

Rapid Identification of *Clostridium difficile*

May 19, 2009

Michael Oliver
Cepheid Contract Specialist

Roberta O'Neil, MS, MT(ASCP)
Cepheid Product Specialist



Agenda

- Overview of Cepheid : In-vitro diagnostics of HAI's
- Toxin vs. non toxin producing *C. difficile*
- Emergence of the virulent strain
- Limitations of current testing methods
- Description of Real Time PCR testing for rapid, sensitive and specific identification of *C. difficile* and differentiation of the hypervirulent strain

Well Established, Innovative Company

\$120M Sales
in 2007

+8 Years
as a Public Company

12 Years
Operating History

+550 Employees
Including 120 R&D Scientists

+60 Countries
with a Distribution Presence

+7 Million
GeneXpert® System Tests Run



What the Post Office wants to avoid...



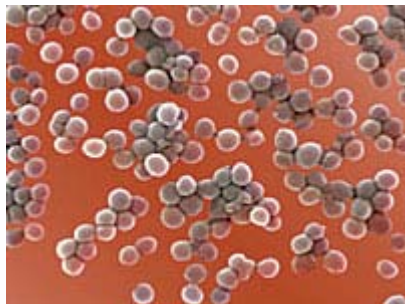
GeneXpert used for Anthrax testing since 2001

280 US Postal Service mail sorting centers:



- 24/7 continuous sampling
- Negative result releases mail batch
- Positive result stops mail, alerts highest authorities, significant fall-out

Labs Assist Infection Control: Critical Issues in Infection Prevention



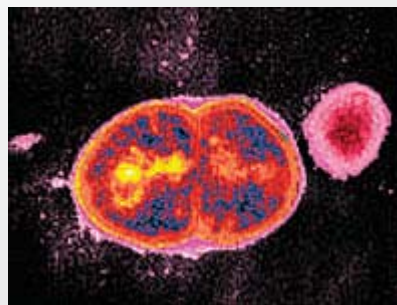
**Methicillin-resistant
*Staphylococcus
Aureus (MRSA)***



