Diagnostic Tests For The Difficult To Diagnose Bacterial Infections

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CASE-1

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Case 1

- A 62-year old female with DM, HT, DLP
  - Admit due to Aspiration pneumonia
  - Treated with ceftriaxone and clindamycin IV
- D7 of admission
  - Developed watery diarrhea 5-10 times/day
  - PE: BT 38 C, mild abdominal distension
  - Stool exam: WBC 5-10, RBC 2-3
  - Stool culture, AFB, stool C. difficile, pending

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Differential Diagnosis of Nosocomial Diarrhea

Infectious Causes	Non-Infectious Causes
1. ***C. difficile***	1. Feeding, Mal-absorption
2. *K. oxytoca*	2. Medications
3. *C. perfringens*	3. Underlying dis: IBS, gut obstruction
4. *S. aureus*	
5. Norovirus	
6. Others: CMV, protozoa, parasites	

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Case-1 (cont.)

- Rx: Metronidazole (500) 1 tab tid pc
- Investigations:
- Not respond to NPO

Could we exclude CDAD?

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CDAD diagnosis

- Stool tests
  - EIA test – ⇩ sens, short turnaround time
  - PCR toxin B - ⇧ sens/spec, fast
  - Cell cytotoxicity assay – ⇧ sens, long turnaround time
- Endoscopy/Pathology
- Imaging: CT-scan

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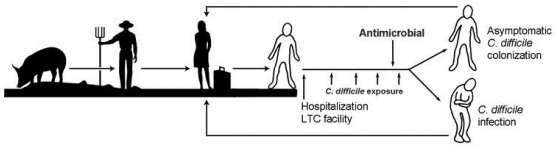
HYPOTHESIS



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The laboratory diagnosis of *C.difficile* infection

- Tissue culture assay
- Latex agglutination test and glutamate dehydrogenase (GHD)
- Enzyme immuno assay (EIA)
- Stool culture
- Molecular technique, polymerase chain reaction

Laboratory Diagnosis

- Enzyme immuno assays (EIA) are commonly used for toxin detection in stool directly: sensitivity issue and expensive
- May use 2-step approach: GDH then EIA
- Molecular technique, polymerase chain reaction (PCR), for detection of toxin genes, *tcdA* and *tcdB,* may be used and be helpful in the diagnosis of CDAD
- Some commercial kits are available or in-house preparation

BD MAX™ Cdiff Assay

- A fully automated sample-to-result platform
- Rapidly identifies patients with CDI, including those caused by hypervirulent strains
  - ✓ Detects the toxin B gene (*tcdB*); the gene essential for CDI
  - ✓ With up to 24 results in < 3 hours

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BD MAX™ Cdiff Performance	
Sensitivity	96.3%
Specificity	92.4%

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BD MAX™ Cdiff Assay

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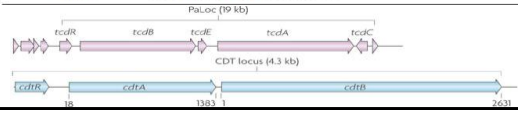
Detection of toxin genes

	Primer name	Primer sequence (5′-3′)	Amplicon size
tcdA	NKV011	TTTTGATCCTATAGAATCTAACTTAGTAAC	2535 bp
	NK9	CCACCAGCTGCAGCCATA	
tcdB	NK104	GTGTAGCAATGAAAGTCCAAGTTTACGC	204 bp
	NK105	CACTTAGCTCT TTGATTGCTGCACCT	
cdtA	cdtAfw	TGAACCTGGAAAAGGTGATG	375 bp
	cdtArev	AGGATTATTTACTGGACCATTTG	
cdtB	cdtBfw	CTTAATGCAAGTAAATACTGAG	510 bp
	cdtBrev	AACGGATCTCTTGCTTCAGTC	



มหาวิทยาลัยมหิดล
Mahidol University

Multiplex PCR method for the detection of *Clostridium difficile* toxin A (*tcdA*) and toxin B (*tcdB*)

16S DNA (1062 bp) Internal control
tcdA (629 bp) Toxin A gene
tcdB (410 bp) Toxin B gene

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PCR ribotyping

Primer name	Primer sequence (5'-3')	Amplicon size
16S	GTGCGGCTGGATCACCTCCT	-
23S	CCCTGCACCCTTAATAACTTGACC	

