

# Basic Diagnostic Microbiology for Infection Preventionists

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**March 16, 2016**

# Outline

- Objective
- Role of the Microbiology Laboratory
- Basic Diagnostic Microbiology for Infection Preventionists\*
  - Pathogen Identification
  - Antimicrobial Susceptibility testing
  - Active Surveillance: MRSA, VRE, CRE
  - Typing: PFGE
- Advanced Diagnostic Microbiology and Molecular Microbiology
- No disclosure
- I have to show slides, articles and mention about commercial instruments and media.

# Objective:

At the end of this lecture the attendee will understand:

- The importance of the microbiology laboratory to the hospital epidemiologist and infection preventionists.
- The basic diagnostic microbiology and emerging technologies in the clinical microbiology laboratory.
- The various techniques available to assist in an epidemiological investigation.



# NATIONAL SUMMARY DATA

Estimated minimum number of illnesses and deaths caused by antibiotic resistance\*:

At least  **2,049,442** illnesses,  
 **23,000** deaths

\*bacteria and fungus included in this report

Estimated minimum number of illnesses and death due to *Clostridium difficile* (*C. difficile*), a unique bacterial infection that, although not significantly resistant to the drugs used to treat it, is directly related to antibiotic use and resistance:

At least  **250,000** illnesses,  
 **14,000** deaths

## WHERE DO INFECTIONS HAPPEN?

Antibiotic-resistant infections can happen anywhere. Data show that most happen in the general community; however, most deaths related to antibiotic resistance happen in healthcare settings, such as hospitals and nursing homes.



U.S. Department of  
Health and Human Services  
Centers for Disease  
Control and Prevention

# ANTIMICROBIAL RESISTANCE

Global Report  
on surveillance  
2014

WHO

**Emergence of  
antimicrobial resistance**

**Inappropriate antibiotic uses**

# Impact of Healthcare Associated Infections (HAIs)

- HAIs affect 648,000 patients (721,800 cases) in U.S. acute care hospitals in 2011 (183 hospitals).
- Of 11,282 patients, 452 had 1 or more HAIs (4.0%).
  - **Pneumonia** (21.8%)
  - **Surgical-site infections** (21.8%)
  - **Gastrointestinal infections** (17.1%)
  - Device-associated infections have traditionally been the focus of programs to HAIs, accounted for 25.6% of such infections.
    - Central-catheter–associated bloodstream Infection (9.9%)
    - Catheter-associated urinary tract infection (12.9%)
    - Ventilator-associated pneumonia (4%)
- *Clostridium difficile* was the most commonly reported pathogen (12.1% of HAIs)

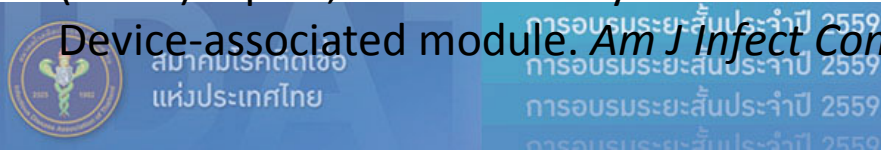


# HAIs in Resource Limited Settings

- INICC conducted a prospective surveillance study from January 2007 through December 2012 in 503 ICUs in Latin America, Asia, Africa, and Europe.  
(605,310 ICU patients for an aggregate of 3,338,396 days)
- Rates of device-associated nosocomial infection were higher in the ICUs of the INICC hospitals than comparable U.S. ICUs.
- CLABSI: 4.9 vs. 0.9 per 1000 central line days
- VAP: 16.8 vs. 1.1 per 1000 ventilator-days
- CAUTI: 5.5 vs. 1.3 per 1000 catheter-days

Rosenthal VD, Maki DG, Mehta Y, et al. International Nosocomial Infection Control Consortium (INICC) report, data summary of 43 countries for 2007-2012.

Device-associated module. *Am J Infect Control*. 2014;42:942-956.



# SSIs in Resource-Limited Settings

- INICC surveillance study performed between January 2005 and December 2010
- 7,523 SSIs associated with 260,973 surgical procedures (SPs) in 82 hospitals of 66 cities from 30 countries in Latin America, Asia, Africa, and Europe.
- SSI rates were significantly higher for most SPs in INICC hospitals compared with NHSN data
- Rates of SSI after hip prosthesis (2.6% vs 1.3%)
  - coronary bypass (4.5% vs 2.9%)
  - abdominal hysterectomy (2.7% vs 1.6%)
  - exploratory abdominal surgery (4.1% vs 2.0%)
  - ventricular shunt (12.9% vs 5.6%)

Rosenthal VD, Richtmann R, Singh S, et al. Surgical site infections, International Nosocomial Infection Control Consortium (INICC) report, data summary of 30 countries, 2005-2010. *Crit Care Med.* 2014;40:3121-3128.

# Clinical Microbiology Laboratory

- **Identification (ID)**
- Isolate and identify causative agents by several methods
- **Antimicrobial susceptibility testing (AST)**
- Basic and conventional biochemical method
- Advance and molecular identification method



# Clinical Microbiology Laboratory

- Bacteriology (Aerobic and anaerobic bacteria) (blood, fluid, urine, respiratory, stool, wound and miscellaneous)
- Mycobacteriology (AFB, Mycobacteria)
- Mycology (Fungus)
- Parasitology
- Virology
- Molecular Microbiology
- Molecular Epidemiology

\*Do not forget!

Specimen accepting and processing area

# Clinical Microbiology Laboratory

- TEAM WORK:
- Microbiologist/ Laboratory director
- Laboratory supervisor
- Laboratory technologist

## “Micro Lab”

- Preactalytic\*– **Analytic**– Post analytic Phase
- **Specimen collection/transportation\*\*\*\*\*** **The most important step!**
- Reporting and interpretation

- **Clinicians\*\*\*\*\***
- Infectious Disease Specialists
- **Infection preventionist (Infection control)**
- Pharmacist
- Antimicrobial stewardship team

**Accurate**  
**Timely**  
**Standard**  
**Impact patient care**  
**Cost effectiveness**



# Role of the Microbiology Laboratory

- **Pre-analytical**

- specimen collection, e.g. blood culture procurement

- **Analytical**

- Grow and/or detect microbial pathogens

- **Diagnostics**

- **Surveillance**

- **Environment**

- Reliably detect antimicrobial resistance

- **Post-analytical**

- Summarize data and trends, e.g. antibiograms

- Communicate with clinicians, HEIC, public health, among others



## Specimens collection is critical for the laboratory testing!

IDSA GUIDELINES

A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2013 Recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM)<sup>a</sup>

Ellen Jo Baron,<sup>1,2</sup> J. Michael Miller,<sup>3</sup> Melvin P. Weinstein,<sup>4</sup> Sandra S. Richter,<sup>5</sup> Peter H. Gilligan,<sup>6</sup> Richard B. Thomson Jr.,<sup>7</sup> Paul Bourbeau,<sup>8</sup> Karen C. Carroll,<sup>9</sup> Sue C. Kehl,<sup>10</sup> W. Michael Dunne,<sup>11</sup> Barbara Robinson-Dunn,<sup>12</sup> Joseph D. Schwartzman,<sup>13</sup> Kimberle C. Chapin,<sup>14</sup> James W. Snyder,<sup>15</sup> Betty A. Forbes,<sup>16</sup> Robin Patel,<sup>17</sup> Jon E. Rosenblatt,<sup>17</sup> and Bobbi S. Pritt<sup>17</sup>

## Emerging Infectious Diseases Guideline (collaboration/ National GL) Require special media/ transportation

Anaerobic blood culture, AFB culture

Culture for *N. gonorrhea*

Anaerobic transport media,

Viral transport media

**CSF culture, ocular specimen, joint fluid, do not refrigerate!**



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# Anaerobic cultures

- Require special transport media
- Accepted specimens: Tissue, aspirated fluid, pus, joint fluid
- Rejected specimens: swabs, urine c/s, sputum, CSF (except from ventricular shunt)



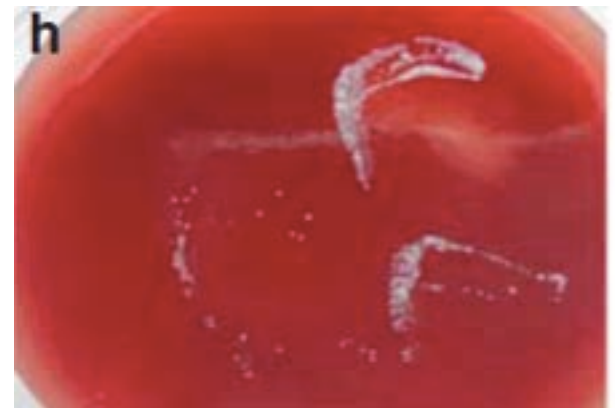
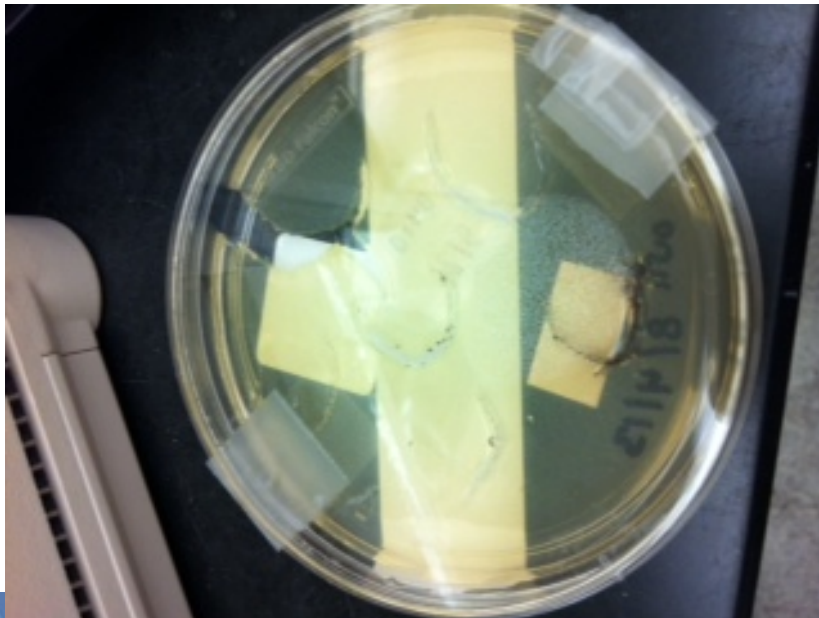
## Viral transport media



Universal Transport  
Medium & patented  
Flocked Swabs



## Corneal scraping



# Blood cultures

- **Automated blood cultures system**  
(continuous monitoring of CO<sub>2</sub> production)
  - BD BACTEC FX (currently use at TUH)
  - BacT/ALERT
  - VersaTREK
- 
- **Collect blood c/s with sterile technique**
  - **BEFORE** antibiotic administration
  - **1 set of blood culture = 2 bottles (10ml/bottle)**





Thailand: 2 Aerobic bottles

USA: 1 Aerobic bottle + 1 Anaerobic bottles\*

Preliminary --- No growth Day 1, Day 2....(based on your labs)

**FINAL REPORT NO GROWTH**

**POSITIVE BLOOD c/s = Critical report**  
**→ Laboratory need to call clinicians**

 **BD BACTEC™ Plus Aerobic/F Culture Vials and BACTEC™ Plus Anaerobic/F Culture Vials**  
**Soybean-Casein Digest Broth**

English: pages 1 – 3      Español: páginas 10 – 13



**Hold for 5 days\***      **For bacteria and yeast (*Candida* spp.) detection except *Brucella* spp. --- hold for 10-14 days**

---

 **BD BACTEC™ Myco/F Lytic Culture Vials**  
**Supplemented Middlebrook 7H9 and Brain Heart Infusion Broth**  
**For Use with BACTEC Fluorescent Series Instruments**

**Hold for 4-6 weeks days for *Mycobacteria* spp. (MAC, MTB), mold, *Cryptococcus* spp. Maybe better for *C. glabrata* detection**



- **POSITIVE Blood c/s → NEXT STEP?**
- **GRAM STAIN!**
- **Cultures and isolation process**
- Inoculate blood on appropriate media
  - Non-selective media
  - Selective/ Differential Media
- Incubate at appropriate condition
- Identification
- Susceptibility Testing and interpretation



## Gram Negative Rods (Aerobic)

(Common)

Growth on MacConkey  
(+)

No Growth on MacConkey (-)  
(Fastidious)

Lactose Fermenters  
(pink)

Non-Lactose  
Fermenters (clear)

TSI: No Reaction Usually  
(Non-Fermenters)

TSI: K/A  
Glucose Fermenters

TSI: A/A

"E.E.C.K"

*Escherichia*  
*Enterobacter*  
*Citrobacter*  
*Klebsiella*

TSI: A/A or K/A  
Fermenters

Some *Escherichia*  
*Enterobacter*  
*Citrobacter*

TSI: K/K  
Non-Fermenters

Oxidase+  
\**Pseudomonas*  
*Burkholderia*  
*Alcaligenes*  
*Achromobacter*  
*Ralstonia*  
*Moraxella* sp.  
*Chryseobacterium*  
etc.