***Pseudomonas
aeruginosa***



***Klebsiella
pneumoniae***



**Vancomycin-resistant
Enterococcus faecium
Enterococcus faecalis
(VRE)**

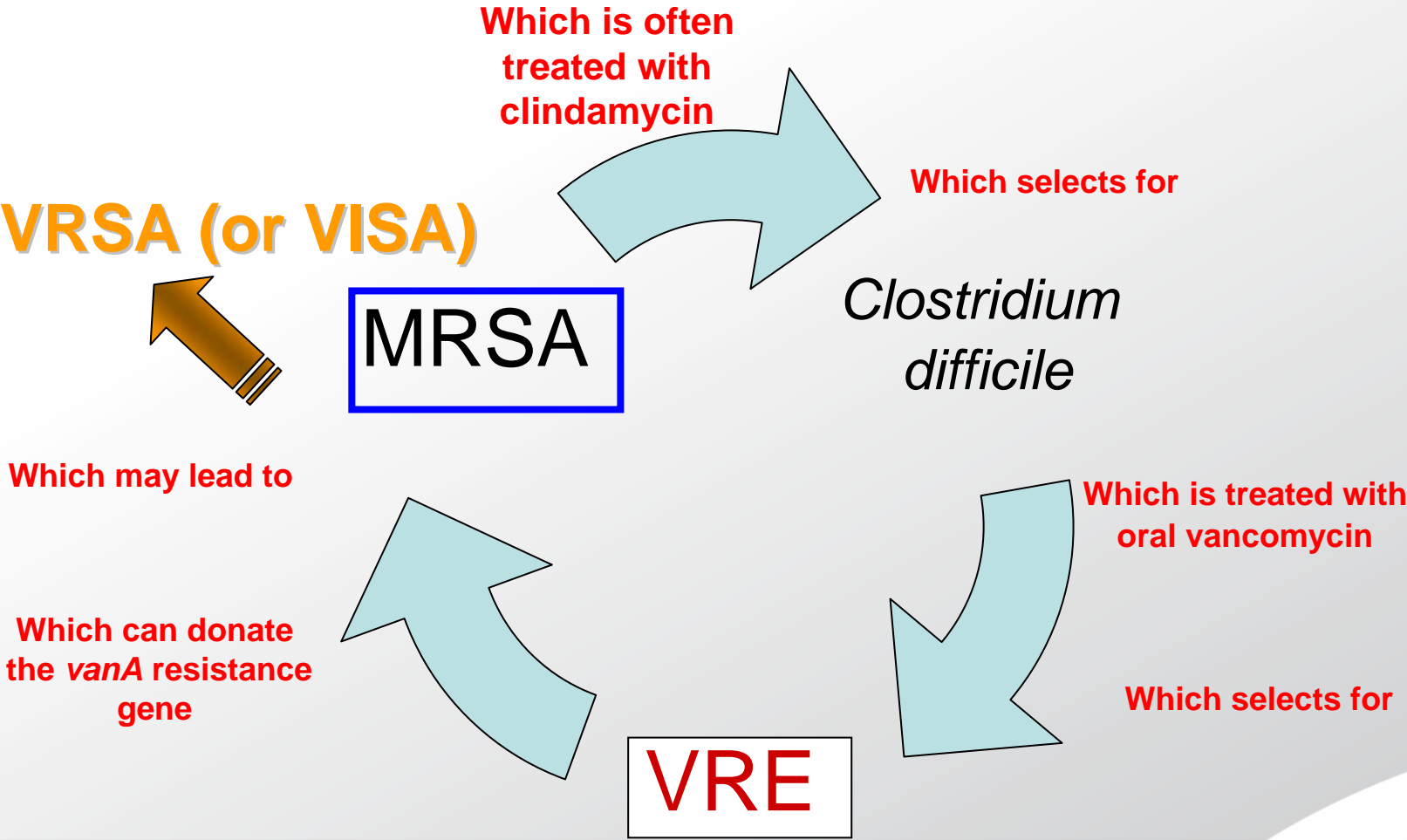


***Acinetobacter
baumannii***



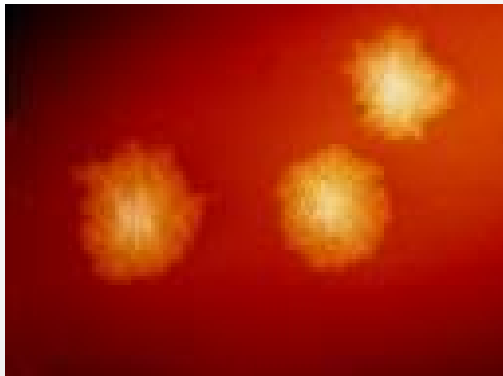
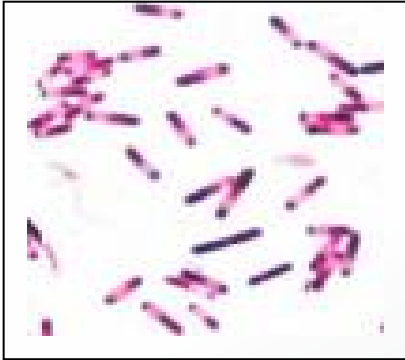
***Clostridium
difficile***