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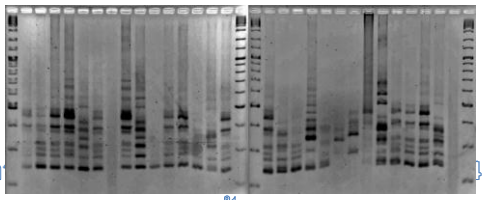
PCR Ribotyping using Intergenic Sequence



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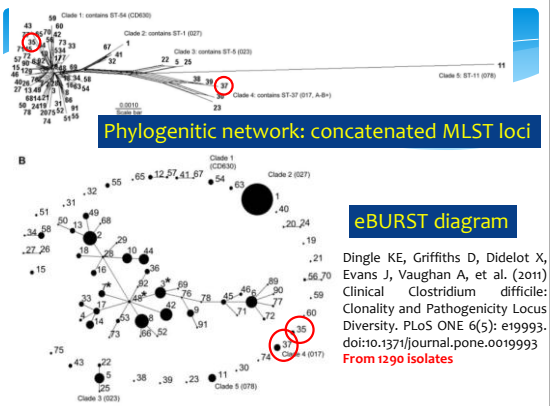
5 Randomly picked *C. difficile*

* MLST for 5 isolates from 5 different wards (year 2006)
1. ST 35
2. New ST, single locus variant (SLV) of ST 35
3. ST 37
4. ST37
5. New ST, SLV of ST37

Both ST 35 and ST37 were originated in UK in 2006



Phylogenitic network: concatenated MLST loci

eBURST diagram

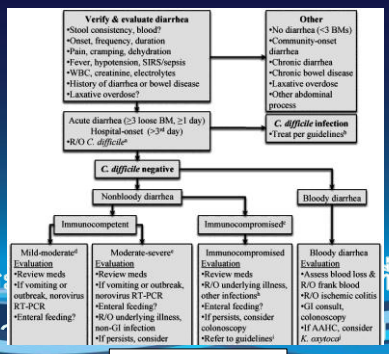
Dingle KE, Griffiths D, Didelot X, Evans J, Vaughan A, et al. (2011) Clinical Clostridium difficile: Clonality and Pathogenicity Locus Diversity. PLoS ONE 6(5): e19993. doi:10.1371/journal.pone.0019993
From 1290 isolates

Take Home Messages

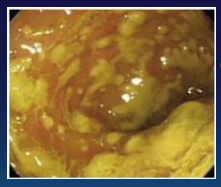
* CDAD may be nosocomial infection
* Infection control has to be in place.
* International clones can spread

* Food-producing animals may be the sources of *C. difficile*

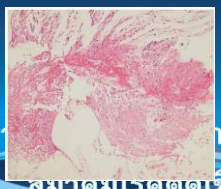
Nosocomial Diarrhea



Clin Infect Dis. Oct 1, 2012; 55(7): 982–989.



Flexible sigmoidoscopy revealed mucosal edema and yellowish exudative plaques



Biopsy showed volcano-like eruption with superficial pseudo-memberane formation adjacent to an area of mucosal necrosis and superficial ulceration

Case-1 (cont.)

- Ceftriaxone/Clindamycin were discontinued.
- Switch from metronidazole PO to Vancomycin PO
- Diarrhea disappeared on D5 of therapy

CASE-2

Case-2

- A 29-year female, GA 16+ weeks
  - 1 wk Lt. ankle arthritis
  - 4 d Rt. ankle arthritis, fever with chill
  - PH: husband had hx of pus per urethra 1 wk ago
  - ROS: No leucorrhea or dysuria
- PE: BT 38.8 C,
  - Both ankle arthritis
  - Tenosynovitis at dorsum of both feet

Approach to Acute Oligo/polyarthritis

Infectious Causes	Non-Infectious Causes
1. Septic arthritis esp. gonococcal arthritis	1. SLE
2. Rheumatic fever	2. Rheumatoid Arthritis
3. Viral arthritis (ie. HBV, parvovirus B-19)	3. Reactive Arthritis
4. Bacterial endocarditis	4. Polyarticular gout
	5. Rare diseases: Still disease, Acute sarcoid arthritis, Mediterranean Fever, Familial Enteropathic Arthropathies

Case-2 (cont.)

