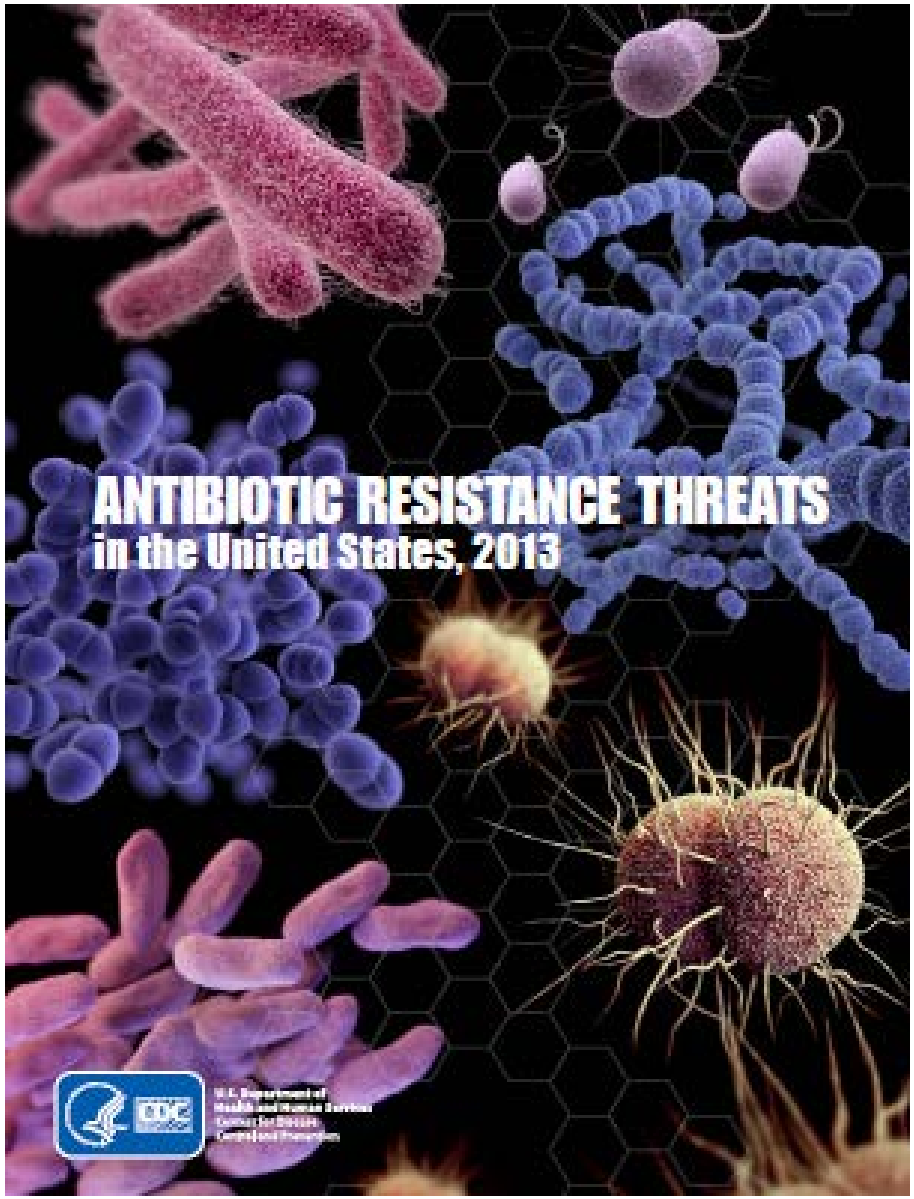


The CDC big three; challenges from the laboratory perspective

Todd F Hatchette MD FRCPC

AMMI Annual Conference 2015

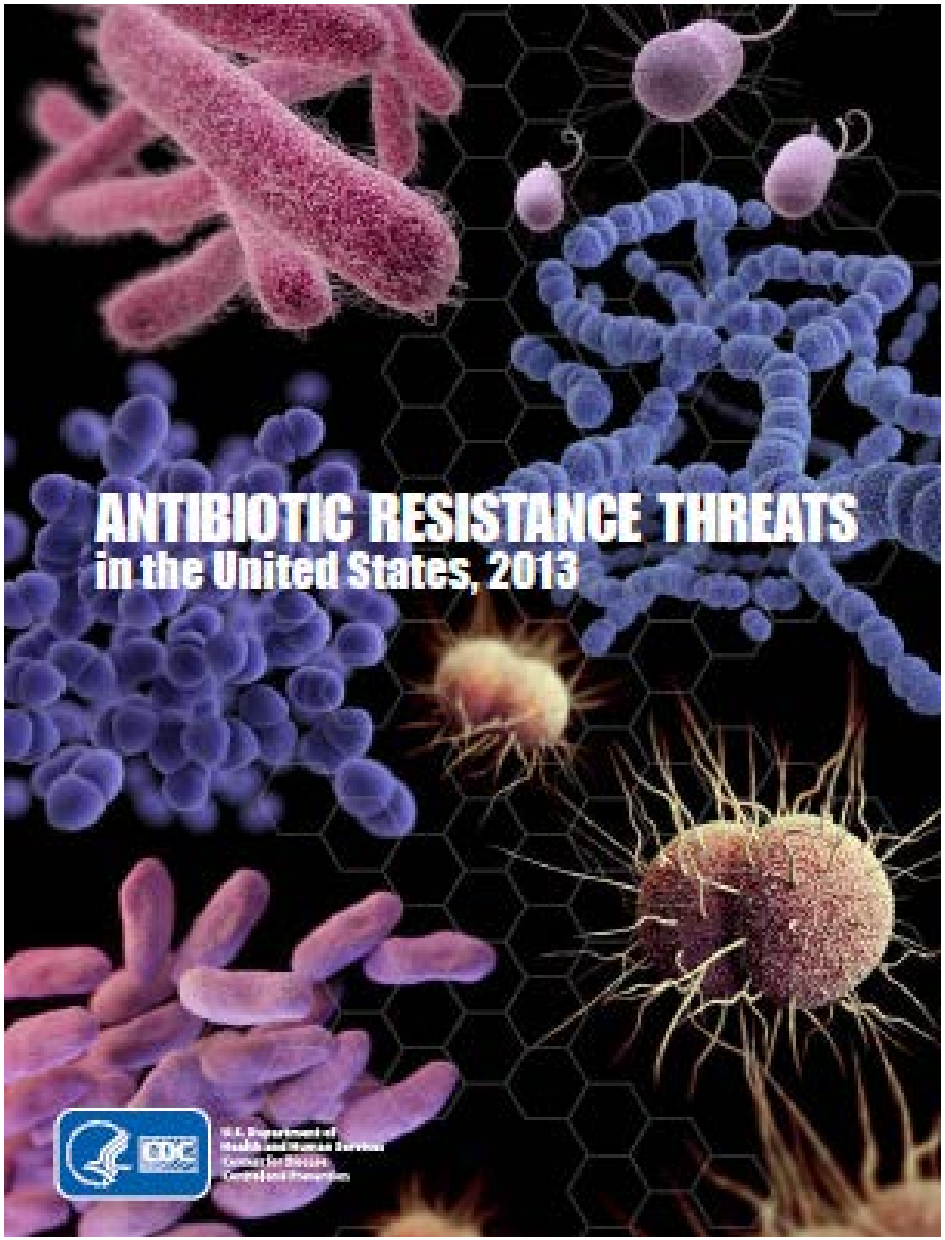
Charlottetown, PEI

Disclosures

- PHAC working groups
- Department of Health and Wellness
- Collaborative research grant with GSK for the SOS network and influenza vaccine effectiveness

Outline

- Describe the capabilities and challenges of novel tools used for the detection of carbapenemase-producing organisms.
- Contrast Clostridium difficile testing algorithms.
  - Are labs using the best strategy?
- Describe impact of current laboratory practices as it relates to GC



Call to Action

"is a snapshot of the complex problem of antibiotic resistance today and the potentially catastrophic consequences of inaction."

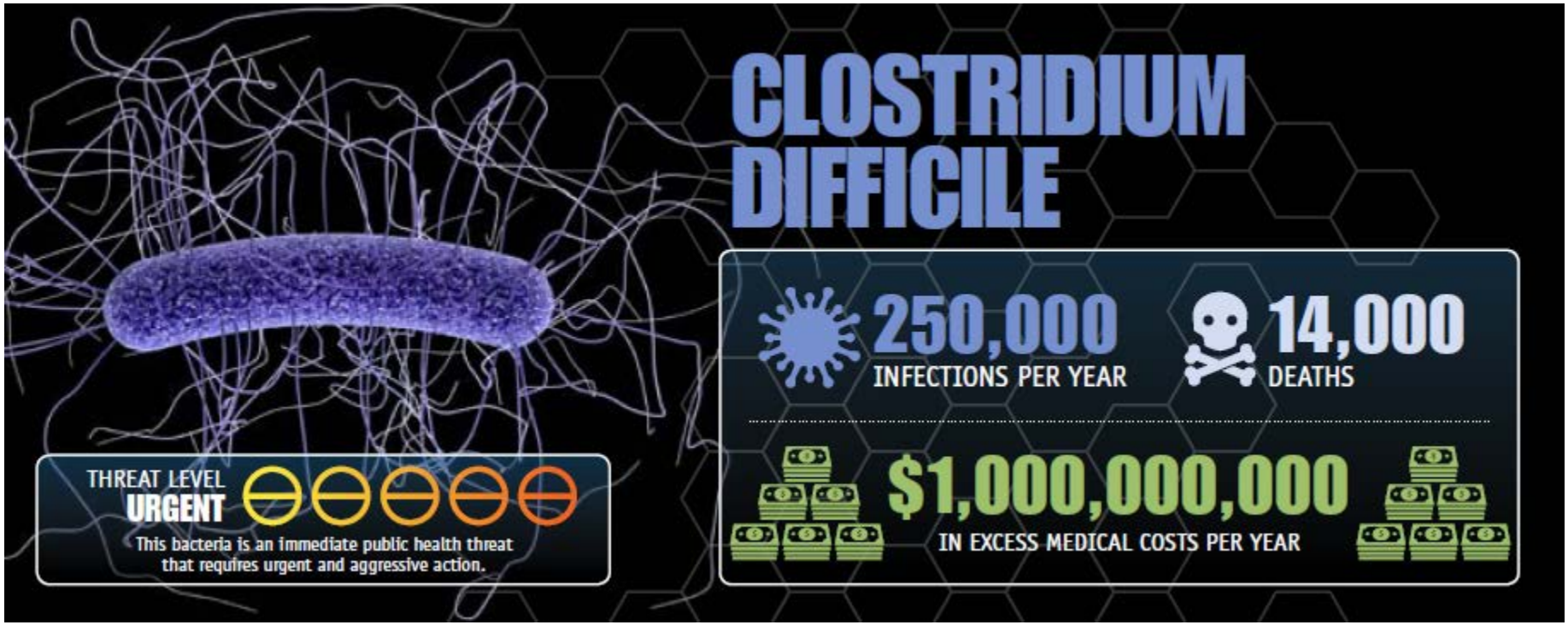
- Urgent Threats
  - Significant risk
  - Limited treatment options
- Serious Threats
  - Reduced incidence or more treatment options
- Concerning Threats

