPS.
Plain CPS vaccines have been used successfully to vaccinate specific groups at risk from meningococcal infection and to control MenA epidemics in the Sub-Saharan African 'Meningitis Belt',

Then it followed the development and widespread use of CPS conjugate vaccines (Gasparini and Panatto 2011).

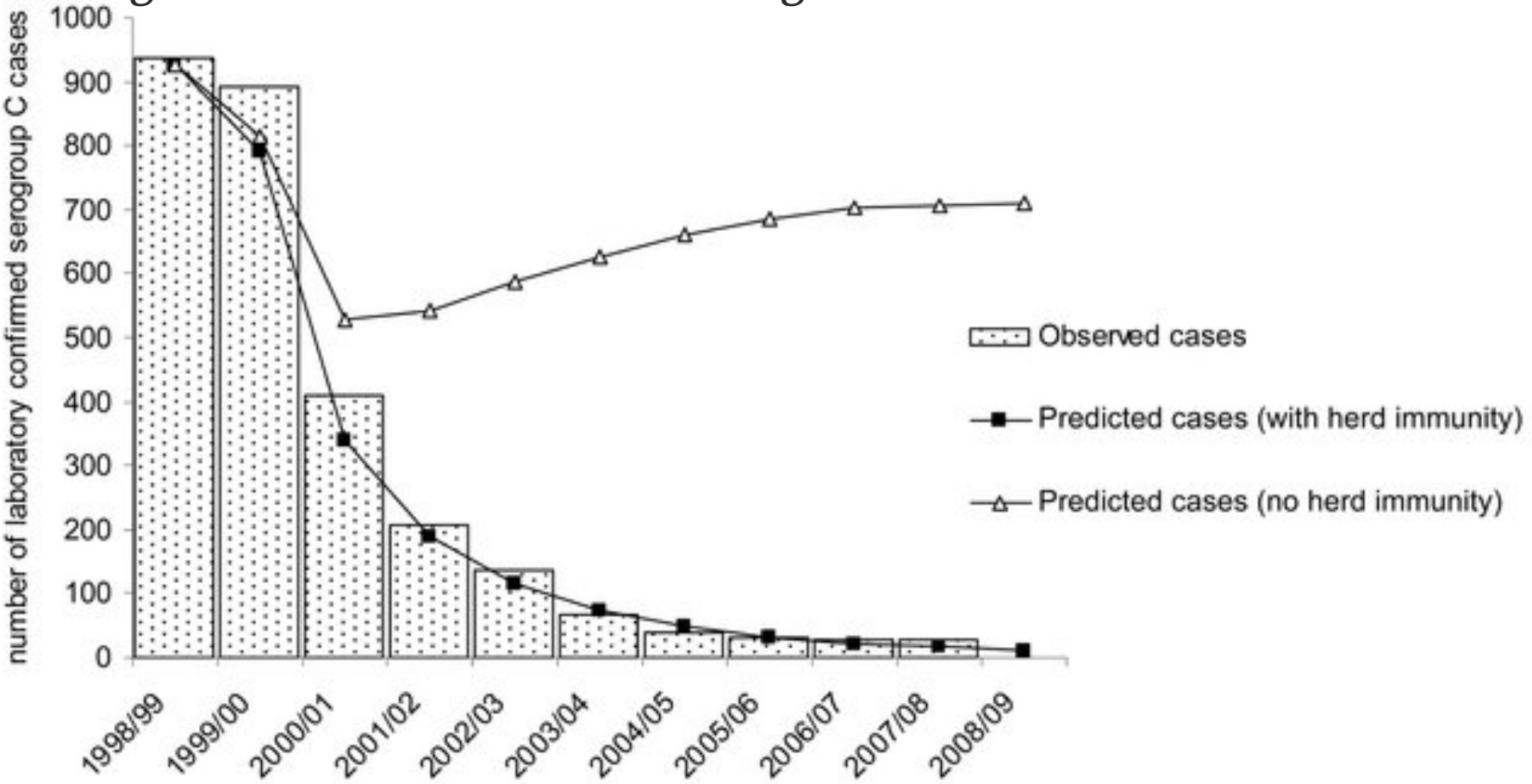
Conjugation of a bacterial CPS to a protein induces stronger antibody responses to the carbohydrate moiety than the corresponding plain CPS.

.....Then Men conjugate vaccines
The role of the adjuvants

What is an adjuvant and why is it added to a vaccine?
An adjuvant is an ingredient used in some vaccines that helps create a stronger immune response in people receiving the vaccine. In other words, adjuvants help vaccines work better. Some vaccines that are made from weakened or killed germs contain naturally occurring adjuvants and help the body produce a strong protective immune response.

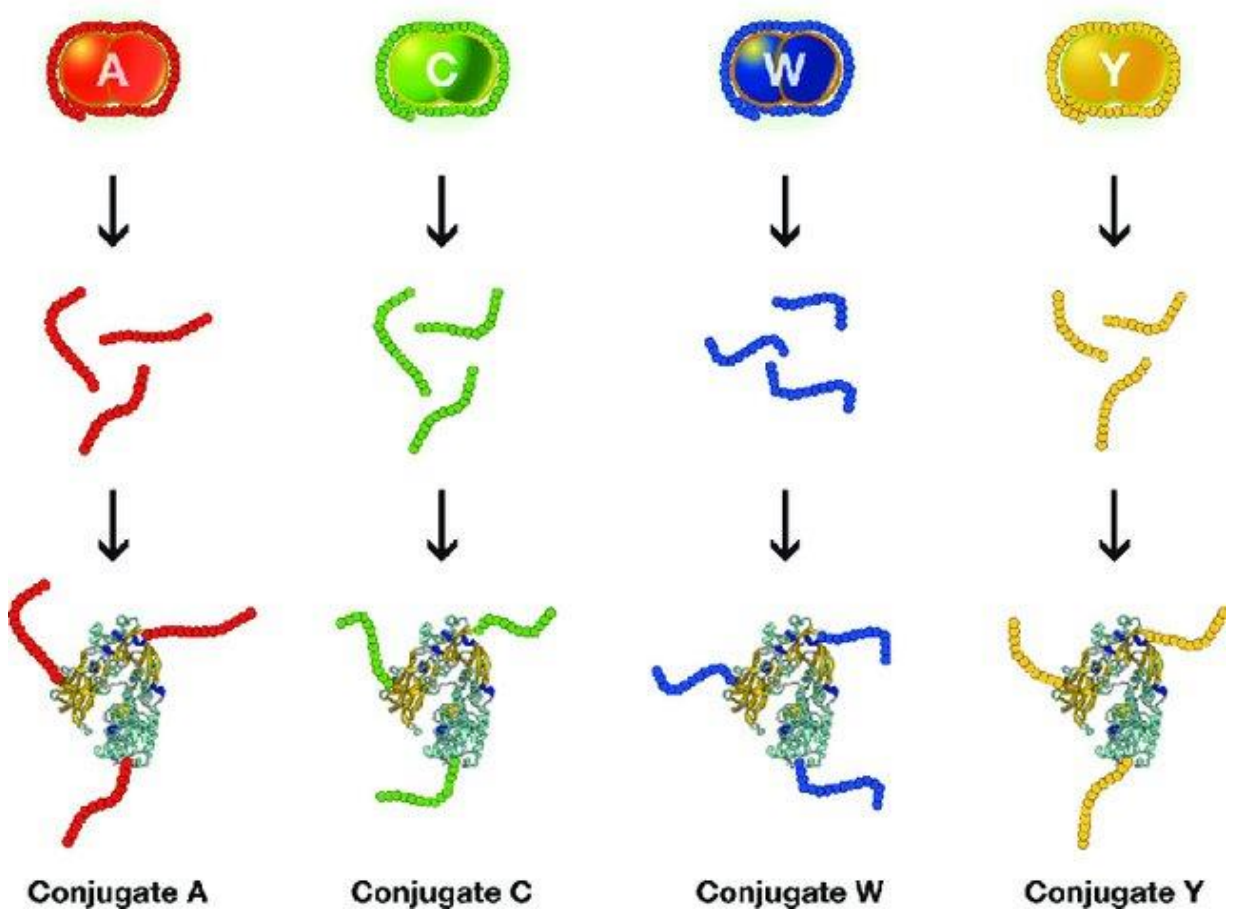
Adjuvant	Composition	Vaccines
Aluminum	One or more of the following: amorphous aluminum hydroxyphosphate sulfate (AAHS), aluminum hydroxide, aluminum phosphate, potassium aluminum sulfate (Alum)	Anthrax, DT, DTaP (Daptacel), DTaP (Infanrix), DTaP-HepB-IPV (Pediarix), DTaP-IPV (Kinrix), DTaP-IPV (Quadracel), 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)

Highly immunogenic proteins including diphtheria and tetanus toxoids and the non-toxic diphtheria toxin variant CRM^{197} have been conjugated to different Men CPS to produce licensed glycoconjugate vaccines. The first meningococcal conjugate vaccine to be used was a MenC conjugate vaccine (MCC) for infants, toddlers and teenagers, introduced into the UK immunisation programme in 1999, in response to ST-11 MenC outbreaks occurring in adolescents and older teenagers



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

Meningococcal quadravalent CPS conjugate vaccines that include MenA, MenC, MenW and MenY CPS have subsequently been developed and licensed (Dull and McIntosh 2012; Vella and Pace 2015). Recently, a MenACWY CPS conjugate vaccine was strongly recommended for vaccination of teenagers in the UK in response to increases in cases of infection caused by MenY and in particular by a hypervirulent clone of MenW (Ladhani et al.2016a).

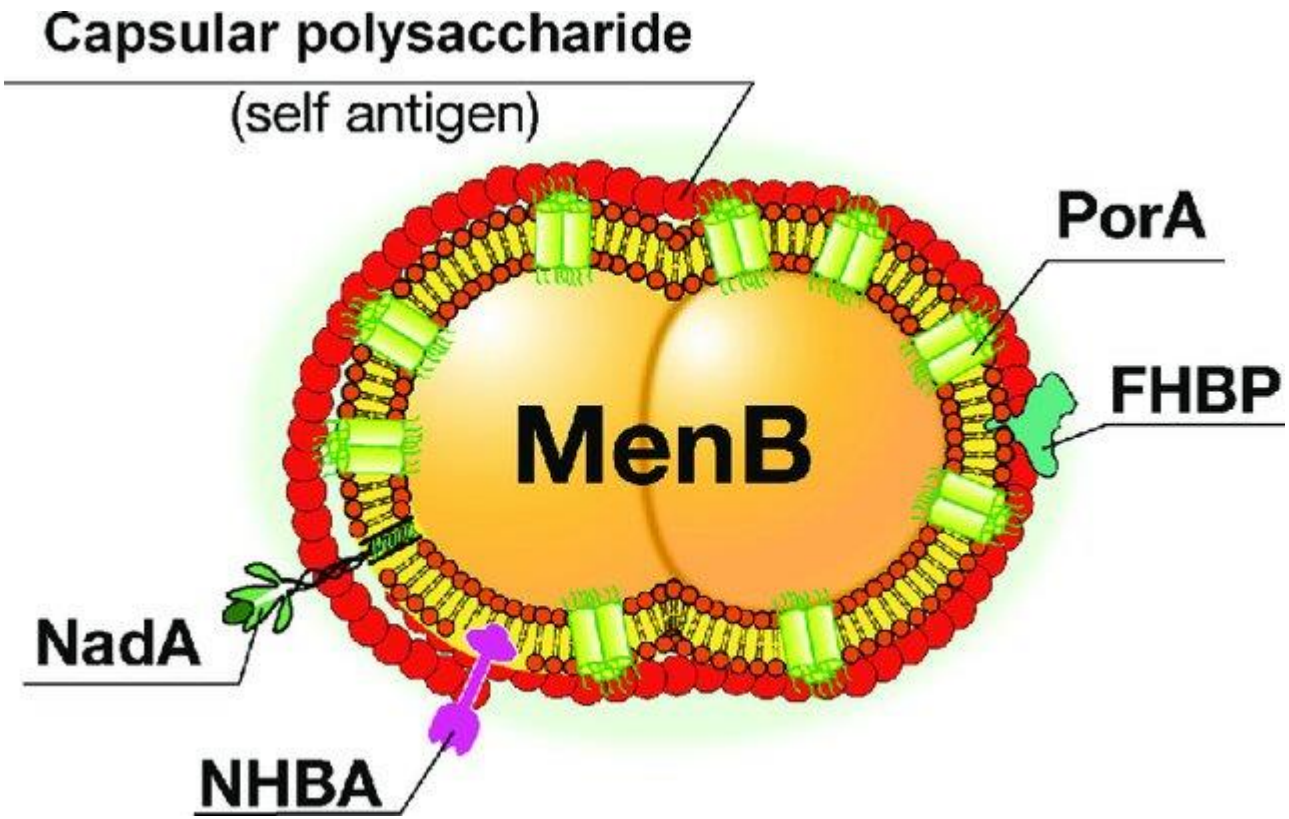


The CPS vaccine approach has not been successful for MenB

Plain MenB CPS was tested in several hundred volunteers, but few individuals showed an antibody response (Wyle et al.1972).
Antibody to MenB CPS has been reported to be an effective opsonin, but a poor bactericidal antibody in a chick embryo model of meningococcal challenge (Frasch et al.1976).

The reluctance to develop MenB CPS vaccines is based on the observation that **the α(2-8) N-acetyl-neuraminic acid linked homopolymer of the polysaccharide is structurally identical to a modification of mammalian neural cell adhesion molecule** (Toikka et al.1998) and thus, **vaccines may induce autoimmune antibodies that potentially cross-react with fetal brain tissue.**

Since the CPS vaccine strategy has been rejected for preventing MenB disease, research has focused intensively on using non-CPS components as potential MenB vaccines.