Oxidase -  
*Acinetobacter*  
*Stenotrophomonas*  
*Xanthomonas*  
etc.

*Proteus*  
*Providencia*  
*Morganella*  
*Hafnia*  
*Serratia*

Stool  
Pathogens

*Salmonella*  
*Shigella*  
*Yersinia*  
*Vibrio* (ox +)  
*Aeromonas* (ox +) – some are lactose fermenters  
*Plesiomonas* (ox +)

Curved, fastidious  
Stool Pathogen

*Campylobacter* (ox +)

"HACEK" Group:

*Haemophilus* sp, not *influenzae*  
*Aggregatibacter*\*\*  
*Cardiobacterium*  
*Eikenella*  
*Kingella*

Common H<sub>2</sub>S +

*Proteus*  
*Salmonella*  
*Citrobacter*

Non-motile "SKY"

*S-Shigella*  
*K-Klebsiella*  
*Y - Yersinia* (37°)

\*\*includes:

*A. aphrophilus* (formerly *Haemophilus aphrophilus* and *H. paraphrophilus*)  
*A. actinomycetemcomitans*

## Gram Negative Cocci

Aerobic

*Moraxella*  
*Neisseria* –

*N. meningitidis*  
*N. gonorrhoeae*

Anaerobic

*Veillonella* sp

Fastidious, Special Media:

*Bartonella*  
*Legionella*  
*Bordetella*

Molecular tests also available

The MacConkey plate illustrates a **lactose fermenter** on the left side, and a non-lactose fermenter on the right side.

Lactose Fermenters  
(pink)

TSI: A/A

"EECK"

Escherichia

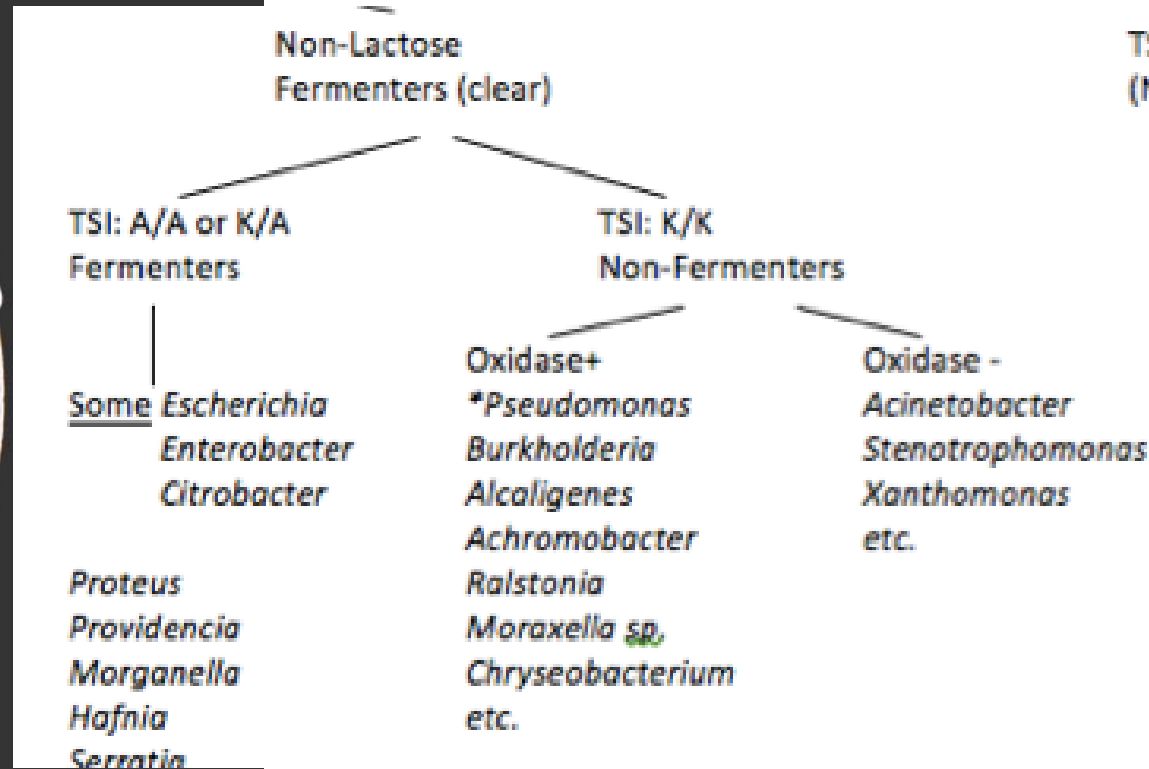
Enterobacter

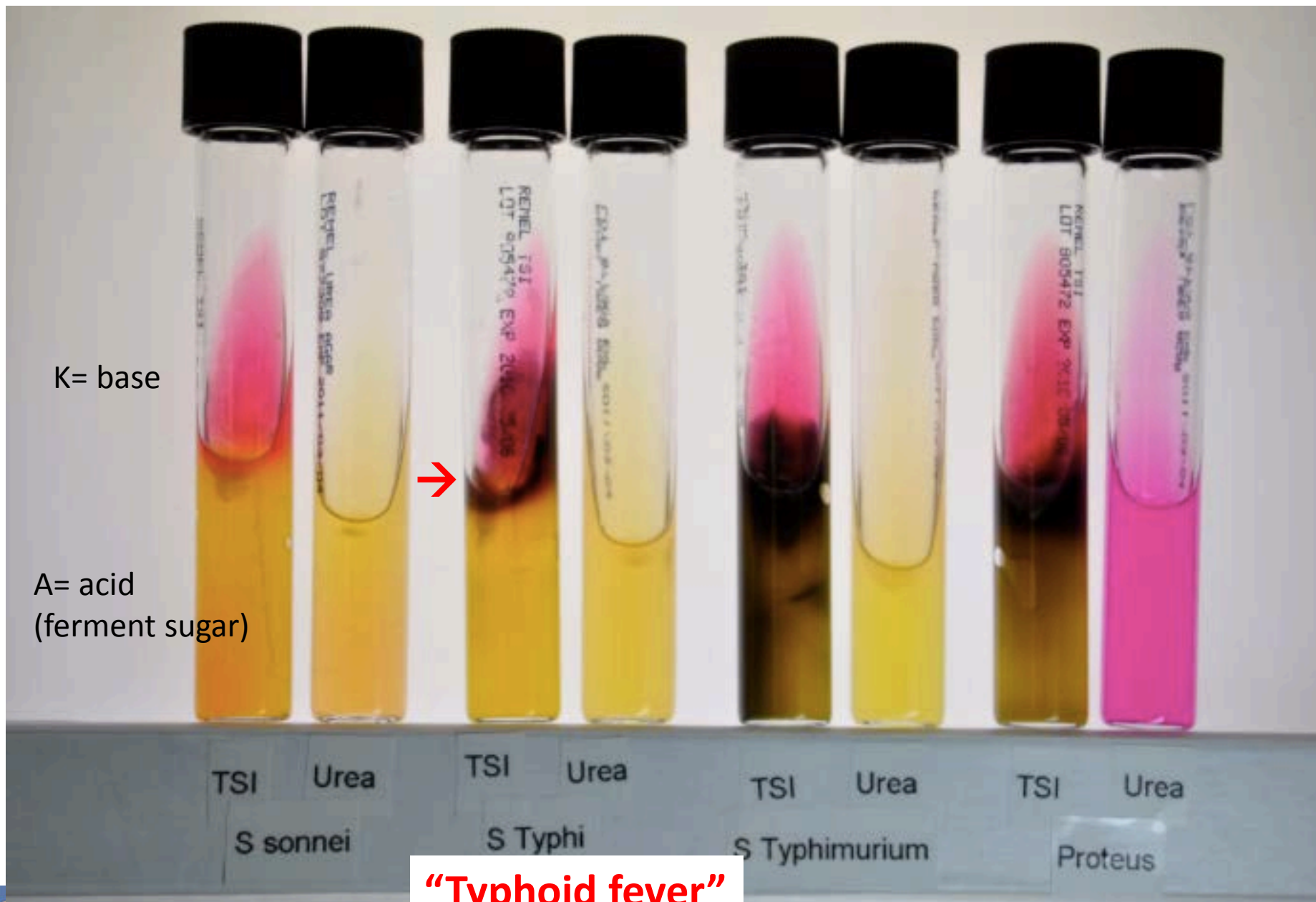
Citrobacter

Klebsiella



This MacConkey plate shows a **non-lactose fermenter**.





**“Typhoid fever”**

**TSI= Triple sugar iron**

(Sucrose, lactose, glucose)

H2S positive = black pigment

**JHU microbiology laboratory**



The oxidase test is often used to help identify gram negative bacteria.

### **POSITIVE OXIDASE**

*Pseudomonas*  
*Burkholderia*  
*Neisseria*



**JHU microbiology laboratory**



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- **Laboratory Diagnosis**

- Specimen collections
- CSF, fluids, blood

- **Gram stain— coccobacilli**

CSF: Ag detection (limited value)

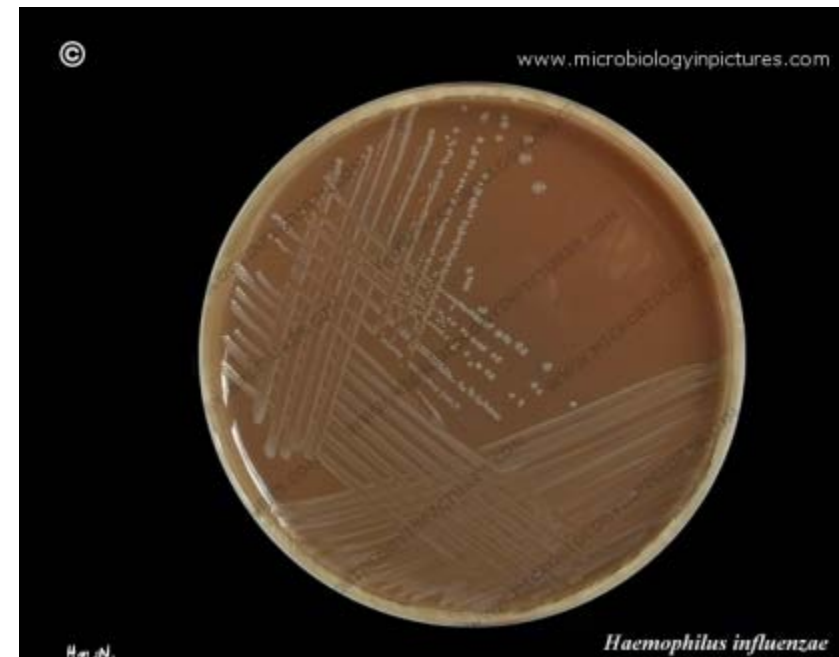
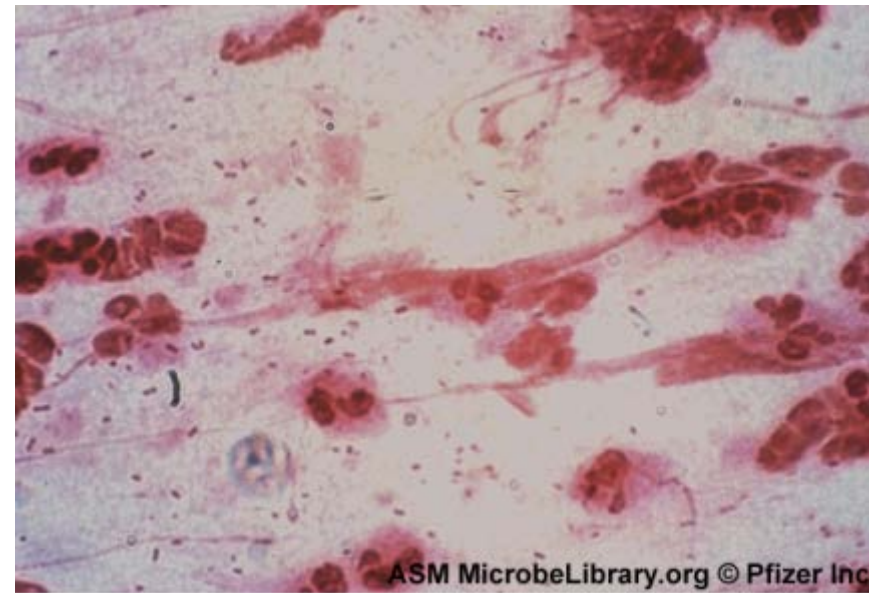
PCR: CSF, blood, genital source

Respiratory flora, ?false positive

- **Isolations**

- **CHOC agar: Colonies –HIB-capsule**

- Satellite phenomenon:
- *SBA/ S. aureus (release factor V)*
- Quad plate
- chemical reactions



# Staphylococci (Catalase +)

Slide (bound) Coagulase  
Staph latex reagent

+/- (equivocal)