Inter-relatedness of Healthcare Associated Pathogens





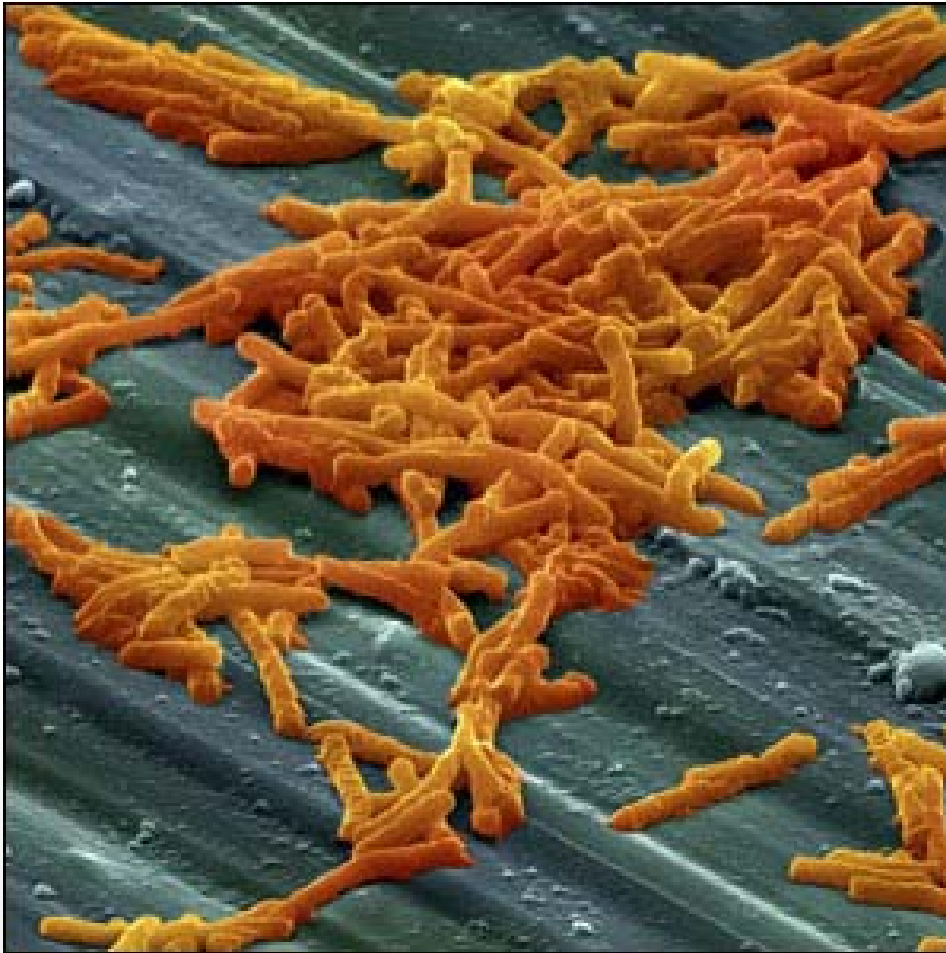
Clostridium difficile - the Organism



- *Clostridium difficile* is a Gram- positive, anaerobic, spore-forming bacillus.
- Spore formation is critical to its prolonged survival in the environment and ability to spread.
- *C. difficile* forms distinctive colonies on selective agar and produces a unique horse barn odor
- Disease is toxin-mediated
- Several strains show resistance to clindamycin and fluoroquinolones

Disease Background

C. difficile pathogenesis



C. difficile can be separated into two main groups: “Toxin Producers” and “Non-Toxin Producers”

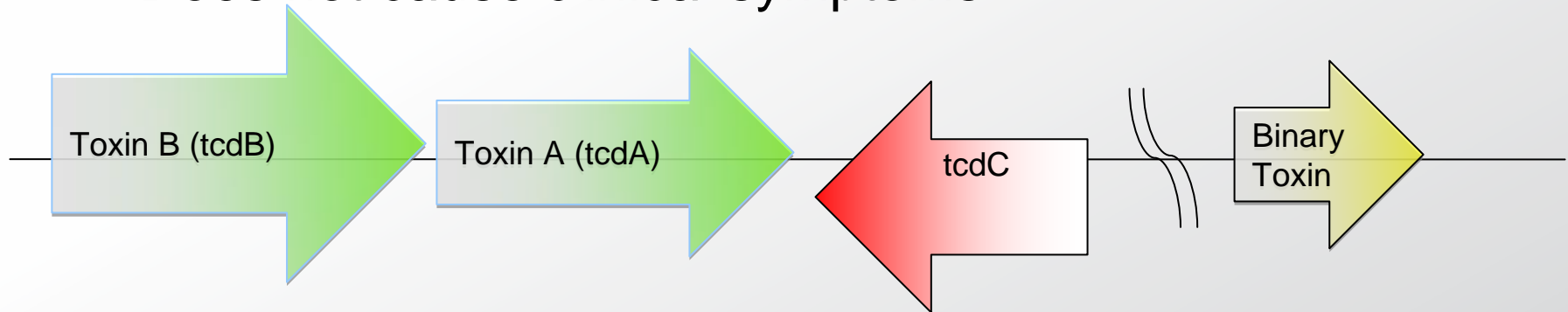
Primary virulence factors are due to two toxins:
Toxin A and **Toxin B**

Disease Background

C. difficile pathogenesis

Non-Toxin Producer:

Does not cause clinical symptoms



Non-toxin producer does not carry genes that can produce toxin!