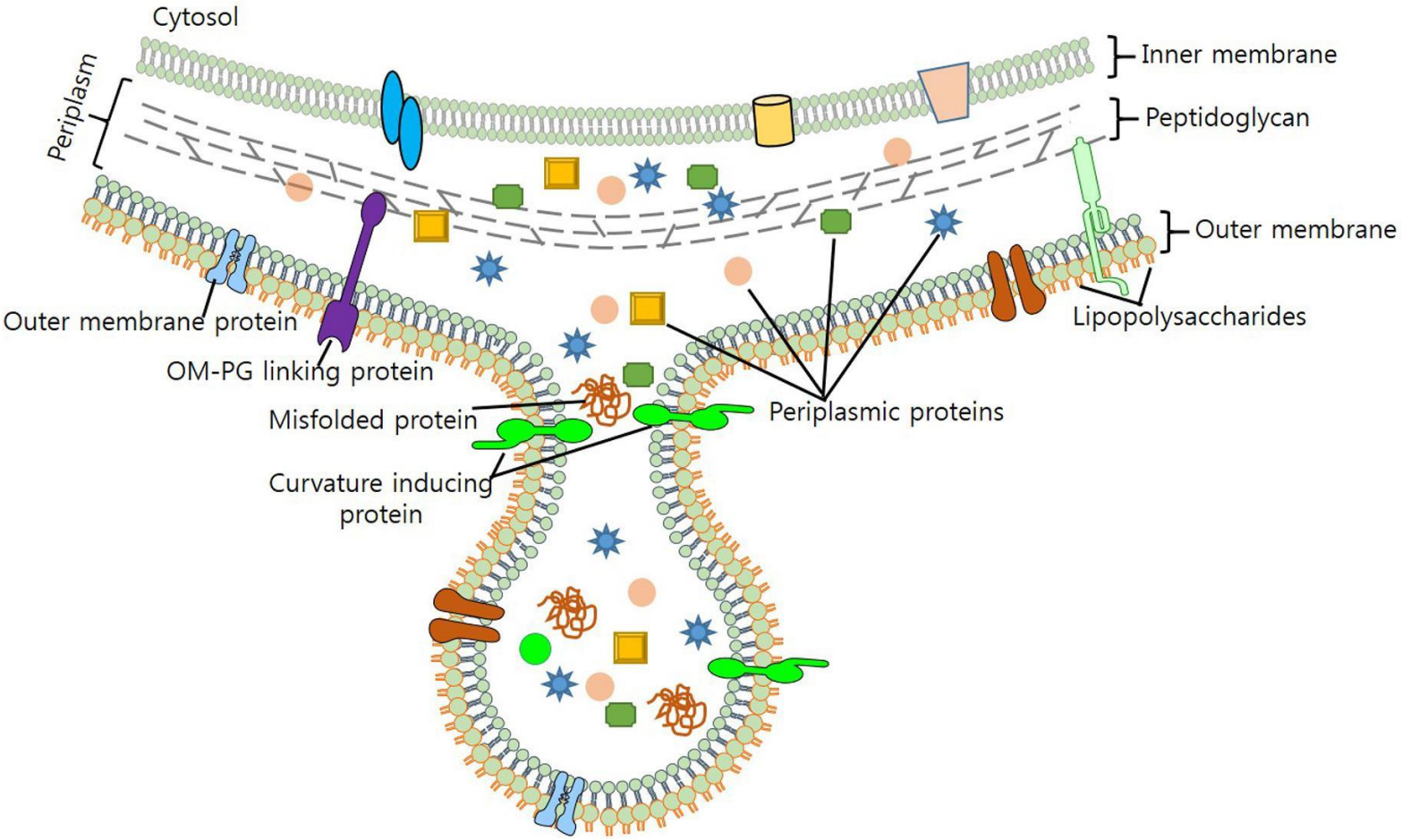
The challenge of developing universal vaccines, August 2011

•F1000 Medicine Reports 3(1):16

Outer Membrane Vesicles (OMVs) of Gram-negative bacteria

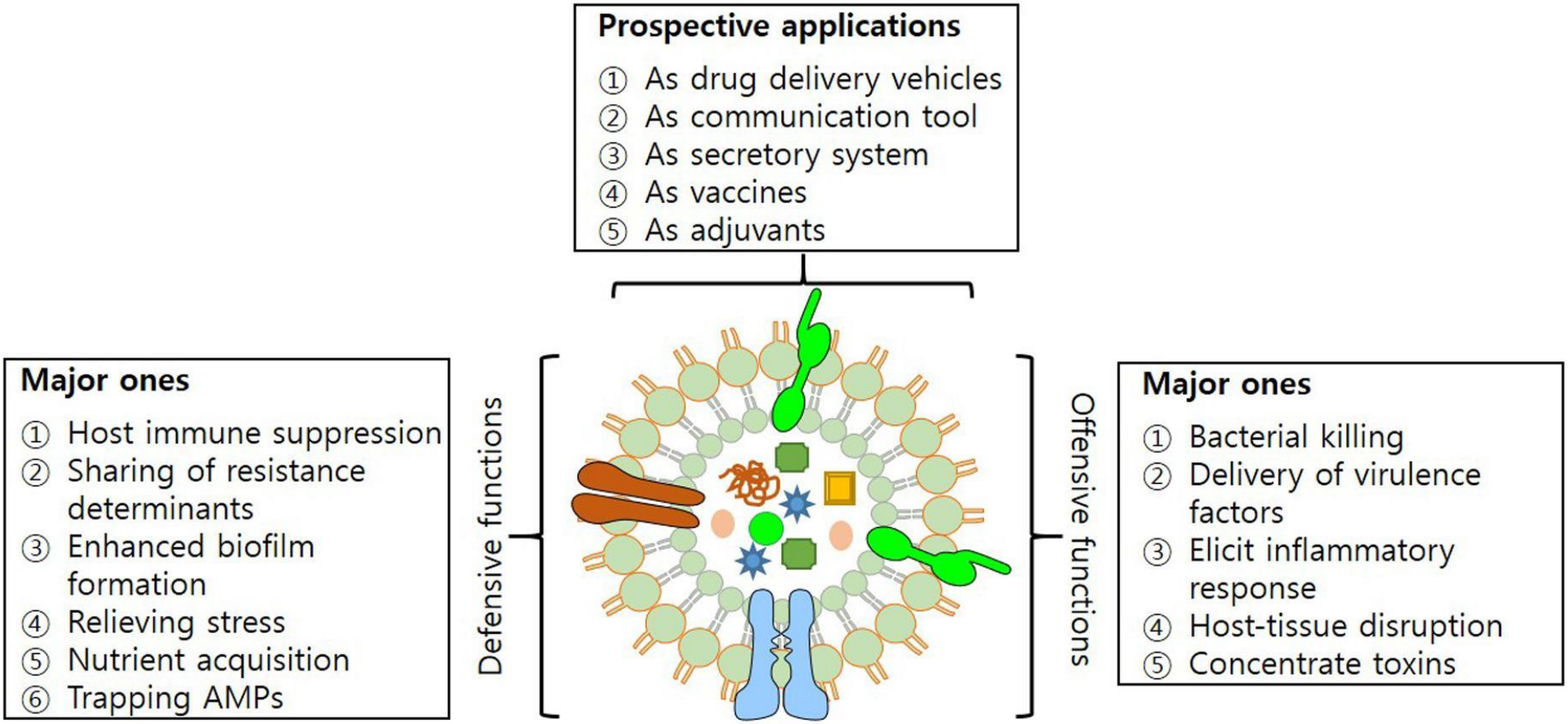
OMVs of Gram-negative bacteria are spherical membrane-enclosed entities of endocytic origin. Reported in the consortia of different bacterial species, production of OMVs into extracellular milieu seems essential for their survival. Enriched with bioactive proteins, toxins, and virulence factors, OMVs play a critical role in the bacteria-bacteria and bacteria-host interactions. Emergence of OMVs as distinct cellular entities helps bacteria in adaptating to diverse niches, in competing with other bacteria to protect members of producer species and more importantly play a crucial role in host-pathogen interaction.

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

Figure illustrating offensive and defensive roles of OMVs utilized in bacteria-bacteria and bacteria-host interactions; and their potential applications.



Study of the OMV production under natural and diverse stress conditions has broadened the horizons, and also opened new frontiers in delineating the molecular machinery involved in disease pathogenesis. Playing diverse biological and pathophysiological functions, OMVs hold a great promise in enabling resurgence of bacterial diseases, in concomitance with the steep decline in the efficiency of antibiotics. Having multifaceted role, their emergence as a causative agent for a series of infectious diseases increases the probability **for their exploitation in the development of effective diagnostic tools and as vaccines against diverse pathogenic species of Gram-negative origin**

For vaccines three types of OMV are defined:

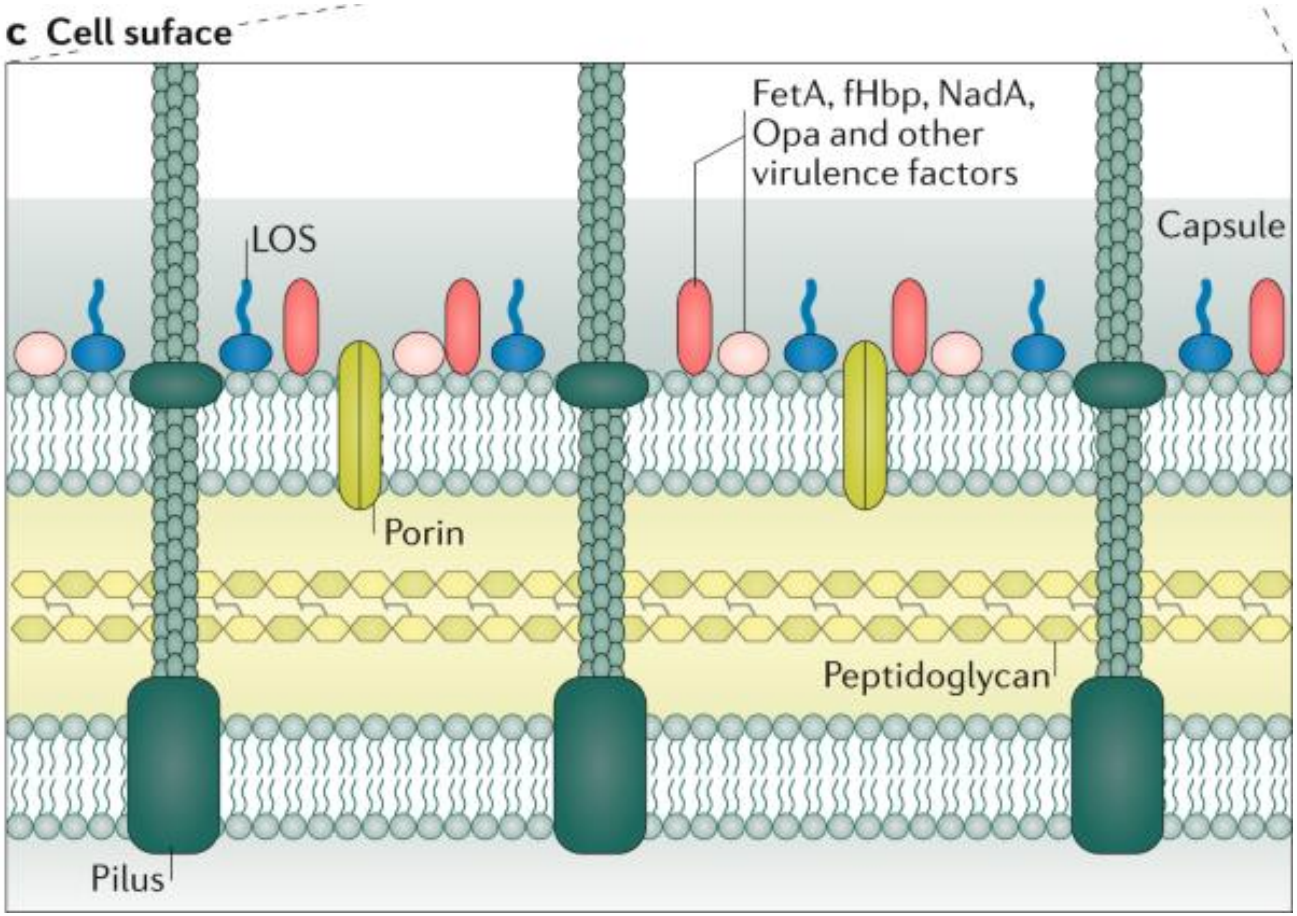
- Naturally shed OMV are native '(n)OMV';
- OMV treated with sodium deoxycholate (NaDOC) and used as vaccines in humans are defined as 'OMV vaccines';
- OMV that are modified by genetic manipulation (with or without NaDOC treatment) are defined as modified '(m)OMV'

The hypothesis for using OMV as vaccines led to the development of two OMV vaccines: VA-MENGOCOC-BC™, developed at the Finlay Institute in Cuba, and MenBvac™ at the Norwegian Institute of Public Health (Holst *et al.*2013). The vaccines were developed from the homologous clonal strains of MenB and used to successfully control ongoing epidemics in both countries.

Various methods are used to manufacture these OMV vaccines (Frasch *et al.*2001; Holst *et al.*2009) including growth under iron (Banerjee-Bhatnagar and Frasch 1990) or zinc-limiting conditions (Stork *et al.*2010) to induce expression of regulated proteins, but extraction with **NaDOC (0.5%) is the common and key step to reduce LOS content.**

Given the complexity of OMV vaccines, the question to be asked is which antigens are directing protective immune responses?

Studies of sera from the Norwegian vaccine trial using western blot showed that antibodies to **the serosubtype-specific PorA protein and Opc protein made the most important single contributions to bactericidal activity against the vaccine strain**



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.