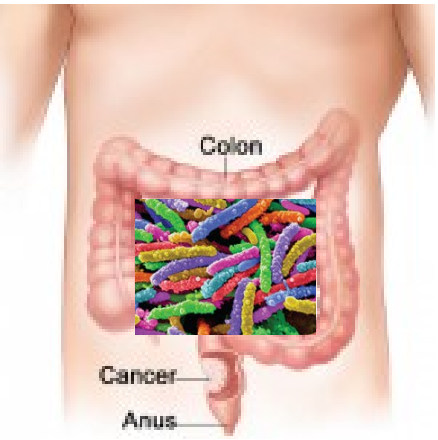
Urgent Threats

Clostridium difficile

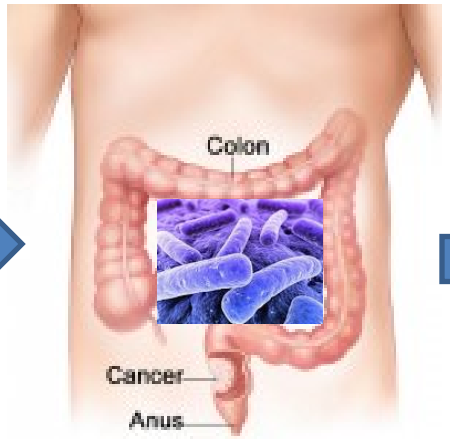
Carbapenem-resistant Enterobacteriaceae

Drug-resistant *Neisseria gonorrhoeae*

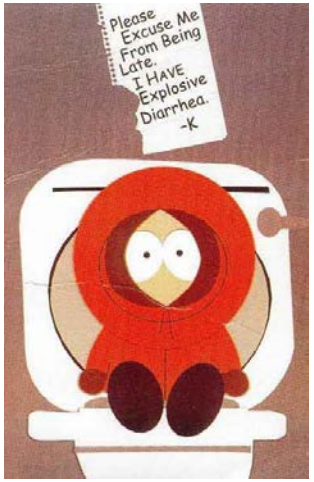




Kills normal flora

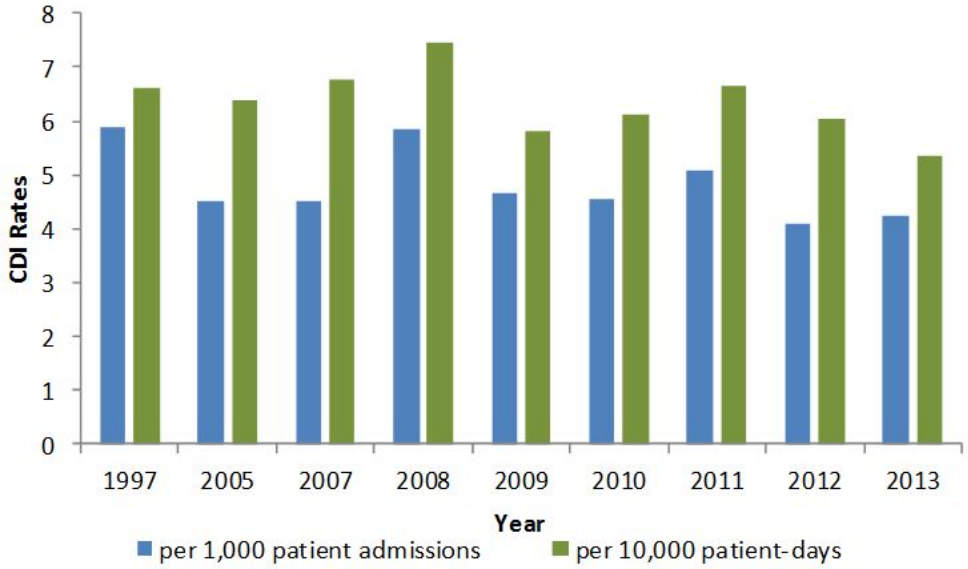


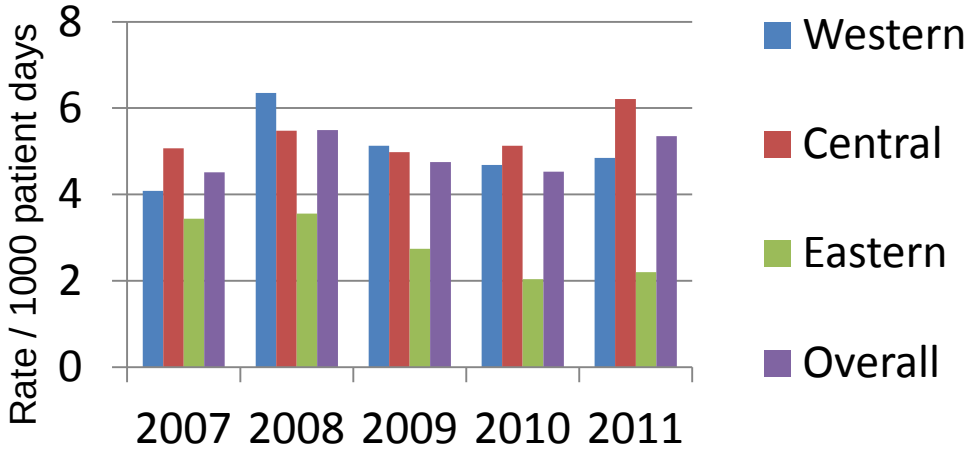
C diff



C.Diff in Canada

(PHAC)





Ideal Assay

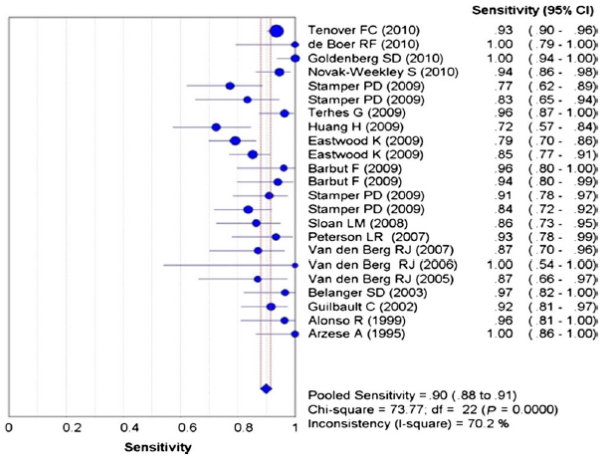
- Want a rapid, accurate inexpensive test
- Tests of limited sensitivity lead to false negatives and potential for further spread and morbidity
- Tests of low specificity lad to unnecessary isolation (or cohorting that could increase risk of exposure) and treatment
- *No Single test fits these requirements*

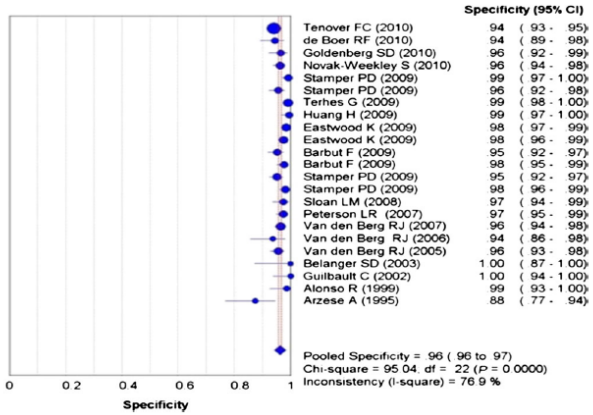
Assay	Pros	Cons	sens	Spec	Cost
Immunoasssay for Toxin A / B	• Rapid • Easy to use	• Lacks sensitivity	69-99% (as low as 38%)	92 -100%	+
glucose dehydrogenase	• Very high NPV • batchable	• Not specific • Positive needs confirmation	88 – 100%	83 -100%	+
Cell Culture Cytotoxic assay	• Identifies presence of the toxin	• Takes 48 hrs for a negative • Requires tissue culture	70-100	90-100	++
Toxigenic culture	• "gold standard"	• Test takes upto 5 days • Cumbersome	90-100	98-100	+++
NAAT	• Can be rapid • Very sensitive	• Expensive • Does not differentiate colonization	88-91%	96-97%	++++

Plancehe et al., 2008 Lancet Infect Dis 8:777 ; Shetty et al., Jl of Hosp Infecti (2011) 1e6
Alfa, and Sepehri. Can J Infect Dis Med Microbiol 2013;24(2):89-92.
Deshpande et al., 2011. Clin Infect Dis 53:e81-e90

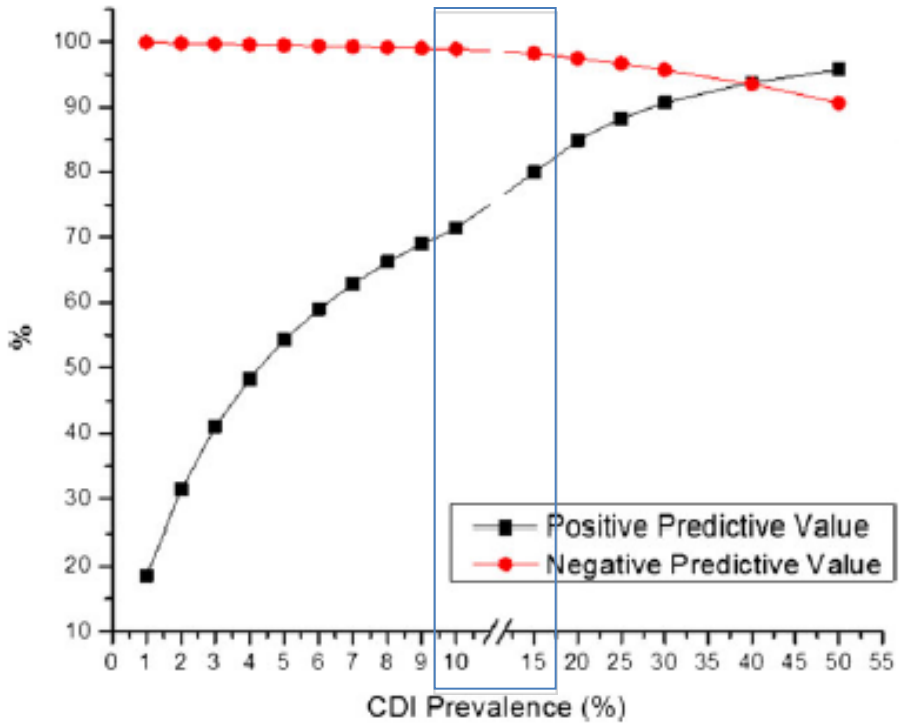
Performance of NAAT a Systemic Review

(Deshpande et al., 2011. Clin Infect Dis 53:e81-e90)



