

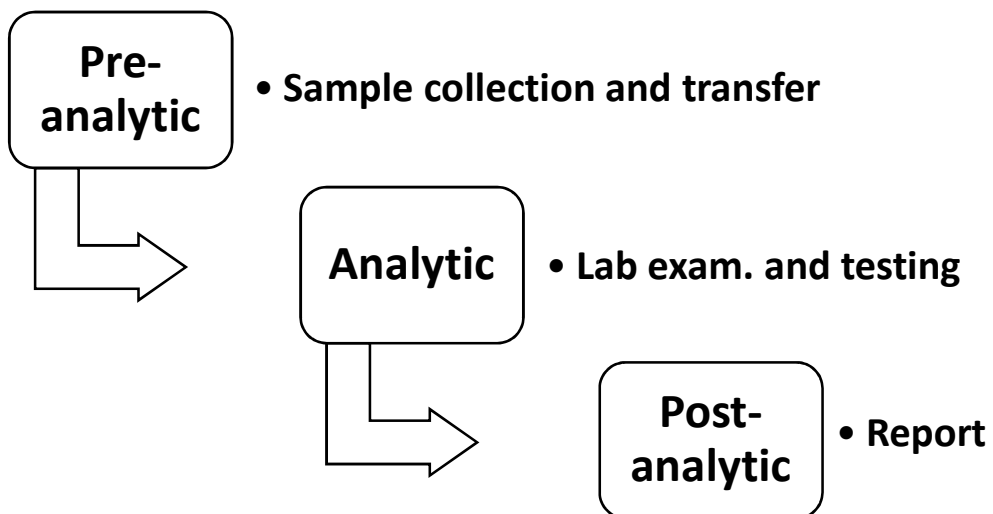


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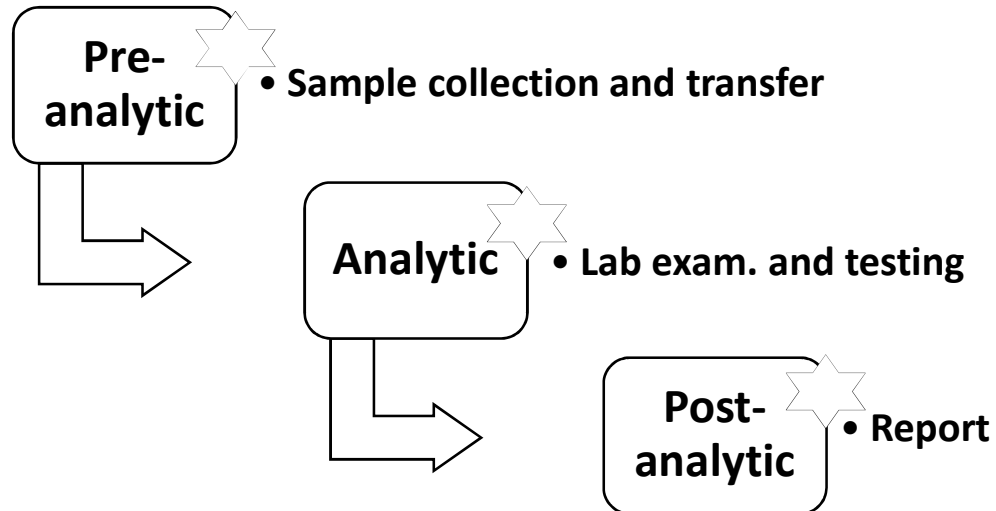
Division of Infectious Disease and Tropical Medicine

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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 cmH₂O
WBC 350 cells/mm³ (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

| | | |
|------------------------|-------------------------|-----------------------|
| <i>S. pneumoniae</i> | <i>S. agalactiae</i> | Gram-negative bacilli |
| <i>H. influenzae</i> | <i>S. suis</i> | |
| <i>N. meningitidis</i> | <i>L. monocytogenes</i> | |

CSF collection and transportation are critical steps!