Examples of strategies for identifying potential meningococcal vaccine antigens

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

MenB protein-based vaccines

Whole genome sequencing paved the way for new approaches to develop meningococcal vaccines by providing information about the complete proteome from which vaccine candidates could be selected by using bioinformatic algorithms to identify putative protective antigens.

This novel *in silico* approach was termed genome- and proteome-based 'reverse vaccinology' (RV) and was first described by Rino Rappuoli and colleagues in 2000 (Rappuoli 2001) **for the discovery of potential antigens in the MenB strain MC58 and led to the development of the Novartis vaccine 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

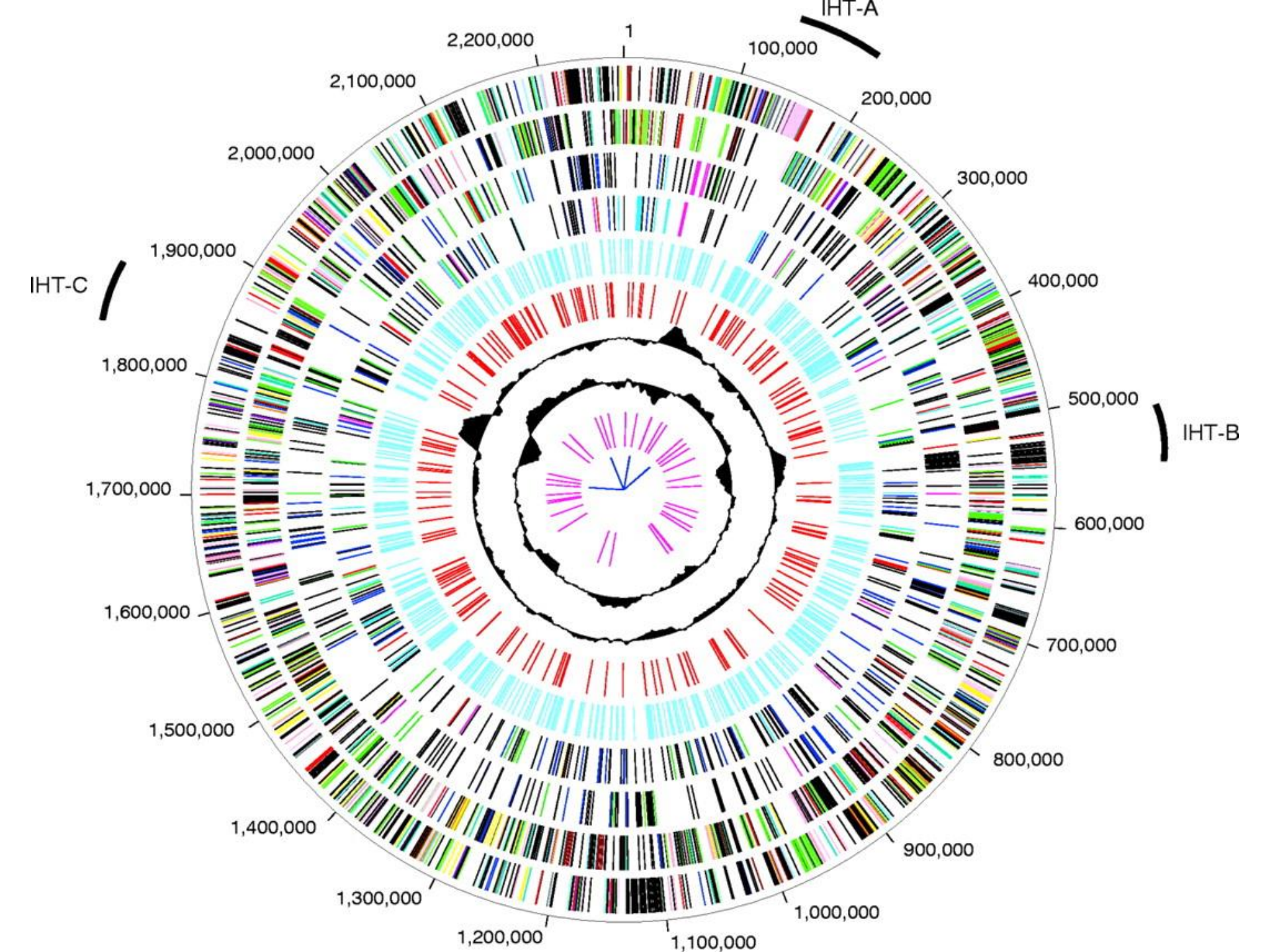
Affiliations + expand

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

https://pubmed.ncbi.nlm.nih.gov/?term=White+O&cauthor_id=10710307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IHT-B
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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.

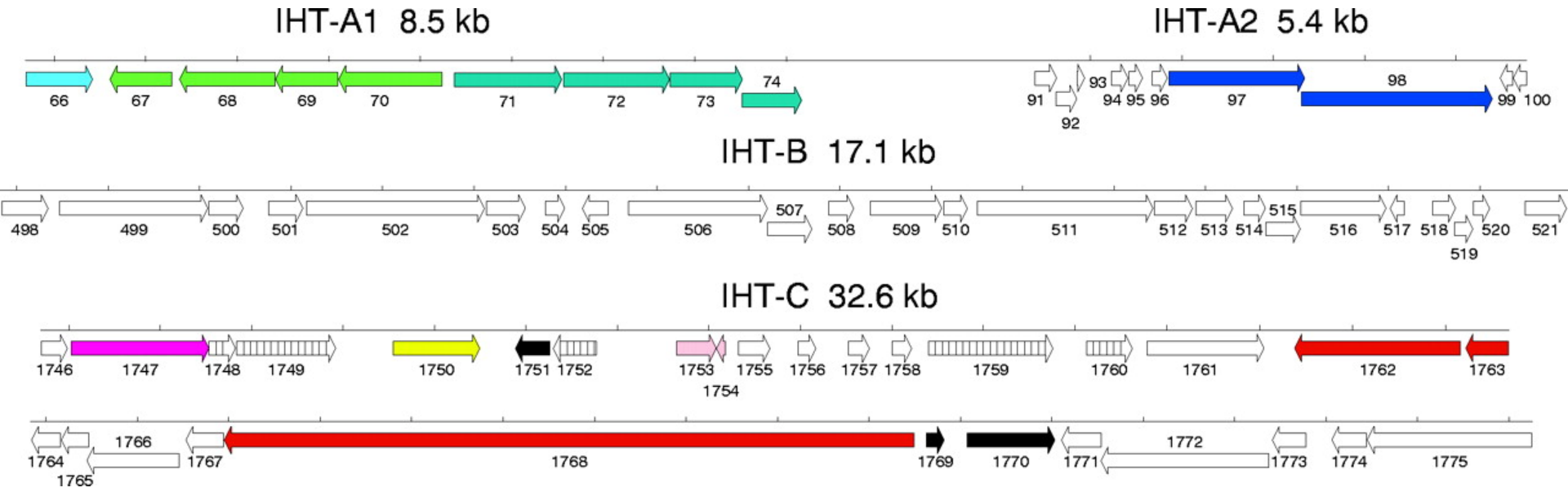
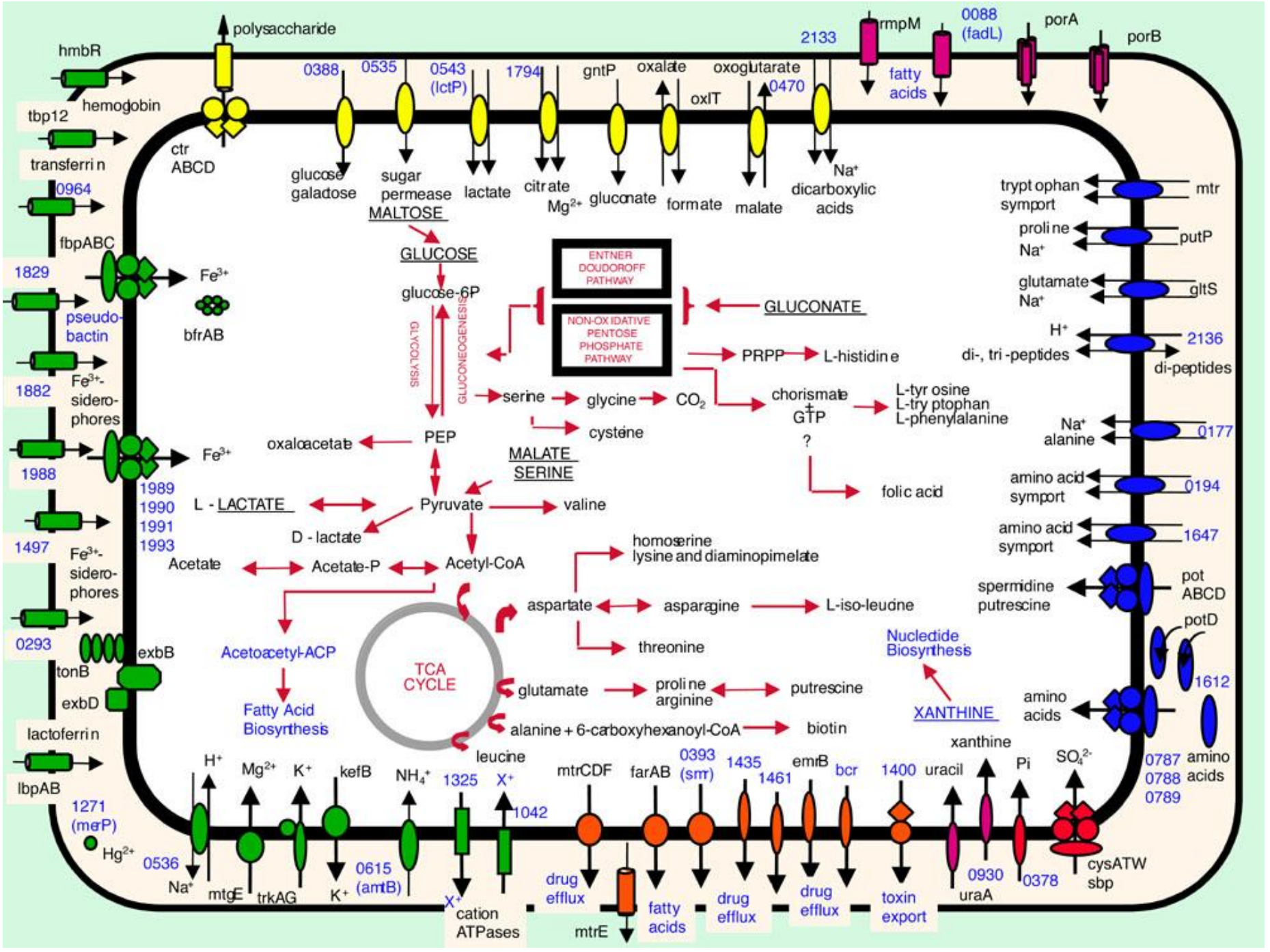


Figure 3 Structure of the putative islands of horizontally tranferred DNA (IHTs) in the N. meningitidis strain MC58 genome. Empty boxes are hypothetical proteins and striped boxes are conserved hypothetical proteins.

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
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1990
1991
1993
0388
0535
0543 (lctP)
1794
gntP
oxalate
oxoglutarate
oxlT
0470
2133
rmpM
fatty acids
0088 (fadL)
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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
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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
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putP
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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

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REPORTS

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

10 Mar 2000
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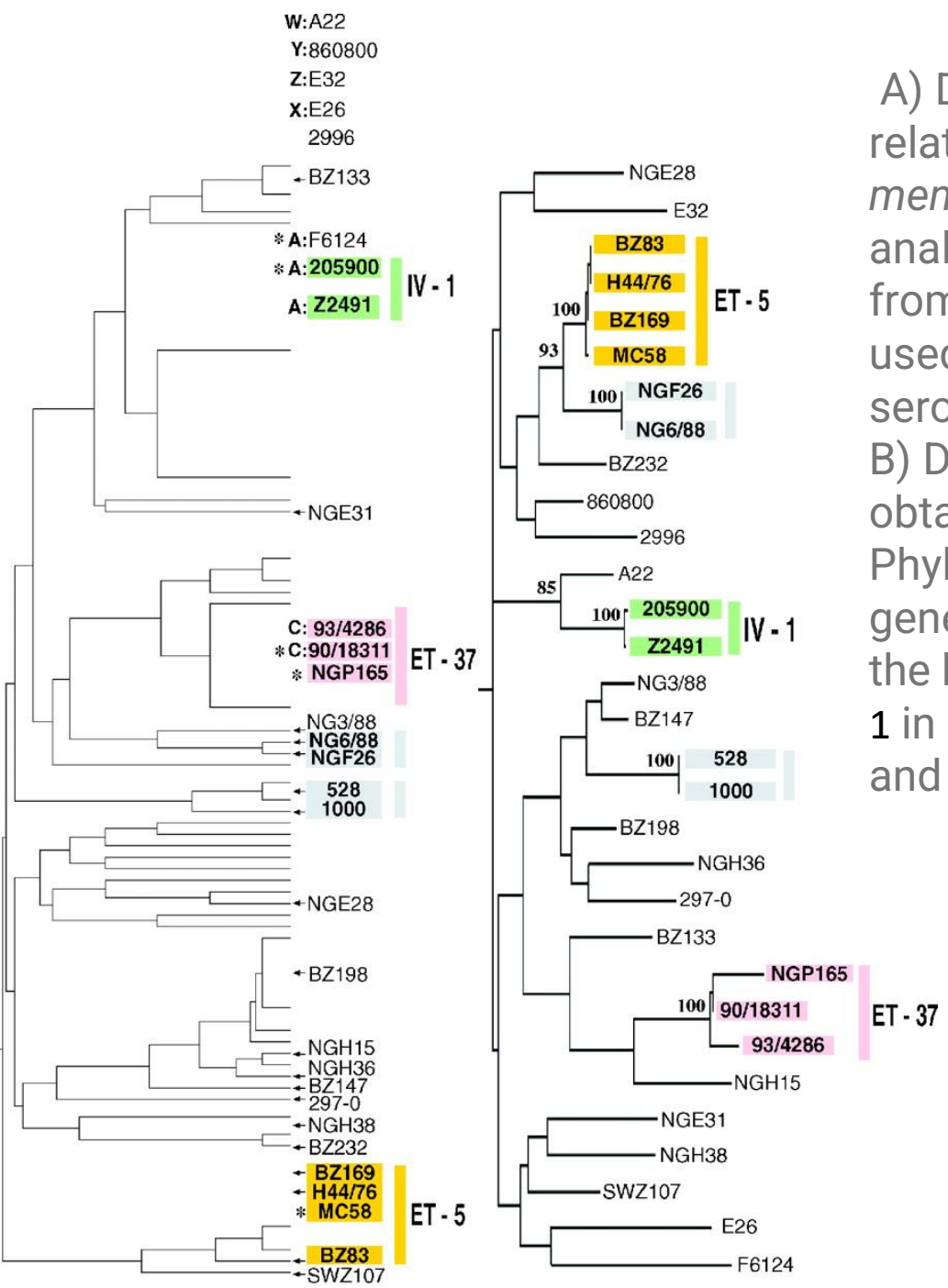
To identify potential vaccine candidates, we determined the genome sequence of the virulent strain MC58 [see (11)]. While the sequencing project was in progress, unassembled DNA fragments were analyzed to identify open reading frames (ORFs) that potentially encoded novel surface-exposed or exported proteins (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%).