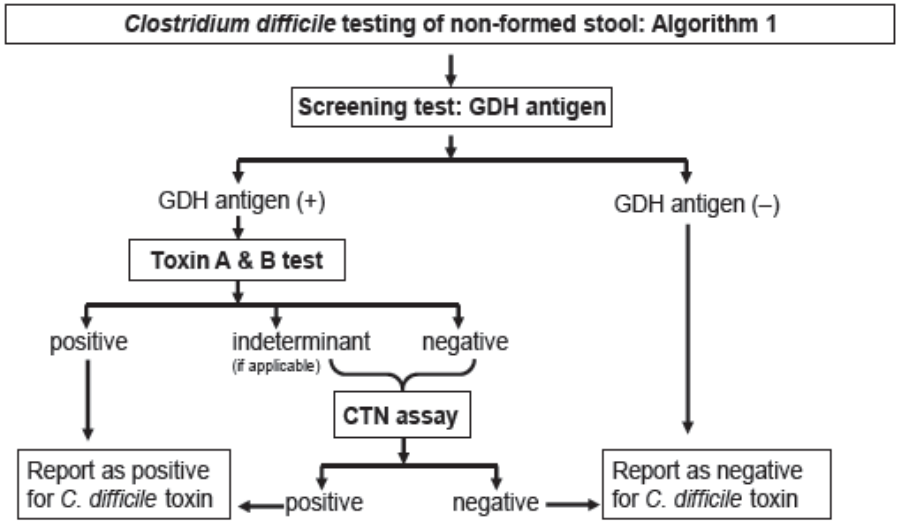


- Pooled sens – 90%
- Pooled spec – 96%



Multi-Step Algorithms

- Options:
- NAAT alone
  - How do you confirm
  - What is the batch size
- Screen with GDH
  - excellent NPV and EIA can be run daily
- But requires confirmation
  - Confirm with Tox A/B EIA
  - confirm with CCCNA
  - Confirm with NAAT



Alfa, and Sepehri. Can J Infect Dis Med Microbiol 2013;24(2):89-92.

How do Multi-step Algorithms Perform?

- Novak-Weekley et al, J Clin Microbiol 2010 48:889-893
  - Prospective study 432 stool samples (72 pos – prevalence 16.7%)

TABLE 1. Summary of algorithm versus stand-alone testing options compared to direct/enriched toxigenic culture

Parameter[a]	Test(s)				
	EIA only	GDH + EIA	GDH + EIA + cytotoxin[b]	GDH + Xpert[c]	Xpert only[d]
No. of specimens	432	432	431	432	428
Sensitivity	58.3 (42/72)	55.6 (40/72)	83.1 (59/71)	86.1 (62/72)	94.4% (68/72)
Specificity	94.7 (341/360)	98.3 (354/360)	96.7 (348/360)	97.8 (352/360)	96.3 (343/356)
Accuracy	88.7 (383/432)	91.2 (394/432)	94.4 (407/431)	95.8 (414/432)	96.0 (411/428)
PPV	68.9 (42/61)	87.0 (40/46)	83.1 (59/71)	88.6 (62/70)	84.0 (68/81)
NPV	91.9 (341/371)	91.7 (354/386)	96.7 (348/360)	97.2 (352/362)	98.8 (343/347)

- Hart et al., Eur J Clin Microbiol Infect Dis (2014) 33:1555–1564
  - Pediatrics n=150 (36% prevalence)

Test	Sensitivity (%)	Specificity (%)
GDH	87	97
Toxin A/B	29	100
Illumigene	89	100
GeneOhm	89	99
CCNA	33	100

Table 2 Statistica

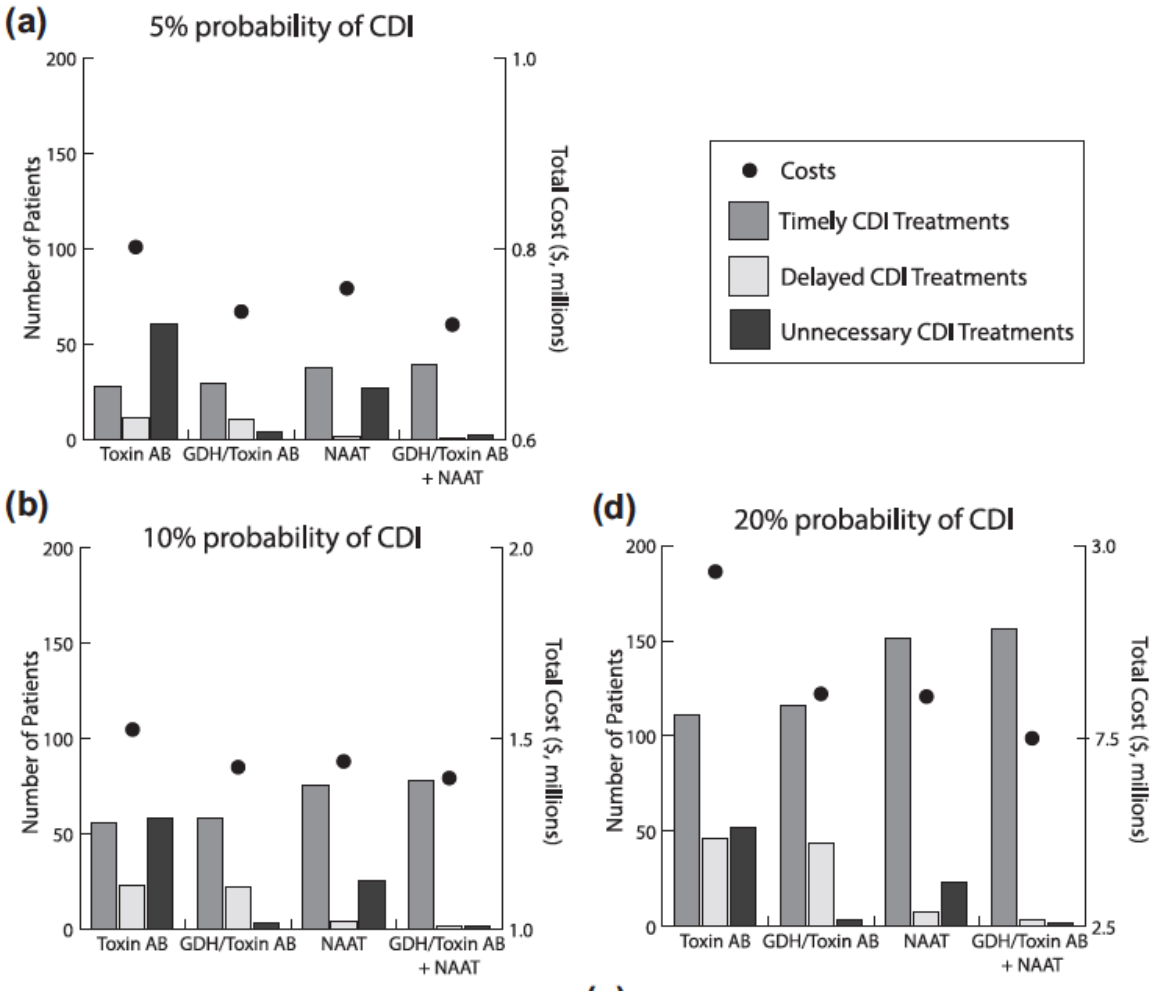
Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
GDH + AB	28	97	81	75
GDH + Illumigen	85	100	100	94
GDH + GeneOhm	83	99	97	93
CCFA + CCNA	30	100	100	76

Which is Cost Effective

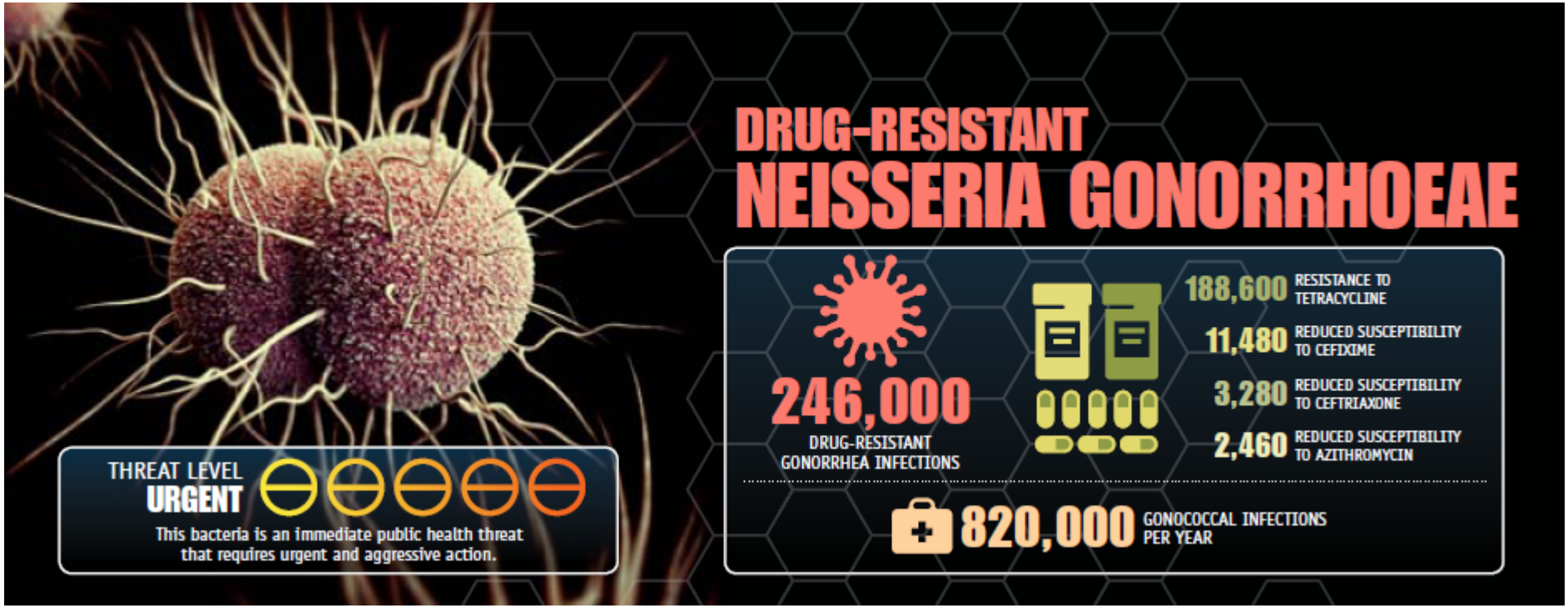
- Bartsch et al., 2015 (Clin Microbiol Infec 21:77e)
  - Modeled the cost of different algorithms
    - Tox A/B
    - GDH + Tox A/B
    - NAAT
    - GDH/TOX A/B + NAAT
  - Factored in isolation costs, treatment delays, inappropriate treatment, potential for secondary cases