Disease Background

Clinical Presentation

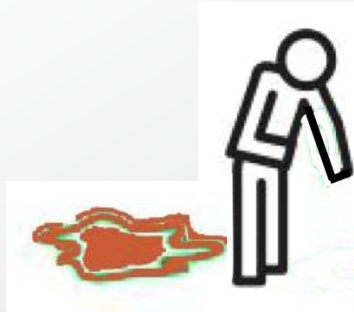


Disease
Onset



Mild
Diarrhea

Abdominal
Cramping



Sever Bloody
Diarrhea

Fever



Colitis

Toxic Mega
Colon



Death

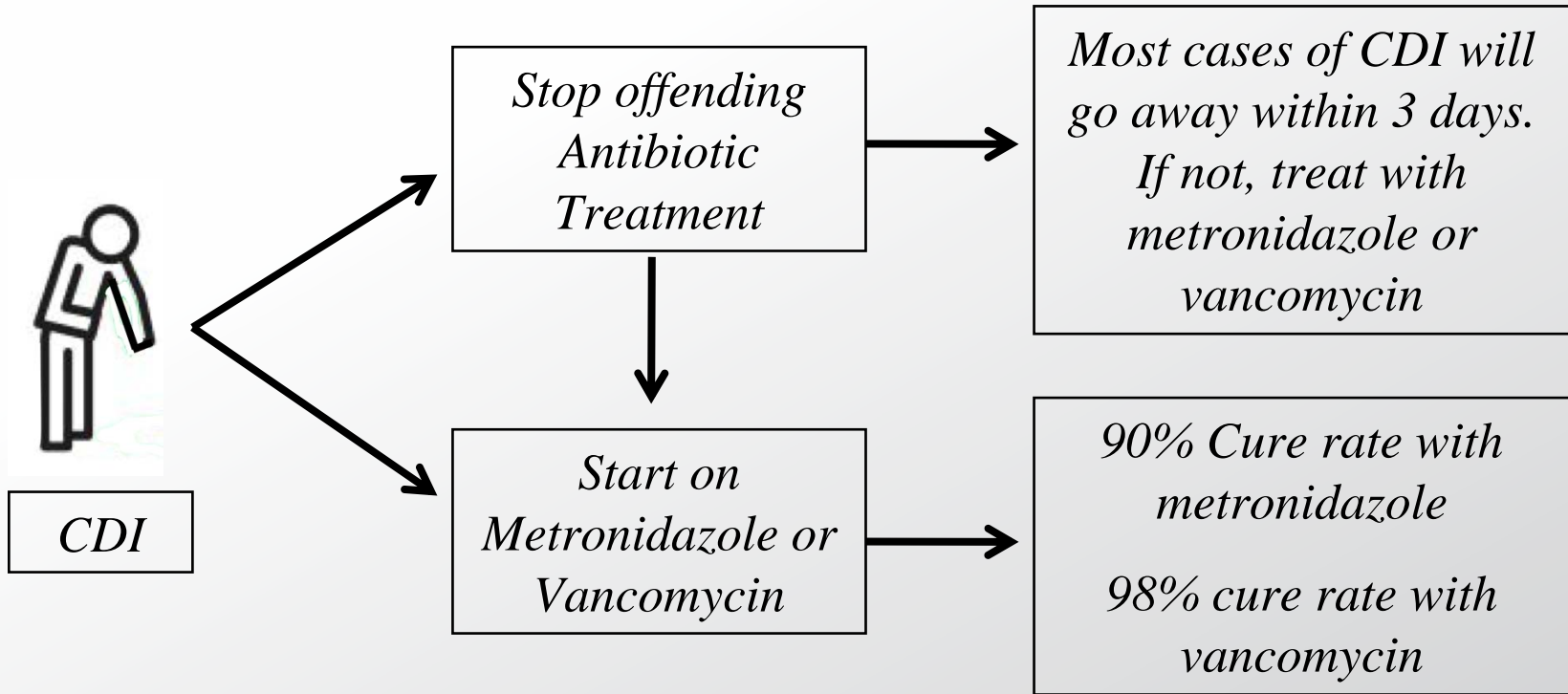
No Medication

Targeted Medication

Surgical Intervention

Treatment Options

Mild Symptoms



Zar, et al., CID 2007;45

New Epidemic Strain of *C. difficile*

- **Name:** BI/NAP1/027, toxinotype III
- Historically uncommon (particularly in U.S. strain collections), now epidemic
- Current strain more resistant to fluoroquinolones
- Carries extra toxin known as binary toxin
- Polymorphism in toxins A and B regulatory gene (*tcdC*) and increased toxin production *in vitro*
- Shows increased spore production

Disease Background

Emerging Hypervirulence

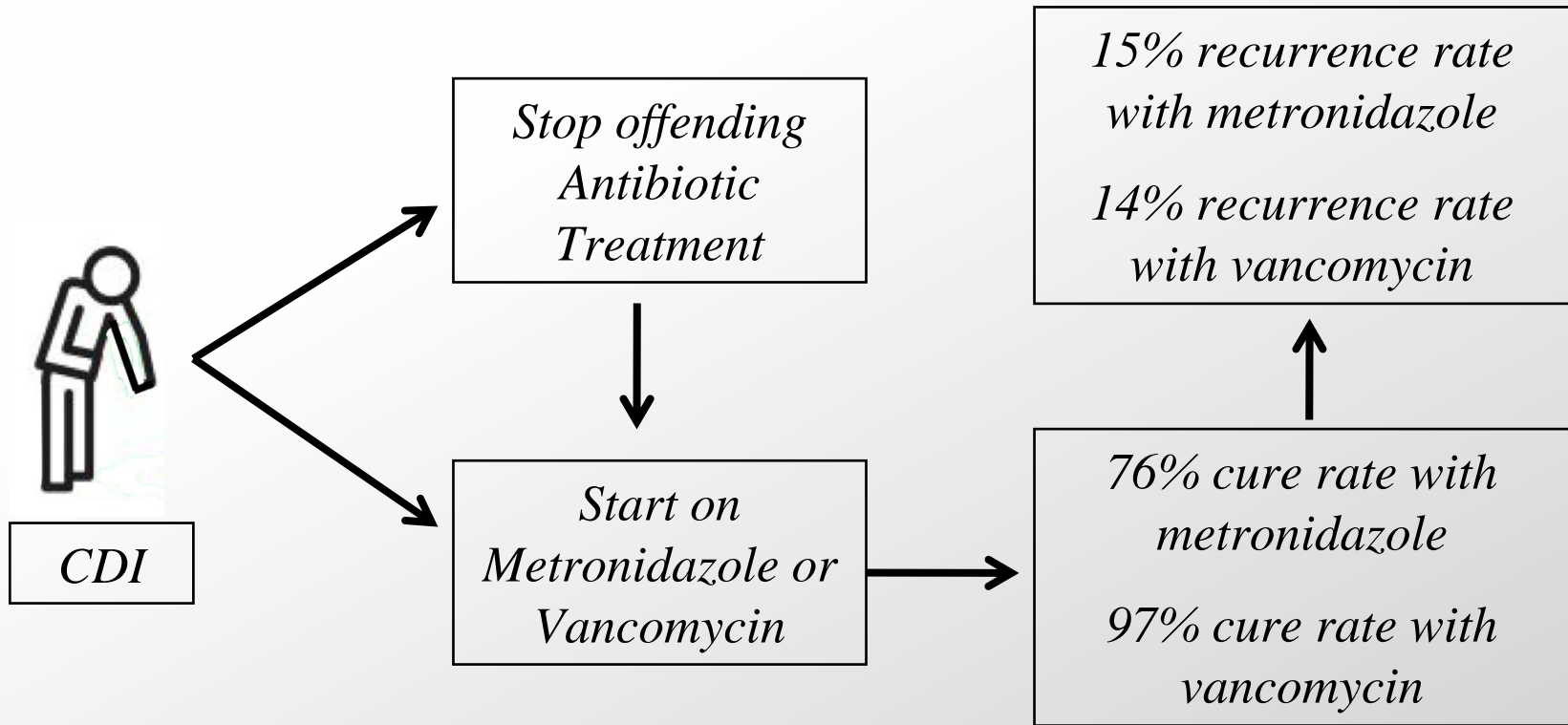
- Incidence of hypervirulent strain related CDI has been increasing in Europe and is now invading US
- Patients with hypervirulent strain tend to have more severe disease