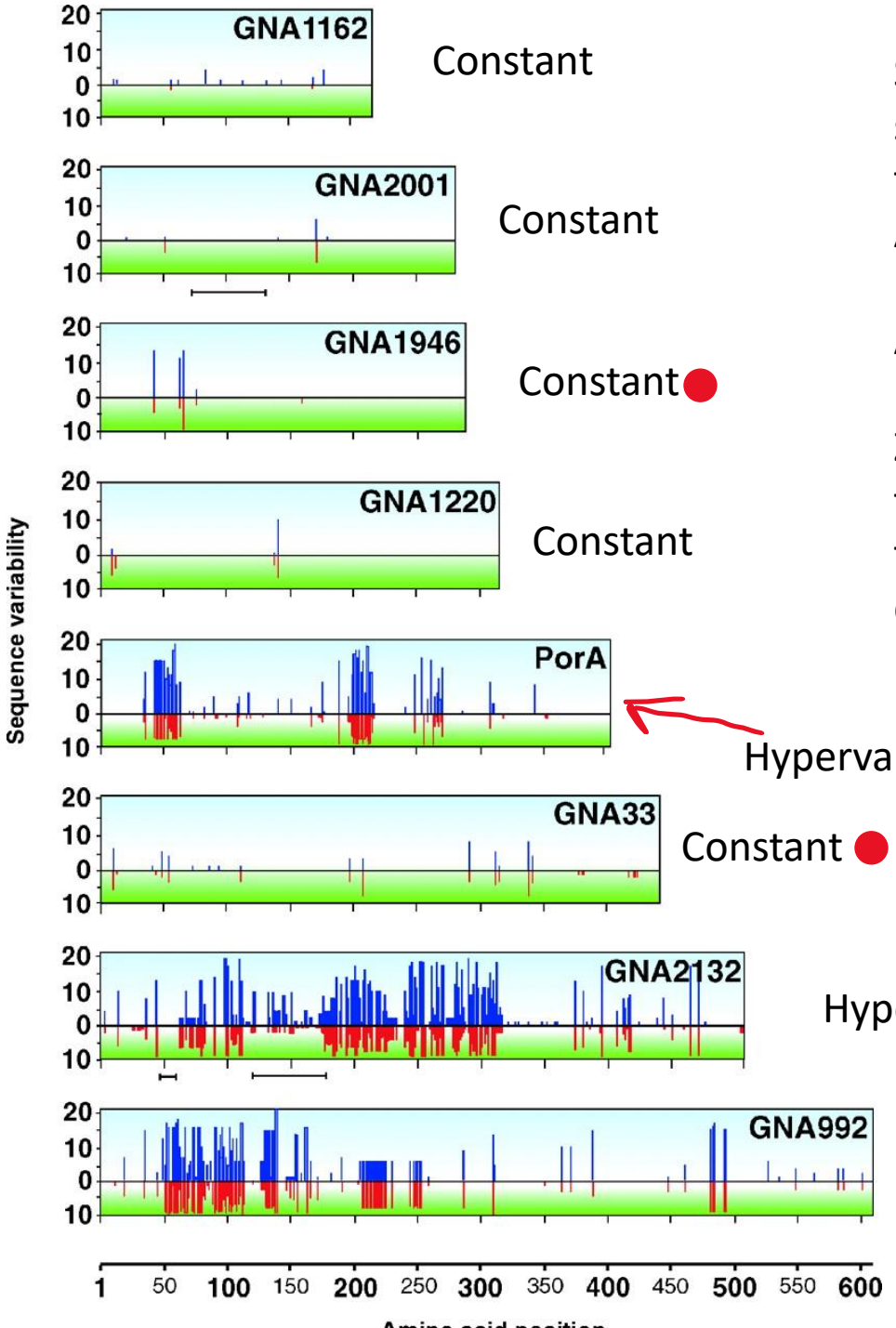
A total of 350 candidate antigens were expressed in *Escherichia coli*, purified, and used to immunize mice. The sera allowed the identification of proteins that are surface exposed, that are conserved in sequence across a range of strains, and that induce a bactericidal antibody response, a property 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)

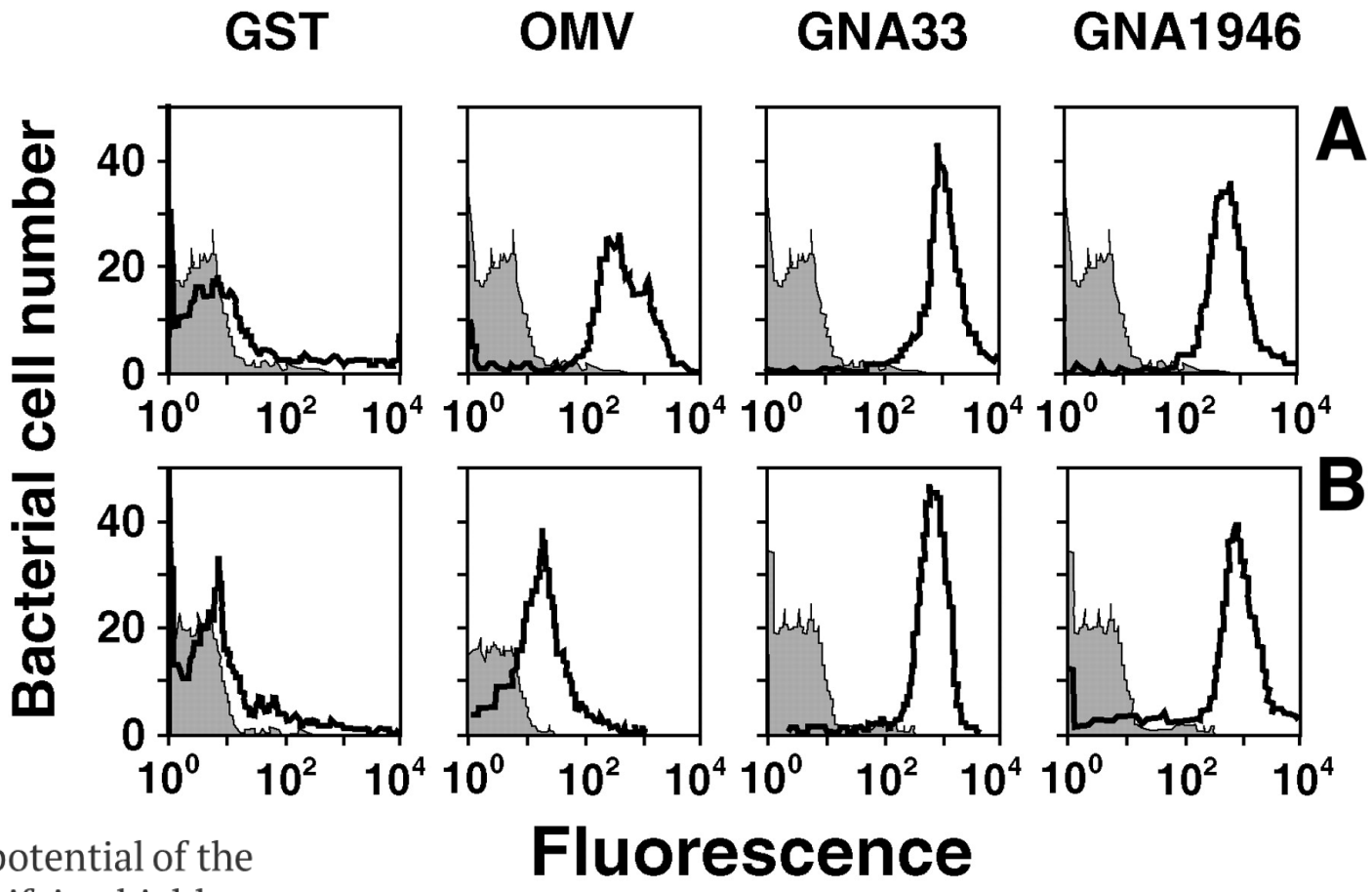
Constant

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*.

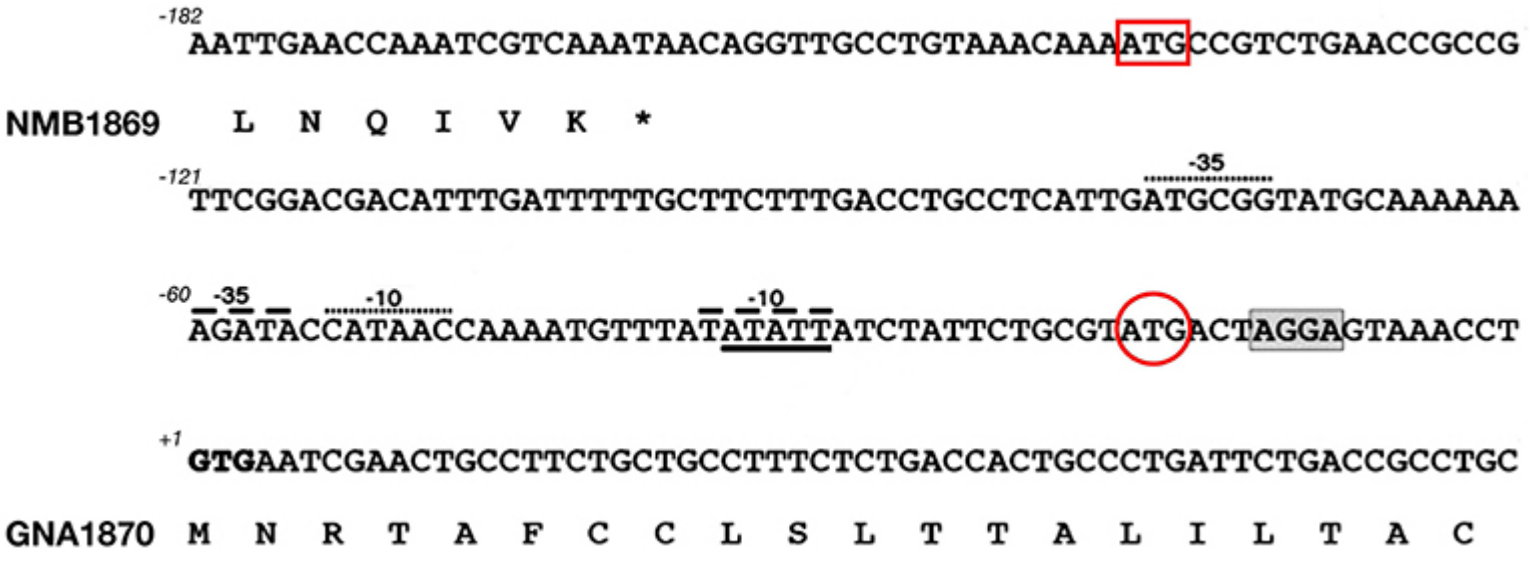
Gene	*N. meningitidis* B (22 strains)	*N. meningitidis* A,C,Y,X,Z,W135 (9 strains)	*N. lactamica* (1 strain)	*N. cinerea* (1 strain)	*N. gonorrhoeae* (3 strains)
*gna*33	+	+	+	+	+
*gna*992	+*	+	+/−†	+/−	−
*gna*1162	+	+	+	+	+
*gna*1220	+‡	+	+/−	+/−	+
*gna*1946	+	+	+	+/−	+
*gna*2001	+	+	+	+/−	+
*gna*2132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

problems

Management and interpretation of about two million base pairs of meningococcal genome sequence data were prone to errors. For instance, prediction of the start codons was based on the identification of the first ATG occurring after a previously identified stop codon. Unfortunately, this did not take into account either the presence of a correctly spaced Shine Dalgarno sequence, or the potential presence of less frequent start codons like TTG or GTG (coding for leucine or valine, respectively).

For example, the annotation of GNA1870 (later renamed **fHbp**) was incorrect as a result of automatic procedures and is now one of the most important meningococcal antigens.