- CBC – leukocytosis
- Right ankle arthrocentesis
  - Direct exam – wbc count 58,000, N 91%, no crystal
  - Gram – no organism
  - Culture – pending
- Pelvic exam: Normal cervix

Diagnosis of DGI

- Culture of Blood, CSF and all mucosal sites
  - Selective media (Chocolate ,Thayer- Martin) for non-sterile site
  - Non-selective media (Blood agar, MacConkey) for sterile site
  - Enriched carbon dioxide environment
- Nucleic Acid Amplification Tests (NAAT)
- PCR
- Co-infection: anti-HIV, HBsAg, C. trachomatis

Case-2 (cont.)

- **Investigation:**
  - Blood and synovial fluid culture – NG
  - NAAT (cervical swab and throat swab) – positive for N. gonorrhea, negative for C. trachomatis
  - PCR (synovial fluid) – N. gonorrhea
  - VDRL, HBS Ag and Anti-HIV – negative
- **Treatment:**
  - ceftriaxone 1 g iv od x 7 days
  - doxycycline 100 mg bid x 7 days

Antimicrobial Susceptibility

Antimicrobial agent	No. (%) of isolates		
	Susceptible	Intermediate	Resistance
PEN	-	17 (13.9)	105 (86.1)
TET	-	6 (4.9)	116 (95.1)
CTX	122 (100)	-	-
CRO	122 (100)	-	-
CIP	2 (1.6)	10 (8.2)	110 (90.2)
OFX	6 (4.9)	5 (4.1)	111 (91)

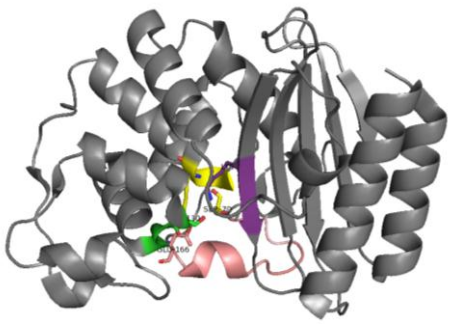
Srifeungfung S et al. Jpn J Infect Dis. 2009

An Extended-Spectrum Beta-Lactamase Production in *Neisseria gonorrhoeae*?: New Evidence Supporting Possible Evolution Pathway

Sasiprapa Prombhul, Makoto Ohnishi, Iyarit Thaipisuttikul, Somporn Srifuengfung, Chanwit Tribuddharat

Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand 10700
Department of Bacteriology I, National Institute of Infectious Diseases, Japan

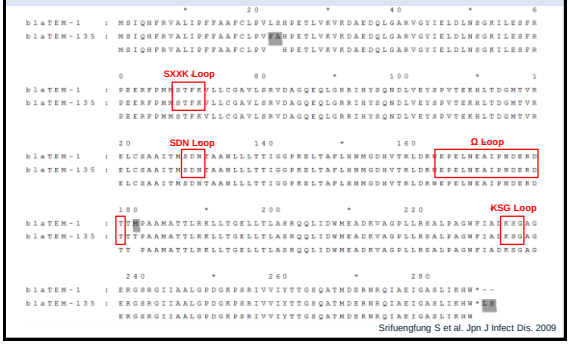
TEM Beta-Lactamases



Prevalence of *bla*$_{TEM}$-carrying GC

Plasmids	Sizes (kb)	TEM-Type	Regions	References
pFA3	7.1	TEM-1	Canada	Gilbride KA et al., 1990
pFA7	-	TEM-1	USA	Sanchez-Pescador R et al., 1993
pJD1	4.2	TEM-1	Sweden	Korch C et al., 1996
pJD9	-	TEM-1	Canada	Dillon JR et al., 1997
pAS84/417	-	TEM-1	Canada	Dillon JR et al., 1997
pJD4	7.4	TEM-1	Canada	Dillon JR et al., 2001
pSJ5.2	5.2	TEM-1	Puerto Rico	Scharbaai-Vazquez R et al., 2007
-	9	TEM-135	Thailand	Srifuengfung S et al., 2009
pCmGFP	6.1	TEM-1	Australia	Srikhanta YN et al., 2009
pNGK	4.2	TEM-1	Korea	Chung GT et al., 2010
pEM1	4.9	TEM-1	South Africa	Muller EE et al., 2010 and 2011

