



Sex Superbug



Gonococcal antimicrobial resistance and vaccines

Magnus Unemo, Assoc. Professor, Director

WHO CC for Gonorrhoea and other STIs

Swedish Reference Laboratory for Pathogenic Neisseria

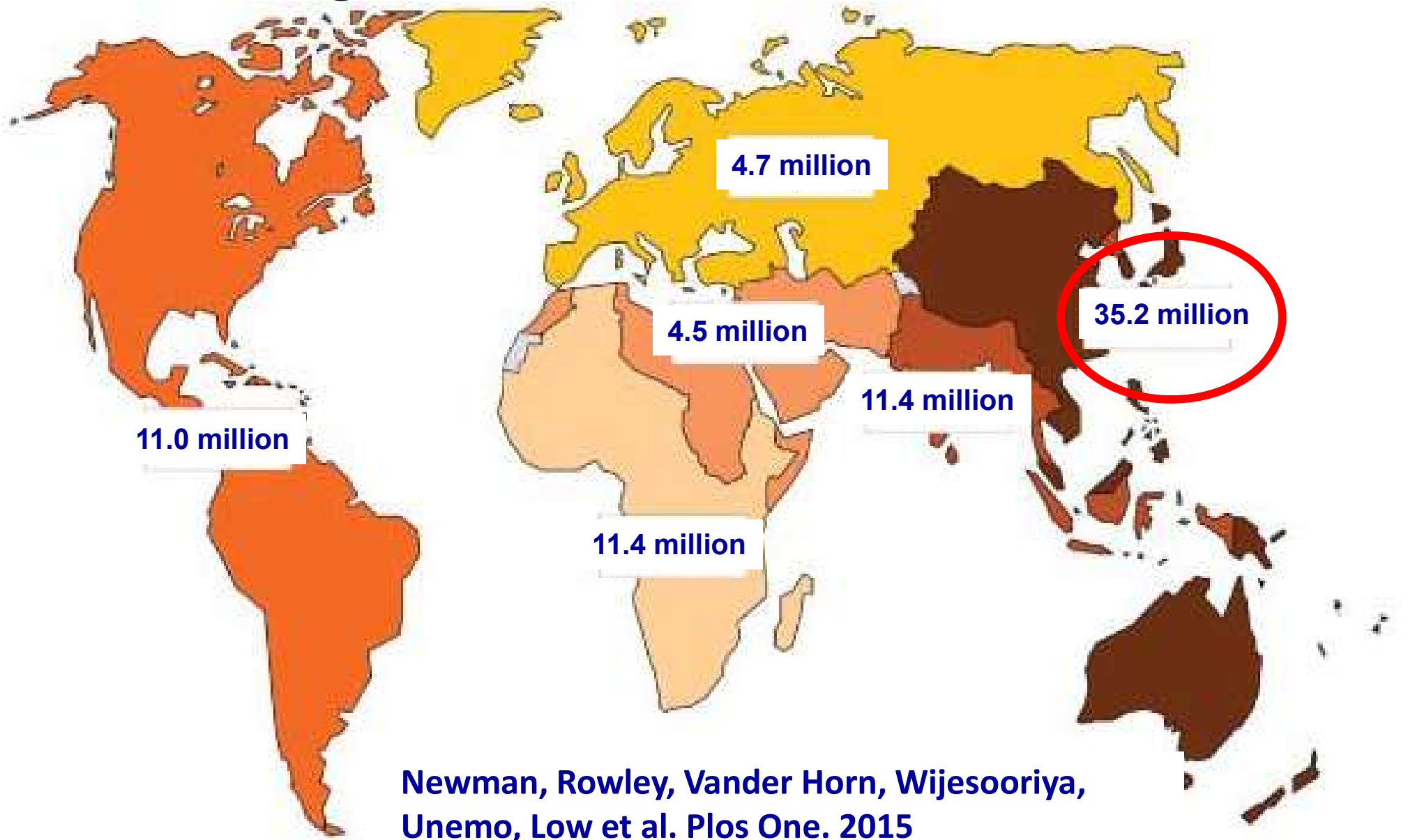
Department of Laboratory Medicine, Microbiology