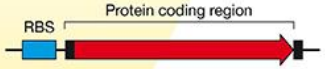


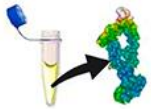
2158

Candidates predicted in the whole genome sequence of *Neisseria meningitidis*

600

In silico predicted surface exposed proteins



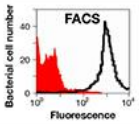


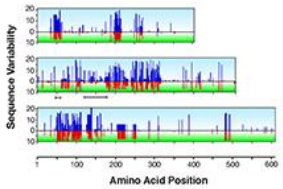
350

Expressed, purified and used to immunize mice

91

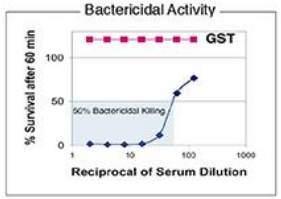
Identified as surface exposed





28

Induced bactericidal antibodies



3

antigens selected

fHbp NadA NHBA

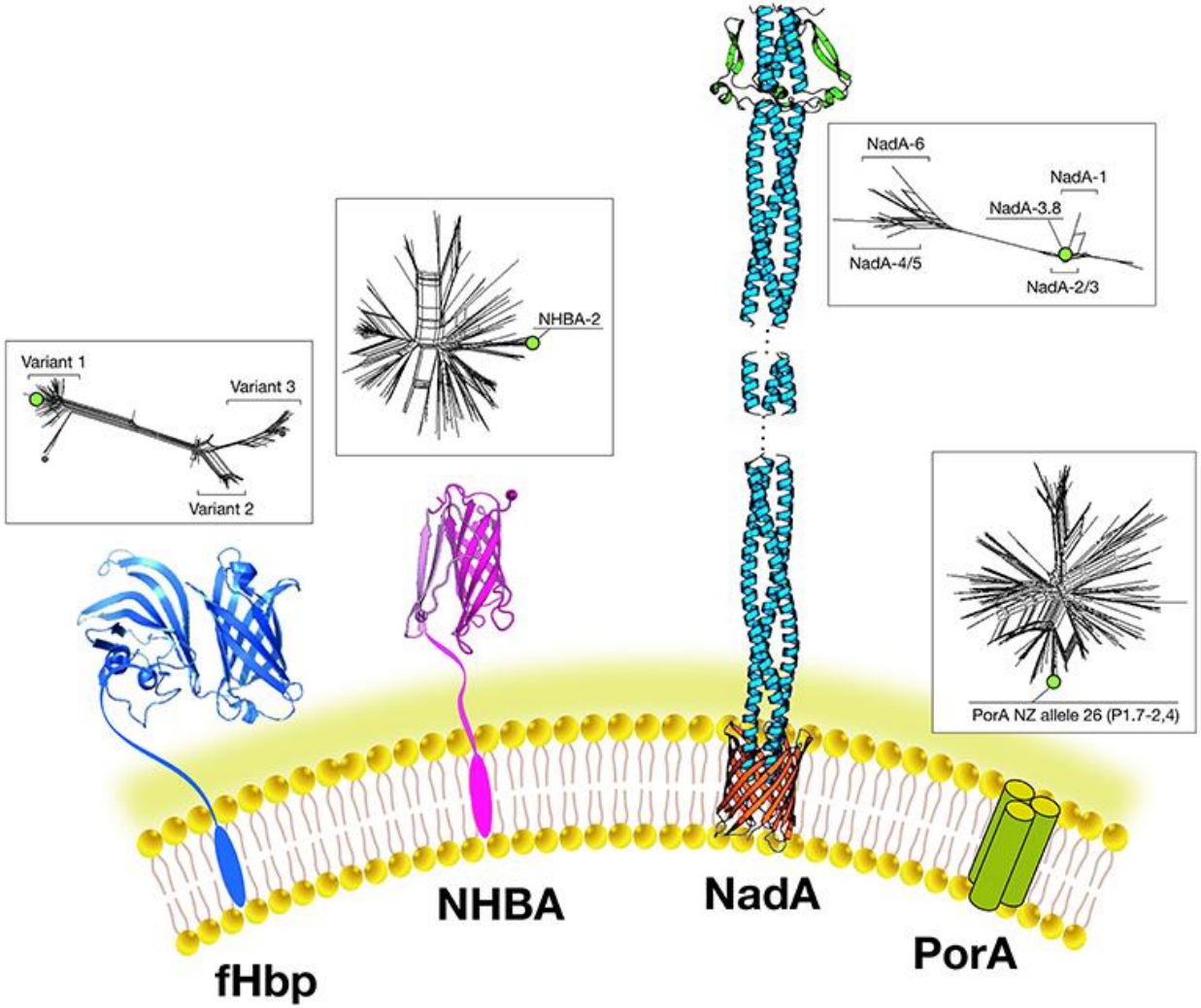
Two protein-based vaccines against MenB:

- the four-component 4CMenB vaccine [Bexsero, GSK]
- the bivalent fHbp2086 vaccine [Trumenba, Pfizer]

The fHbp2086 vaccine consists of equal amounts of two factor H binding protein (fHbp) variants belonging to subfamilies A and B, which were identified by biochemical approaches.
The 4CMenB vaccine contains three components that were identified by reverse vaccinology based on the complete genome sequence of a pathogenic reference MenB strain (MC58 strain):

(1) the fHbp variant 1.1 (subfamily B) fused to the genome-derived Neisseria antigen (GNA) 2091,
(2) the Neisseria adhesin A (NadA),
(3) the neisserial heparin binding antigen (NHBA) peptide 2 fused to GNA1030
Immunization with fHbp and NHBA fused to GNA2091 and GNA1030, respectively, resulted in increased bactericidal activity compared to immunization with the unfused proteins.
(4) Besides these three recombinant surface-exposed protein antigens, the vaccine contains OMVs from the New Zealand strain NZ98/254 containing porin A (PorA) P1.4

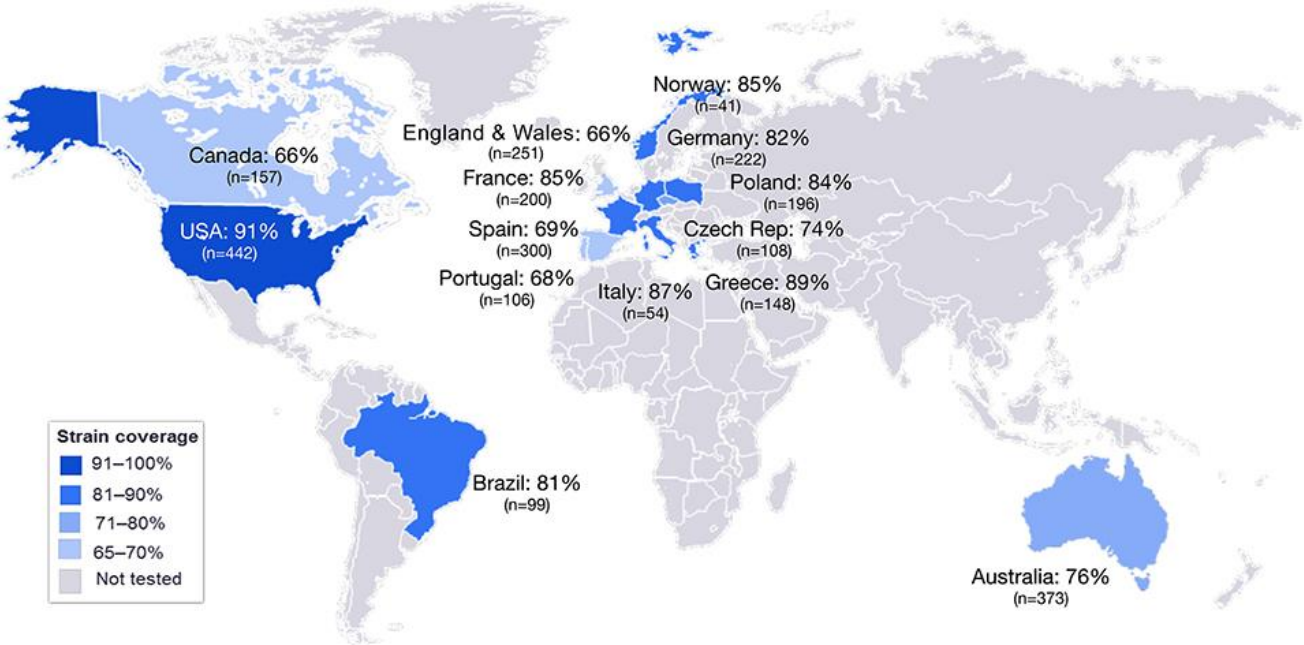
Ruiz García, Y., Sohn, WY., Seib, K.L. *et al.* Looking beyond meningococcal B with the 4CMenB vaccine: the *Neisseria* effect. *npj Vaccines* **6**, 130 (2021). https://doi.org/10.1038/s41541-021-00388-3



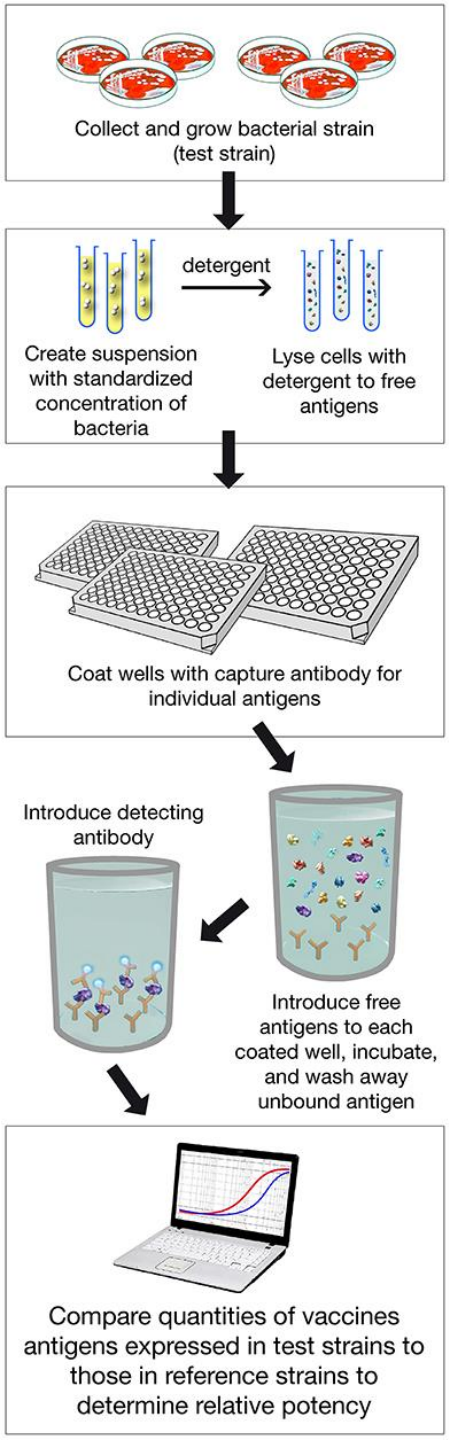
Main components of the 4CMenB vaccine:

- x-ray structure of fHbp, depicted in dark blue
- x-ray structure of the C-terminal domain of NHBA depicted in purple
- X-ray structures of the trimeric head and stalk domains of NadA molecule shown in light blue; the anchor region (in red)
- Schematic model of the trimeric PorA molecule (the immunodominant antigen of the OMV) is depicted in green

An innovative method was developed to assess coverage and predict effectiveness of the 4CMenB vaccine. This assay, called MATS (Meningococcal Antigen Typing System), correlated information on the quantity and quality of the antigens expressed by individual MenB strains and the potency of the immune response elicited by the vaccine based on bactericidal assays.



MATS coverage estimates by geographic region. This map schematically represents the results of MATS coverage estimates generated on MenB isolates from different countries colored in blue and scaled according to coverage estimates.

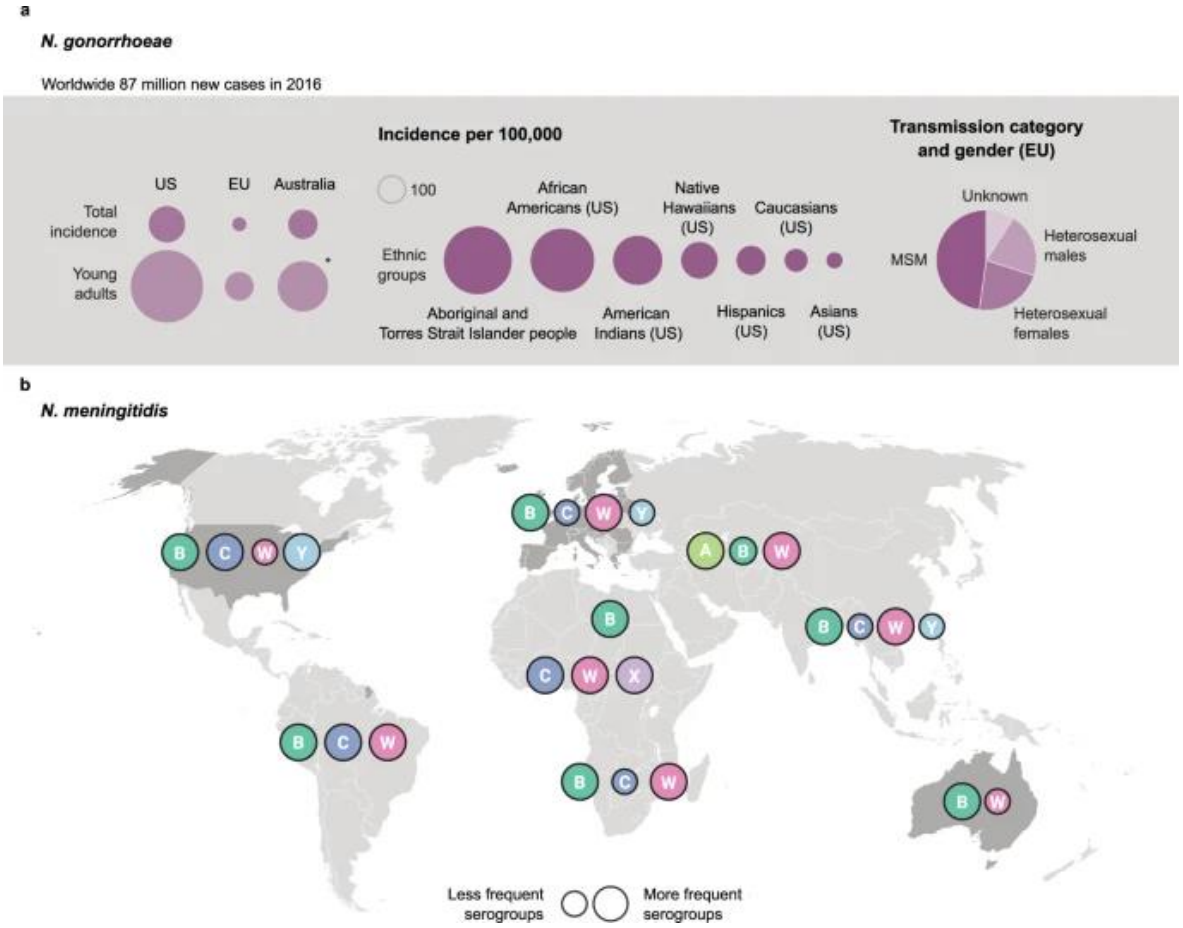


4CMenB represents a striking departure from the successful research and development platform that resulted in several, highly safe and effective conjugate meningococcal vaccines (against meningococcal serogroups A, C, W, and Y strains) formulated through covalent chemical linkage of different serogroup capsular polysaccharides to proteins. Although each of the meningococcal capsular polysaccharides shows strikingly distinct chemical compositions, each is an invariant structure whose target epitopes do not change over time or region.