Four Conserved Domains of Class A beta-lactamase

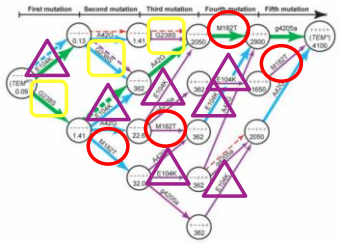


Srifuengfung S et al. Jpn J Infect Dis. 2009

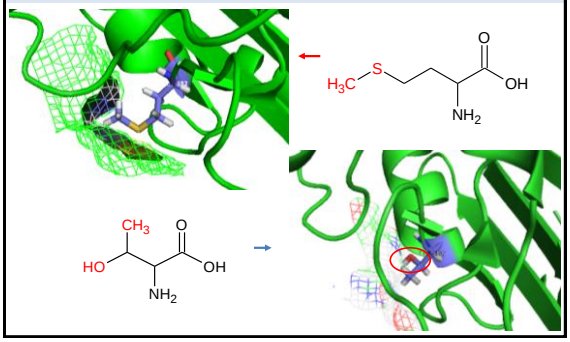
Darwinian Evolution Can Follow Only Very Few Mutational Paths to Fitter Proteins

Daniel M. Weinreich,* Nigel F. Delaney,† Mark A. DePristo, Daniel L. Hartl

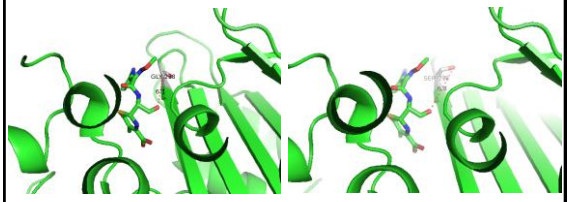
www.sciencemag.org **SCIENCE** VOL 312 7 APRIL 2006



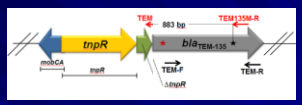
Improvement of Protein Stability



G238S on *bla*$_{TEM}$ Allowing CTX Resistance Phenotype

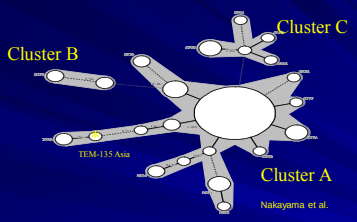


Global Dissemination of IncP Plasmid Containing Two *bla*$_{TEM-1}$ and *bla*$_{TEM-135}$ Alleles Conferring Antibiotic Resistance in *Neisseria gonorrhoeae*



- MLST Study: Collaboration with the National Institute of Infectious Diseases, Japan
- International spread among the UK, Greece, Japan, and Thailand
- *N. gonorrhoeae* is transforming to ESBL producer! Sexual networks exist!

Gonococci Cluster Analysis: MLST



MLST revealed some correlations of the isolates between Thailand, Japan, the United Kingdom, and Greece

Molecular Analyses of TEM Genes and Their Corresponding Penicillinase-Producing Neisseria gonorrhoeae Isolates in Bangkok, Thailand

Shu-ichi Nakayama, Chanwit Tribuddharat, Sasiprapa Prombhul, Ken Shimuta, Somporn Srifuengfung, Magnus Unemo and Makoto Ohnishi
Antimicrob. Agents Chemother. 2012, 56(2):916. DOI: 10.1128/AAC.05665-11.

CASE-3

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Case-3

- A 20-year male, vet student
  - Low-grade fever, DOE for 1+ month
  - MR murmur, sign of CHF
  - Bilat. axillar lymphadenopathy
  - No embolic phenomenon
  - Not responded to ampicillin + gentamycin
- **Investigation:**
  - Echo – MR with vegetation
  - Hemoculture x IV – no growth at D5

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Modified Duke's criteria

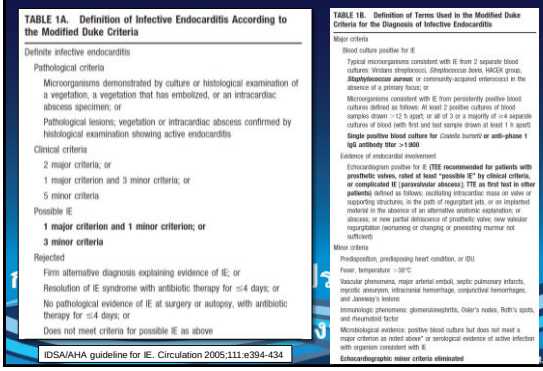

TABLE 1A. Definition of Infective Endocarditis According to the Modified Duke Criteria