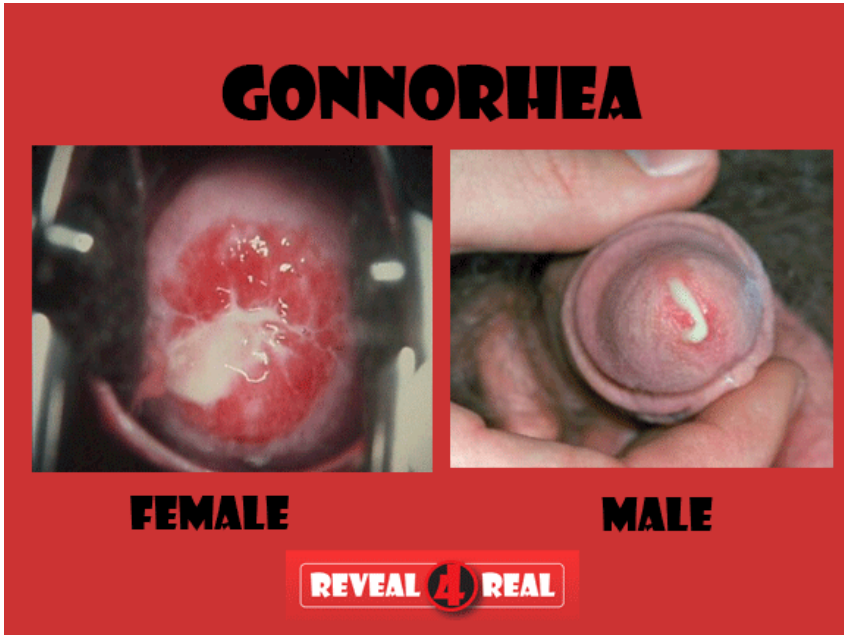
GDH-Tox A/B + NAAT is Cost Effective

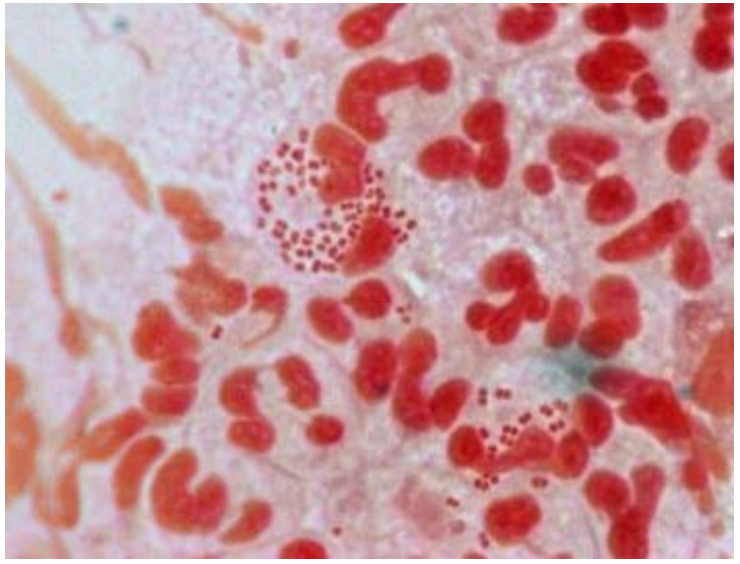
- GDH/ToxA/B + NAAT also had fewest unnecessary bed delays

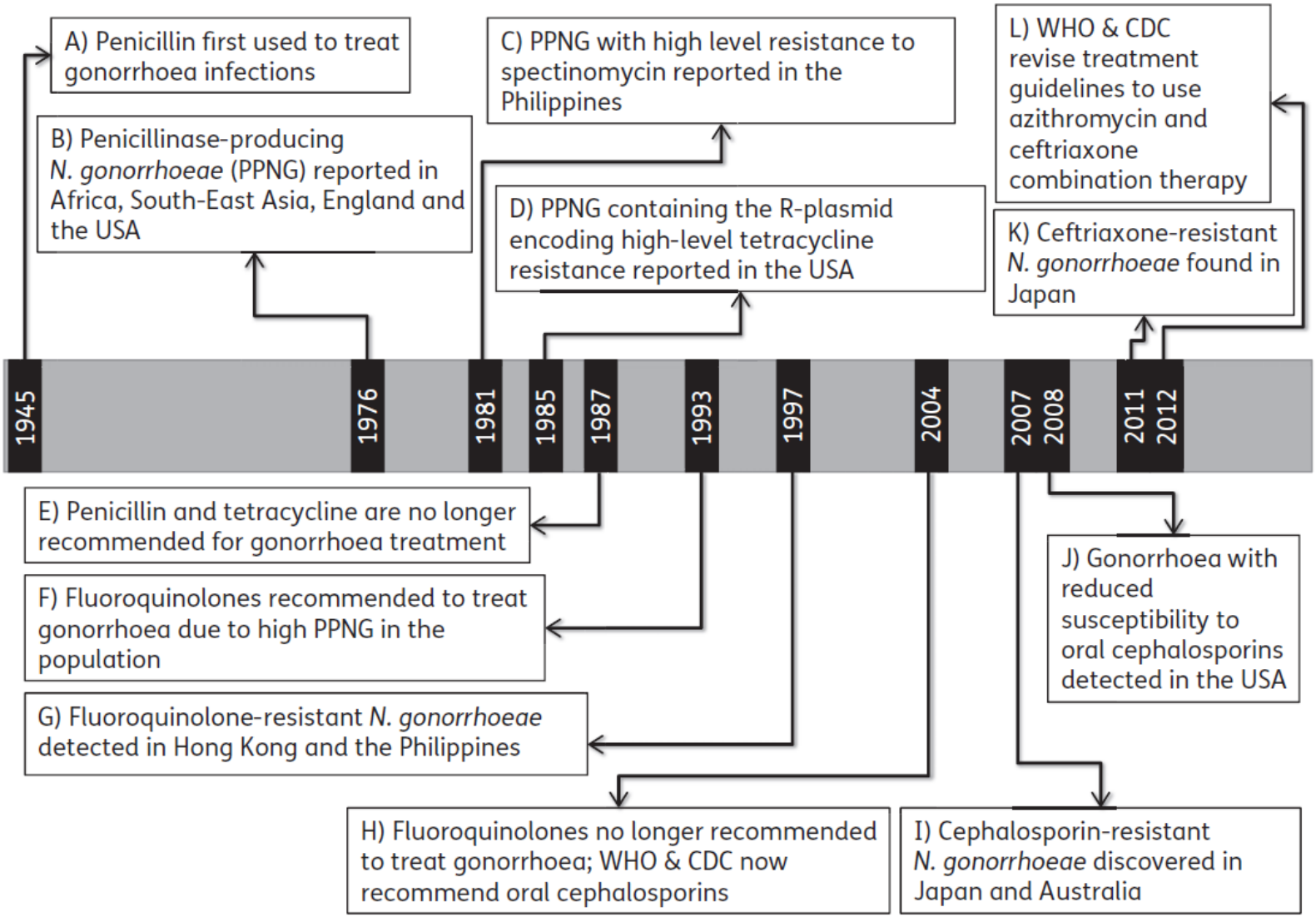


What about antimicrobial resistance?


DRUG-RESISTANT
NEISSERIA GONORRHOEAE
THREAT LEVEL
URGENT
This bacteria is an immediate public health threat that requires urgent and aggressive action.
246,000
DRUG-RESISTANT GONORRHEA INFECTIONS
188,600 RESISTANCE TO TETRACYCLINE
11,480 REDUCED SUSCEPTIBILITY TO CEFIXIME
3,280 REDUCED SUSCEPTIBILITY TO CEFTRIAXONE
2,460 REDUCED SUSCEPTIBILITY TO AZITHROMYCIN
820,000 GONOCOCCAL INFECTIONS PER YEAR


