

# Efflux pumps in *Neisseria gonorrhoeae*: cause of resistance and targets for therapeutics/vaccines?

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No financial disclosures or conflicts

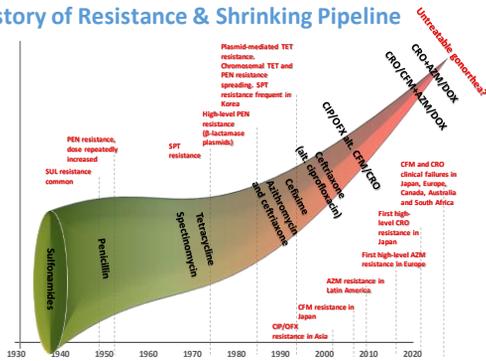
## The Emerging Threat of Untreatable Gonococcal Infection

Gail A. Bolan, M.D., P. Frederick Sparling, M.D., and Judith N. Wasserheit, M.D., M.P.H.  
**FEBRUARY 9, 2012**



“It is time to sound the alarm. During the past 3 years, the wily gonococcus has become less susceptible to our last line of antimicrobial defense, threatening our ability to cure gonorrhea and prevent severe sequelae.”

### History of Resistance & Shrinking Pipeline

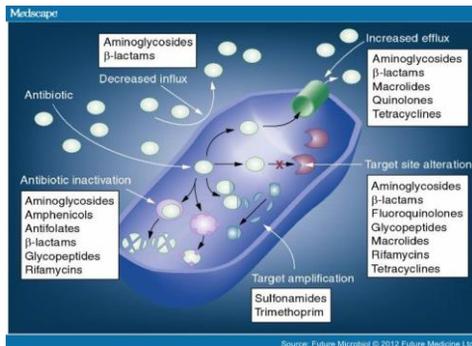


Nemo and Shafer, 2014

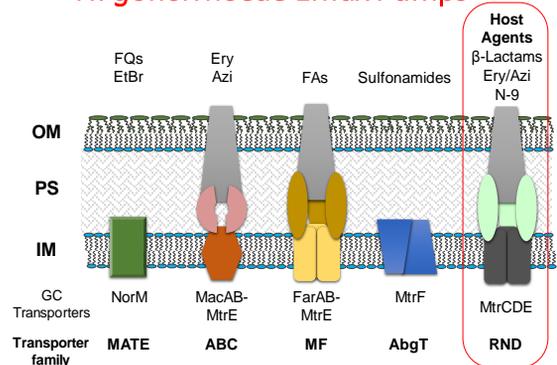
2007: Super Bug Status!

To Effectively Counteract Resistance With New Drug Development, It Is Important To Understand Mechanisms of Resistance

### Mechanisms of Antibiotic Resistance



### *N. gonorrhoeae* Efflux Pumps

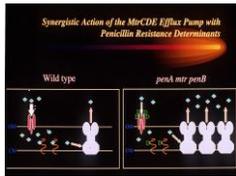
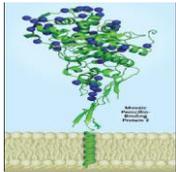


ANNALS OF THE NEW YORK ACADEMY OF SCIENCES  
 Issue: The Evolution of Infectious Agents in Relation to Sex

**Antibiotic Resistance in *Neisseria gonorrhoeae*: origin, evolution, and lessons learned for the future**

Magnus Unemo<sup>1</sup> and William M. Shafer<sup>2</sup>

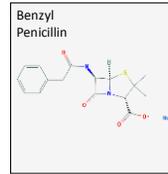
<sup>1</sup>National Reference Laboratory for Pathogenic Neisseria, Department of Laboratory Medicine, Microbiology, Örebro University Hospital, Örebro, Sweden. <sup>2</sup>Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, Georgia and Laboratories of Bacterial Pathogenesis, Veterans Affairs Medical Center, Decatur, Georgia



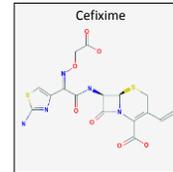
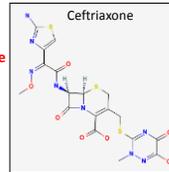
For ceftriaxone resistance: mosaic PBP2 and overproduction of the MtrCDE efflux pump

**Important β-Lactams Previously or Currently Used to Treat Gonorrhea: Clinically Resistant Strains of Historical Importance**

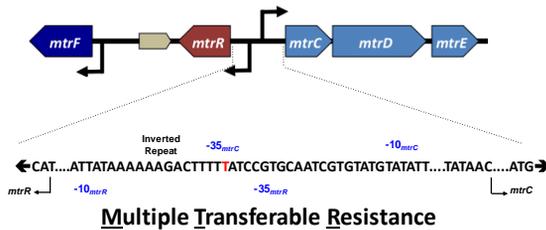
NC epidemic Isolate-1983 FA6140



Japan isolate 2009 (HO41)