Definite infective endocarditis

Pathological criteria

Microorganisms demonstrated by culture or histological examination of a vegetation, a vegetation that has embolized, or an intracardiac abscess specimen; or

Pathological lesions; vegetation or intracardiac abscess confirmed by histological examination showing active endocarditis

Clinical criteria

2 major criteria; or

1 major criterion and 3 minor criteria; or

5 minor criteria

Possible IE

1 major criterion and 1 minor criterion; or

3 minor criteria

Rejected

Firm alternative diagnosis explaining evidence of IE; or

Resolution of IE syndrome with antibiotic therapy for ≤4 days; or

No pathological evidence of IE at surgery or autopsy, with antibiotic therapy for ≤4 days; or

Does not meet criteria for possible IE as above

TABLE 1B. Definition of Terms Used in the Modified Duke Criteria for the Diagnosis of Infective Endocarditis

Major criteria

Blood culture positive for IE

Typical microorganisms consistent with IE from 2 separate blood cultures: Viridans streptococci, *Streptococcus bovis*, HACEK group, ***Staphylococcus aureus***; or community-acquired enterococci in the absence of a primary focus; or

Microorganisms consistent with IE from persistently positive blood cultures defined as follows: At least 2 positive cultures of blood samples drawn >12 h apart; or all of 3 or a majority of ≥4 separate cultures of blood (with first and last sample drawn at least 1 h apart)

Single positive blood culture for *Coxiella burnetii* or anti-phase 1 IgG antibody titer >1:800

Evidence of endocardial involvement

Echocardiogram positive for IE **(TEE recommended for patients with prosthetic valves, rated at least "possible IE" by clinical criteria, or complicated IE [paravalvular abscess]; TTE as first test in other patients)** defined as follows: oscillating intracardiac mass on valve or supporting structures, in the path of regurgitant jets, or on implanted material in the absence of an alternative anatomic explanation; or abscess; or new partial dehiscence of prosthetic valve; new valvular regurgitation (worsening or changing or preexisting murmur not sufficient)

Minor criteria

Predisposition, predisposing heart condition, or IDU

Fever, temperature >38°C

Vascular phenomena, major arterial emboli, septic pulmonary infarcts, mycotic aneurysm, intracranial hemorrhage, conjunctival hemorrhages, and Janeway's lesions

Immunologic phenomena: glomerulonephritis, Osler's nodes, Roth's spots, and rheumatoid factor

Microbiological evidence: positive blood culture but does not meet a major criterion as noted above* or serological evidence of active infection with organism consistent with IE

Echocardiographic minor criteria eliminated

IDSA/AHA guideline for IE. Circulation 2005;111:e394-434

Major Criteria – Blood culture Positive for IE

1. Typical microorganisms consistent with IE from 2 separate blood cultures (Viridians Strep., S. bovis, HACEK, S. aureus or CA-enterococci in the absence of primary focus
2. Microorganism
   - At least 2 culture >12 hrs apart
   - +ve 3 from 4+ blood culture (at least > 1 hr apart)
3. Single Hemoculture of C. burnetii or Anti-phase 1 IgG antibody titer >1:800

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IDSA/AHA guideline for IE. Circulation 2005;111:e394-434

Culture Negative IE

1. Previous antibiotic therapy
2. Inadequate microbiological techniques
3. Non-bacterial pathogens
4. Fastidious bacteria
   - HACEK *(H. parainfluenzae, H. aphrophilus, H. paraphrophilus, H. influenza, Actinobacillus actinomycetemcomitans, Cardiobacterium hominis, Ekenella corrodens, Kingella kingae and K. denitrificans)*
   - Bartonella spp., Chlamydia spp., Brucella spp.
   - Tropheryma whipplei, Legionella spp.