**WHO Collaborating Centre for
Gonorrhoea and other Sexually
Transmitted Infections**

Örebro University Hospital, Sweden

WHO global estimates: 78 million new cases of gonorrhoea in adults in 2012



Newman, Rowley, Vander Horn, Wijesooriya, Unemo, Low et al. Plos One. 2015

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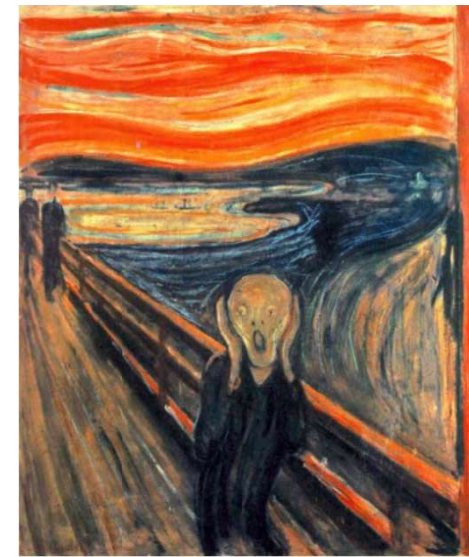
Gonorrhoea – major public health concern!

1. High incidence
2. Severe complications and sequelae, including infertility, blindness, and ↑ acquisition and transmission of HIV
3. High cost – especially as “disability-adjusted life years” (DALY; Ebrahim. STI. 2005)
4. Suboptimal diagnostics, testing, case reporting, surveillance in many countries
5. **Antimicrobial resistance (AMR) high in *Neisseria gonorrhoeae* (NG) and mainly empiric “blind” treatment**

Newman, Rowley, Vander Horn, Wijesooriya, Unemo, Low et al. Plos One. 2015



The Scream
(Edvard Munch,
1893)



70-80 years

Options for empiric antimicrobial monotherapy

~~Penicillins~~

~~Tetracyclines~~

~~Aminoglycosides~~

~~Fluoroquinolones (ciprofloxacin, ofloxacin etc.)~~

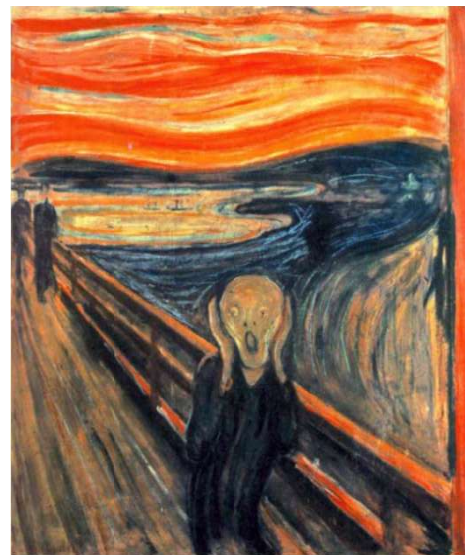
~~Macrolides (erythromycin, azithromycin)~~

~~Spectinomycin (Resistance selection! Not available!)~~

Only left \Rightarrow Ceftriaxone \Rightarrow BUT first superbugs with high-level resistance found!



The Scream
(Edvard Munch, 1893)



70-80 years

Option

Pharmaceutical industry has not kept up with the evolution of NG
⇓
Dual antimicrobial therapy (ceftriaxone plus azithromycin) recommended in USA, Canada, Europe, Australia....

therapy

→

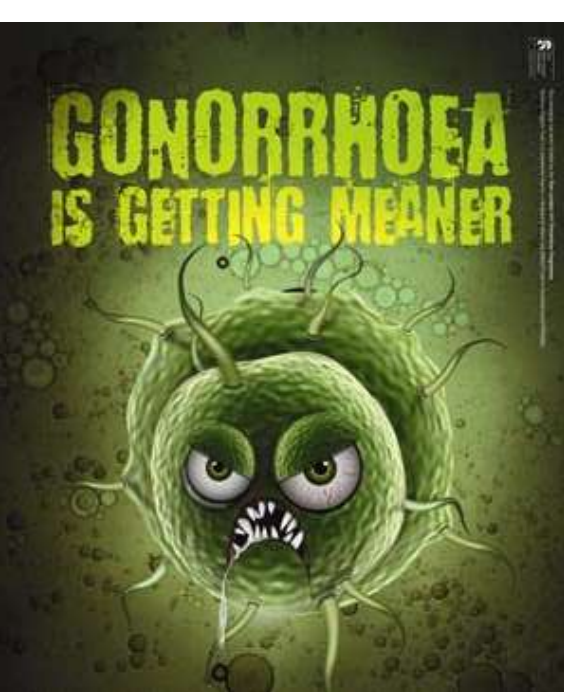
able!)