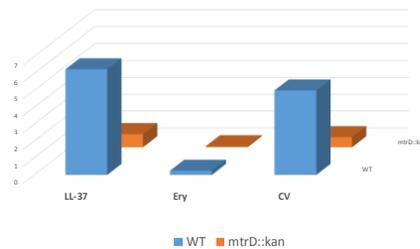
**The *mtrCDE* locus**



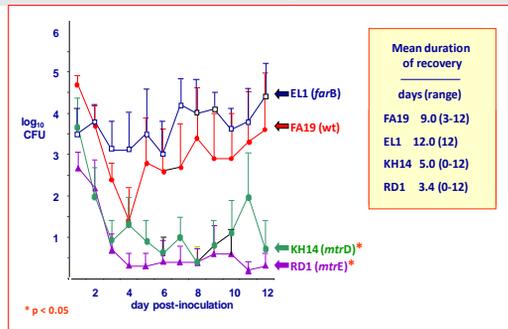
**Multiple Transferable Resistance**

Maness & Sparling, 1973. *J. Infect. Dis.*  
 Pan & Spratt, 1994. *Mol. Microbiol.*  
 Hagman et al. 1995, 1997. *Microbiol.*  
 Delahay et al. 1997. *Microbiology*  
 Veal & Shafer, 2003. *JAC.*

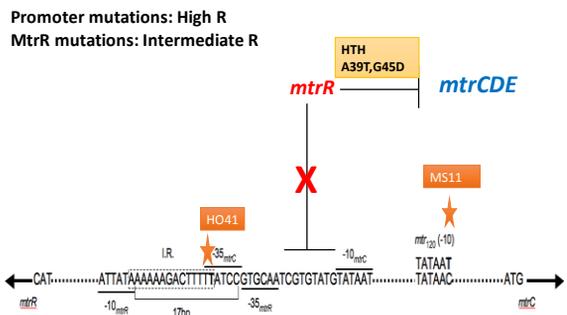
**Loss of the MtrCDE Efflux Pump Increases Gonococcal Susceptibility to Antimicrobials**



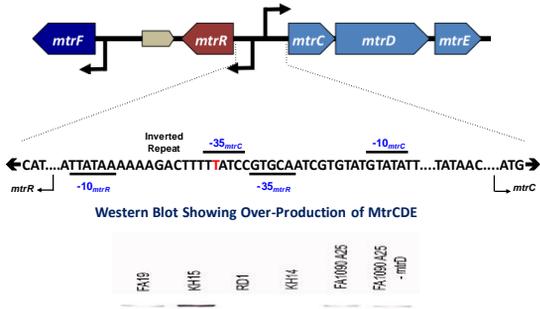
**Mtr Efflux Pump-Deficient Gonococci Have a Survival Defect in a Murine Model of Lower Genital Tract In Females (Jerse et al. 2003)**



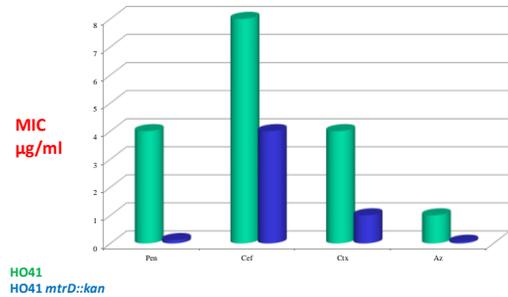
**Cis- and Trans-Acting Mutations That Increase Resistance**



**A Single Nucleotide Change Can Account for the Mtr Phenotype in High Level Resistant Strains**



**Loss of the MtrCDE Efflux Pump Enhances Gonococcal (resistant strain HO41) Susceptibility to Antibiotics**



Golparian et al. 2014 Antimicrob. Agents Chemother.

**The Emerging Threat of Untreatable Gonococcal Infection**

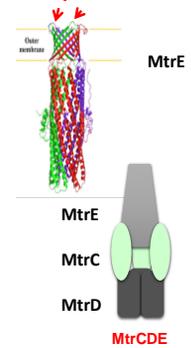
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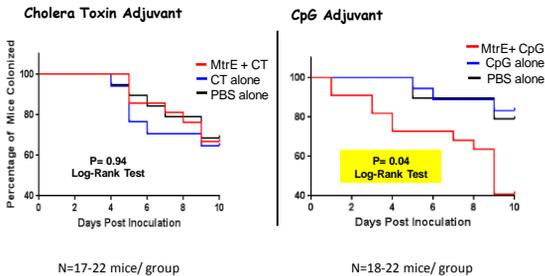
**Can We Exploit the MtrCDE Efflux Pump In Efforts to Counteract Antibiotic Resistance Expressed by Gonococci?**

**Efforts By Two Groups**

- Ann Jerse Ph.D. (USUHS, Bethesda, MD, USA): vaccine development targeting surface exposed domains of MtrE, the OMP channel of the pump
- Edward Yu Ph.D. (Iowa State University, Ames, IA, USA): developing peptides that target MtrC, the periplasmic adapter protein of the pump, and block assembly of a functional pump



**Comparison of MtrE<sub>3-467</sub> Immunization/Challenge Results Using CT versus CpG as the Adjuvant**



Data from Ann Jerse Ph.D.

**Conclusions**

- The MtrCDE efflux pump is an important part of how gonococci develop resistance to antibiotics
- This pump is also important for bacterial survival during an experimental infection
- Expression of the *mtrCDE* operon is regulated by *cis*- and *trans*-acting factors
- Propose that the MtrCDE efflux is a target for new drug discovery or an antigen for a future vaccine

## Special Thanks To Collaborators

- **Ann Jerse Ph.D. and her lab group** (Bethesda, USA): all mouse work and antibiotic resistance collaborations for nearly 15 years
- **Magnus Unemo Ph.D. and Daniel Golparian** (Orebro, SE): clinical significance of mtr mutations.
- **Edward Yu Ph.D. and his lab group** (Ames, USA): structural biology of Mtr efflux pump proteins MtrD and MtrE and EPIs.
- **Fred Sparling M.D.** (Chapel Hill, USA)

Shafer Lab 2015



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