GONNORHEA
FEMALE
MALE
REVEAL 4 REAL

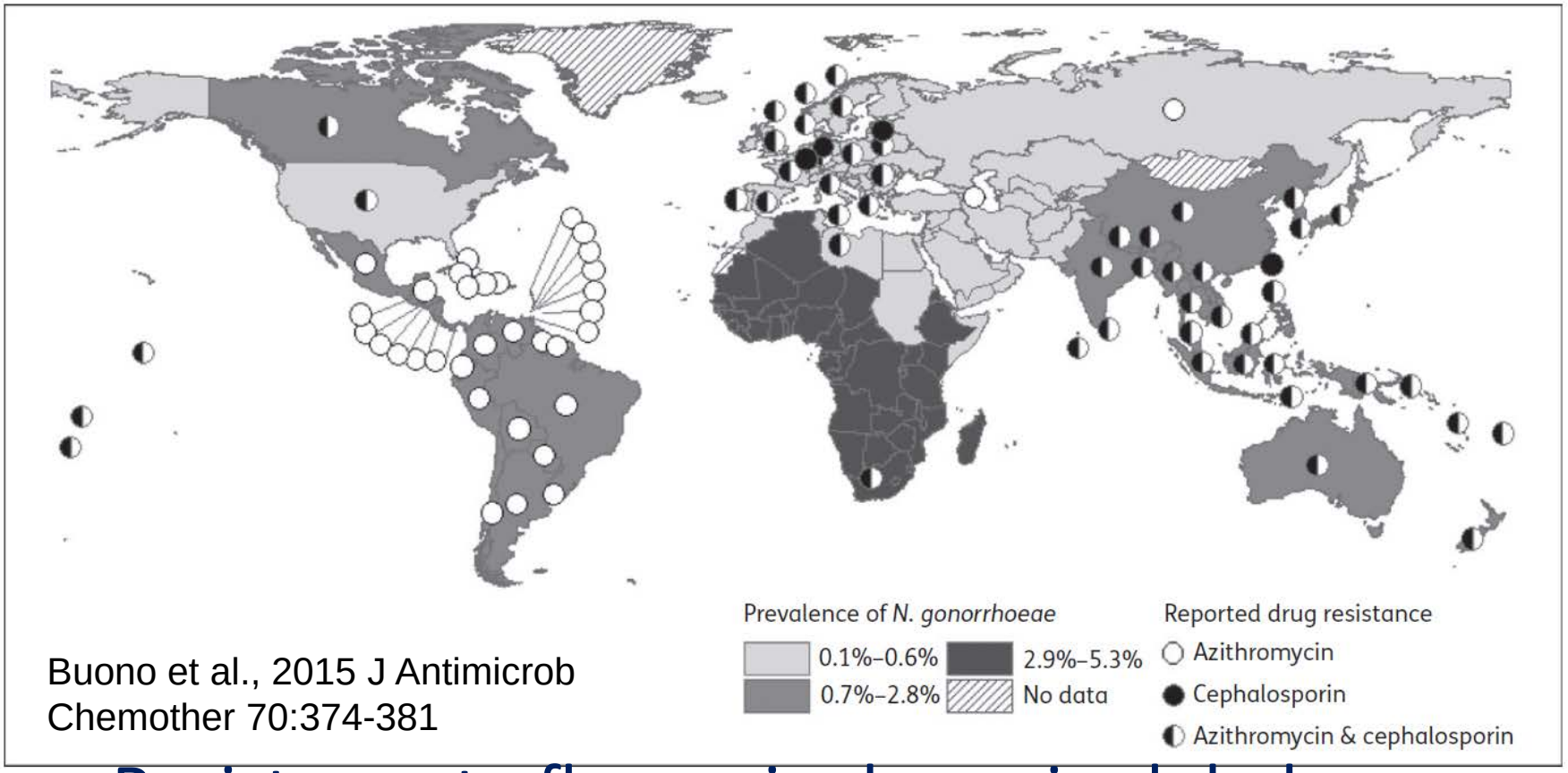




Buono et al., 2015 J Antimicrob Chemother 70:374-381

- Selection Pressure allows for Horizontal gene transfer form non GC Neisseria particularly in the throat
- WHO recommends only drugs with >95% efficacy be used as first line rx
- Ideally we could have individualized treatment to ensure narrowest spectrum used

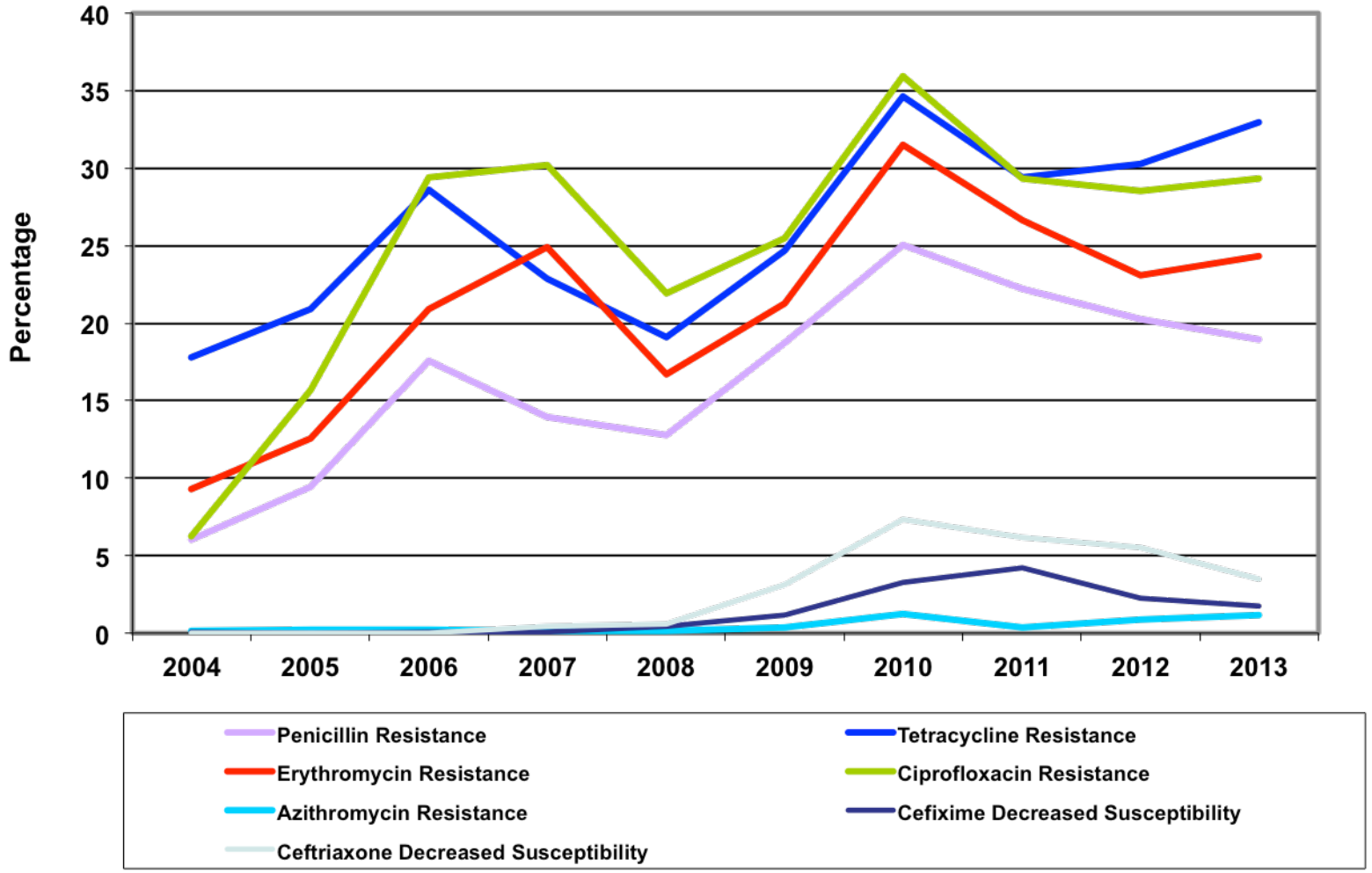
Resistance to Azithromycin and Cephalosporins is a global Problem



Buono et al., 2015 J Antimicrob Chemother 70:374-381

- Resistance to fluorquinolones is global

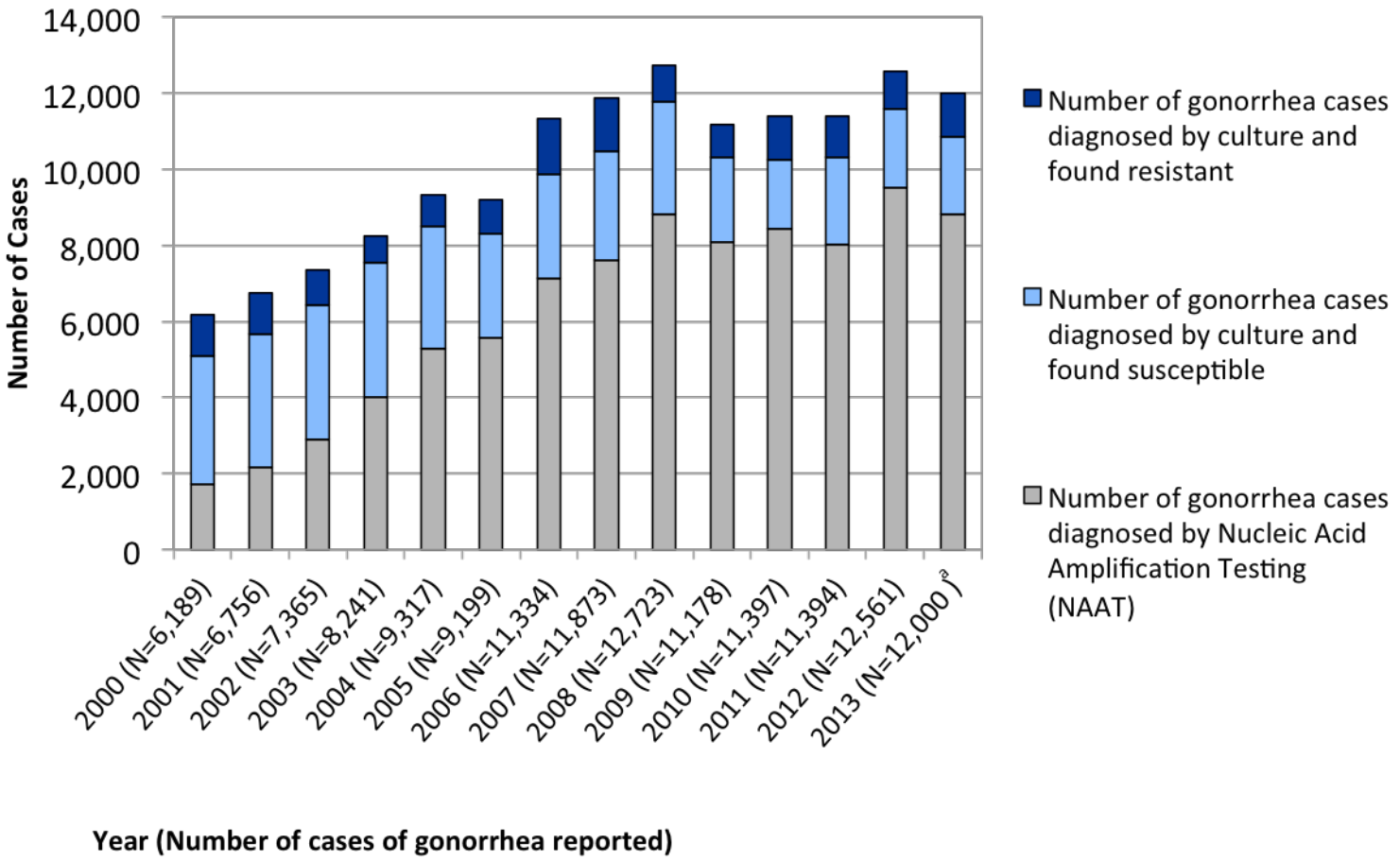
GC Resistance Rates in Canada are Increasing



Source: Irene Martin NML (National Surveillance of Antimicrobial Susceptibilities of Neisseria gonorrhoeae Annual Summary 2013)