level

Only left →

resistance found!

ceftriaxone → BUT not superbug with high level



'Worse than AIDS' - sex 'superbug' discovered in Japan called disaster in waiting

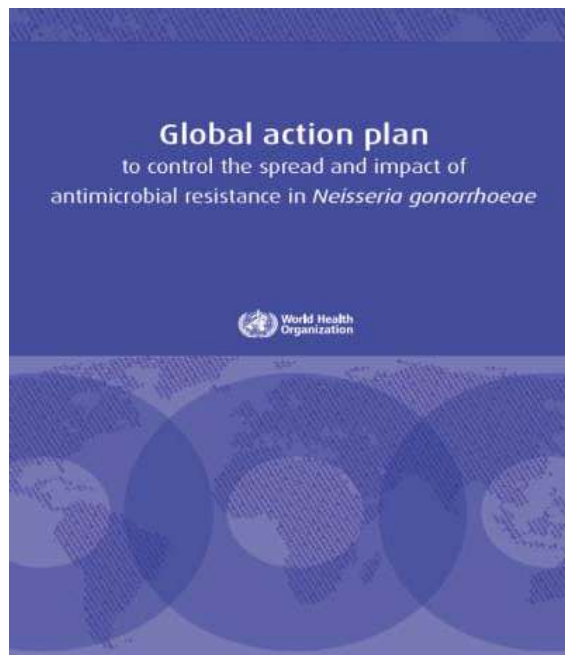
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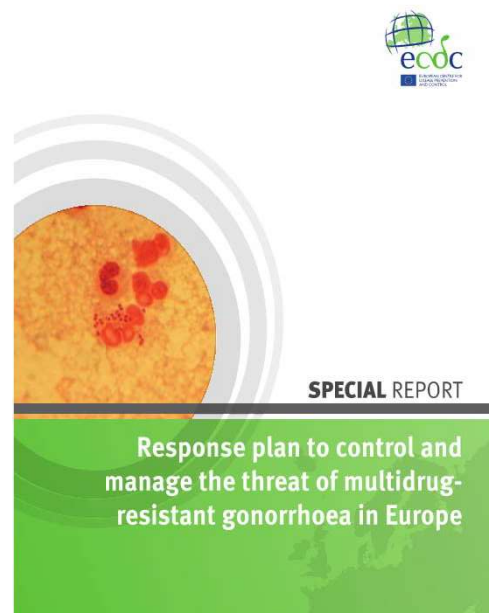
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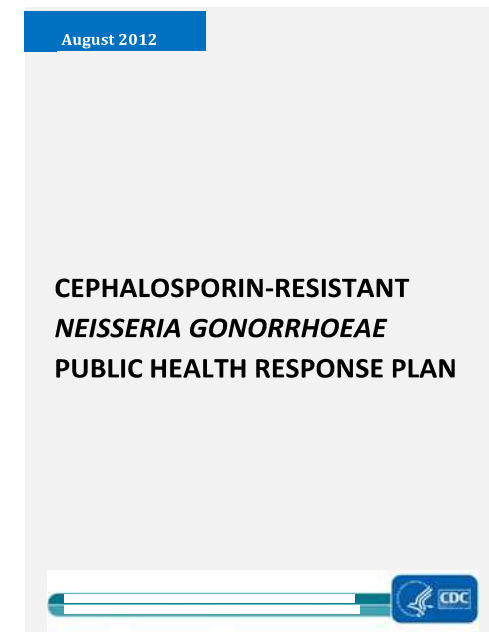
WHO 2012



ECDC for EU/EEA



US CDC for USA



Essentials

- **Novel antimicrobials for treatment!**
- Enhanced surveillance of gonococcal antimicrobial resistance and treatment failures globally
- Point of care testing, rapid genetic resistance testing, and genome-based characterisation
- **Only sustainable solution for control of gonorrhoea: vaccine covering global diversity of clinical strains (multiple antigens)!**

Early gonorrhoea vaccine field studies and identified challenges

- Parenteral heat-killed whole cell (Inuits, Canada) – **NO protection** (Greenberg et al. CJPH. 1974)!
- Parenteral intradermal purified pilin (US military, Korea) - **NO protection** (Boslego et al. Vaccine. 1991)!