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IDSA/AHA guideline for IE. Circulation 2005;111:e394-434

Brucellosis

Clinical description

An illness characterized by acute or insidious onset of fever, night sweats, undue fatigue, anorexia, weight loss, headache, and arthralgia

Laboratory criteria for diagnosis

- Isolation of *Brucella* sp. from a clinical specimen, or
- Fourfold or greater rise in *Brucella* agglutination titer between acute- and convalescent-phase serum specimens obtained greater than or equal to 2 weeks apart and studied at the same laboratory, or
- Demonstration of *Brucella* sp. in a clinical specimen by immuno-fluorescence

Case classification

- **Probable**: a clinically compatible case that is epidemiologically linked to a confirmed case or that has supportive serology (i.e., *Brucella* agglutination titer of greater than or equal to 160 in one or more serum specimens obtained after onset of symptoms)
- **Conf**

CDC. (1990). Case Definitions for Public Health Surveillance. *MMWR*, 39(RR-13), 1-43.

Micro lab for Infective Endocarditis

- Standard culture
  - New automated hemoculture systems are fine to grow
  - However, species identification is difficult
- Additional investigation for culture negative IE
  - Some organisms need at least two days to show their colonies on the plate
  - *Brucella* do not grow on MacConkey agar
  - On blood agar, colonies look like Gram-positive
  - RapID, Vitek1-2, API, and Bruker's MALDI-TOF may not correctly identify *Brucella*

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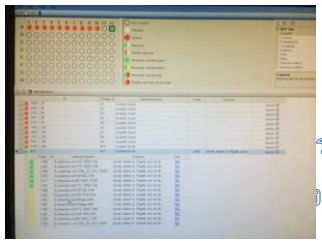
FAST-TRACK COMMUNICATION

Importance of Using Bruker's Security-Relevant Library for Biotyper Identification of *Burkholderia pseudomallei*, *Brucella* Species, and *Francisella tularensis*

Scott A. Cunningham,[a] Robin Patel[a,b]
Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology,[a] and Division of Infectious Diseases, Department of Medicine,[b] Mayo Clinic, Rochester, Minnesota, USA



JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2011, p. 1165–1168 Vol. 49, No. 3
0095-1137/11/$12.00 doi:10.1128/JCM.01131-10

CASE REPORTS

Ribosomal RNA Sequence Analysis of *Brucella* Infection Misidentified as *Ochrobactrum anthropi* Infection[▽]

Rebecca T. Horvat,[1]* Wissam El Atrouni,[2] Kassem Hammoud,[2] Dana Hawkinson,[2] and Scott Cowden[3]

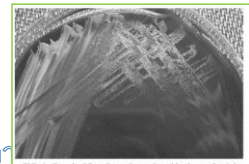


FIG. 1. Growth of *Brucella* species on sheep blood agar after 1 day of incubation in an aerobic environment.

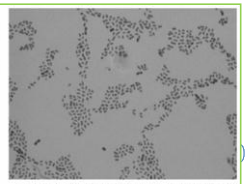


FIG. 2. Gram stain of colonies from the blood agar plate. Note the variable sizes of the bacteria and inconsistent staining properties of the cells.

Case-3

- *Brucella* slide agglutination (rose Bengal): positive
- *Brucella* IgM (ELISA) : > 1:100
- *Brucella* IgG (ELISA) : negative

- Blood PCR was positive for *Brucella melitensis*
- Blood culture was negative after incubation for 30 days.

- Responded well to rifampicin, doxycycline, streptomycin

CASE-4

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Case-4

A 72- year old male, BPH, DM, HT, Hx of multiple course of antibiotic therapy

- 2 days after TURP
  - Fever breakthrough ceftriaxone
  - Hypotension, responded to IV fluid
- Meropenem was initiated
- Hemoculture grew GNB → *E. coli*

Susceptibility Pattern

Antibiotics	Susceptibility
Cefazolin	R
Ceftriaxone	R
Ertapenem	S
Amikacin	S
Ciprofloxacin	S

New CLSI criteria: ESBL or Non-ESBL?

Should we de-escalate antibiotic therapy?

This is an ESBL Producer- Definitely!!!

- Old CLSI criteria for identifying ESBLs in (3-)4 species
- New CLSI criteria: No more ESBL test!
- MIC will be used instead
- Year 2010: New MIC breakpoints for Enterobacteriaceae was introduced
- A lot of debate: CLSI keeps changing further
- In year 2014: the new "SDD: Susceptible dose dependent" is introduced!!