But for vaccines, such as 4CMenB, where the antigens inducing protective immunity are proteins, the scenario is fundamentally different. The amino acid sequence of each of the protein antigens is highly variable, a consequence of their location on the bacterial surface where exposure to immune responses drives selection and fixation of diversity in circulating strains of meningococci.

Genetic variation in meningococci occurs predominantly through recombination, not intra-genomic mutations. Thus, within the natural population of meningococci, there is frequent horizontal transfer of DNA, mainly through DNA transformation, not only between distinct genotypes of N. meningitidis, but also from other sub-species of Neisseria and, rarely, other distinct bacterial species. For example, conserved homologs of the *nhba* gene have been found in commensal Neisseria species, such as *N. lactamica, N. polysaccharea,* and *N. flavescens*. This finding is relevant because of the potential "selective impact" that a NHBA-containing vaccine could have not only on encapsulated meningococcal strains, which are potentially pathogenic, but also on the commensal flora. This rampant recombination has major implications in that to be an effective vaccine, 4CMenB must elicit antibodies that protect against an enormous diversity of circulating meningococcal

Varying degrees of strain coverage were estimated depending on the non-B meningococcal serogroup and antigenic repertoire. 4CMenB elicits immune responses against non-B meningococcal serogroups and N. gonorrhoeae. Real-world evidence showed risk reductions of 69% for meningococcal serogroup W clonal complex 11 disease and 40% for gonorrhea after 4CMenB immunization. In conclusion, functional antibody activity and real-world evidence indicate that 4CMenB has the potential to provide some protection beyond MenB disease.



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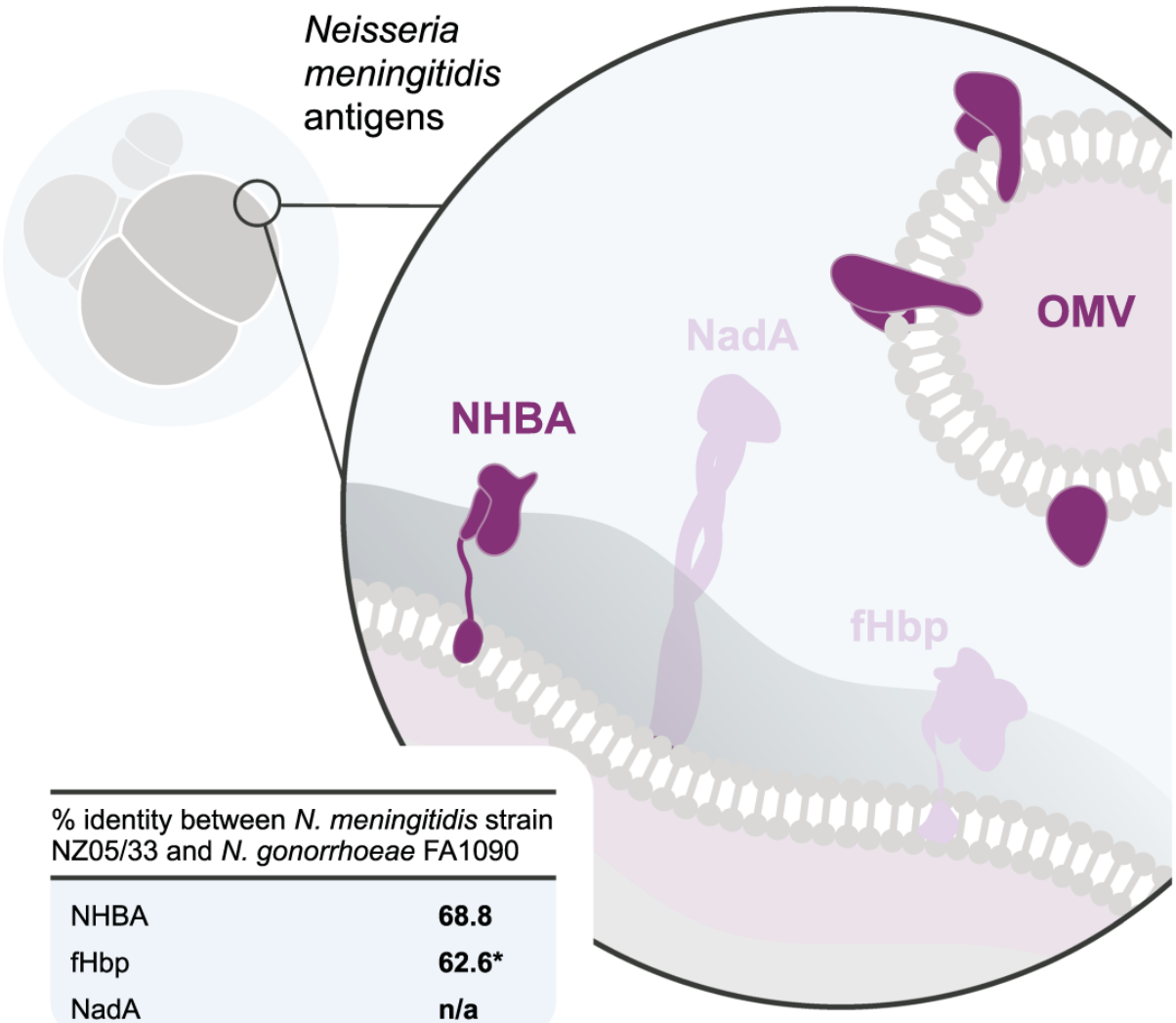

% identity between *N. meningitidis* strain NZ05/33 and *N. gonorrhoeae* FA1090	
NHBA	**68.8**
fHbp	**62.6***
NadA	**n/a**

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**

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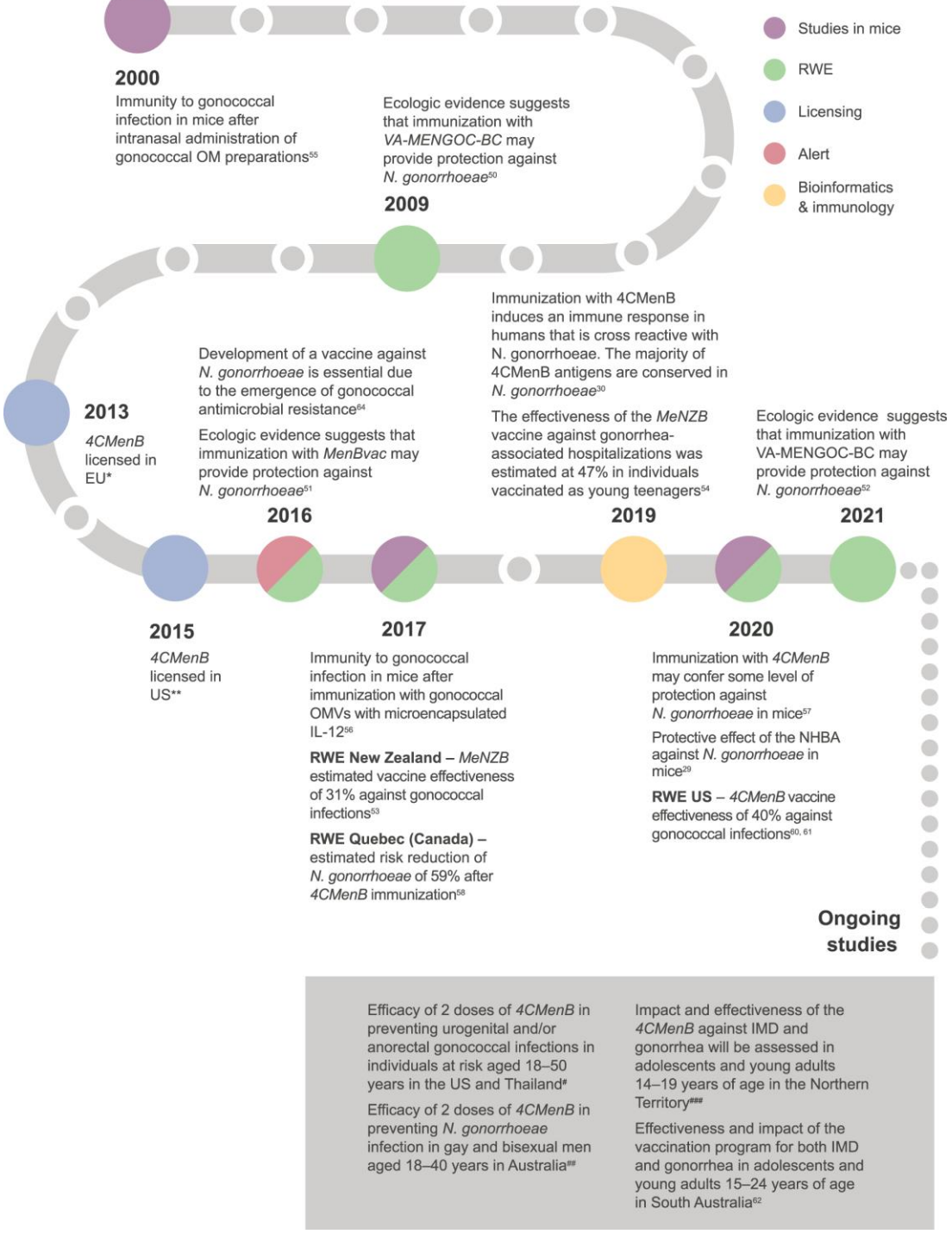
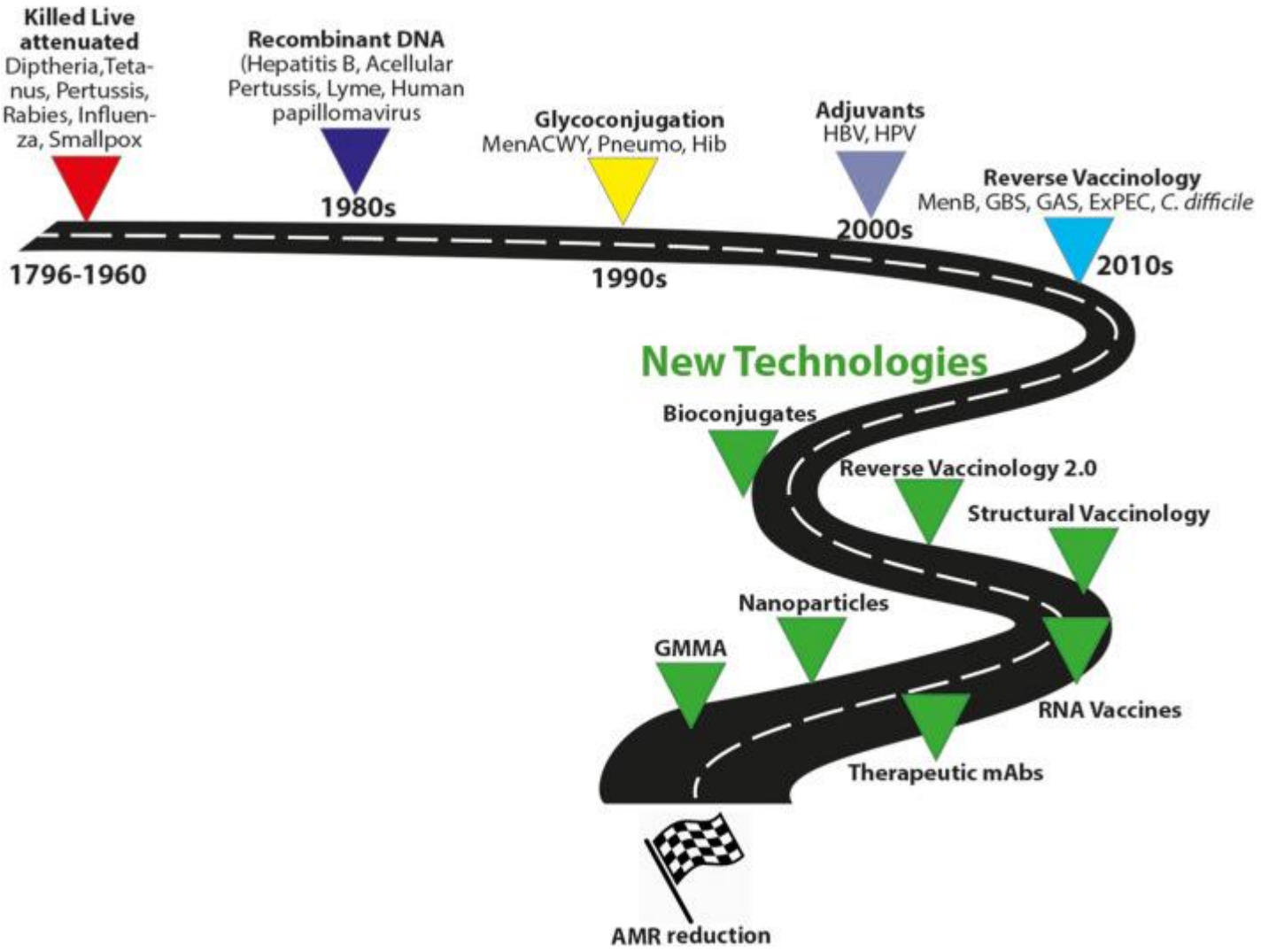
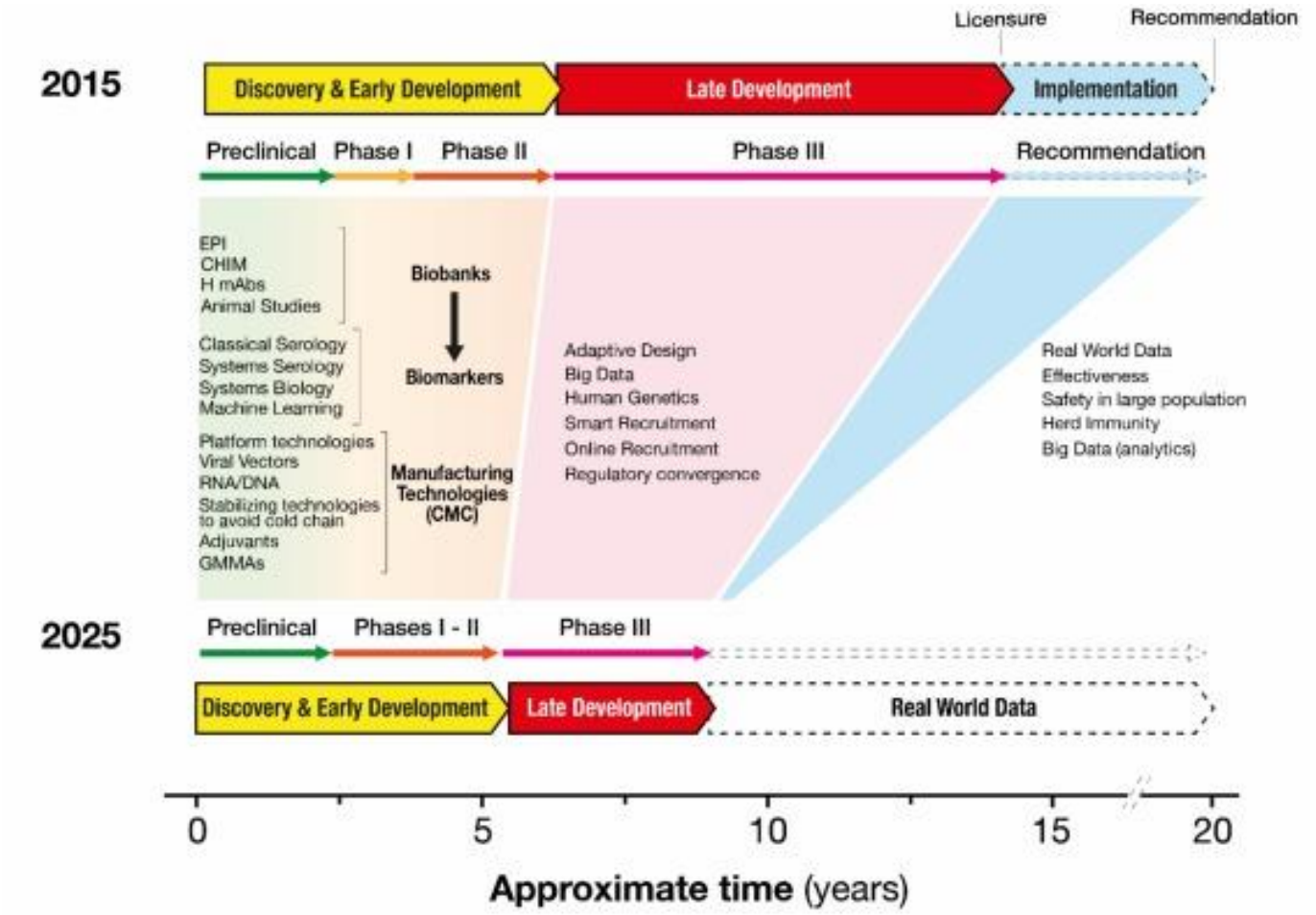