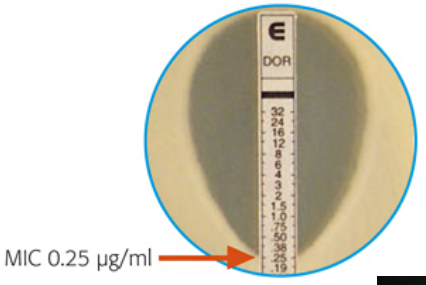
Canadian Data

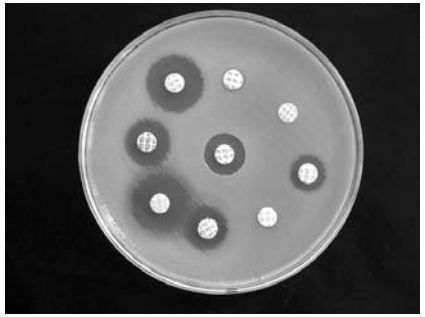
Molecular testing more common



Source: Irene Martin NML (National Surveillance of Antimicrobial Susceptibilities of Neisseria gonorrhoeae Annual Summary 2013)

Resistance Detection Methods





- Agar dilution methods are CLSI recommended standard
  - Time consuming
  - Labor intensive
- E test / disc diffusion have been used

Images:
•Prajna Sharma and Vishwanath 2012
•www.biomerieux-diagnostics.com/etest
•wikipedia

Molecular identification of resistance

- No commercially available method
- In house methods are available
  - Quinolone – gyrA and parC
  - Azithromycin - 23s rRNA and mtrR mutations
  - Cephalosporin – mosaic penA gene

Challenge

- Rapid evolution
- NAAT requires a known target
- Acquisition of plasmid and chromosomally mediated resistance
- Variability between penA alleles can lead to different MICs
- Wont pick up “unknown” mechanisms like phenotypic testing
- Mechanisms are shared with commensal organisms

Challenges

Multiplexing is possible but not all mechanisms are well characterized

NAAT Good

- TEM-1 [penicillin]
- Tet(M) [tetracycline]
- parC/gyrA [quinolone]
- mtrR [azithromycin]

NAAT not so good

- Cephalosporins
  - penA – high sequence variability
- Azithromycin
  - 23SrRNA allele availability

Data Starting to Emerge for NAAT from Residual Specimens

- Nicol et al., 2015 (Sex Transm Infect 91:91-93)
  - Three real time assays to detect gyrA, PPNG, and sequence for mosaic penA on residual specimen from Cobas 4800 CT/GC assay
  - 94% of specimens had enough DNA for amplification

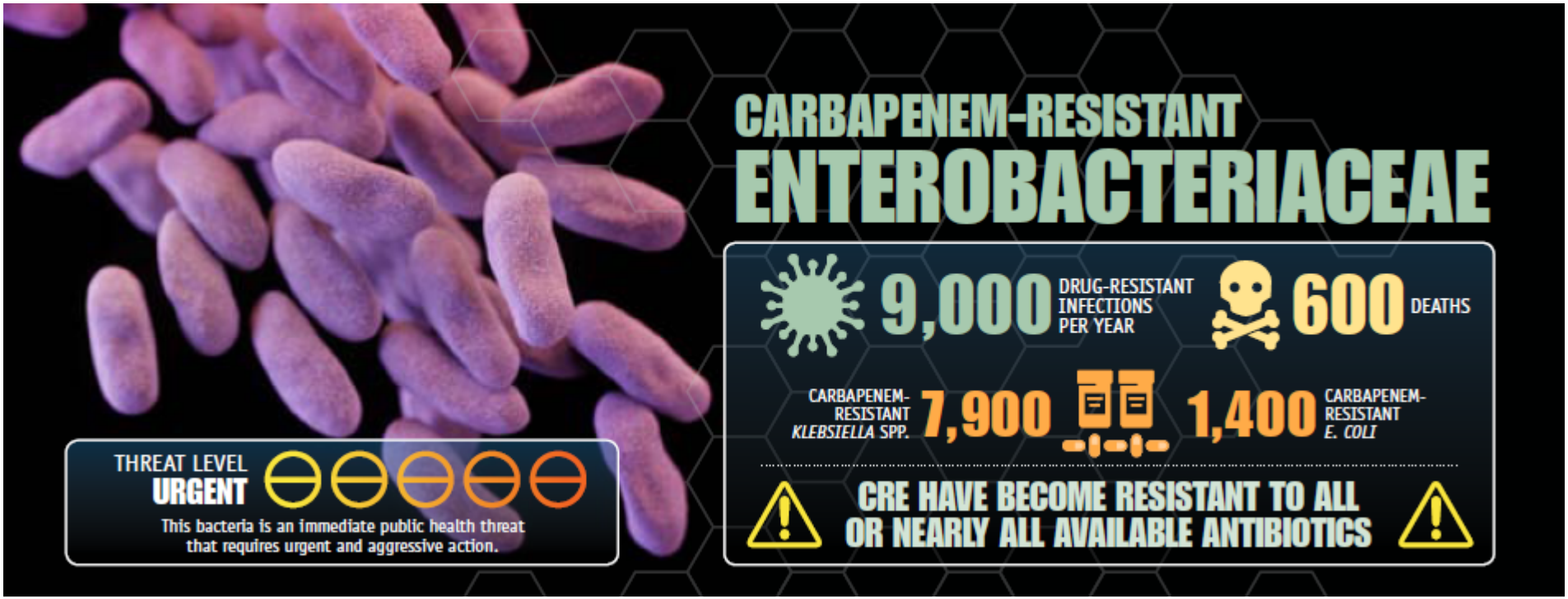
Culture Based Surveillance

Canada - Enhanced surveillance of antimicrobial-resistant gonorrhea program (ESAG)

- NS has 3 clinics and callbacks,
- MB has 5 engaged service
- AB has 2 STI clinics (Edmonton and Calgary)
- Other P/Ts are showing interest but not fully participating as yet.

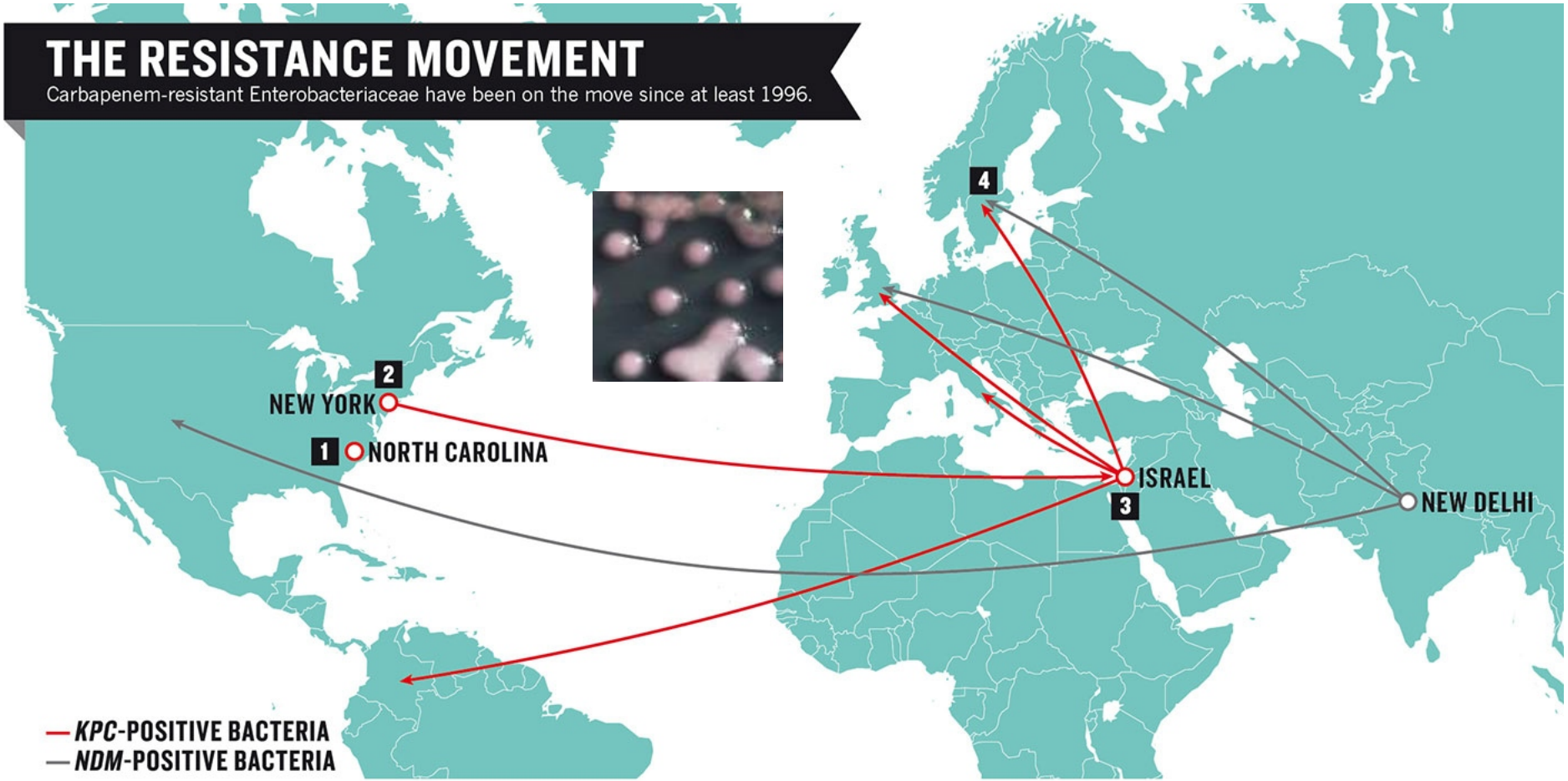
US - Gonoccoccal Isolate Surveillance Project (GISP)