Summary of Major Changes in CLSI 2014: M100-S24

Why were the cefepime breakpoints reconsidered?
The issue of new breakpoints for cefepime became apparent for several reasons:

- Previous breakpoints were based on a higher dose of cefepime than is often used.
- Clinical failures were noted for isolates with cefepime MICs of 4 and 8 µg/mL, especially when lower doses of cefepime were used.
- There are limited new drugs in the pipeline that show activity against multidrug-resistant gram-negative bacteria; thus, there is a need to optimize use of drugs currently available. Designing susceptibility reports to correlate better with dosages of the drug used is one way to help accomplish this goal.

What does "susceptible-dose dependent" (SDD) mean?
SDD interpretation is a new interpretive category for antibacterial susceptibility testing, although it has been applied for interpretation of antifungal susceptibility test results for several years.

Definition:
The "susceptible-dose dependent" category implies that susceptibility of an isolate is dependent on the dosing regimen that is used in the patient. In order to achieve levels that are likely to be clinically effective against isolates for which the susceptibility testing results (either MICs or disk diffusion) are in the SDD category, it is necessary to use a dosing regimen (ie, higher doses, more frequent doses, or

Cefepime Breakpoint Change for *Enterobacteriaceae*

Previous – 2013

Method	Susceptible	Intermediate	Resistant
MIC	≤8 µg/mL	16 µg/mL	≥32 µg/mL
Zone Diameter (Disk Diffusion)	≥18 mm	15–17 mm	≤14 mm

Revised – 2014

Method	Susceptible	Susceptible-Dose Dependent	Resistant
MIC	≤2 µg/mL	4–8 µg/mL	≥16 µg/mL
Zone Diameter (Disk Diffusion)	≥25 mm	19–24 mm	≤18 mm

Abbreviation: MIC, minimal inhibitory concentration.

Cefepime Therapy for Monomicrobial Bacteremia Caused by Cefepime-Susceptible Extended-Spectrum Beta-Lactamase–Producing *Enterobacteriaceae*: MIC Matters

Nan-Yao Lee,[1,2] Ching-Chi Lee,[1,2] Wei-Han Huang,[4] Ko-Chung Tsui,[5,8] Po-Ren Hsueh,[6,7,a] and Wen-Chien Ko[1,2,3,a]

Clinical Infectious Diseases 2013;56(4):488–95

Conclusions. Based on the current Clinical and Laboratory Standards Institute susceptible breakpoint of cefepime (minimum inhibitory concentration ≤8 µg/mL), **cefepime definitive therapy is inferior to carbapenem therapy in treating patients with so-called cefepime-susceptible ESBL-producer bacteremia.**

ESBL and treatment options

Antimicrobial agents	Serious infection	Non-serious infection
3rd & 4th Ceph	X	✓ (UTI)
βL-βI	✓	✓
Fluoroquinolones	X	?
Aminoglycosides	X	?
Carbapenems	✓	✓

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Take Home Message

- *Clostridium difficile* infection can be a nosocomial infection - Need good infection control
- Most difficult diagnoses are from incomplete tests - Lack of budget/insight
- Emerging resistance is inevitable
- ESBL producers and "clinical" evidence - Need more clinical studies

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Discussion and Questions

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A comparison of rapid *C. diff* tests[12-20]

Manufacturer/Product	Sensitivity	Specificity	Assay Time	Number of Steps
Meridian ImmunoCard® Toxins A/B	95.2%	98.5%	15 min.	5
Wampole® Toxin A/B Quik Chek*	90.2%	99.7%	25 min.	8
Wampole® *C. diff* Quik Chek Complete*	87.8%	99.4%	25 min.	8
Remel Xpect® *Clostridium difficile* Toxin A/B†	86.3%	96.2%	20 min.	6
Meridian Premier™ Toxins A/B	94.7%	97.3%	60 min. (30 min. with Stat Fax™ -2200)	8
Wampole® *C. difficile* Tox A/B II	92.0%	100%	60 min. (30 min. with Stat Fax™ -2200)	8
Remel Prospect Toxin A/B EIA†	90.3%	96.2%	100 min.	10
Biomerieux Vidas *C. difficile* Toxin A&B‡	81.3%	99.5%	75 min.	7
BD Geneohm™ *C. diff*§	93.8%	95.5%	75 to 90 min.	20
Prodesse™ Progastro CD‡	91.7%	94.7%	180 min.	60
Cepheid® Xpert® *C. diff*§	93.5%	94.0%	60 min.	7