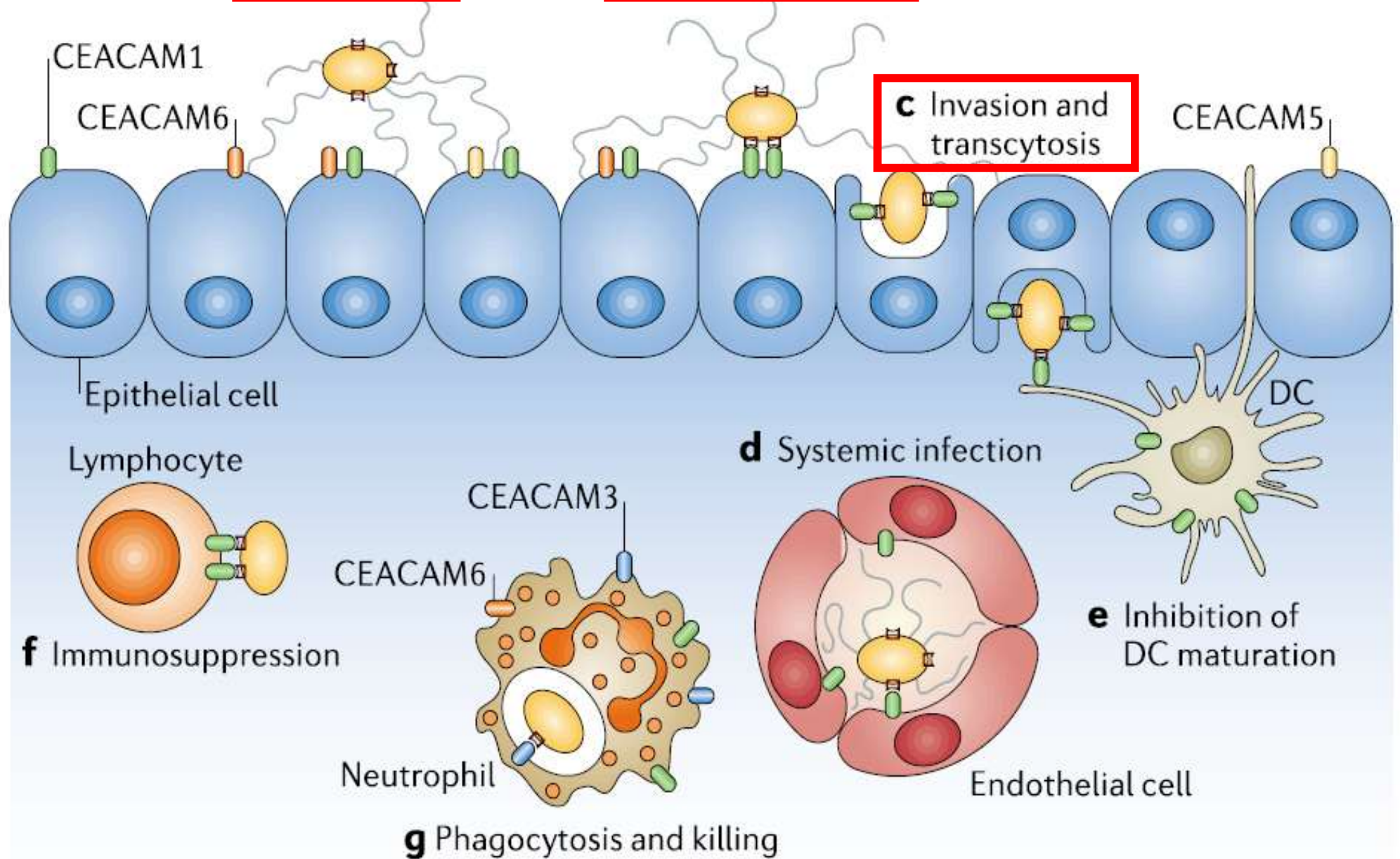
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- **Antigenic variability** and phase variation of NG surface molecules, reinfection with identical strain
 - No known correlates of protection, **weak mostly local and transient immune response** of uncomplicated infection, little immunological memory
 - **Lack of small laboratory animal model** to examine candidate antigens and immune response