*Staph aureus*  
H<sub>2</sub>O Control -

Tube (free) coagulase  
Exogenous DNA (SE) or *Thermonuclease*  
*Mannitol/Salt*

*Staph spp. (coag neg)*  
CoNS

If H<sub>2</sub>O control +:  
*R/O S. schleiferi* and  
*S. lugdunensis*  
(Other *biochemicals*  
Must be set up,  
See Staph charts)

*Staph aureus*

*Staph coag neg*

*Novobiocin* (from Phoenix)

R

*S. saprophyticus*  
If Phoenix ID < 95%  
Confirm Xylose -  
*Trehalose* +

S

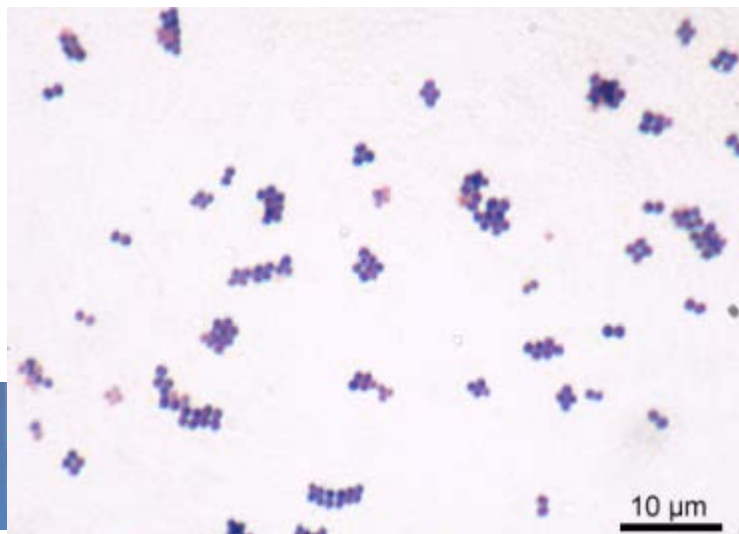
*Staph spp (coag neg)*  
(Not *speciated* routinely)

If Micro oxidase +:

*Micrococcus/Rothia* species

*Alloiococcus spp*

JHU microbiology laboratory

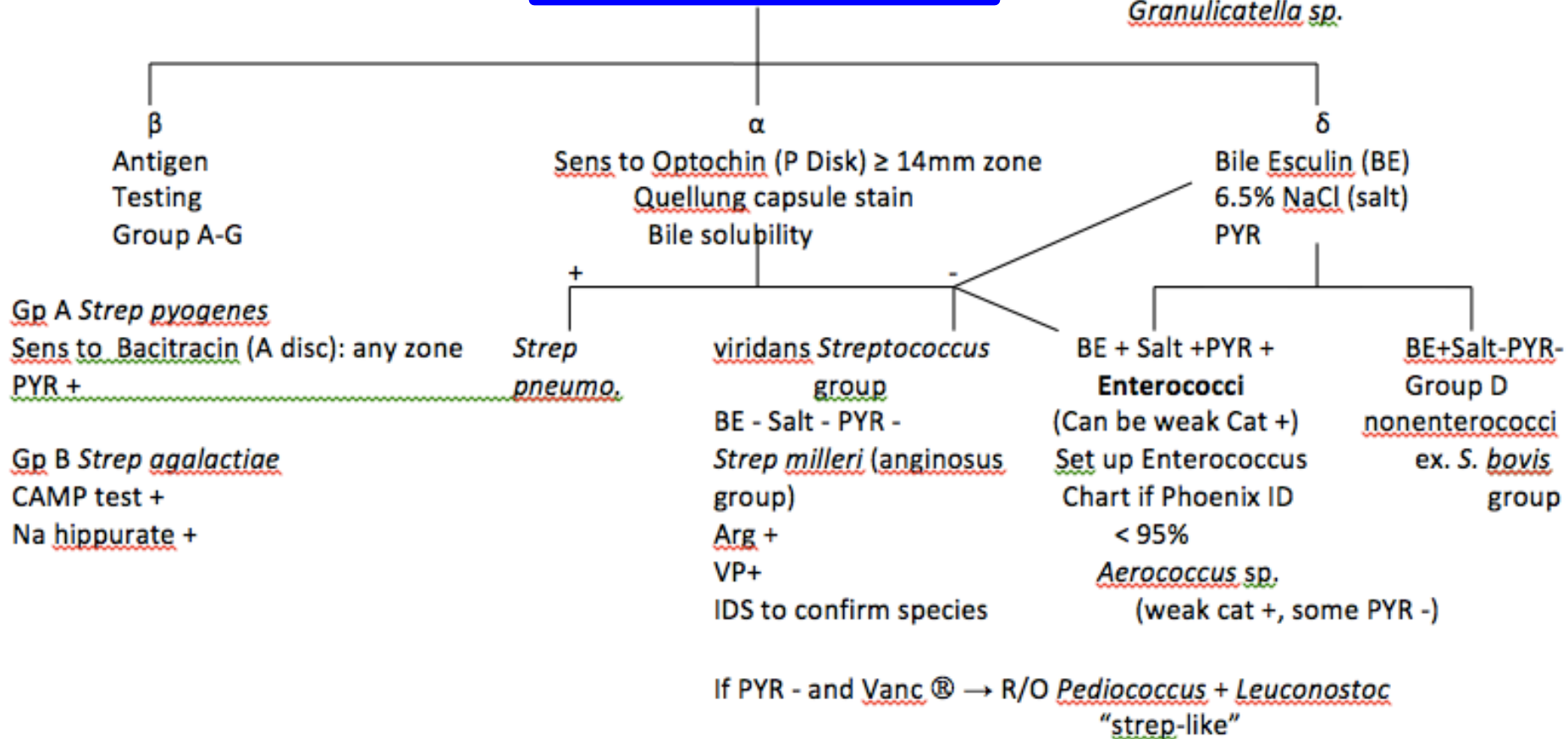


10 μm

## Gram Positive Cocci

### Streptococci (Catalase -) Hemolysis on SBA

if no growth on SBA:  
R/O *Abiotrophia sp.*  
*Granulicatella sp.*

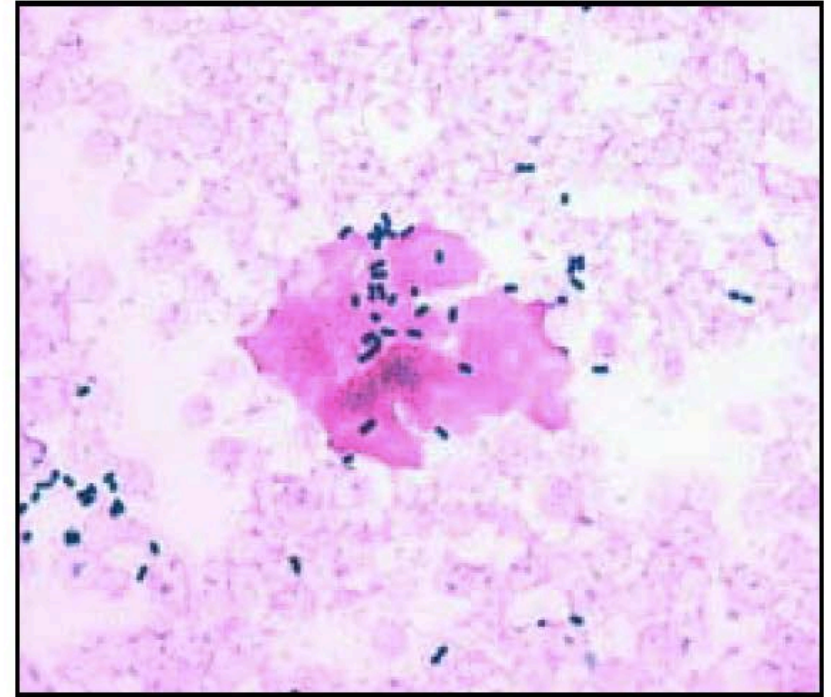


# Errors in Interpretation of Gram Stains From Positive Blood Cultures

Kenneth H. Rand. MD.<sup>1</sup> and Maria Tillan. MD<sup>2\*</sup>



**Image 1** *Bacillus* species staining gram-negative from a positive blood culture. Although this smear could be interpreted as gram-variable, it is easy to understand how it could be read as gram-negative ( $\times 1,000$ ).



**Image 2** *Acinetobacter* staining gram-positive from a positive blood culture ( $\times 1,000$ ).

Error in conventional biochemical detection method



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*Am J Clin Pathol* 2006;126:686-690

# Bacterial Misidentification in a Resource-Limited Microbiology Laboratory Setting and Quality Improvement Strategies

Nuntra Suwantararat, MD,\*† Sasinuj Rutjanaweche, MD,‡ Aubonphan Buppajarntham, MD,‡  
Karen C. Carroll, MD,\*† Thana Khawcharoenporn, MD, MSc,‡ and Anucha Apisarnthanarak, MD‡

ID problem = Nonfermenters Error in conventional biochemical detection method

**TABLE 1.** Summary of Bacterial Misidentification, Patient Outcome, and QI Strategies

Patient	Source	Correct Identification	Misidentification	Outcome	Identify Problem	QI
1	Blood	<i>A. baumannii</i>	<i>Stenotrophomonas maltophilia</i>	Died*	Morphology/biochemical identification	Nonfermenter identification protocol
2	Blood	<i>A. baumannii</i>	Gram-positive cocci	Died*	Gram stain interpretation	Gram stain protocol
3	Blood	<i>Burkholderia pseudomallei</i>	<i>A. baumannii</i>	Died†	Biochemical identification	Nonfermenter identification protocol
4	Sputum	<i>Burkholderia cepacia</i>	<i>B. pseudomallei</i>	Survived	Biochemical identification	Nonfermenter identification protocol
5	Blood	<i>P. aeruginosa</i>	Gram-positive bacilli	Died‡	Gram stain interpretation	Gram stain protocol
6	Blood	<i>Streptococcus mutans</i>	<i>Corynebacterium</i> species	Survived	Gram stain interpretation, morphology identification	Gram stain protocol
7	Joint fluid	<i>Streptococcus suis</i>	<i>Bacillus</i> species	Survived	Gram stain interpretation, morphology identification	Gram stain protocol

\*Sepsis, inadequate antibiotic coverage for extensively drug-resistant *A. baumannii* bacteremia.

†Sepsis, pneumonia, inappropriate antibiotic treatment for *B. pseudomallei* infection.

‡Sepsis, persistent fever with vancomycin treatment, inappropriate antibiotic treatment for *P. aeruginosa* infection.

Mis-ID = 1.3% (7/550) — In 1 month  
2013 Mis-ID = 0.08% (6/7554), (P < 0.001)

Suwantararat N, J Patient Saf 2014 (in press)

# Automated biochemical identification and susceptibility method

MicroScan system

Phoenix system

VITEK 2 system



(a) MicroScan instrument



(b) MicroScan® panel

**MicroScan**

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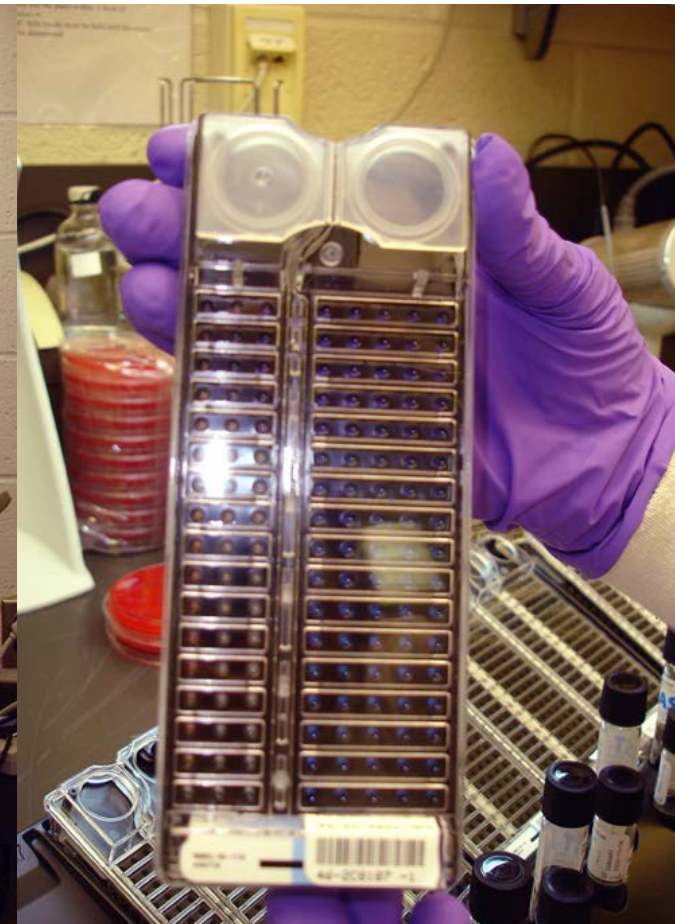
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The BD Phoenix system is presently used in our Microbiology laboratory for routine identification and susceptibility testing of commonly isolated aerobic bacteria from patient cultures. Susceptibility testing is done by the microbroth dilution method



