

## 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

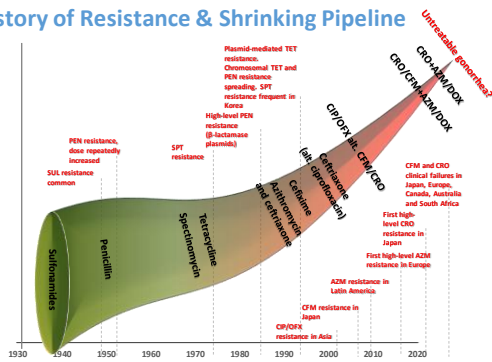
Gail A. Bolan, M.D., P. Frederick Sparling, M.D., and Judith N. Wasserheit, M.D., M.P.H.

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

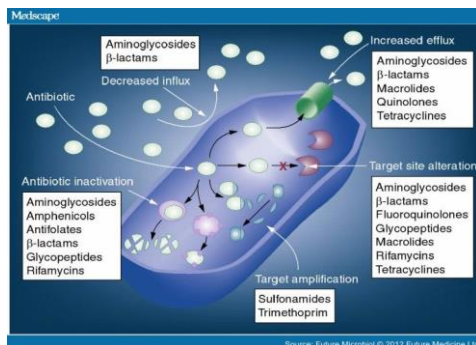


Unemo and Shafer, 2014

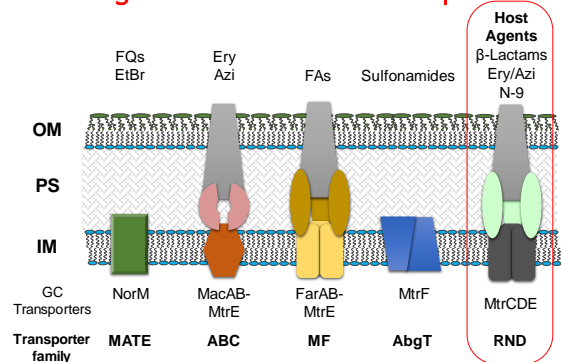
2007: Super Bug Status!

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

## Mechanisms of Antibiotic Resistance



## *N. gonorrhoeae* Efflux Pumps

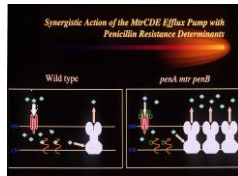
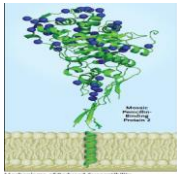


ANNALS OF THE NEW YORK ACADEMY OF SCIENCES  
Issue: The Evolution of Infectious Agents in Relation to Sex

## 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>

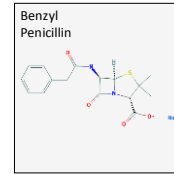
<sup>1</sup>National Reference Laboratory for Pathogenic Neisseria, Department of Laboratory Medicine, Microbiology, Örebro University Hospital, Örebro, Sweden. <sup>2</sup>Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, Georgia and Laboratories of Bacterial Pathogenesis,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



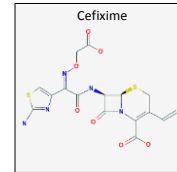
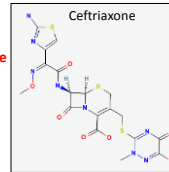
For ceftriaxone resistance: mosaic PBP2 and overproduction of the MtrCDE efflux pump

Important  $\beta$ -Lactams Previously or Currently Used to Treat Gonorrhea: Clinically Resistant Strains of Historical Importance

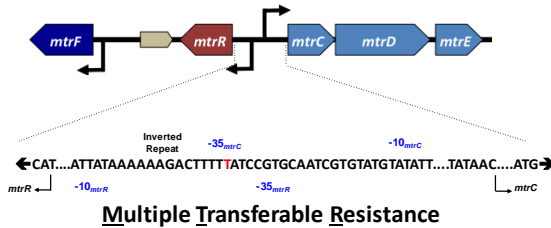
NC epidemic  
Isolate-1983  
FA6140



Japan isolate  
2009  
(HO41)