Pathogenesis of gonococcal infections

a Anchorage

b Tight adherence

c Invasion and transcytosis



Gonorrhoea vaccine candidate antigens

Functional class	Description
------------------	-------------

Colonization

- | | |
|--------------|--|
| PilC | Pilus-associated adhesin; phase variable expression, variable and conserved regions |
| PilQ | Outer membrane channel through which pili are extruded; stable expression and antibodies against meningococcal PilQ are bactericidal |
| PorB | Major porin, two serogroups (PorB1A and PorB1B), stable expression; involved in gonococcal invasion of cervical cells through the C3R integrin; PorB1A molecules directly mediate uptake through the SREC-1 receptor |
| Opa proteins | Phase variable; 8–10 antigenically distinct Opa proteins per strain; peptide antigens may be used to avoid immunosuppressive domains; a cyclic peptide corresponding to the semivariable (SV) loop recognises Opa proteins with as many as 6–8 amino acid differences in this loop |
| OmpA | Surface-exposed, stably expressed, highly conserved. Mediates invasion of cervical and endometrial cells |

Nutrient acquisition

- | | |
|------------|--|
| TbpA, TbpB | Transferrin receptor; TbpA and TbpB are highly and semiconserved, respectively. Purified TbpA or TbpB induce bactericidal antibodies in mice that block growth in the presence of Tf as a sole iron source |
| LbpA, LpbB | Lactoferrin receptor; antibodies against <i>N meningitidis</i> homologues are bactericidal |
| TdfJ | Iron-induced zinc transporter; antibodies against the meningococcal homologue (ZnuD) are bactericidal |

Evasion of innate defenses

MtrE	Surface-exposed channel of the MtrC-MtrD-MtrE and FarA-FarB-MtrE active efflux pumps; stable expression and highly conserved; antibodies to recombinant MtrE are bactericidal
Lst	α 2,3 sialyltransferase; catalyses the addition of host-derived sialic acid to the LNT species of LOS; protects gonococci from complement, non-opsonic uptake by neutrophils and antimicrobial peptides. Antibodies to purified Lst reduce sialylation
PorB	In serum resistant strains, PorB binds soluble negative regulators of the complement cascade (C4b-binding protein, factor H) to down-regulate complement activation at the gonococcal surface

Other

2C7 epitope	Bactericidal LOS epitope; phase variable but expressed by >95% of isolates. Antibodies to a 2C7 peptide mimetic are bactericidal and opsonophagocytic and active and passive protection was demonstrated in mice
AniA	Nitrite reductase; surface-exposed, conserved; induced by low O ₂ tension and the presence of nitrite. Required for anaerobic growth and biofilm formation; plays a role in serum resistance. A truncated AniA protein that lacked the glycosylated C-terminus induced antibodies that inhibited nitrite reductase activity
OpcA	Stably expressed in <i>N gonorrhoeae</i> . OMV from <i>N meningitidis</i> with a phase-locked 'on' <i>opcA</i> gene is a candidate meningococcal vaccine
NspA	Stably expressed, highly conserved. Meningococcal NspA is protective in mouse model of meningococcal infection
Outer membranes	Can be engineered to stabilise the expression of phase variable or regulated antigens and increase the diversity of antigenic variants present. An outer membrane preparation was protective against <i>N gonorrhoeae</i> in a mouse model

Ev

Potential protection (conserved antigens):