# VITEK 2™ — technology

## Identification card

- GP
- GN
- Fastidious bacteria  
(*Hemophilus sp*, *Nisseria sp*)
- Anaerobes
- Yeast



PC & Software



VITEK® 2 Compact



VITEK® 2

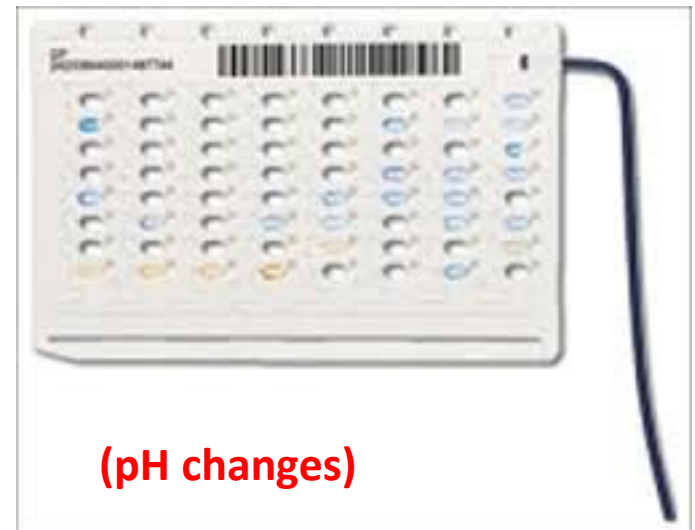


Smart Carrier™

## AST cards

- GP-AST
- GN-AST
- Yeast-AST

## Abx list (18 drugs, MICs)



(pH changes)

# Clinical Microbiology 2015

- Standard
- Accurate result
- Rapid detection  
(blood, sterile site)
- Short turnaround time
- Cost effectiveness
- Impact patient care
- Laboratory consolidation
- Collaboration
- **TEAM WORK!**

Mass spectrometry  
(MALDI-TOF MS)

Molecular detection (PCR)

Automated laboratory system



- **Molecular study**
- **MALDI-TOF (Mass spectrometry)**
- Bacterial 16SrRNA sequencing (500bp, 1000bp, 1500bp)
- Fungal DNA sequencing
  
- Bacterial resistance genes detection –PCR
  
- **Rapid detection:**
- **Multiplex PCR – Blood, Stool, respiratory, CSF**
  
- **Epidemiological studies**
- Bacterial Typing
- PFGE
  
- **Whole genomes sequencing**

# Active Surveillance MRSA

- **Recommended by SHEA/CDC**

Legislated in some states in the US

- **Methods**

- Chromogenic agar media

- Molecular methods

- Variety of platforms

- Sensitivity: 88-100%;

- Specificity : 92-99 %

- Problems

- *mecA* dropouts

- Some MR-CoNs may test positive

- False negatives have also been reported due to emergent strains with unusual genotypes



***S. aureus***

**= Coagulase test positive**

Muto CA, et. al. *Infect Control Hosp Epidemiol* 2003;24:362. Peterson LR, et al. *J Clin Microbiol* 2010;48:1661; Arbefeveille SS, et al 2011; *J Clin Microbiol* 49:2996; Malhotra-Kumar et al 2010; *J Clin Microbiology* 48:4598



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# *Staphylococcus aureus*

- **Isolation procedure**
- 5% Sheep blood Agar (broth may enhance recovery)
- Incubation: 18-24 hr

## **Selective agars:**

- **CNA** (Columbia Colistin-nalidixic acid agars)
- **Mannitol salt agar**
- Lipase-salt-mannitol agar (Remel)
- CHROMagar *Staph aureus*  
(CHROMagar, Paris, France)
- **BBL CHROMagar *Staph aureus*\***  
(BD-Diagnostics)
- *S. aureus* ID (bioMerieux, France)



# Active Surveillance

## Vancomycin Resistant Enterococci (VRE)

### Recommended by SHEA

- Active surveillance for high risk institutions
- Vigorous infection control practices
  - Isolation of colonized patients
  - Use of barrier precautions
  - Hand hygiene
  - Control antibiotic pressure

### •Methods

- Stool culture
  - BHI with 6 µg/ml (or higher) of vancomycin
  - Chromogenic agars
  - Molecular methods are non-specific



# Carbapenem Resistant *Enterobacteriaceae* (CRE)

- CREs are usually resistant to all Beta-lactamase antibiotics and other classes of antimicrobial agents
- Hence an infection control concerns
- Numerous mechanism of resistance-carbapenamase production

## Variability in geographic distribution

USA: KPC (most common), MBLs such as NDM-1

Asia: Less KPC, more MBLs (NDM-1)

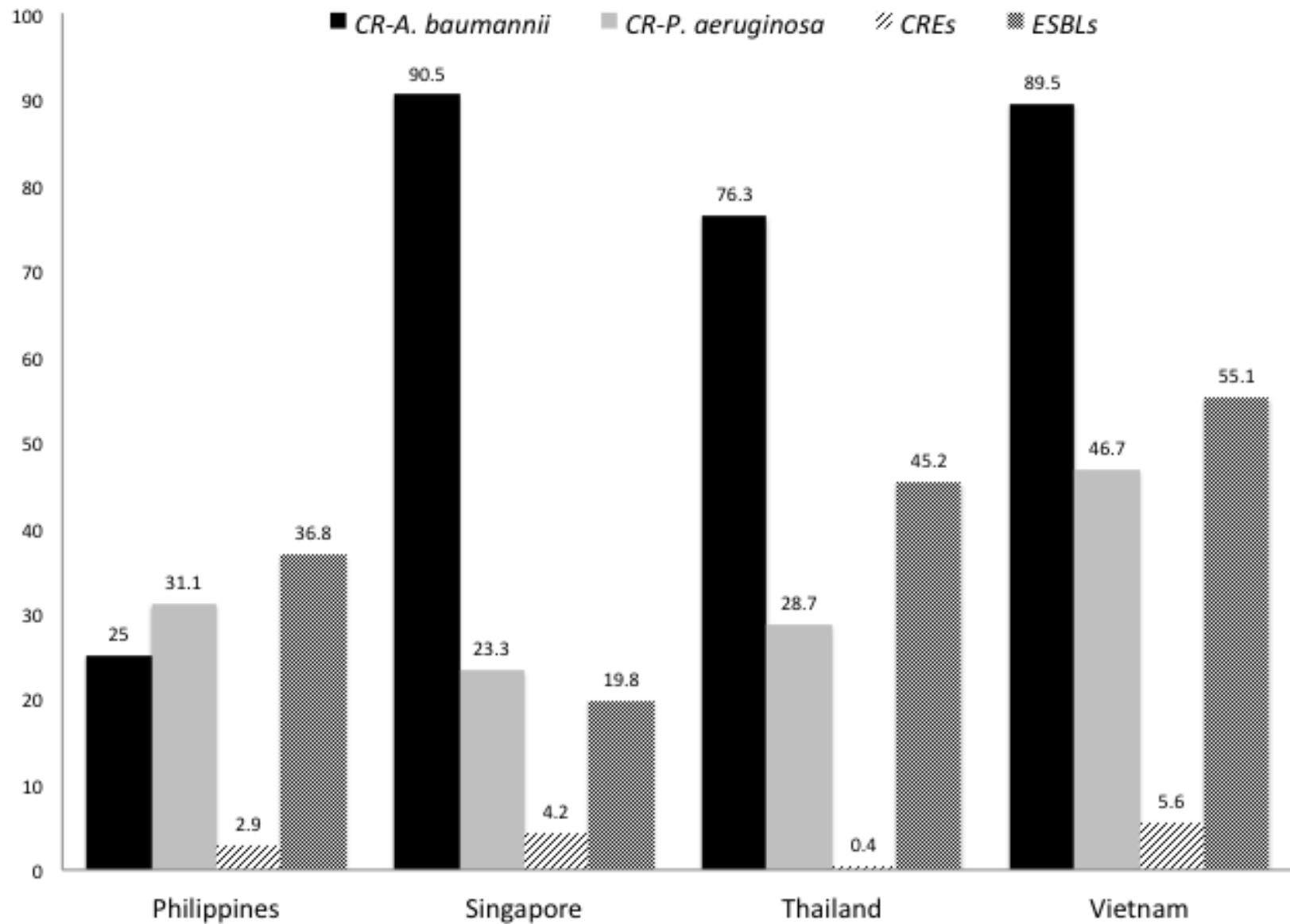
Europe: Greece VIMs

South America: KPC, MBLs

## • Methods for screening

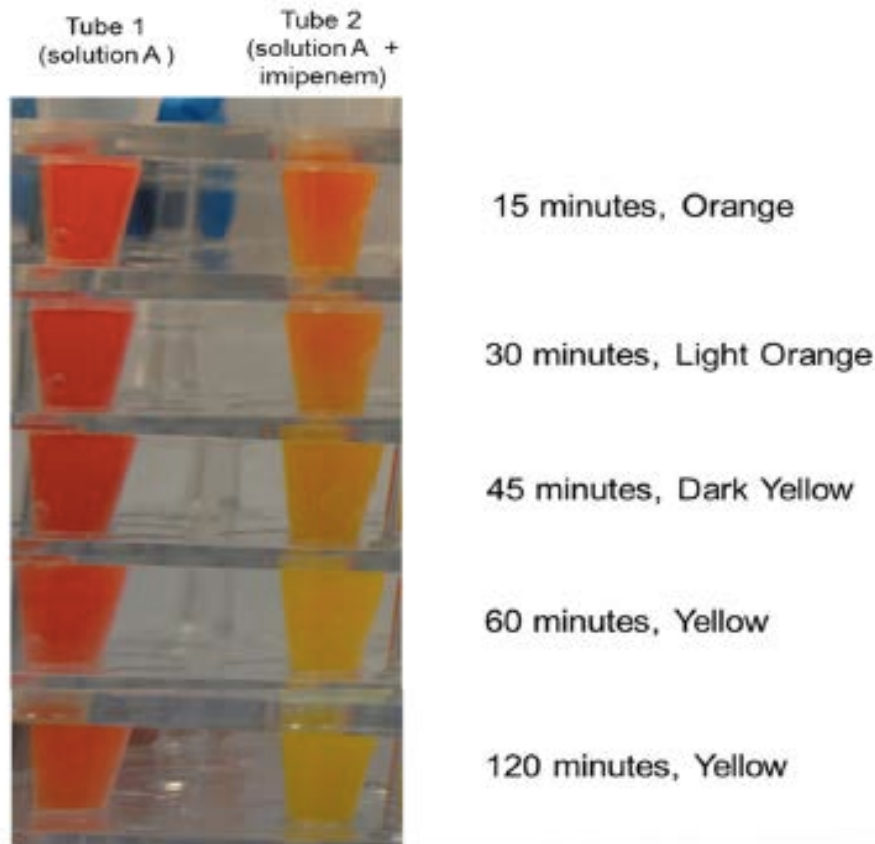
- CDC broth enriched method (US)
- Chromogenic agars
- Molecular assays
- NP Carba





Prevalence (%) of extended-spectrum  $\beta$ -lactamases (ESBLs) and carbapenem-resistant organisms by country in Southeast Asia, adopted from Kiratisin P, et al. COMPACT II

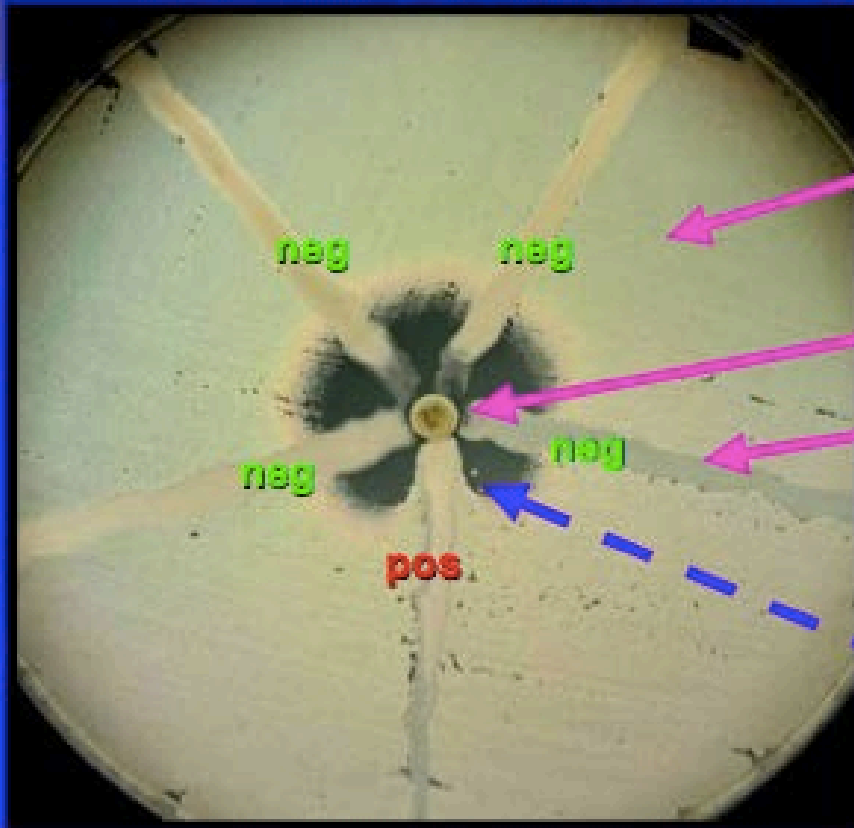
## KPC *Providencia stuartii*



## Comparison of a Novel, Rapid Chromogenic Biochemical Assay, the Carba NP Test, with the Modified Hodge Test for Detection of Carbapenemase-Producing Gram-Negative Bacilli

Shawn Vasoo,<sup>a</sup> Scott A. Cunningham,<sup>a</sup> Peggy C. Kohner,<sup>a</sup> Patricia J. Simner,<sup>a</sup> Jayawant N. Mandrekar,<sup>b</sup> Karen Lolans,<sup>c</sup> Mary K. Hayden,<sup>c,d</sup> Robin Patel<sup>a,e</sup>

# Modified Hodge Test



1. Swab *E. coli* ATCC 25922 onto plate to create lawn (1:10 dilution of McF 0.5).
2. Place imipenem disk in center.
3. Streak test isolates from edge of disk to end of plate.
4. Incubate overnight.
5. Look for growth of *E. coli* around test isolate streak - indicates carbapenem-hydrolyzing enzyme.