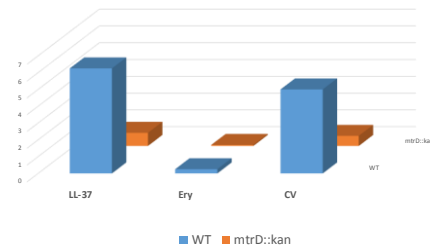


## The *mtrCDE* locus

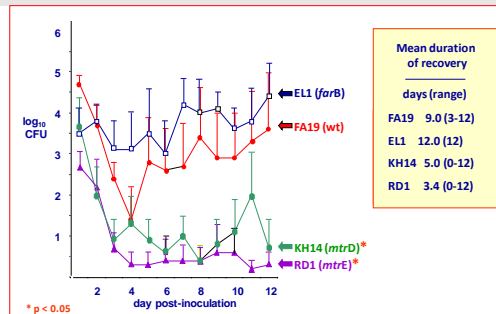


Maness & Sparling, 1973. *J. Infect. Dis.*  
Pan & Spratt, 1994. *Mol. Microbiol.*  
Hagman et al. 1995, 1997. *Microbiol.*  
Delahay et al. 1997. *Microbiology*  
Veal & Shafer, 2003. *JAC*.

Loss of the MtrCDE Efflux Pump Increases Gonococcal Susceptibility to Antimicrobials

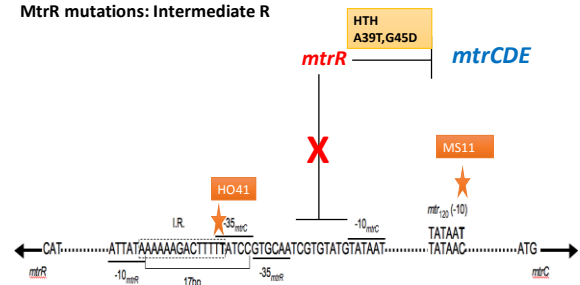


## Mtr Efflux Pump-Deficient Gonococci Have a Survival Defect in a Murine Model of Lower Genital Tract In Females (Jerse et al. 2003)

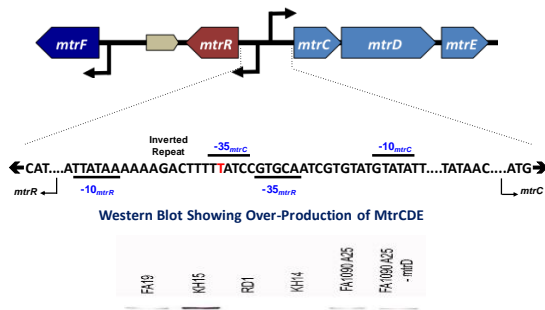


## Cis- and Trans-Acting Mutations That Increase Resistance

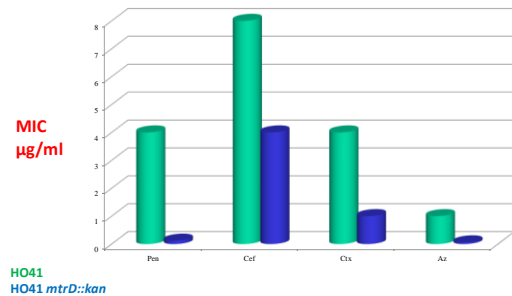
Promoter mutations: High R  
MtrR mutations: Intermediate R



## 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

Gail A. Bolan, M.D., P. Frederick Sparling, M.D., and Judith N. Wasserheit, M.D., M.P.H.

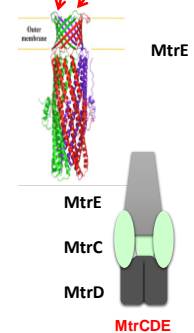
FEBRUARY 9, 2012



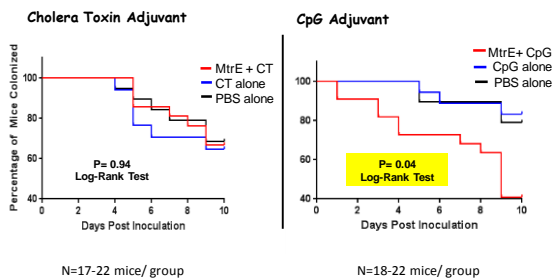
## Can We Exploit the MtrCDE Efflux Pump In Efforts to Counteract Antibiotic Resistance Expressed by Gonococci?

## 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



Many thanks to the NIH and VA  
for funding support