With the appearance of the hypervirulent strain
(NAP1/027/BI),

C. difficile is becoming more virulent, prompting the need
for early diagnosis and immediate therapy

Treatment Options

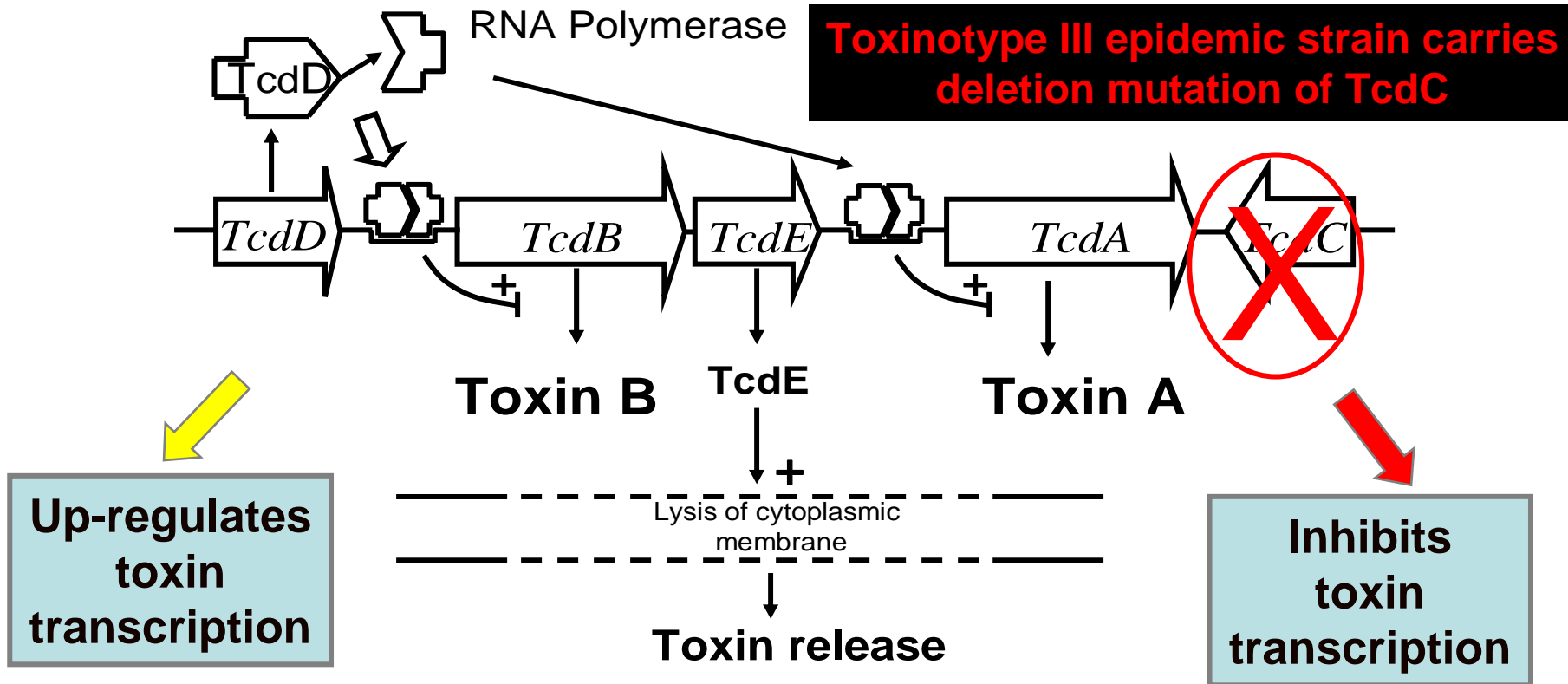
Severe Symptoms (O27/BI/NAP1)