26 sites



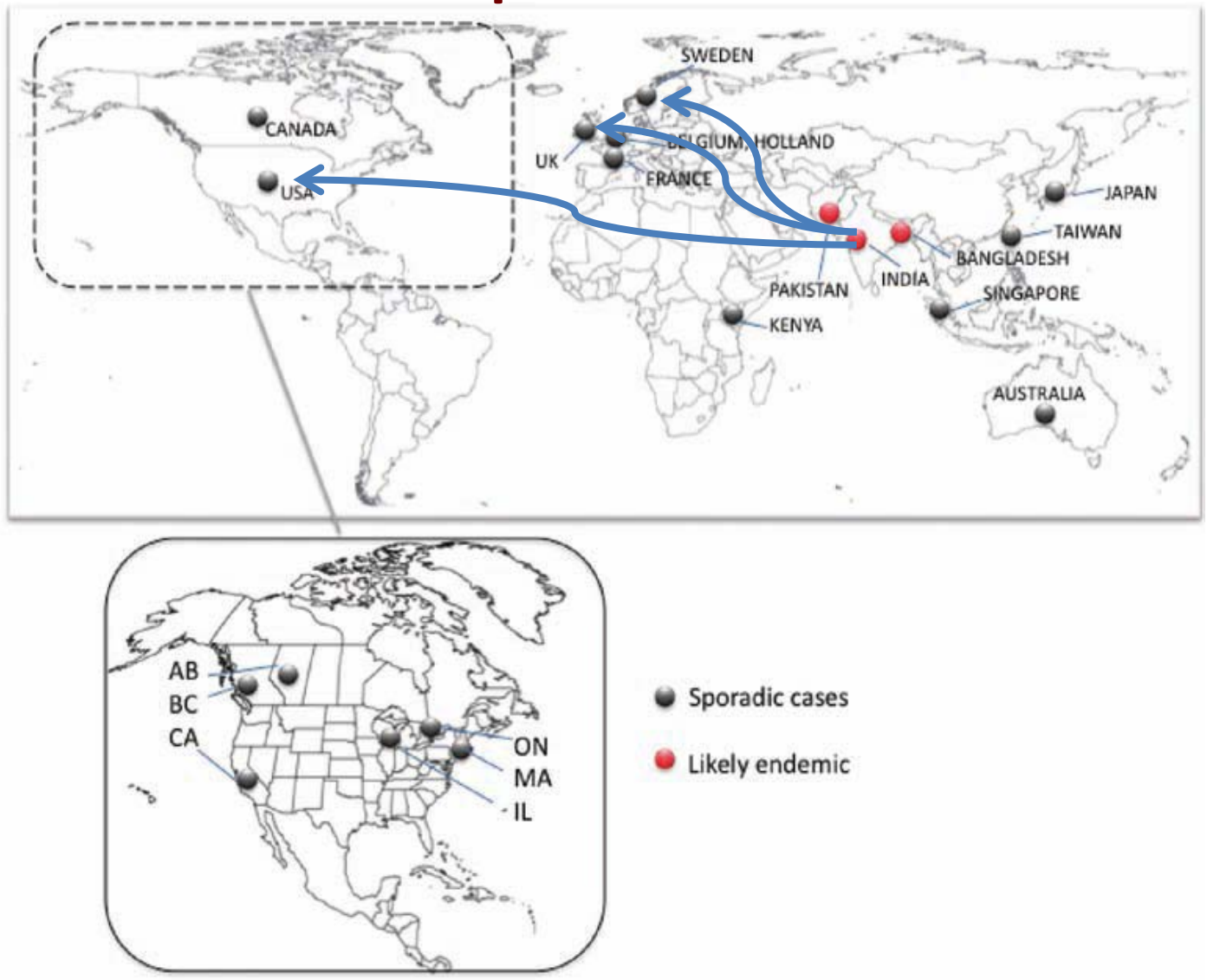
- Latest Canadian Data

Global Spread



MCKENNA, Nature, 2013

Carbepenem Resistance- Global Spread



CRE

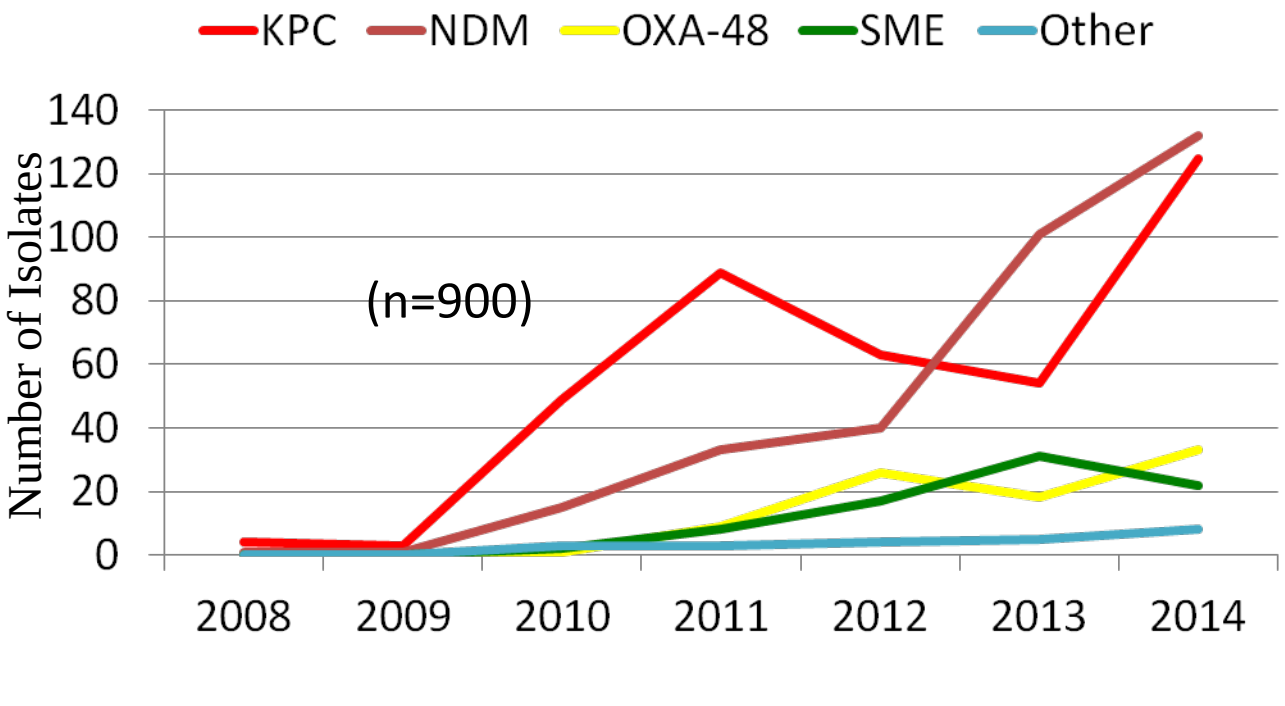
Variability in Mechanisms

1. A beta lactamase with a Porin mutation
2. Specific Carbepenamase enzymes

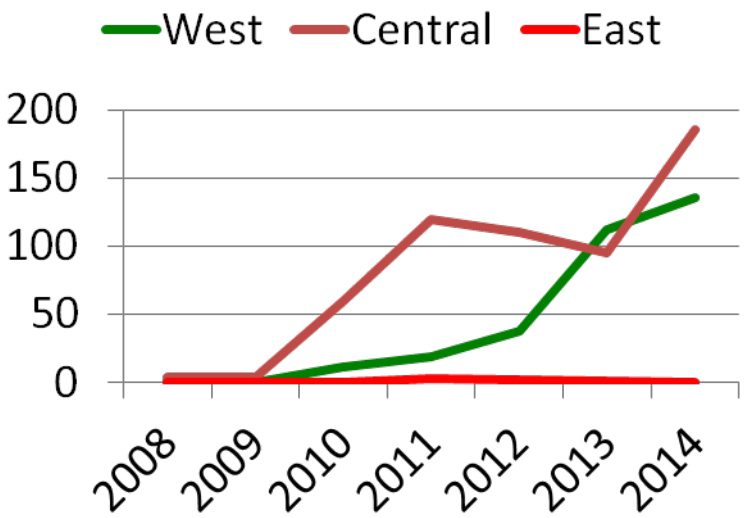
Enzyme Class / Characteristics	Different Types
Class A beta lactamase enzymes •Hydrolyze all beta lactams •Inhibited by boronic acid and partially by calvulanic acid	**KPC**, SME , IMI, NMC, GES
Class B beta lactamase enzymes •Highest carbapenemase activity •Generally only spare monbactam •Not inhibited by BL inhibitors •Required Zinc	Often named by place of origin **NDM, IMP, VIM,** GIM, SPM, SIM
Class D beta lactamase enzymes •Spares ceftazidime •Often require another enzyme (ESBL) for complete resistance	**OXA-48** ; OXA-181

Nordman et al., 2012 Clin Microbiol Infect 18: 432-438

CPE in Canada: CPHLN Data



Source: Mike Mulvey, NML



CRE Detection

- Automated systems may not always be able to detect CRE

	MIC (mg/L)		
	Imipenem	Meropenem	Ertapenem
KPC	0.5 to >32	0.5 to >32	0.5 to >32
IMP/VIM/NDM	0.5 to >32	0.5 to >64	0.38 to >32
OXA-48/OXA-181	0.25 to 64	0.38 to 64	0.38 to >32

- CLSI have lowered breakpoints for better detection

Good Candidate
May lack specificity

	CLSI		EUCAST	
	S (≤)	R (≥)	S (≤)	R (>)
Imipenem	1	4	2	8
Meropenem	1	4	2	8
Ertapenem	0.5	2	0.5	1
Doripenem	1	4	2	8

Nordman et al., 2012 Clin Microbiol Infect 18: 432-438

Is Confirmation Necessary?

