



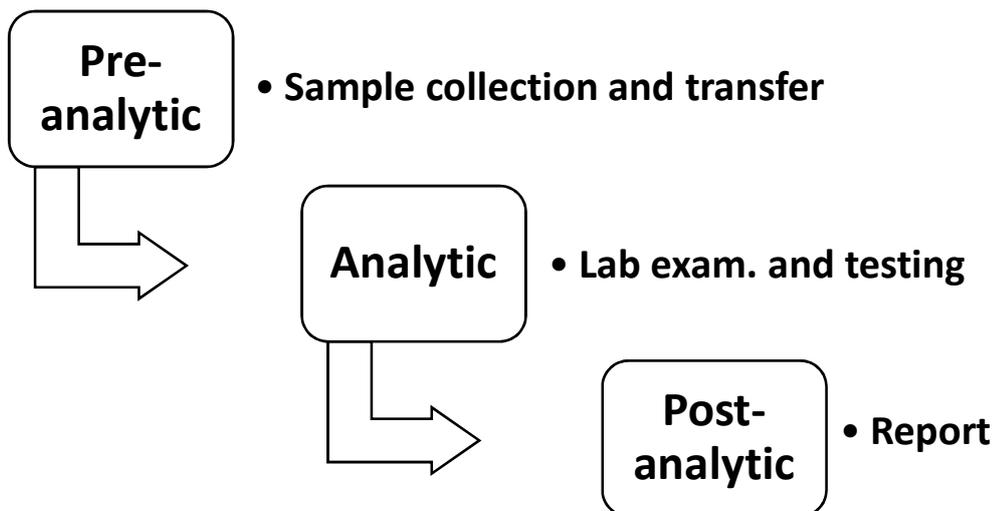
# Pitfalls in Clinical Microbiology

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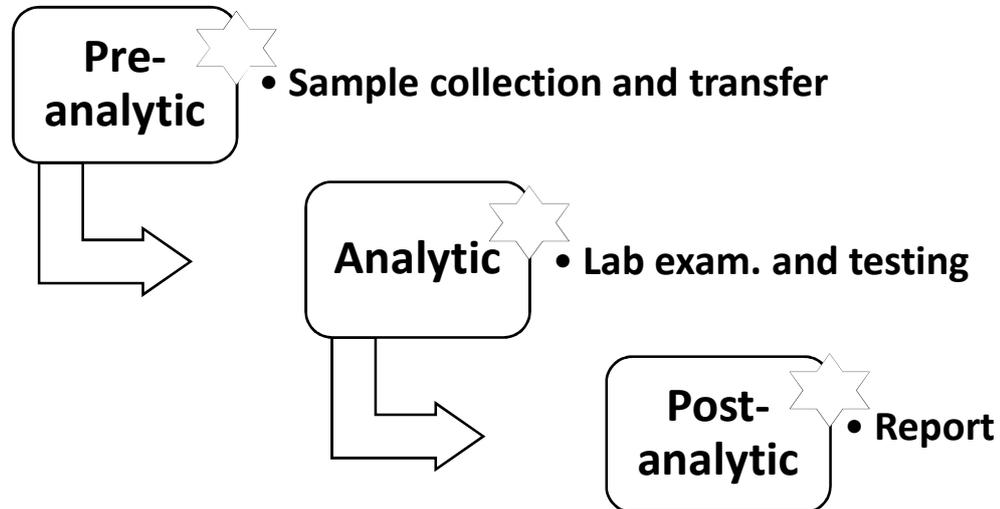
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## Clinical Microbiology



## Pitfalls are everywhere...



### Case 1 FAQs from residency trainees

- A 68-year-old man presented with fever, headache and altered mental status for 3 days
- PE: Drowsy, stiffness of neck- positive
- CT brain: Diffuse leptomenigeal enhancement
- CSF exam: Opening pressure 25 cmH<sub>2</sub>O  
WBC 350 cells/mm<sup>3</sup> (PMN 85%, L 15%),  
glucose 24 , protein 280 mg/dL