Photo courtesy of J. Patel 49



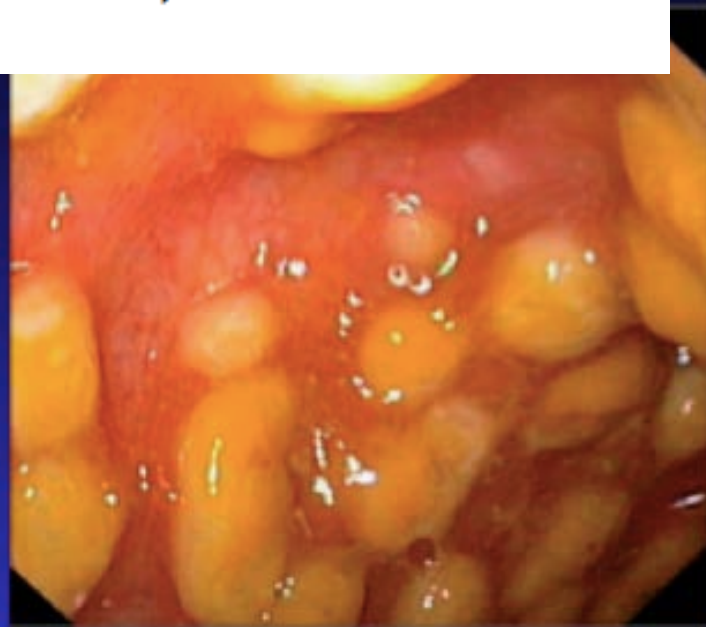
# Diagnosis of *Clostridium difficile* Infection: an Ongoing Conundrum for Clinicians and for Clinical Laboratories

Carey-Ann D. Burnham,<sup>a</sup> Karen C. Carroll<sup>b</sup>

Departments of Pathology & Immunology and Pediatrics, Washington University School of Medicine, St. Louis, Missouri, USA<sup>a</sup>; Departments of Pathology and Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA<sup>b</sup>

Clinical Microbiology Reviews p. 604–630

July 2013 Volume 26 Number 3



Lancet 371:1486, 2008

วิธีการตรวจ/ทดสอบ

id amplification tests 77 - 100  
(NAATs/PCR)

ตารางที่ 1 ความสามารถของวิธีการตรวจหาเชื้อ *C. difficile*

แบบต่างๆ (ปรับปรุงจากเอกสารอ้างอิงที่31)



## Kirby-Bauer Method

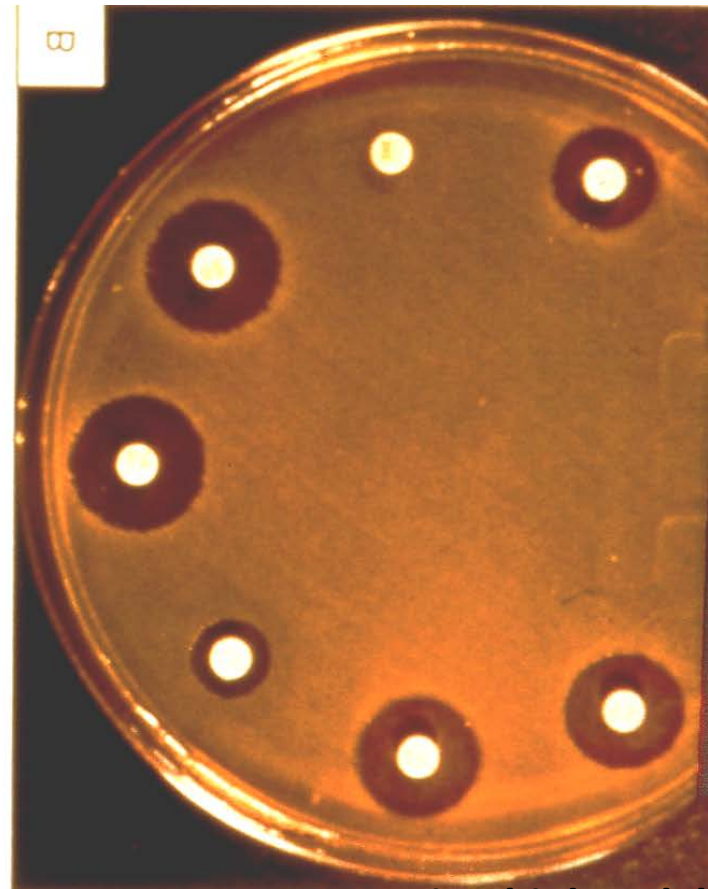
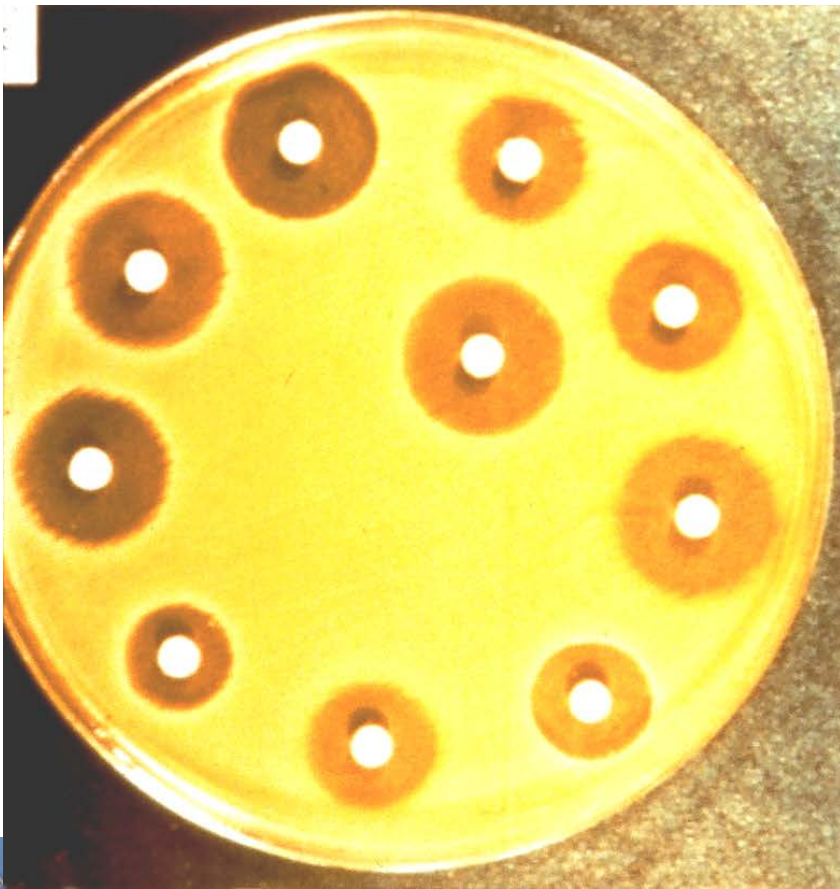
## Susceptibility testing

### 1. Disk Diffusion

Antibiotic susceptibility testing in which disks containing various antibiotics are placed on a plate swabbed with the organism.

**Zones of inhibition** are measured to determine whether the organism is **susceptible, intermediate or resistant**.

(Based on interpretation guideline, CLSI (USA), EUCAST)



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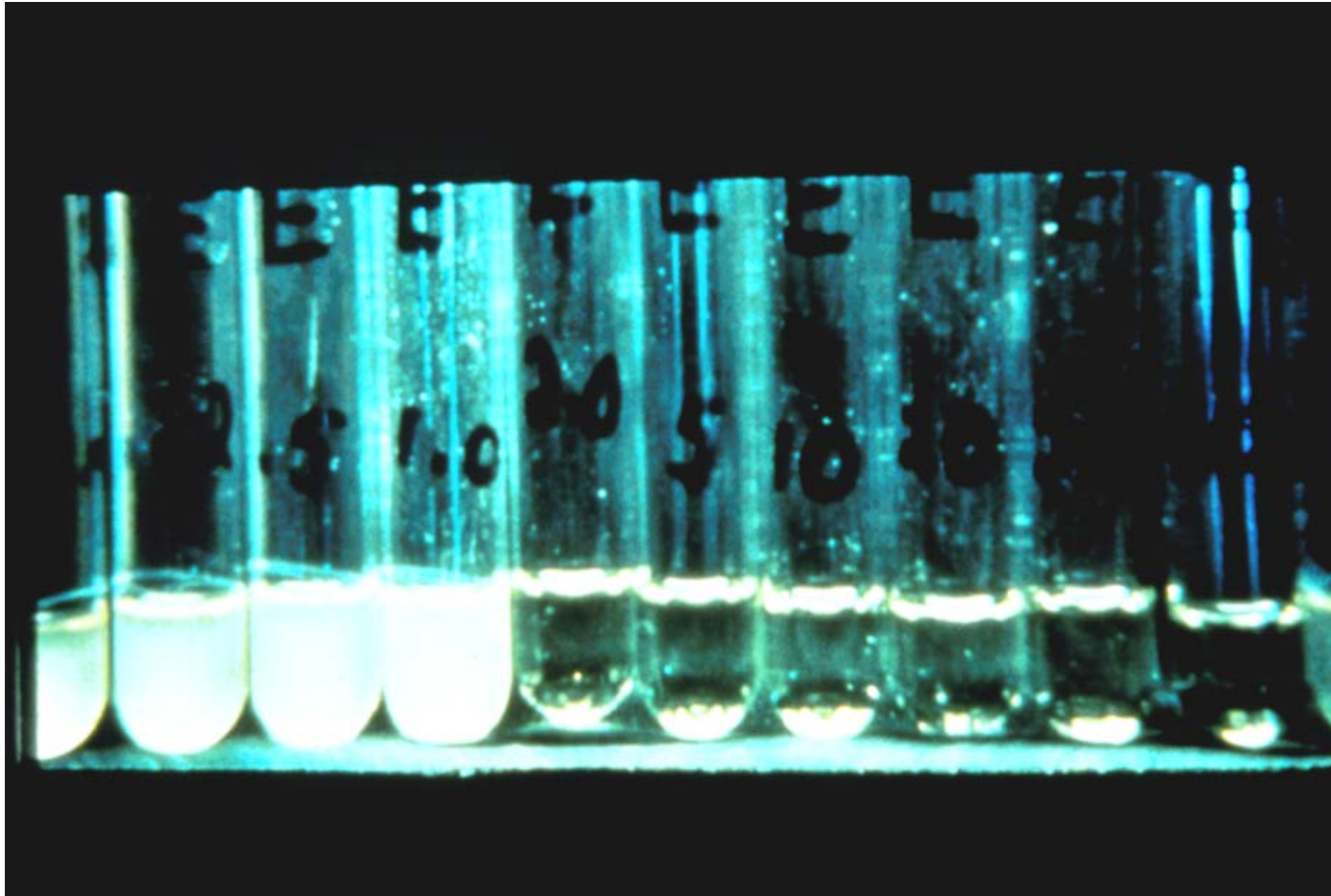
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## Susceptibility testing

## 2. Tube Dilution Method

The first tube in which there is no visible growth is the **MIC** level of the antibiotic for the organism tested.