2C7 mimetics: by active and passive immunization

TbpB – TbpA: Antibodies (Abs) block uptake of iron

MtrE: by active immunization

Ot

PorB: Loop specific peptides induce cross-reactive, bactericidal Abs

AniA: Abs block nitrite reductase function

Lst: Abs reduce surface sialylation

OmpA: Bactericidal Abs

OpcA: Bactericidal Abs

Outer
membranes

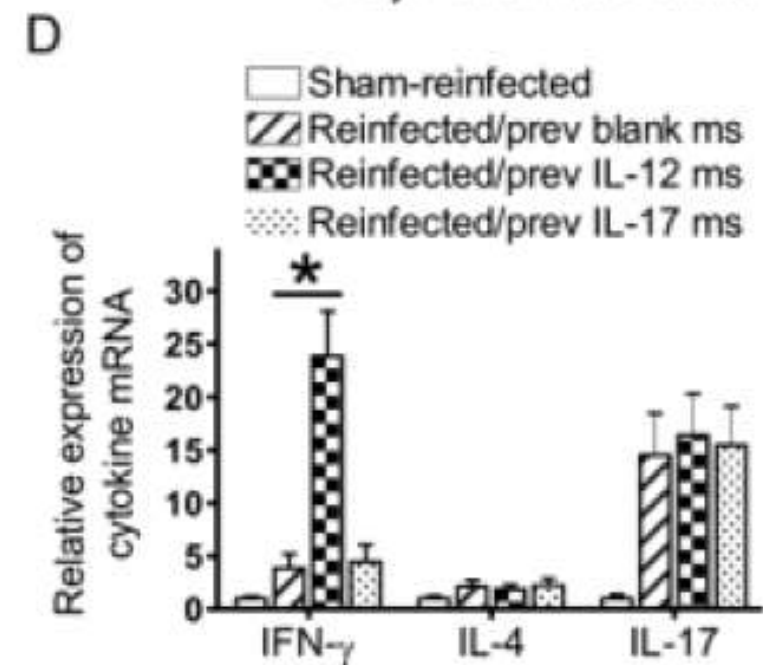
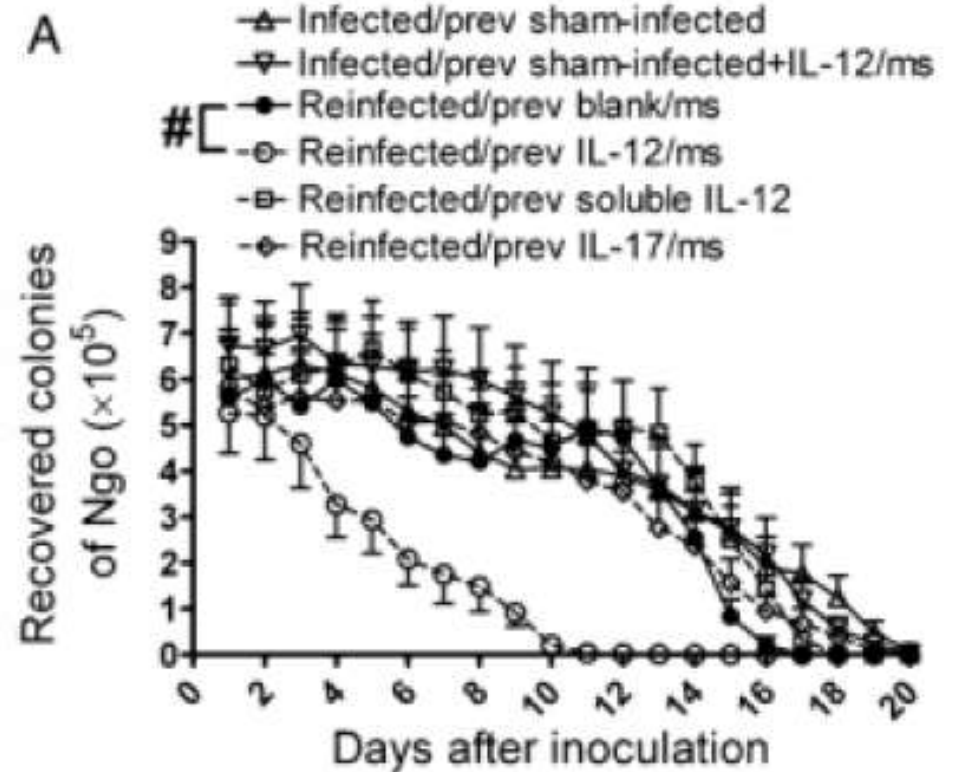
Can be engineered to stabilise the expression of phase variable or regulated antigens and increase the diversity of antigenic variants present. An outer membrane preparation was protective against *N gonorrhoeae* in a mouse model

Enhancement of Adaptive Immunity to *Neisseria gonorrhoeae* by Local Intravaginal Administration of Microencapsulated Interleukin 12

Yingru Liu, Nejat K. Egilmez, and Michael W. Russell

JID 2013 208:1821-9

- Vaginal administration of microencapsulated IL-12 administered during primary infection leads to more rapid immune-mediated clearance to secondary infection
- Correlates with heightened Th1 response, and generation of gonococcal-specific serum IgG and mucosal IgA and IgG
- Microencapsulated anti-IL-10 or anti-TGF β had a similar effect