Zar, et al., CID 2007;45

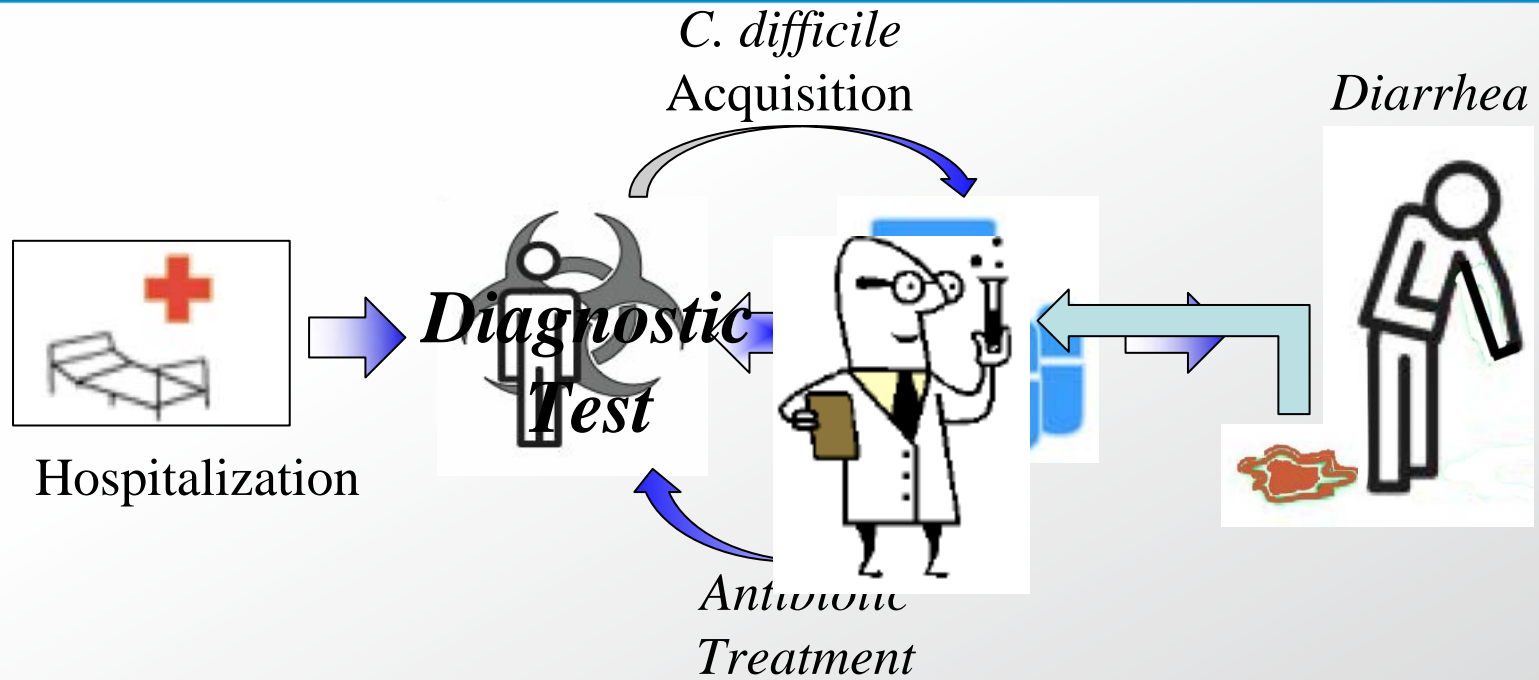
The pathogenicity locus of toxigenic *Clostridium difficile* (PaLoc)

Toxin production under control of a negative regulatory gene *tcd C*



Disease Background

Nosocomial Infection



Laboratory Diagnosis of *C. difficile* Infection- Current Problems

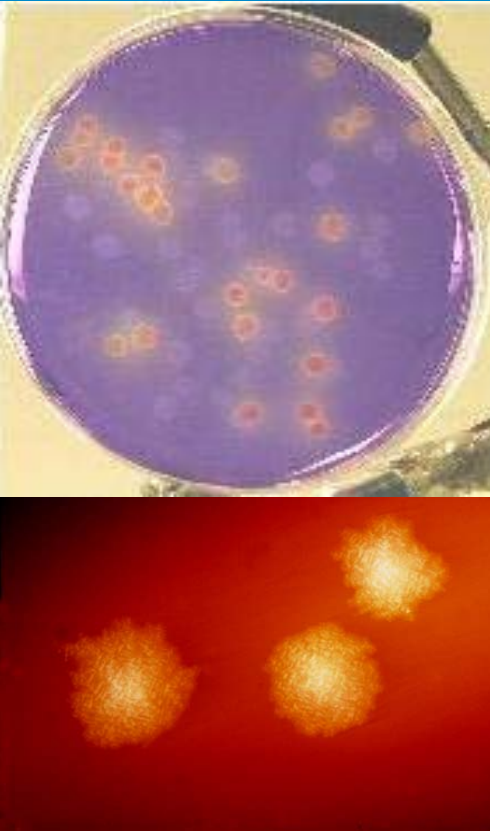


Laboratory manager

- "We have rapid and sensitive tests for *C. difficile*
- Which one do you want?"
- Rapid
- Or sensitive