It Depends

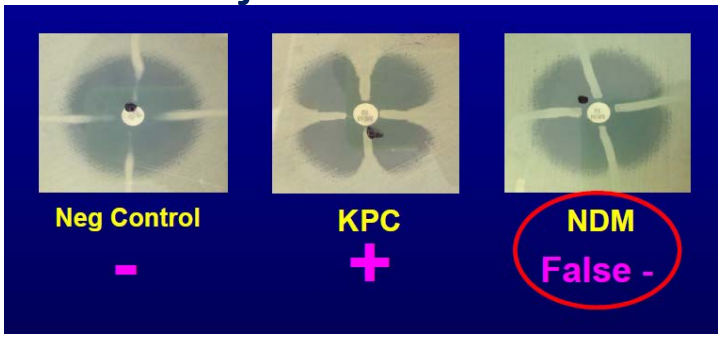
- CLSI does not recommend confirmation
  - Breakpoints all that is necessary for treatment decisions
  - But not a lot of treatment data out there
  - Some carbapenemases are susceptible or intermediate to carbapenems (OXAs)
    - How do you screen for theses
  - Only necessary for epidemiology and infection control reasons

The Ideal System

- Rapid (same day results)
- Sensitive and Specific
- Easy to perform
- Easy to interpret results
- Identify the different resistance mechanisms
- Identify the different genetic variants
- In expensive

CRE Confirmation

- Phenotypic tests - None has 100% sensitivity or specificty
- Not good for OXA types
- Modified Hodge Test
  - Good for KPC and OXA less for NDM
  - Lacks specificity
  - Time consuming
  - subjective



Source: Janet Hindlre CLSI webinar update Feb 2015

- Addition of inhibitors
- EDTA / boronic acid

www.biomerieux-diagnostics.com

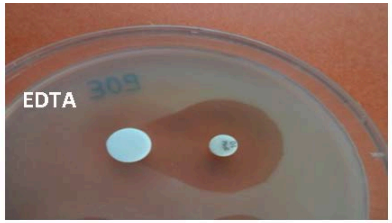


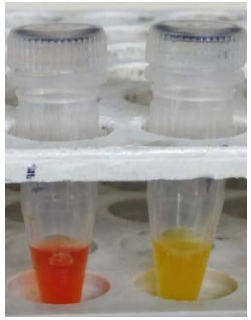
Photo 9 : DDST test +ve

Photo 10 : Disc potentiation test +ve

Basak and Monali-
www.intechopen.com/books/trends-in-infectious-diseases

CARBA NP Test

- Mix suspect colony
- decrease in pH from hydrolysis of carbapenem
- Reagent must be fresh and takes time to prepare
- False negative for OXA
- Can give invalid results (subjective)

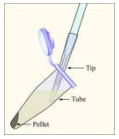


phenol red indicator

Source: Janet Hindlre CLSI webinar update Feb 2015

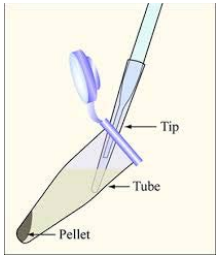
Source: Janet Hindlre CLSI webinar update Feb 2015

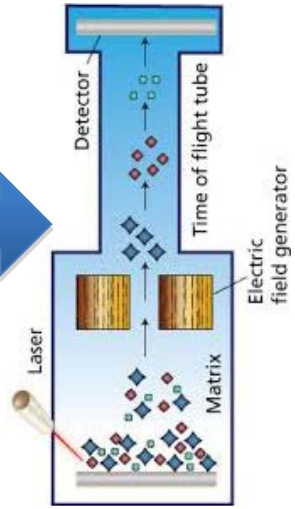
MALDI-TOF Identification of CRE

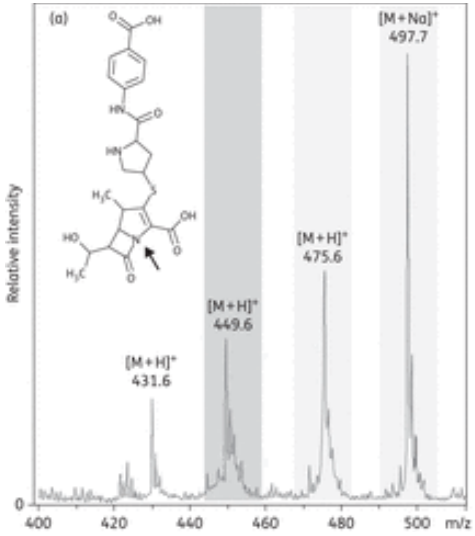


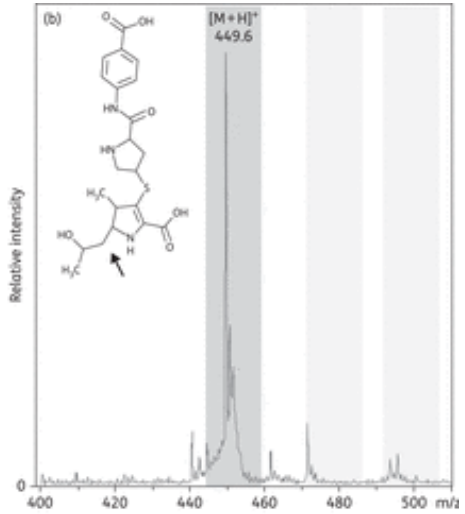
Washed pellet

2-4 hrs

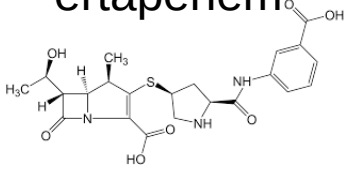




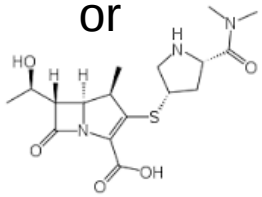




ertapenem



or



merpenem

Carvalhaes et al., 2014. J Antimicrob Chemother. 69(8):2132-6.

MALDI-TOF Identification of CRE

- Carvalhaes et al.., 2014. J Antimicrob Chemother 69: 2132-2136
  - Direct detection of CRE from 100 randomly selected blood cultures
  - 21 isolates were CRE
  - All KPCs and one SM1 detected after 4 hours of incubation
  - 3/11 OXA required testing of bacterial colonies in detect carbapenemase activity
- Papagiannitsis et al 2015 J Clin Microbiol. 2015 Feb 18.
  - Addition of NH_4HCO_3 improved detection of OXA-48

- MALDI TOF
  - Can detect CRE independent of the enzyme produced, including novel enzymes
  - rapid
  - Requires molecular to characterize

Molecular Detection (NAAT)

- Biofire (FDA approved)
  - KPC
- Nanosphere (FDA approved)
  - KPC, NDM, OXA, IMP, VIM
- NucliSENS EasyQ VKPC
- Cepheid
  - KPC, NDM, OXA-48, IMP-1, VIM
- BD Max
  - KPC, NDM, OXA-48
- Check-Points
  - KPC, NDM, OXA-48, IMP, VIM
- Amplex - Hyperplex Superbug ID
  - all variants of VIM, IMP, KPC, OXA-48 NDM-1

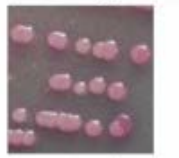
- Expensive
- Requires molecular expertise
- Sensitivity dependant on amount of DNA
  - may require growth first
- Need to target the gene

Source: Janet Hindlre CLSI webinar update Feb 2015

CRE Screening

- Lots of questions that depend on local epidemiology
  - Who, how often etc
- Stools/rectal swabs most common specimen
- None will detect the type of carbapeneamase
- Broth enrichment step may increase KPC detection (delays TAT)
- Direct to screening media
  - CRE specific

www.chromagar.com/clinical-microbiology-chromagar-kpc-focus-on-kpc-resistance-32.html



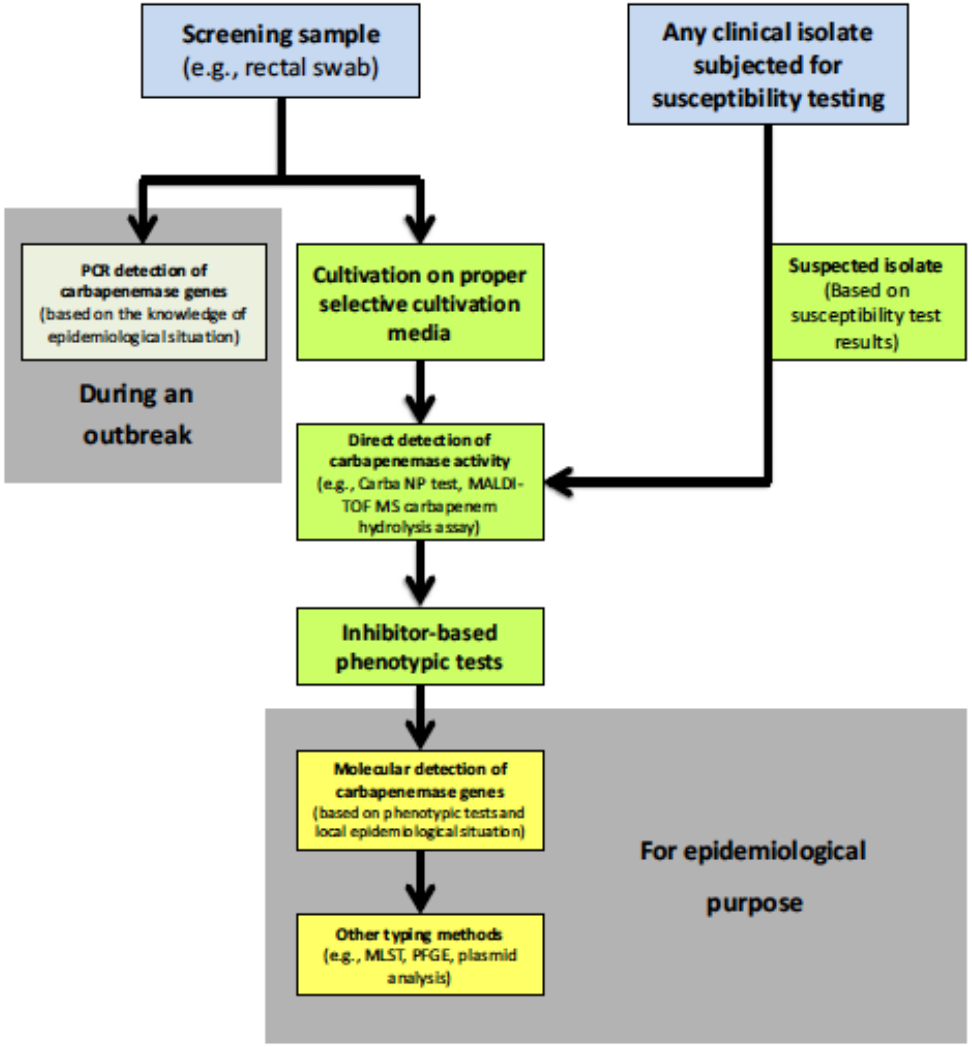
  - ESBL surrogate screening

- Next gen / whole genome sequencing
- Microarray
- MALDI –ToF MS
  - Detect degradation products

Methods of Detection

	Modified Hodge Test	Carba NP	Molecular Detection	MALDI-TOF
Strengths	Relatively simple	rapid	Determines type of carbapenemase	Rapid Inexpensive Detects variety of MBL
Weaknesses	•Can be subjective •False positives due to other mechanisms (ESBL or AMPC + porin mutation) •Some false negatives (NDM – can add zinc)	•Can give invalid results (subjective) •Reagent preparation takes time •False negative for OXA	•Expensive •Requires molecular expertise •Need to target the gene (if it is not included it will not be detected)	•Generate own spectral library •Requires molecular differentiation of types of resistance

Potential Algorithm



Hrabak et al., 2014 Clin Micrbiol Infec 20:839-853

Conclusion

- Resistance is a problem
- Many different options for detecting resistance
- Must be tailored to your local context

Questions