BALB/c mouse

In vivo models



"Humanized" mouse

- **17 β -estradiol treated female BALB/c (or C57/BL6) mice model** (to examine antigens, adjuvants (incl. formulations), immunization routes, and correlates of protection (or immunosuppression) in a whole model system) (Jerse et al. Front Microbiol. 2011)
- **Transgenic mice expressing human CEACAMs, transferrin, C4B-binding protein, and factor H** (even mouse lines with multiple transgenes) that **relieve some of the host restrictions** for the obligate human pathogen NG (Scott Gray-Owen, personal communication)
- **Experimental human male volunteer urethral gonorrhoea model** (>300 subjects have proven safety; natural course of infection, including signs and symptoms) (Hobbs et al. Front Microbiol. 2011)

NG vaccine - current situation

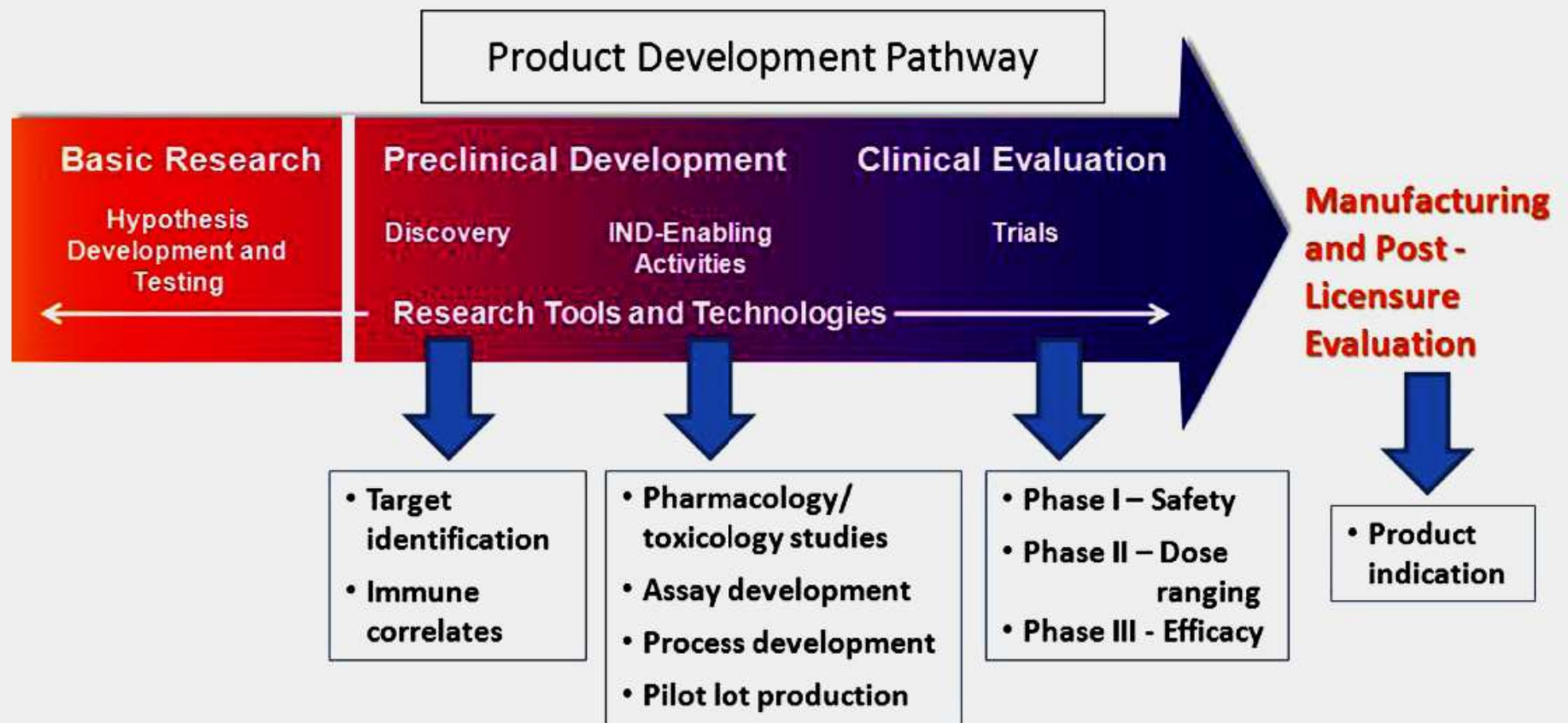
- Improved **understanding of molecular pathogenesis**
- Detailed **knowledge regarding many potential NG vaccine antigens**, including conserved regions
- **Whole genome sequencing, transcriptomics, proteomics etc.** can simultaneously in detail examine many vaccine antigens (diversity, variability, expression...), population studies
- **Improved understanding of immune responses incl. suppressive responses**, e.g. Rmp Abs block bactericidal PorB and LOS Abs, NG suppression of protective Th1 (and Th2) adaptive immune response via TGF- β , IL-10 and Tr1 cells, and elicitation of Th17-driven innate responses driving the neutrophil response (Liu et al. JID. 2013) etc.