## **Case 1      Microbiological examination**

**CSF Gram stain:** Numerous PMN, no organisms

**CSF culture:** No growth

**CSF latex agglutination:** Negative

**Blood cultures:** Gram-positive cocci in pairs and short chains

**Final blood C/S: “ *Streptococcus pneumoniae* ”**

**Why was CSF sample not able to isolate *S. pneumoniae*?**

## CSF examination for diagnosis bacterial meningitis

### Common pathogens

*S. pneumoniae*

*S. agalactiae*

Gram-negative bacilli

*H. influenzae*

*S. suis*

*N. meningitidis*

*L. monocytogenes*

### CSF collection and transportation are critical steps!

Inappropriate collection and transfer cause some bacteria unable to grow

*S. pneumoniae*

*H. influenzae*

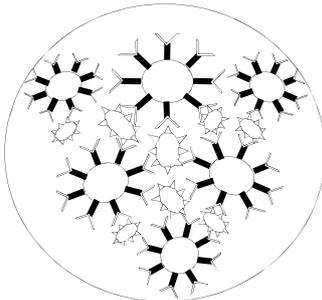
*N. meningitidis*

## Common pitfalls in CSF examination and diagnosis

- Delayed specimen transfer and plating
- Refrigerate CSF sample before bacterial culture
- Unintentionally discard the left-over sample
- Specimen contamination

**Why was latex agglutination testing not able to detect *S. pneumoniae* antigen from CSF?**

## CSF for latex agglutination testing

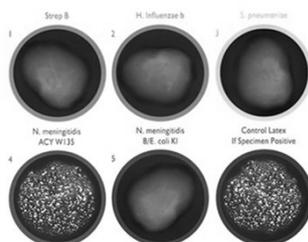


### Antigen-based testing: Latex agglutination

- Rapid test
- Detect capsular surface Ag
- High specificity
- Use for cases with prior antibiotic Rx

### Limitations

- Poor sensitivity in low amount of Ag
- False negativity in acapsular strain



## CSF exam for diagnosis bacterial meningitis

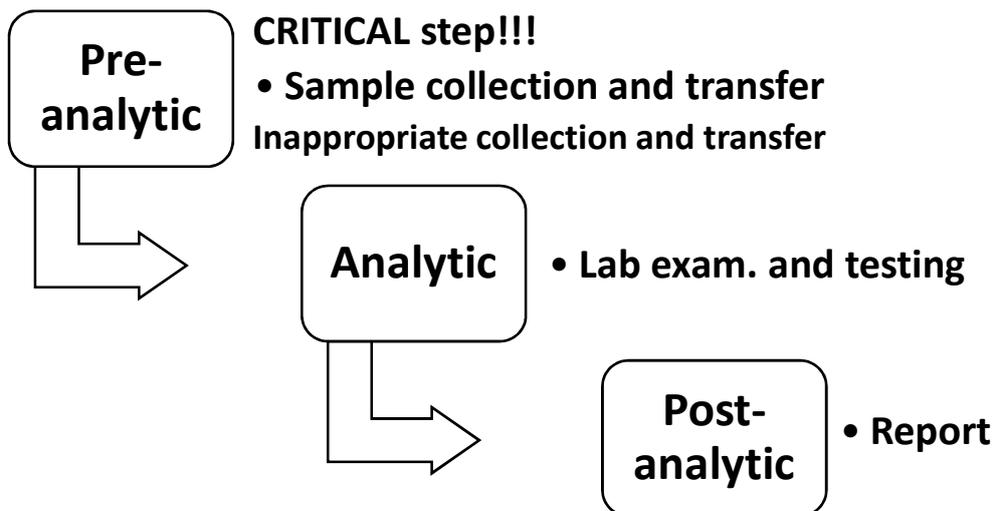
### Recommendation

- Sterile container : bottle No. 2 or 3 for microbiology laboratory
- Immediately send to Laboratory (< 15 min.)
- Store at room temperature (not in 4°C), < 24 h.

### The left-over sample can be refrigerated and used for molecular identification

- $\leq 4\text{ }^{\circ}\text{C}$  ( $\leq 1$  week)
- $-20\text{ }^{\circ}\text{C}$  ( $\leq 1$  month)

## Summary: Pitfall in pre-analytic step



## Case 2, frequent & everyday practices

- A 75-year-old man presented with fever, dyspnea & increased airway secretion
- Old CVA status bed-ridden, on NG tube feeding, and long term Foley catheter
- Physical exam and CXR are suggestive of aspiration pneumonia
- Sputum culture grew mixed bacteria
- Urine culture from Foley catheter was also sent due to cloudy urine seen
- Rx: IV amoxi-clavulanate was started