Rosini R, Nicchi S, Pizza M, Rappuoli R. Vaccines Against Antimicrobial Resistance. Front Immunol. 2020 Jun 3;11:1048. doi: 10.3389/fimmu.2020.01048.



Black S, Bloom DE, Kaslow DC, Pecetta S, Rappuoli R. Transforming vaccine development. Semin Immunol. 2020 Aug;50:101413. doi: 10.1016/j.smim.2020.101413. Epub 2020 Oct 28

frontiers in Microbiology

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

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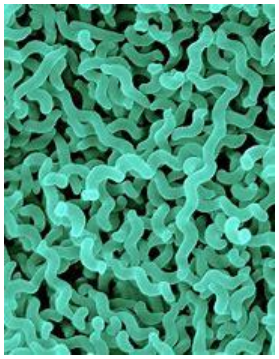
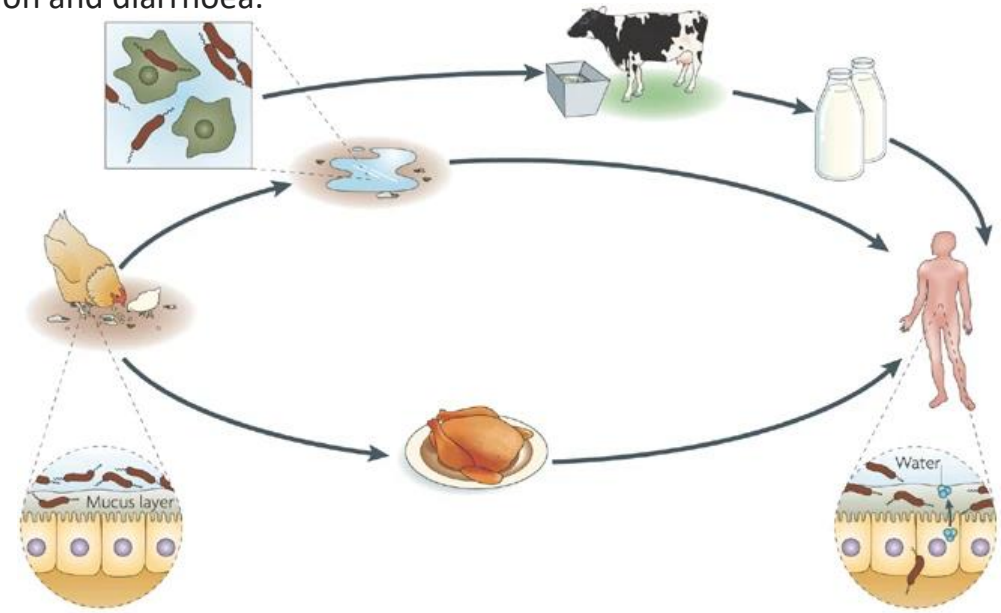


Thermotolerant campylobacters are the most frequent cause of bacterial infection of the lower intestine worldwide. *C. jejuni* belongs to the epsilon class of proteobacteria, in the order *Campylobacteriales*; this order includes two other genera, *Helicobacter* and *Wolinella*. Like *C. jejuni*, members 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.



Young KT, Davis LM, Dirita VJ. Campylobacter jejuni: molecular biology and pathogenesis. Nat Rev Microbiol. 2007 Sep;5(9):665-79. doi: 10.1038/nrmicro1718. PMID: 17703225.

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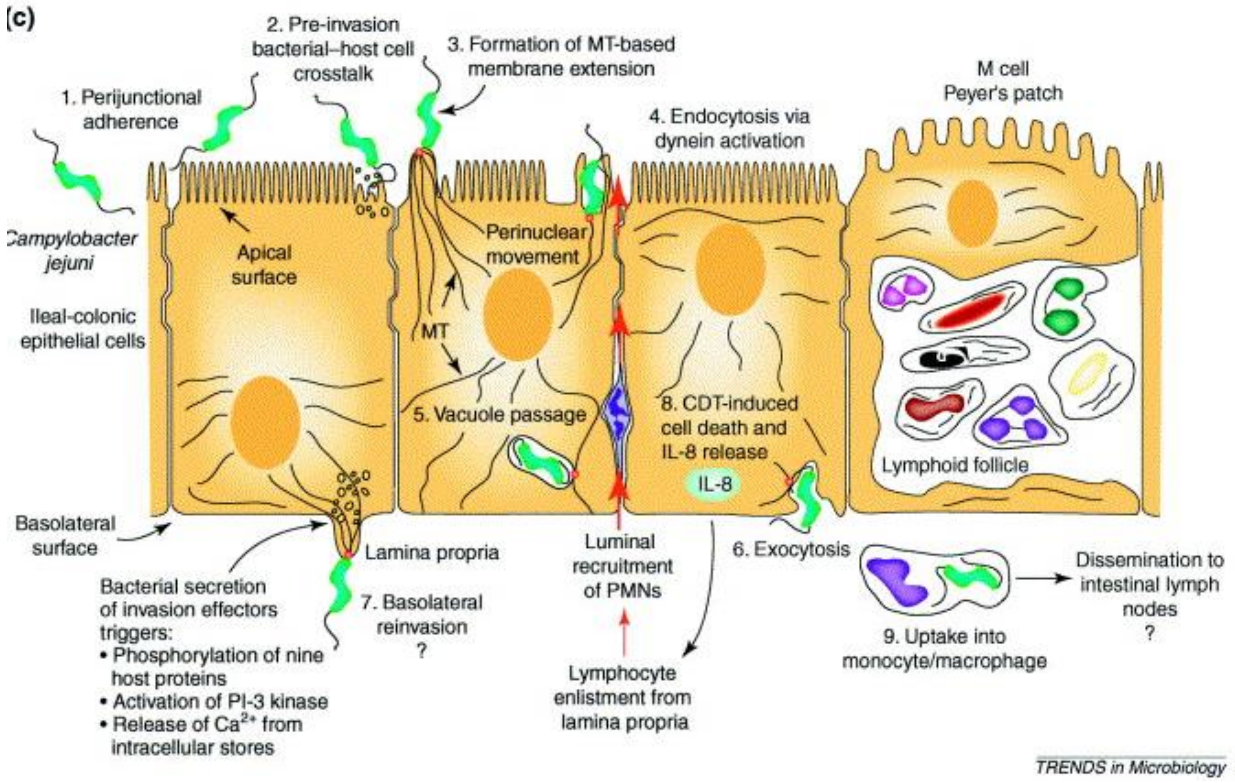
The determination of the complete genome sequence of several *C. jejuni* strains and plasmids has heralded the beginning of a new era of *C. jejuni* research. These projects have revealed the potential mechanisms by which *C. jejuni* associates with the host; for example, the complete sequencing of **pVir**, 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

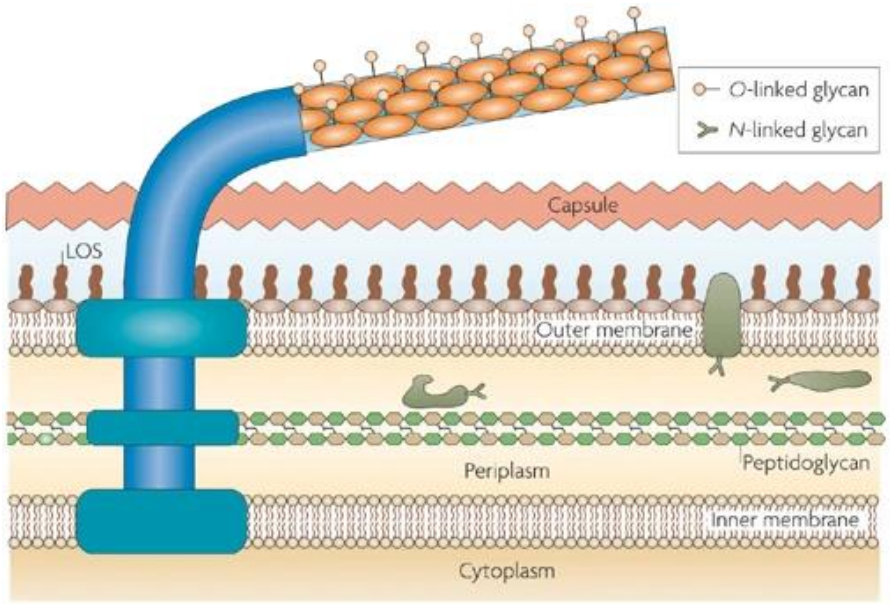
The mechanism of pathogenesis comprises four main stages: **adhesion to intestinal cells, colonization of the digestive tract, invasion of targeted cells, and toxin production**.