Toxigenic Culture: “The Gold Standard”

Sensitive but not rapid



Toxigenic Culture Assay

- Culture could be either traditional or Chromogenic Media
- Cytotoxin assay is a way to detect the presence of toxin by placing sample on a media and assessing cell growth/death

Strengths:

- Low in Price
- High sensitivity and specificity
- Regarded as the Gold Standard for toxigenic *C.difficile*

Weaknesses:

- Time consuming (at least 72hrs)
- Needs Toxin Confirmation for culture
- Labor intensive
- Longer LOS due to slow TAT
- Extended period of spore dissemination by patient(s)

GDH: Glutamate dehydrogenase: Sensitive but not specific



Glutamate Dehydrogenase (GDH)

- GDH is a common bacterial enzyme and is produced by all strains of *Clostridium difficile*
- Antigen based test which uses monoclonal antibodies specific to *C. difficile* to detect this “common antigen”

Strengths

- Low Price - if batched
- Easy to operate
- Fast Turn Around Time
- High negative predictive value – could be cost effective as initial screen

Weaknesses

- Indirect Test usually run in “batch mode”
- Detects GDH from both toxic and non-toxic *C. difficile* strains
 - Positive result requires confirmation for toxin production
- Many bacterial species product GDH, tests with poor specificity for *C. difficile* specific GDH are prone to false positive results (80-96%)
 - Un-necessary isolation with GDH pre-emptive approach increases cost burden
 - “Group Isolation” likely leads to infection of “innocent”
 - Single Room Isolation: Decrease in quality health-care due to disease stigma

EIA : Rapid but not sensitive

Enzyme Immuno Assay (EIA)

Strengths:

- Low in Price (per test – not per reportable)
- Easy to operate
- Fast Turn Around Time

Weaknesses:

- Low sensitivity
 - High False Negative Rate
- Lack of confidence from the Physicians
- Multiple tests to confirm
- Increased reagent cost
- Increased labor cost and stool handling
- Extended period of spore dissemination by patient(s)
- Additional cost due to continuous CDI incidences



Recent Mayo Clinic Assessment of Multiple Tests

- Used toxigenic culture as the “gold standard”

<u>Test name</u>	<u>Sensitivity/Specificity</u>
• Premier™ Toxins A & B	48% / 98%
• ImmunoCard® Toxins A & B	48% / 99%
• Xpect® <i>C. difficile</i> Toxin A/B	48% / 84%
• Triage <i>C. difficile</i> Panel (toxin A)	33% / 100%
• Home-brew PCR (for <i>tcdC</i>)	86% / 97%

LM Sloan et al, JCM, 2008 Jun;46(6):1996-2001

Why use RT – PCR?

1. Current rapid EIA tests exhibit poor sensitivity
2. “Two-Step” GDH Antigen-based/culture algorithms require result confirmation and time consuming
3. None of screening methods can distinguish the hypervirulent strain

Only the GeneXpert *C. difficile* Assay provides accurate and timely identification of toxin producing as well as hypervirulent strain of *C. difficile*:

Culture vs Rapid RT-PCR

Microbiology Culture

Waiting 2-3 days for the bugs to grow



Real time PCR

Test results in few hours

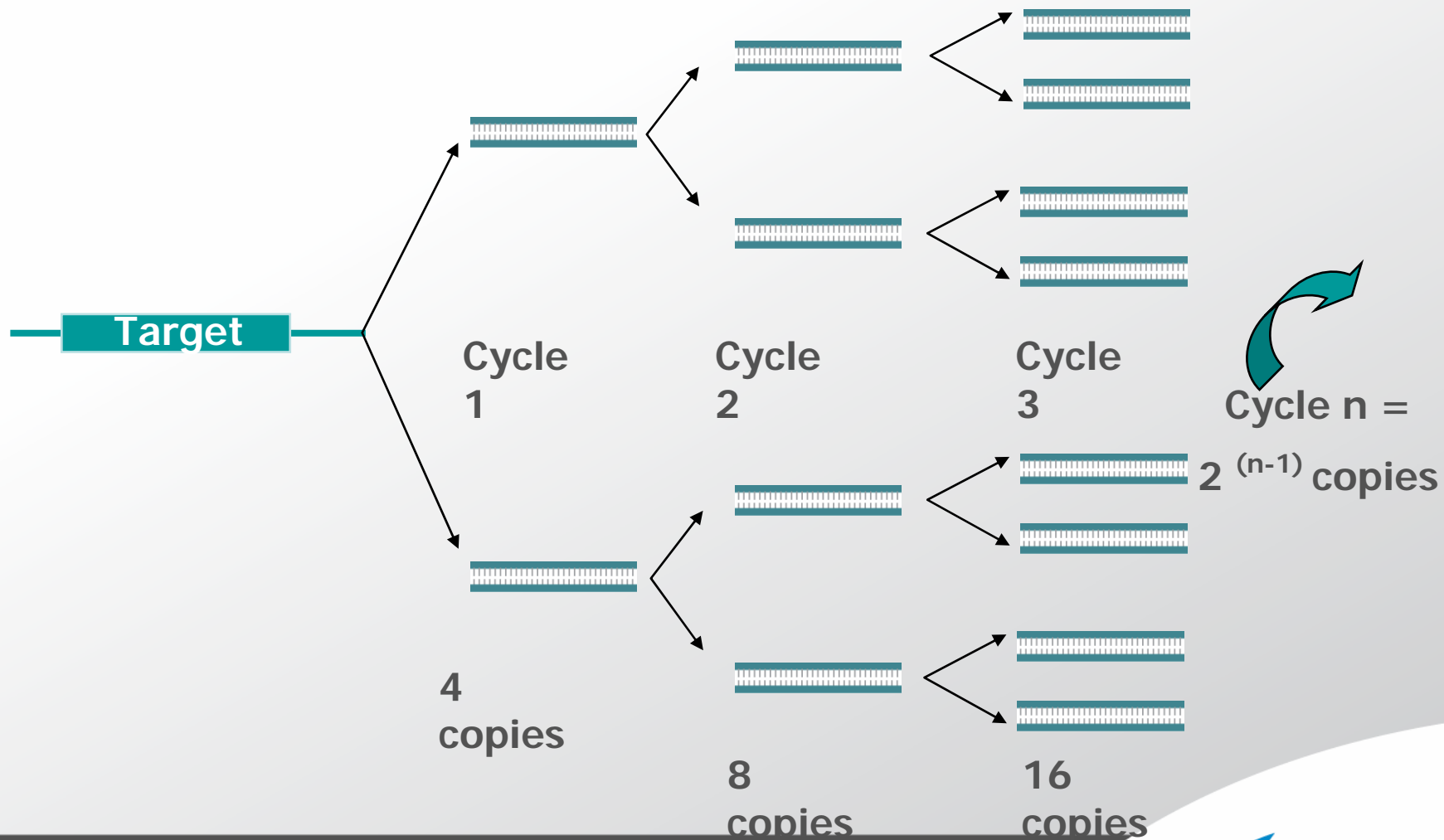


What it Does - Targets Nucleic Acids

Chromosomal DNA



PCR Cycles : “Xeroxing” of DNA



GeneXpert *C. difficile*



- Rapid detection of *C. difficile* in stool (**less than 50 minutes**)
- Detection of three targets plus control will yield the following two results:
 - Toxigenic *C. difficile* present
 - Presumptive epidemic strain 027:NAP1:BI

GeneXpert *C. difficile*



- Sensitivity : 96%
- Specificity : 96%
- **Negative Predictive Value: 99.4%**

“Once and done”

No reason to test multiple stool samples

WHAT IS NEXT?

TB / MDR : **Available NOW “rest of world”**

Rapid test: < 2 hours

Direct from sputum

Sensitivity of culture

Simultaneous identification of drug resistance

FLU / RSV: Expected 2010

Including identification of H1N1 swine flu

Long Island Area Hospitals using GeneXpert

- NY Hospital Queens
- John T Mather Memorial
- Stony Brook University
 - Long Island Jewish
 - St. Francis Hospital
- Mercy Medical Center
 - Good Samaritan
- Winthrop University
 - VA Northport

GeneXpert Clinical Assay Development (Current Menu & Active Projects)

Product

Xpert MRSA (<1hr tat)
 MRSA/SA nasal combo (pre-surgical)
 Flu / RSV
 Group B strep (<1hr tat)
 Enterovirus
 MRSA/SA for Skin & Soft Tissue (<1hr tat)
 MRSA/SA for pos. blood culture (<1hr tat)
 VRE(vanA/vanB) (<1hr tat)
C. Difficile : detects virulent strain 027 (<1hr tat)
 GC-CT
 HPV Panel with typing
 Sepsis Panel
 CMV and EBV
 MRSA/VRE combo surveillance
 Norovirus
 KPC (klebsiella)
 Oncology markers (bladder, lung)

Status

FDA approved
 RUO-Available 4/1/09
 Expected 2010
 FDA approved
 FDA approved
 FDA approved
 FDA approved
 RUO-Available
 RUO-Available
 Dec ('09)
 2010
 2010
 2010
 Late 2009
 Development
 Development
 Development

TB/rifampicin resistance

Available ROW

NASDAQ: CPHD



defining *on-demand* molecular diagnostics

 **Cepheid[®]**
Bring answers to life.