## Case 2 Urine culture from Foley catheter

### Urine culture (Foley catheter)

1. *Enterobacter cloacae*  $\geq 10^4$ - $10^5$  CFU/mL
2. *Enterococcus faecalis*  $\geq 10^4$ - $10^5$  CFU/mL
3. Yeasts  $\geq 10^3$ - $10^4$  CFU/mL

	Isolate 1	Isolate 2		Isolate 1	Isolate 2
Ampicillin	R	R	Meropenem	S	-
Amoxi-clav	R	-	Gentamicin	R	S
Ceftriaxone	R	-	Amikacin	S	-
Ceftazidime	R	-	Ciprofloxacin	R	R
Cefepime	S	-	Doxycycline	R	R
Pip-tazobactam	S	-	SMX-TMP	R	-
Ertapenem	S	-	Vancomycin	-	S
Imipenem	S	-	Nitrofurantoin	-	S

## Regarding urine culture report...

- |  |   |
|--|---|
| <b>Urine culture</b><br>(Foley catheter) | <ul style="list-style-type: none"><li>▪ <i>Enterobacter cloacae</i> <math>\geq 10^4</math>-<math>10^5</math> CFU/mL</li><li>▪ <i>Enterococcus faecalis</i> <math>\geq 10^4</math>-<math>10^5</math> CFU/mL</li><li>▪ Yeasts <math>\geq 10^3</math>-<math>10^4</math> CFU/mL</li></ul> |
|--|---|

## Were those isolates really pathogens or colonizations?

## What is your management?

- |  |   |
|--|---|
| <b>Urine culture</b><br>(Foley catheter) | <ul style="list-style-type: none"><li>▪ <i>Enterobacter cloacae</i> <math>\geq 10^4</math>-<math>10^5</math> CFU/mL</li><li>▪ <i>Enterococcus faecalis</i> <math>\geq 10^4</math>-<math>10^5</math> CFU/mL</li><li>▪ Yeasts <math>\geq 10^3</math>-<math>10^4</math> CFU/mL</li></ul> |
|--|---|

1. Add vancomycin
2. Switch to pip-tazobactam and vancomycin
3. Switch to pip-tazobactam, vancomycin, and fluconazole
4. Do nothing, ask for whom sent this urine sample
5. Consult Infection Prevention and Control team

## Patients with urinary catheterization

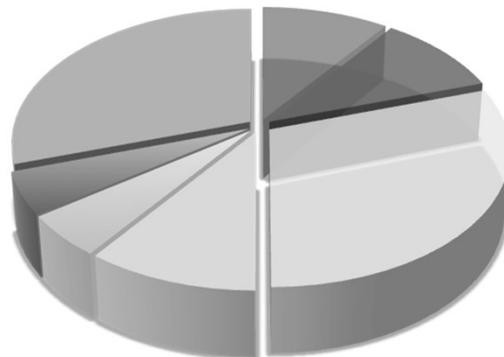


- Bacteriuria rate is based on duration of catheter placement
- The rate will be increasing 3-8% per catheter day
- In  $\geq 30$  catheter days, the rate is almost 100%

Hooton TM, et al. *Clinical Infectious Diseases* 2010;50:625-63.

## Epidemiology of bacteriuria in long term urinary catheterization

- E. coli*
- K. pneumoniae*
- Other *Enterobacteriaceae*
- P. aeruginosa*
- Enterococci
- Yeasts
- Polymicrobial



*Int J Antimicrob Agents* 2008; 31(Suppl 1):S68-78., *Clinical Infectious Diseases* 2010; 50:625-63.

## Fever in patients with long term urinary catheterization

### Difficulties

- Infectious vs. Non-infectious causes
- Catheter associated UTI (CA-UTI) vs. Other sites of infection
- Diagnosis of CA-UTI

## Catheter associated UTI (CA-UTI)

### Diagnosis

#### Symptoms and signs

“ Non-specific ”

Fever, malaise, altered mental status, sepsis syndrome

Local urinary symptoms & signs: infrequent

#### Urine exam

Color, cloudiness, nitrite  
Number of WBC counts } **Poor specificity**

**\* Exclude other causes of infection \***

## Catheter associated UTI (CA-UTI)

### Diagnostic criteria

#### “ Urine quantitative culture ”

Patients who are retained catheter for  $\geq 1-2$  weeks,  
urine C/S should be taken from a newly replaced catheter