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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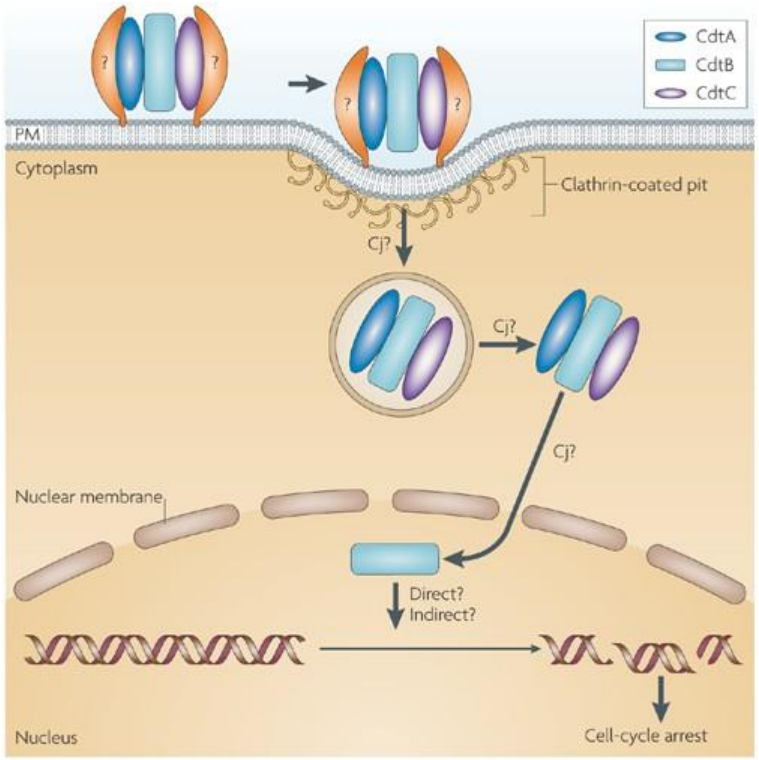
Cytolethal distending toxin.

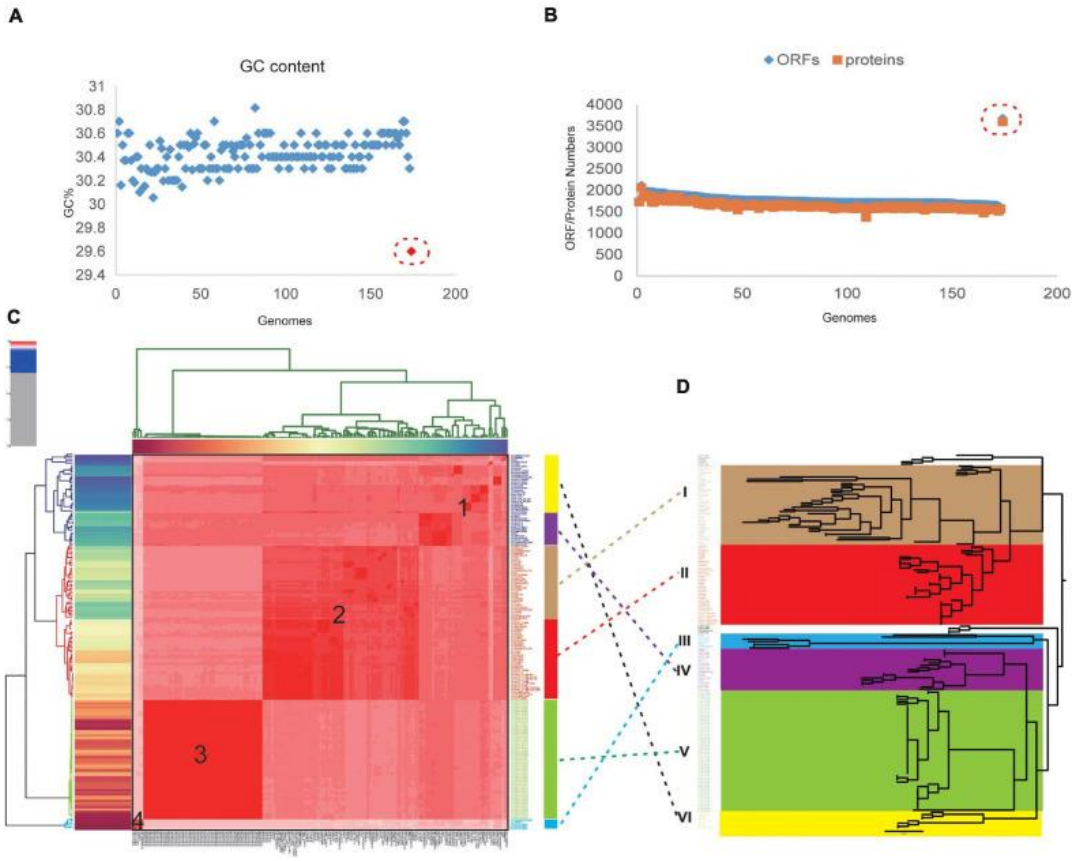
C. jejuni produces cytolethal distending toxin (CDT), which is also produced by a diverse group of other bacterial species. The toxin causes arrest at the G_1/S or G_2/M transition of the cell cycle, depending on the cell type. The active holotoxin is a tripartite complex of CdtA, CdtB and CdtC, although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



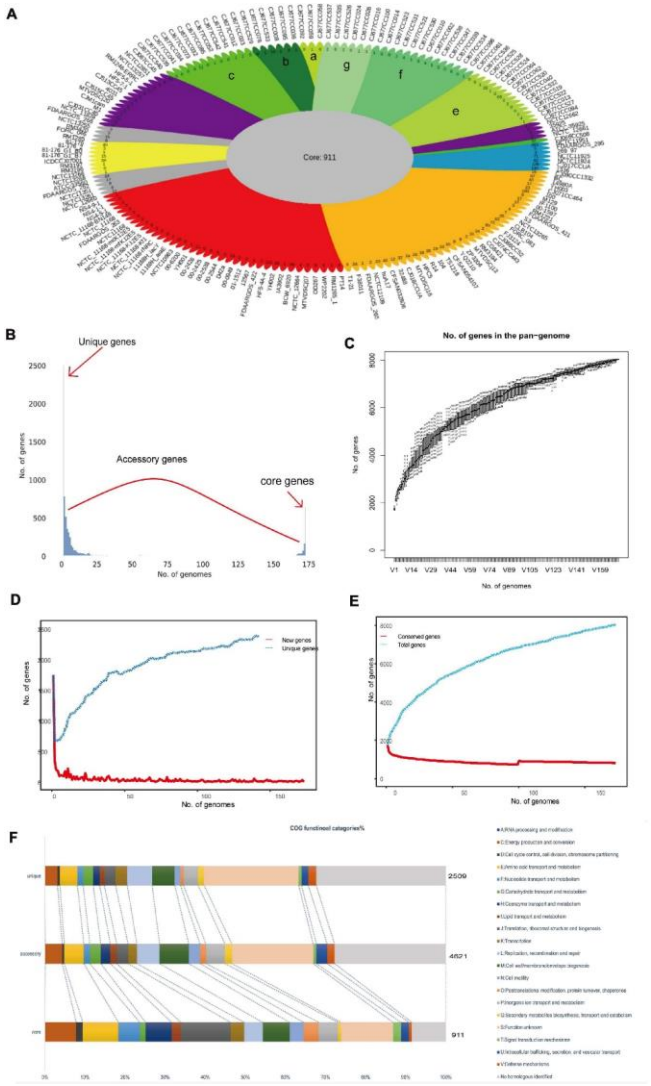
Campylobacter jejuni genomics

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.

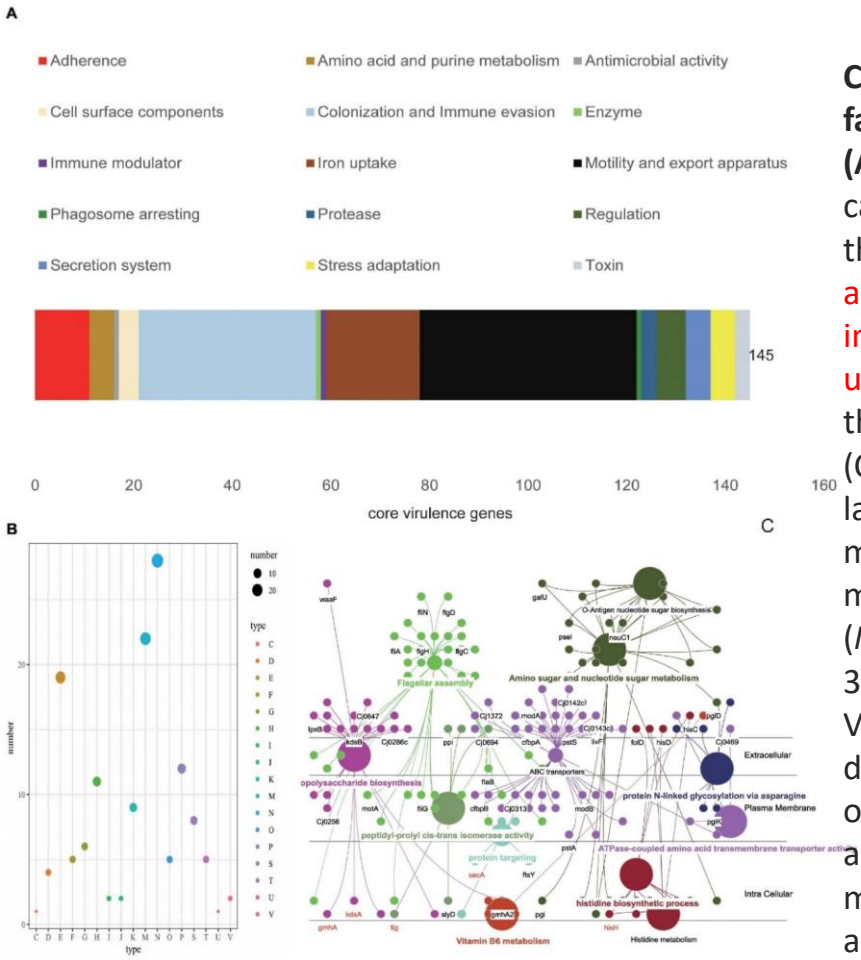


Using the whole-genome and core genome alignment concatenation approach, phylogenetic trees for the set of 173 genomes were constructed, the core genome tree could be divided into six main clades, in which nine strains were diverged independently of the other members.



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. Seventy-four 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.

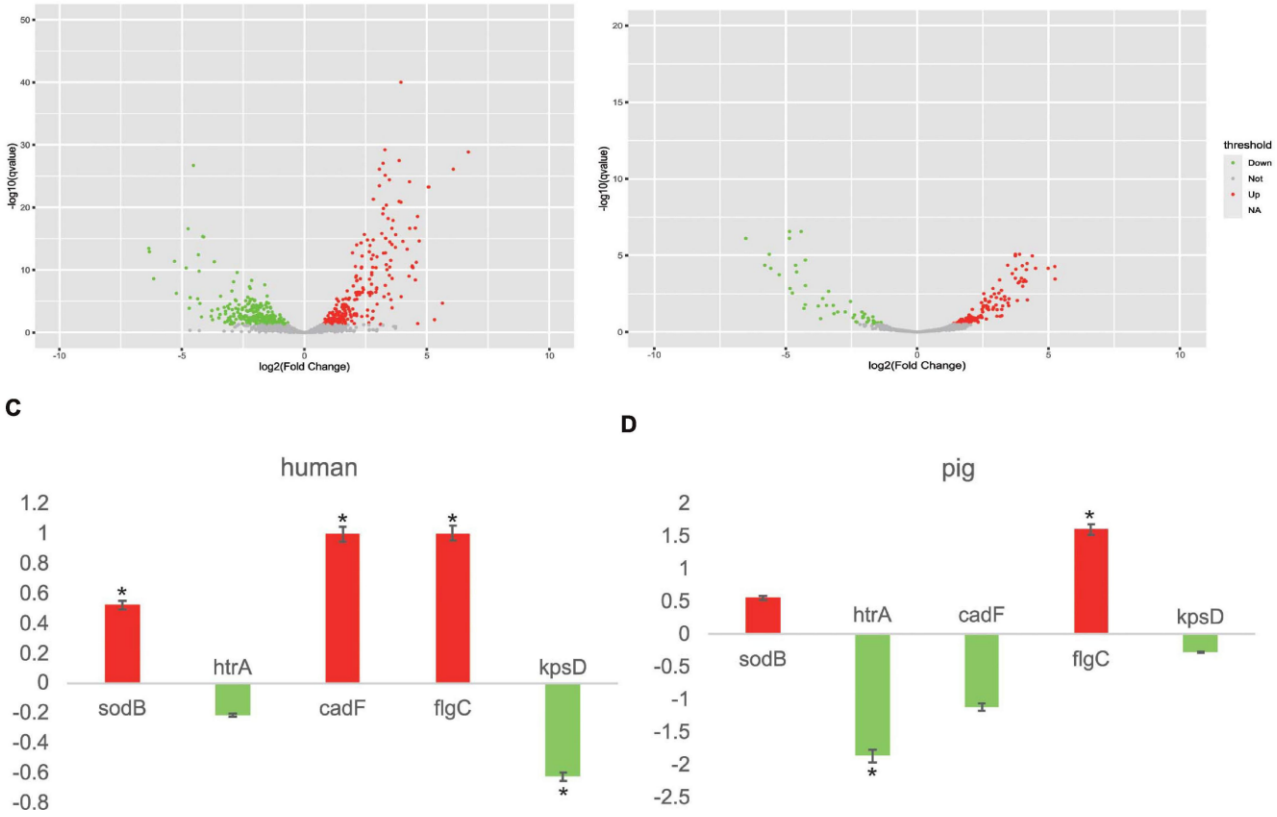
The core VF proteins for subcellular location revealed that 47 proteins were cytoplasmic, 19 were located in the cytoplasmic membrane, one was in the outer membrane, three were unknown, and four were periplasmic

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*