Antibiotic Concentration Low -----High

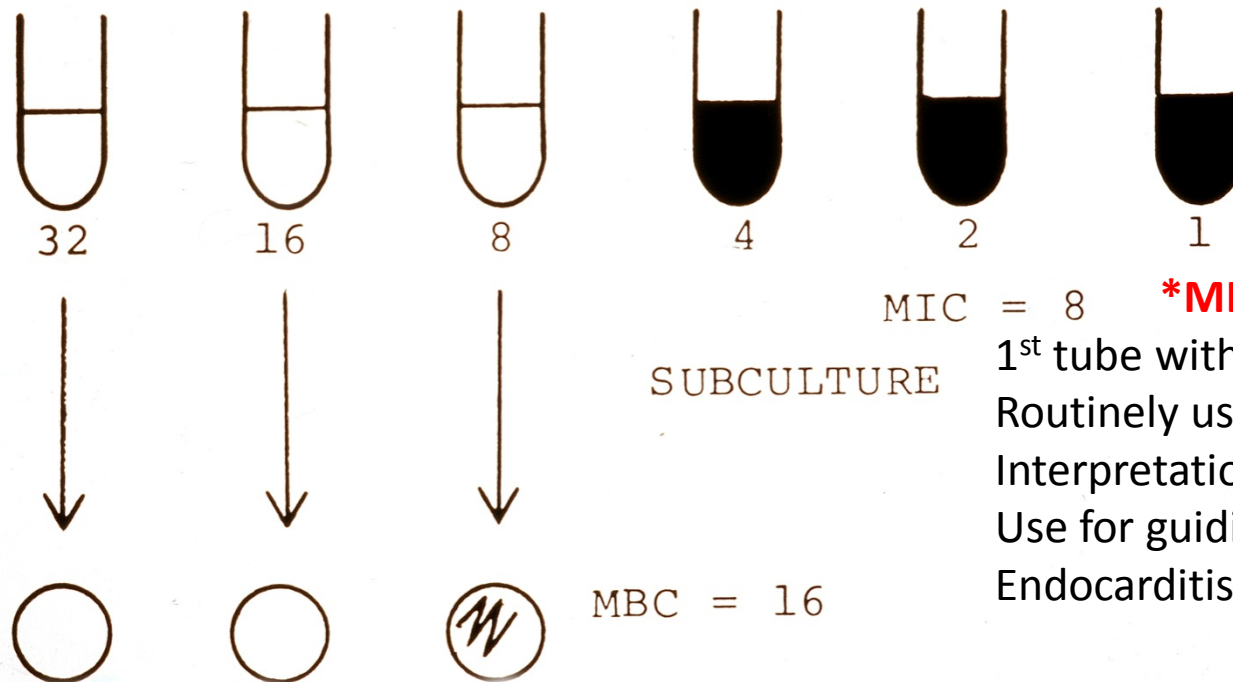


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# Illustration of the difference between **MIC**, minimum inhibitory concentration, and **MBC**, minimum bactericidal concentration, of an antibiotic.



MIC = 8 \*MIC=

SUBCULTURE

1<sup>st</sup> tube with no visible growth  
Routinely use, CLSI and EUCAST  
Interpretation  
Use for guiding treatment\*  
Endocarditis, meningitis

MBC = 16

**MBC** = 99% bacterial growth inhibition  
(Currently use only for research,  
previously use for endocarditis treatment)

**Broth microdilution:** manual or commercial system ie MicroScan, TREK panels  
Phoenix system, VITEK2 system - Give **MIC** interpretation



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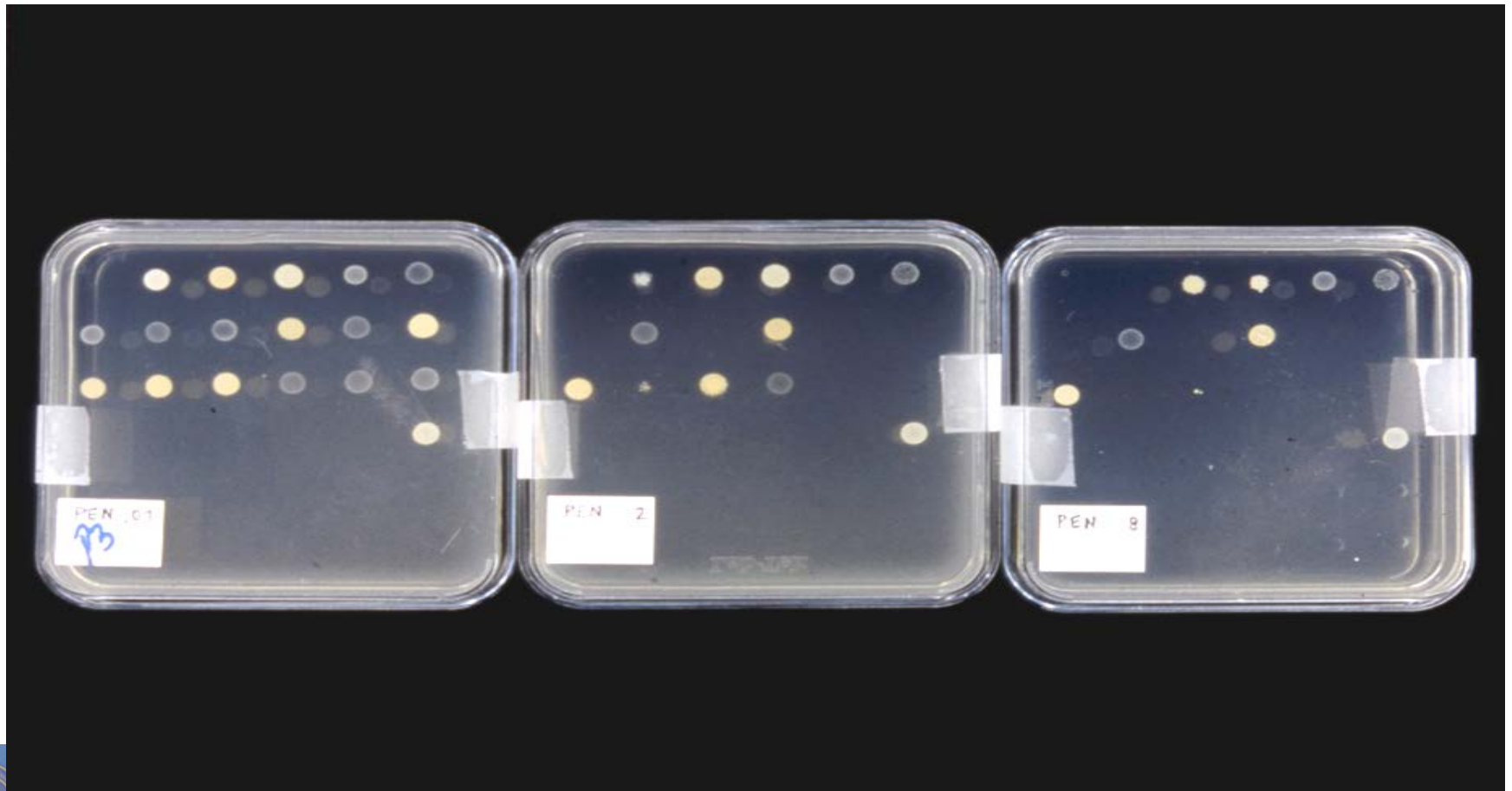
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### 3. Agar Dilution Method

Formerly used at JHH, this method measures MICs of antibiotics by comparing growth of colonies on plates of increasing antibiotic concentrations.

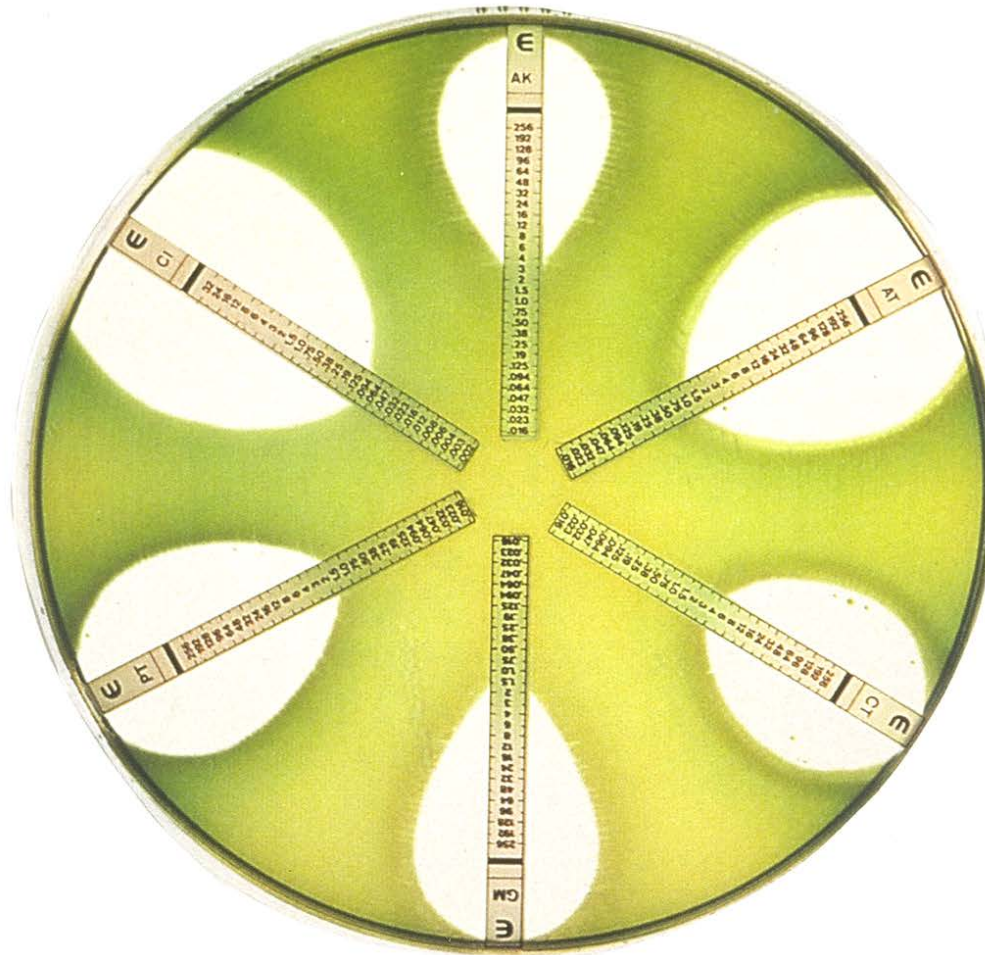
Labor intensive/ rarely use in routine labs

For research: Standard for Anaerobic bacteria AST, *N. gonorrhea* AST



## 4. E-test Method

Each strip is impregnated with increasing concentrations of a different antibiotic ; strips are placed on a plate swabbed with the organism to be tested, and incubated overnight. The **MIC** level for each antibiotic is at the line crossed on the strip where the organism is inhibited from growing.



E= elliptical shape

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# M100S

## Performance Standards for Antimicrobial Susceptibility Testing

### CLSI breakpoints interpretation Example

## Clinical breakpoints

## Organization

## EUCAST structure, committees and statutes

EUCAST News

### Clinical breakpoints

### Information on breakpoint tables

### Antibiotics lacking clinical data

## Setting breakpoints

### Expert rules

### Resistance mechanisms

### MIC distributions ECOFFs

### Zone distributions ECOFFs

### AST of bacteria

### AST of fungi

### AST of veterinary pathogens

## Frequently Asked Questions (FAQ)

## Meetings

## EUCAST Presentations

## Documents

## Translations

### Information for industry

## Links


## Contacts


 Website changes

## Clinical breakpoints

See → information on Clinical breakpoint tables.

### Breakpoint table for bacteria

 Clinical breakpoints - bacteria (v 5.0) - pdf file for printing (2015-01-01 and uploaded again 2015-01-26 after correction of internal links)

 Clinical breakpoints - bacteria (v 5.0) - excel file for screen (2015-01-01 and uploaded again 2015-01-26 after correction of internal links)

Breakpoint table 5.0 (2015-01-01) includes ceftobiprole and telavancin. Updates from the preliminary table published 2014-12-05 are

- a link to the EUCAST guidance on topical agents have been added in the list of contents

- comments for cephalosporins in tab "Staphylococci" have been modified.

- the intended change in amoxicillin/clavulanic acid zone diameter breakpoints for *Haemophilus influenzae* is temporarily cancelled. An extended evaluation is needed.

- the system of presenting notes was changed.

- the system for highlighting changes from one table to the next was partly changed - changes in breakpoints are still highlighted in yellow, changes in comments/notes are highlighted by underlining new or changed text and by crossing out text which was removed.

Addendum -

 clinical breakpoints and QC recommendations for new agents dalbavancin, oritavancin and (19 April 2015).

Breakpoints published in Addendum during the year will be part of the next version of the full Clinical breakpoint tables valid from early January each year.

## Interpretive Criteria

Interpretive criteria are the MIC or zone diameter values used to indicate susceptible, intermediate, and resistant breakpoints.