NG vaccine - current situation

- **17 β -estradiol treated female BALB/c mice model and transgenic “humanized” mice**
- **Protection described in mice:** PorB/VRPs, MAP1 2C7 LOS epitope peptide, MtrE given with CpG, NG OMV and microencapsulated IL-12 induce protection (serum and vaginal IgA and IgG, and Th1 response)
- **Experimental human male volunteer urethral gonorrhoea model**
- **New vaccine and adjuvant technologies available:** vector-based, DNA vaccines, genetic engineering, improved delivery system (protein-coated microcrystals, nanoparticles..), immunization routes (oral or nasal for mucosal immunization?)....

Future priorities

- **Now is the time** to develop the only sustainable solution for gonorrhoea control (spare a lot of antimicrobials)!
- **Leadership, multidisciplinary consortium (coordination, collaboration, communication), funding – see opportunities, not only obstacles!**
- **Start with male urethral infection** (homologous followed by heterologous strains) to obtain proof of principle?
- **Increase the research** on pathogenesis (natural course), antigens (polyvalent vaccine), adjuvants, host response (protective and suppressive in males and females), surrogate measures for assessing immunity, and improve (inclusion criteria, knock out/in mutants....) and enhance the availability of harmonized and quality assured assays, preclinical and clinical models



The product development pathway for a potential gonococcal vaccine.

The potential impact of vaccination on the prevalence of gonorrhea

Andrew P. Craig^a, Richard T. Gray^a, Jennifer L. Edwards^b, Michael A. Apicella^c, Michael Jennings^d, David P. Wilson^{#a}, and Kate L. Seib^{#d,*} [Vaccine. 2015](#)

A partially efficacious vaccine could have a significant impact on GC prevalence, if coverage is high and protection lasts over the highest risk period (*i.e.* most sexual partner change) among young people

Vaccine efficacy duration		Reduction in GC prevalence	Population coverage
100%	20 y	50% in 7 y	100% All 13 year olds
		> 90% in 13 y	
>70%	10 y	50% in 10 y	
100%	7.5 y	> 90% in 20 y	100% All 13 year olds
80%	10 y		
50%	20 y		
100%	20 y	> 90% in 20 y	50% All 100% Male 100% Female 75% Core (5%)
50%	20 y	50% in 20 y	

Rapid

Efficacy vs. duration

Target

The potential impact of vaccination on the prevalence of gonorrhoea

Important considerations

- 1. Modeling of impact and cost-effectiveness.** HPV a good example, but NG vaccine could be administered also after sexual debut!
- 2. Endpoint** (inhibit transmission, infection, disease (e.g. PID), sequelae (infertility)....)?
- 3. Target population:** adolescents but also adults, both males and females, not only high-risk/vulnerable groups
- 4. Community-based**
- 5. Enhance coverage:** Social marketing for acceptability (parents, adolescents, adults, health care providers...)

50%	20 y	50% in 20 y	100% Male 100% Female 75% Core (5%)
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Target

Acknowledgements

- **Carolyn Deal and Tom Hiltke, NIH/NIAID**
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- **Gonococcal Vaccine Consortium (GVC)**
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 - Peter Rice (University of Massachusetts Medical School, USA)
 - Lee Wetzler (Boston University, USA)
 - Ian Feavers (NIBSC, United Kingdom)
 - Scott Gray-Owen (University of Toronto, Canada)
 - Additional GVC participants (international)