Clinical manifestation	Quantitative culture (CFU/mL)
Catheter-associated UTI (CA-UTI)	$\geq 1-2$ microorganism(s), $\geq 10^3$ CFU/mL + symptoms and signs
Catheter-associated asymptomatic bacteriuria (CA-ASB)	$\geq 1-2$ microorganism(s), $\geq 10^5$ CFU/mL <u>without</u> symptoms and signs

## Catheter associated UTI (CA-UTI)

Clinical manifestation	Quantitative culture (CFU/mL)
Catheter-associated UTI (CA-UTI)	$\geq 1-2$ microorganism(s) <sup>@</sup> , $\geq 10^3$ CFU/mL* + symptoms and signs
Catheter-associated asymptomatic bacteriuria (CA-ASB)	$\geq 1-2$ microorganism(s), $\geq 10^5$ CFU/mL** <u>without</u> symptoms and signs

<sup>@</sup> ถ้าพบแบคทีเรีย > 2 ชนิดขึ้นไปมักเกิดจากการปนเปื้อน การตรวจต่ออาจไม่มีความคุ้มค่า

\* ค่า  $\geq 10^3$  CFU/mL เป็นค่าที่เหมาะสมที่สุดทั้งความไวของการวินิจฉัยและการนับจำนวนโคโลนี บางคำแนะนำอาจใช้ค่า  $\geq 10^5$  CFU/mL

\*\* ค่า  $\geq 10^5$  เป็นค่าที่มีความจำเพาะสูงและลดการใช้ยาต้านจุลชีพอย่างไม่เหมาะสม

Hooton TM, et al. *Clinical Infectious Diseases* 2010;50:625-63., Raz R, et al. *J Urol* 2000; 164:1254-8., Tenke P, et al. *Int J Antimicrob Agents* 2008; 31(Suppl 1):S68-78.

## **Recommendation for clinicians**

### **Patients with long term urinary catheterization**

- Color and turbidity of urine are not suggestive of CA-UTI
- Should know
  - When catheterized urine should be or should not be sent
  - How to send appropriately
  - How to interpret CA-UTI vs. Colonization

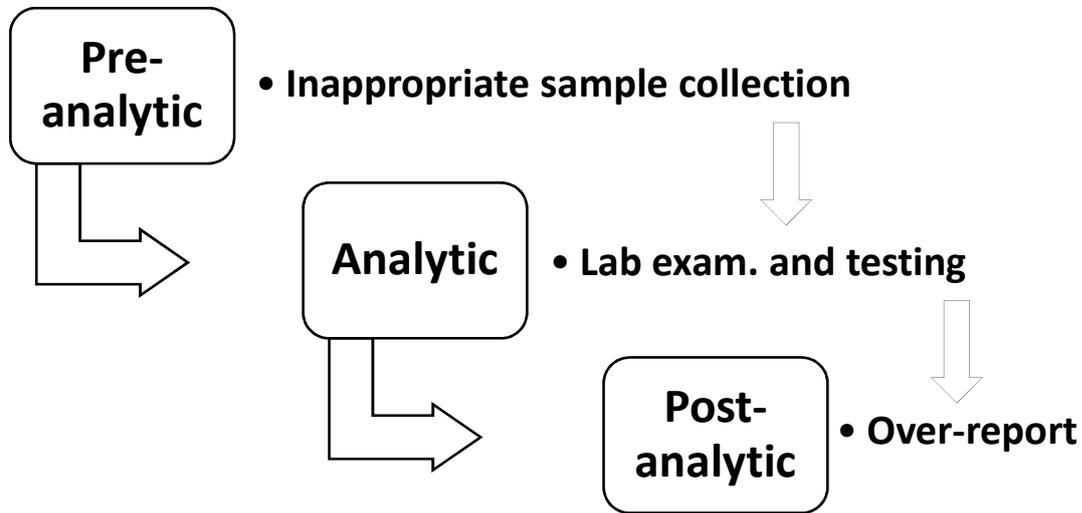
\*Nurses should specify source or where the urine sample is taken from\*

## **Recommendation for Micro. Lab personnel**

### **When multiple type of isolates grew on urine culture**

- Know source of urine sample
- Routine antibiotic susceptibility testing should not be tested or reported
- Discuss with patient care team and IPC team