Antimicrobial Agent	Disk Content	Zone Diameter Interpretive Criteria (nearest whole mm)			MIC Interpretive Criteria ( $\mu\text{g/mL}$ )		
		S	I	R	S	I	R
X	30 $\mu\text{g}$	$\geq 20$	15–19	$\leq 14$	$\leq 4$	8–16	$\geq 32$
Y	—	—	—	—	$\leq 1$	2	$\geq 4$
Z	10 $\mu\text{g}$	$\geq 16$	—	—	$\leq 1$	—	—

For example, for antimicrobial agent X with interpretive criteria in the table above, the susceptible breakpoint is 4  $\mu\text{g/mL}$  or 20 mm and the resistant breakpoint is 32  $\mu\text{g/mL}$  or 14 mm.



Table 2A. Zone Diameter and Minimal Inhibitory Concentration Interpretive Standards for *Enterobacteriaceae*

Testing Conditions	Routine QC Recommendations (See Tables 4A and 5A for acceptable QC ranges.)
<b>Medium:</b> Disk diffusion: Mueller-Hinton agar (MHA) Broth dilution: cation-adjusted Mueller-Hinton broth Agar dilution: MHA <b>Inoculum:</b> Growth method or direct colony suspension, equivalent to a 0.5 McFarland standard <b>Incubation:</b> 35°C ± 2°C; ambient air Disk diffusion: 16 to 18 hours Dilution methods: 16 to 20 hours	<i>Escherichia coli</i> ATCC®* 25922 <i>Pseudomonas aeruginosa</i> ATCC® 27853 (for carbapenems) <i>Escherichia coli</i> ATCC® 35218 (for $\beta$ -lactam/ $\beta$ -lactamase inhibitor combinations)  When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

\* ATCC® is a registered trademark of the American Type Culture Collection.

Refer to Tables 3A, 3B, and 3C for additional testing recommendations, reporting suggestions, and QC.

### General Comments

- (1) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see M02, Subchapter 3.6). Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. Strains of *Proteus* spp. may swarm into areas of inhibited growth around certain antimicrobial agents. With *Proteus* spp., ignore the thin veil of swarming growth in an otherwise obvious zone of growth inhibition. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.
- (2) When fecal isolates of *Salmonella* and *Shigella* spp. are tested, only ampicillin, a fluoroquinolone, and trimethoprim-sulfamethoxazole should be reported routinely. In addition, for extraintestinal isolates of *Salmonella* spp., a third-generation cephalosporin should be tested and reported, and chloramphenicol may be tested and reported if requested. Susceptibility testing is indicated for typhoidal *Salmonella* (*S. Typhi* and *Salmonella* Paratyphi A–C) isolated from extraintestinal and intestinal sources. Routine susceptibility testing is not indicated for nontyphoidal *Salmonella* spp. isolated from intestinal sources.
- (3) The dosage regimens shown in the comment column below are those required to achieve plasma drug exposures (in adults with normal renal and hepatic functions) on which breakpoints were based. When implementing new breakpoints, it is strongly recommended that laboratories share this information with infectious diseases practitioners, pharmacists, pharmacy and therapeutics committees, and infection control committees.

NOTE: Information in boldface type is new or modified since the previous edition.

Table 2A. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Zone Diameter Interpretive Criteria (nearest whole mm)				MIC Interpretive Criteria (µg/mL)				Comments
			S	SDD	I	R	S	SDD	I	R	
PENICILLINS											
A	Ampicillin	10 µg	≥17		14–16	≤13	≤8		16	≥32	(4) Results of ampicillin testing can be used to predict results for amoxicillin. See comment (2).
B	Piperacillin	100 µg	≥21		18–20	≤17	≤16		32–64	≥128	
O	Mecillinam	10 µg	≥15		12–14	≤11	≤8		16	≥32	
O	Ticarcillin	75 µg	≥20		15–19	≤14	≤16		32–64	≥128	(5) For testing and reporting of <i>E. coli</i> urinary tract isolates only.
β-LACTAM/β-LACTAMASE INHIBITOR COMBINATIONS											
B	Amoxicillin-clavulanate	20/10 µg	≥18		14–17	≤13	≤8/4		16/8	≥32/16	
B	Ampicillin-sulbactam	10/10 µg	≥15		12–14	≤11	≤8/4		16/8	≥32/16	
B	Piperacillin-tazobactam	100/10 µg	≥21		18–20	≤17	≤16/4		32/4–64/4	≥128/4	
B	Ticarcillin-clavulanate	75/10 µg	≥20		15–19	≤14	≤16/2		32/2–64/2	≥128/2	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)											
(6) <b>WARNING:</b> For <i>Salmonella</i> spp. and <i>Shigella</i> spp., first- and second-generation cephalosporins and cephamycins may appear active <i>in vitro</i> , but are not effective clinically and should not be reported as susceptible.											
(7) Following evaluation of PK-PD properties, limited clinical data, and MIC distributions, revised interpretive criteria for cephalosporins (cefazolin, cefotaxime, ceftazidime, ceftizoxime, and ceftriaxone) and aztreonam were first published in January 2010 (M100-S20) and are listed in this table. Cefuroxime (parenteral) was also evaluated; however, no change in interpretive criteria was required for the dosage indicated below. When using the current interpretive criteria, routine ESBL testing is no longer necessary before reporting results (ie, it is no longer necessary to edit results for cephalosporins, aztreonam, or penicillins from susceptible to resistant). However, ESBL testing may still be useful for epidemiological or infection control purposes. For laboratories that have not implemented the current interpretive criteria, ESBL testing should be performed as described in Table 3A.											
Note that interpretive criteria for drugs with limited availability in many countries (eg, moxalactam, cefonicid, cefamandole, and cefoperazone) were not evaluated. If considering use of these drugs for <i>E. coli</i> , <i>Klebsiella</i> , or <i>Proteus</i> spp., ESBL testing should be performed (see Table 3A). If isolates test ESBL positive, the results for moxalactam, cefonicid, cefamandole, and cefoperazone should be reported as resistant.											
(8) <i>Enterobacter</i> , <i>Citrobacter</i> , and <i>Serratia</i> may develop resistance during prolonged therapy with third-generation cephalosporins as a result of derepression of AmpC β-lactamase. Therefore, isolates that are initially susceptible may become resistant within three to four days after initiation of therapy. Testing of repeat isolates may be warranted.											
A	Cefazolin	30 µg	≥23		20–22	≤19	≤2		4	≥8	(9) Interpretive criteria are based on a dosage regimen of 2 g every 8 h. See comment (7).
C	Ceftaroline	30 µg	≥23		20–22	≤19	≤0.5		1	≥2	For UTI interpretive criteria, see below under CEPHEMS (ORAL).
											(10) Interpretive criteria are based on a dosage regimen of 600 mg every 12 h.



# Carbapenem-Resistant *Enterobacteriaceae*: Epidemiology and Prevention

Neil Gupta,<sup>1,2</sup> Brandi M. Limbago,<sup>2</sup> Jean B. Patel,<sup>2</sup> and Alexander J. Kallen<sup>2</sup>

<sup>1</sup>Epidemic Intelligence Service and <sup>2</sup>Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention, Atlanta, Georgia

**Clinical Infectious Diseases 2011;53(1):60–67**

**Table 2. Clinical and Laboratory Standards Institute Interpretive Criteria for Carbapenems and *Enterobacteriaceae* [41]**

Agent	Previous breakpoints (M100-S19)MIC (μg/mL)			Revised breakpoints (M100-S20)MIC (μg/mL)		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Doripenem	...	...	...	≤1	2	≥4
Ertapenem	≤2	4	≥8	≤0.25	0.5	≥1
Imipenem	≤4	8	≥16	≤1	2	≥4
Meropenem	≤4	8	≥16	≤1	2	≥4

**NOTE.** MIC, minimum inhibitory concentration.

**New breakpoints (Jan 2010) vs old breakpoints + confirmation test \*\*\*  
(ie; MHT, NP carba)**



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CLINICAL AND  
LABORATORY  
STANDARDS  
INSTITUTE\*

January 2014

# M39-A4

## Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data; Approved Guideline—Fourth Edition

### Antibiogram



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# Appendix E1. Cumulative Antimicrobial Susceptibility Report Example – Antimicrobial Agents Listed Alphabetically (Hypothetical Data)

Memorial Medical Center  
1 January – 31 December 2012 Cumulative Antimicrobial Susceptibility Report\*  
Percent Susceptible

Gram-Negative Organisms	No. Strains	Amikacin	Ampicillin	Cefazolin	Cefotaxime	Ceftazidime	Ciprofloxacin	Nitrofurantoin†	Gentamicin	Meropenem	Piperacillin- tazobactam	Trimethoprim- sulfamethoxazole	Tobramycin
<i>Acinetobacter baumannii</i>	32	80	R	R	34	52	51	†	60	80	46	58	59
<i>Citrobacter freundii</i>	49	100	R	R	72	67	90	78	100	99	67	67	100
<i>Enterobacter aerogenes</i>	31	100	R	R	68	69	92	85	91	99	74	95	91
<i>Enterobacter cloacae</i>	76	99	R	R	61	62	92	81	90	99	77	84	90
<i>Escherichia coli</i>	1433	99	36	68	96	94	72	98	91	99	51	65	92
<i>Klebsiella pneumoniae</i>	543	99	R	72	91	92	84	74	94	95	86	81	94
<i>Morganella morganii</i>	44	100	R	R	85	81	99	R	100	99	64	75	100
<i>Proteus mirabilis</i>	88	100	87	80	99	99	89	R	90	100	70	73	93
<i>Pseudomonas aeruginosa</i>	397	97	R	R	R	76	75	R	80	80	85	R	83
<i>Salmonella</i> spp.	32	–	88	–	97	97	90	–	–	100	91	86	–
<i>Serratia marcescens</i>	50	100	R	R	82	94	95	R	94	99	94	91	89
<i>Shigella</i> spp.	33	–	64	–	100	100	95	–	–	100	84	69	–
<i>Stenotrophomonas maltophilia</i>	72	R	R	R	R	63	6	R	R	R	–	98	R

\* The percent susceptible for each organism/antimicrobial combination was generated by including the first isolate of that organism encountered on a given patient.

† Nitrofurantoin data from testing urine isolates only.

‡ (–) drug not tested or drug not indicated.

Abbreviations: No., number; R, intrinsic resistance.

**Table H1. 95% CIs for Selected Sample Sizes\***

Sample Size	Susceptible or Resistant Rate																	
	10%		20%		30%		40%		50%		60%		70%		80%		90%	
10	0	43	5	52	10	61	17	69	24	76	31	83	39	90	48	95	57	100
20	2	31	7	42	14	52	22	61	30	70	39	78	48	86	58	93	69	98
30	3	26	9	38	17	48	25	58	33	67	42	75	52	83	62	91	74	97
40	3	24	10	35	18	46	26	55	35	65	45	74	54	82	65	90	76	97
50	4	22	11	33	19	44	28	54	37	63	46	72	56	81	67	89	78	96
60	4	20	12	32	20	43	29	53	38	62	47	71	57	80	68	88	80	96
70	5	20	12	31	20	42	29	52	39	61	48	71	58	80	69	88	80	95
80	5	19	13	30	21	41	30	51	39	61	49	70	59	79	70	87	81	95
90	5	18	13	30	21	40	30	50	40	60	50	70	60	79	70	87	82	95
100	5	18	13	29	22	40	31	50	40	60	50	69	60	78	71	87	82	95
200	7	15	15	26	24	37	33	47	43	57	53	67	63	76	74	85	85	93
400	7	13	16	24	26	35	35	45	45	55	55	65	65	74	76	84	87	93
600	8	13	17	23	26	34	36	44	46	54	56	64	66	74	77	83	87	92
1000	8	12	18	23	27	33	37	43	47	53	57	63	67	73	77	82	88	92

\* CIs were calculated using the Agresti-Coull interval.

If the number of isolates is below 30, the Agresti-Coull approximation is not as accurate as other recommended approaches. Alternative estimates which can be applied include the Wilson interval without continuity correction (Agresti-Coull was designed as an approximation to the Wilson interval, so the CIs are very similar), the more conservative Wilson interval with continuity correction, or the even more conservative Clopper-Pearson method. Conservative CIs are a bit wider than nonconservative CIs to improve the likelihood that the 95% CI estimated from the data indeed includes the true 95% CI. So, with a given dataset, a conservative 95% CI may in fact be a 97% or 98% CI. Calculations for all of the methods mentioned can be found in the below references.<sup>1-10</sup>

# Direct Infection-Control

## Related Functions: Micro Lab

- **Participate as a member of the infection control committee**
  - Ensures communication
  - Enhances education
  - Allows for allocation of resources
- **Collaborate with IC personnel on outbreak investigations**
- Strain typing
- Store isolates



# Application of Typing Techniques

- Recognize and confirm an outbreak
  - Clusters of patients within hospitals
  - Track spread between hospitals over time
- Document nosocomial transmission
- Measure impact of intervention strategies
- Distinguishing relapse from re-infection in individual patients

Pfaller MA. 2001. *Emerg Infect Dis* 7:312-318.



# Epidemiologic Typing Methods

## Basic Principles



- Perform only with clear objectives
- Variability exists in all methods
  - evaluate all implicated isolates simultaneously
  - compare to epidemiologically unrelated control isolates
- Demonstrate not only relatedness of clustered isolates, but differences from isolates not involved epidemiologically



# Typing Questions and Suitable Methods

Questions	Suitable Methods	Discriminatory power	Time span
Outbreak investigations Short-term/local surveillance Control of hygiene measures	PFGE, RFLP, AFLP, RA-PCR, VNTR, SLST, micro-array	high	weeks-months
Long-term/global epidemiological studies Population genetics Analysis of population-based interventions, e.g. vaccination	MLST, micro-array, in part SLST, whole genome sequencing*	Low *(high)	years

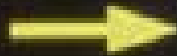
# PFGE Principles

- Variation of conventional agarose gel electrophoresis
- Suspension of organism is embedded in agarose plugs to minimize shearing of DNA
- DNA is cut with restriction enzymes that have infrequent recognition sites
- Larger pieces of DNA are separated by shifting direction of current frequently
- 5-20 fragments ranging in size from 10kb to 800 kb in length are generated



# Pulsed Field Electrophoresis (CHEF)

Preparation of unsheared  
Chromosomal DNA  
in agarose blocks

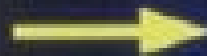


Digestion of DNA with  
rare cutting enzyme

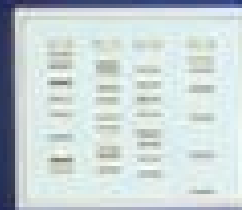


ตัดตำแหน่งของสาร  
พันธุกรรม ใน DNA  
ด้วย restriction enzyme

Agarose gel electrophoresis



Visualization under UV



ถ่ายภาพรูปแบบ  
สารพันธุกรรมที่จำแนกได้  
ภายใต้แสง UV

สกัด DNA จาก  
สารละลาย  
ของเชื้อ  
แบคทีเรียใน  
เจล agarose  
(DNA ที่ขนาด  
น้อยที่สุด)

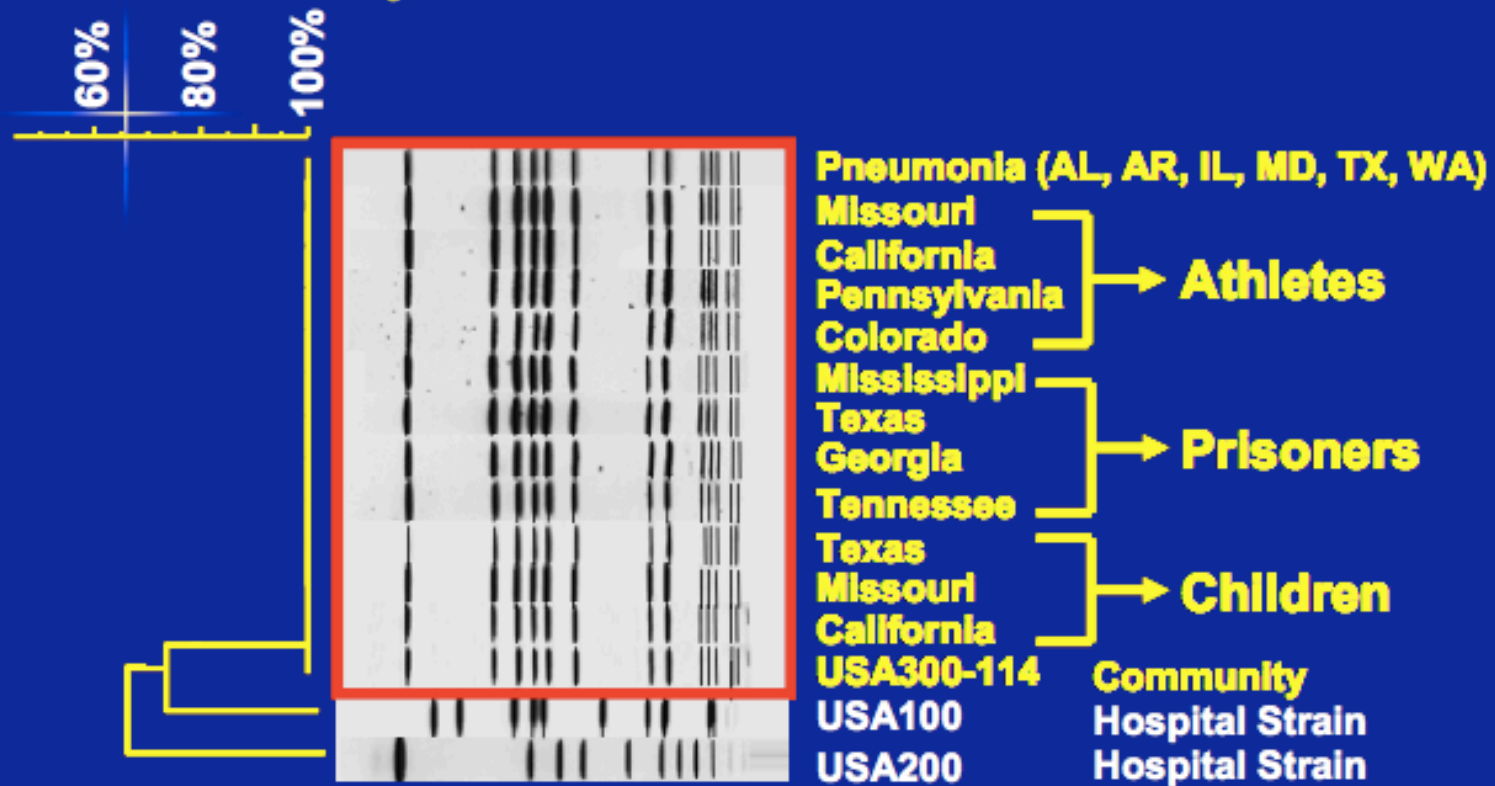
จำแนกรูปแบบ  
สารพันธุกรรม  
ด้วยการใช้  
กระแสไฟฟ้าใน  
ทิศทางต่าง ๆ บน  
agarose gel



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## A Single Pulsed-Field Type (USA300) has Accounted for Most Community-Associated MRSA Infections in the U.S.



# Modified Tenover Criteria



- $\leq 3$  differences in restriction-fragment positions
  - could have occurred by a single genetic event
  - may represent subtypes of the same strain
- $>3$  restriction differences in restriction fragment positions
  - less likely to be epidemiologically related

Goering RV. In *Rapid Detection of Infectious Agents*, Specter, et.al. (eds), Plenum Press, New York, 1998, p.131.



# PFGE



## Advantages

- Patterns easier to interpret compared to other techniques
- Highly reproducible
- Excellent discriminatory power
- Theoretically all bacteria are typeable, some fungi as well

## Disadvantages

- Cost of equipment
- Tedious
- Slow
- Certain organisms may not be typeable e.g.  
*C. difficile*, *Aspergillus sp.*
- May be too sensitive in detecting differences

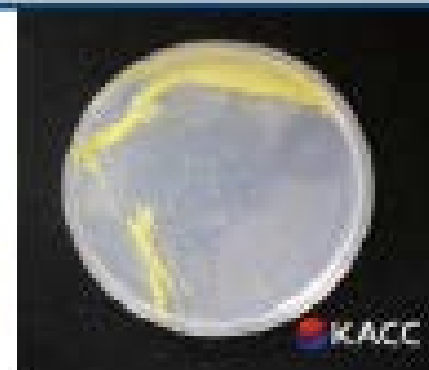


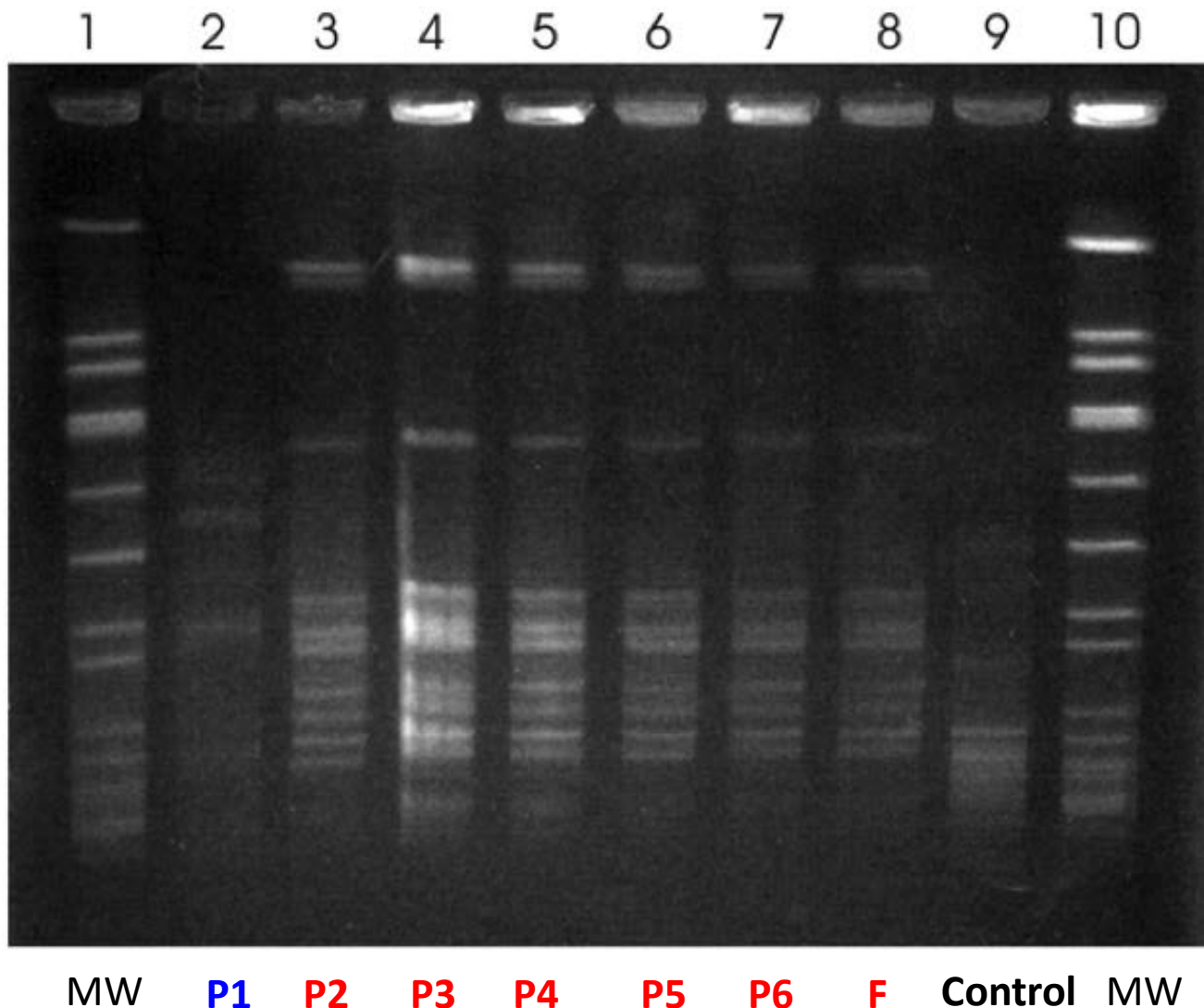
# Sphingomonas Bacteremia

## Laboratory Contamination or Hospital Outbreak?



- Over a two week period, blood bench technologist reported 6 pts with *Sphingomonas paucimobilis* bacteremia
- All 6 patients had positive BacT/Alert FA positive bottles (charcoal containing media)
- All isolates appeared phenotypically identical: yellow pigmented, weakly oxidase positive, aerobic GNR
- Infection control was notified
- All FA bottles from one of the implicated units (about 50) were removed and incubated without inoculation—no positives





Maragakis LL, Chaiwarith R, Srinivasan A, Torriani FJ, Avdic E, Lee A, et al. *Sphingomonas paucimobilis* bloodstream infections associated with contaminated intravenous fentanyl. Emerg Infect Dis 2009;15(1):12-8.



สมาคมโรคติดต่อ  
แห่งประเทศไทย

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# Control of HAIs Requires



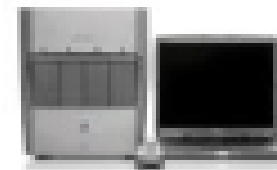
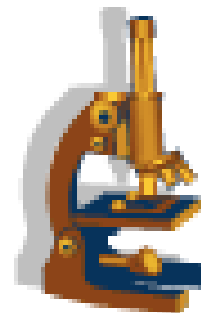
From all:

- Hand Hygiene
- Compliance with infection control
- Vaccination
- Stay at home when ill
- Antibiotic stewardship



• From the Lab:

- Quality Microbiology
- Active surveillance
- Communication



# Acknowledgement

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# Questions and Discussion

**Thank you for your attention**

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