## Summary: Pitfalls in analytic step



### Case 3 A patient with gram-negative rod bacteremia receiving empirical imipenem Rx

**Blood C/S: *Salmonella* non-Typhi group D**

Agents	Results	Agents	Results
Amoxicillin-clavulanate	S	Ciprofloxacin	R
Cefazolin	S	Levofloxacin	S
Ceftriaxone	S	Gentamicin	S
Cefepime	S	Amikacin	S
Piperacillin-tazobactam	S	TMP-SMX	S
Imipenem	S	Tigecycline	S
Meropenem	S		

AST by automated system

**If you'd like to step down imipenem to a narrower spectrum agent.**

**“Which one(s) is/are safe for the patient?”**

## Common pitfalls in *Salmonella* AST report

### Micro. Lab



- Do not follow standard guideline
- Report every agents from the panel
- No pre-authorization

### Clinician



- Choose the antibiotic based on “S” only (feeling >> evidence-based data)

## CLSI recommendation for *Salmonella* AST

### Intestinal source

AST only for ampicillin, FQs and SMX/TMP

### Extra-intestinal source

AST as above + 3<sup>rd</sup> cephalosporins +/- chloramphenicol

For FQ AST: Use specific breakpoint for *Salmonella*, different from other *Enterobacteriaceae*

AST for AMGs, 1<sup>st</sup> and 2<sup>nd</sup> cephalosporins may be active *in vitro*, but show Rx failure

→ **these agents should not be tested or reported**

### Case 3 A patient with *Salmonella* non-Typhi bacteremia

#### Corrected AST report

Agents	Results	Agents	Results
Ampicillin	R	Ciprofloxacin	R
Ceftriaxone	S	SMX/TMP	S

### Case 4 A patient with CKD stage 3 had gram-negative rod bacteremia

Blood C/S: *K. pneumoniae*, carbapenem-resistant

Agents	Results	Agents	Results
Ampicillin	R	Imipenem	R
Amoxi-clavulanate	R	Meropenem	R
Cefazolin	R	Ciprofloxacin	R
Cefoxitin	R	Levofloxacin	R
Ceftriaxone	R	Gentamicin	I
Ceftazidime	R	Amikacin	S
Cefepime	R	Doxycycline	R
Pip-tazobactam	R	TMP-SMX	R
Ertapenem	R		

## Common pitfalls in CRE antibiotic susceptibility testing

### Clinician



Clinician'd like to know AST results of the last resort antibiotics

- Colistin
- Tigecycline
- Fosfomycin

Those agents should be reported as “ S ” or “ R ”

## Common pitfalls in CRE antibiotic susceptibility testing

### Micro. Lab



- Use non-standardized AST methods for the last resort agents e.g. performing disk diffusion or E-test
- Use mixed CLSI and non-CLSI breakpoint criteria
- **Lack of communication between patient care team and microbiology lab personnel**

## Antibiotic susceptibility testing for *Enterobacteriaceae*

Agents	CLSI 2018	EUCAST 2018	Remarks
<b>Colistin</b>	No clinical breakpoint	Breakpoint available	MIC testing by broth microdilution only
<b>Tigecycline</b>	No clinical breakpoint	Breakpoint available (For complicated- SSTI or IAI only)	MIC testing by broth microdilution
<b>Fosfomycin</b>	Available for <i>E. coli</i> urine isolate only	Available for <i>E. coli</i> and other <i>Enterobacteriaceae</i>	Disk diffusion or MIC by agar dilution Contain glucose-6-phosphate

## Recommendation

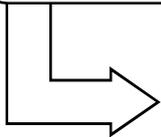
### Carbapenem-resistant *Enterobacteriaceae*

- AST for colistin, tigecycline and fosfomycin
  - Prefer MIC method, but no CLSI breakpoint
  - Non-standardized AST should not be performed or reported
- Discuss with patient care team
- Therapeutic options are based on case series & non-RCT studies

## Summary: Pitfall in post-analytic step

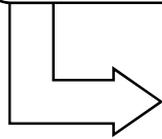
**Pre-analytic**

- Sample collection and transfer



**Analytic**

- Lab exam. and testing



**Post-analytic**

**CRITICAL step!!!**

- Report
- Mis-or over-report  
→ Mislead clinician