Inappropriate collection and transfer cause some bacteria unable to grow

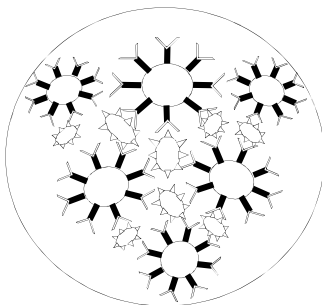
S. pneumoniae
H. influenzae
N. meningitidis

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

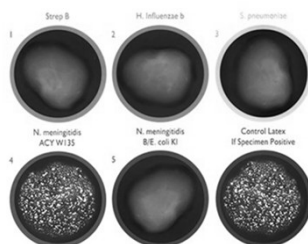


Antigen-based testing: Latex agglutination

- 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

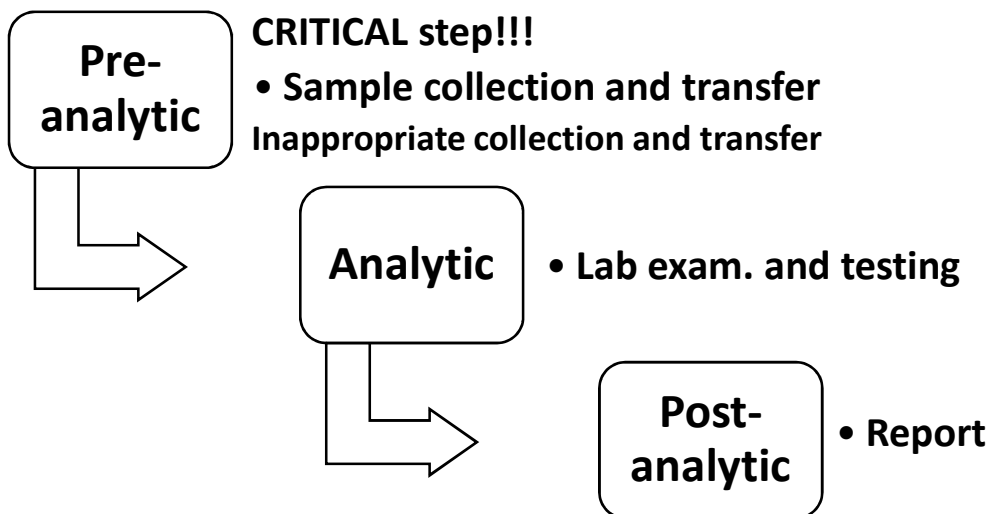
Recommendation

- 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

≤ 4 °C (≤ 1 week)
-20 °C (≤ 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...

Urine culture (Foley catheter)

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

Were those isolates really pathogens or colonizations?

What is your management?

Urine culture (Foley catheter)

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

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

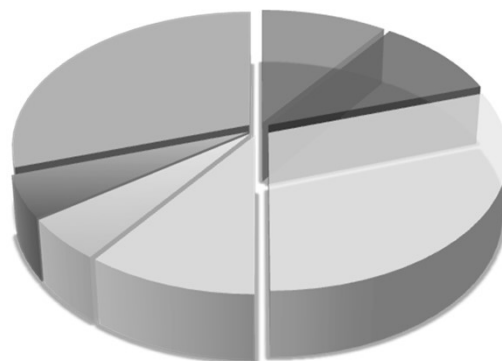


- Bacteriuria rate is based on duration of catheter placement
- The rate will be increasing 3-8% per catheter day
- In ≥ 30 catheter days, the rate is almost 100%

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) [@] , $\geq 10^3$ 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 |

[@] ถ้าพบแบคทีเรีย > 2 ชนิดขึ้นไปมักเกิดจากการปนเปื้อน การตรวจต่ออาจไม่มีความคุ้มค่า

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

** ค่า $\geq 10^5$ เป็นค่าที่มีความจำเพาะสูงและลดการให้ยาต้านจุลชีพอย่างไม่เหมาะสม

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

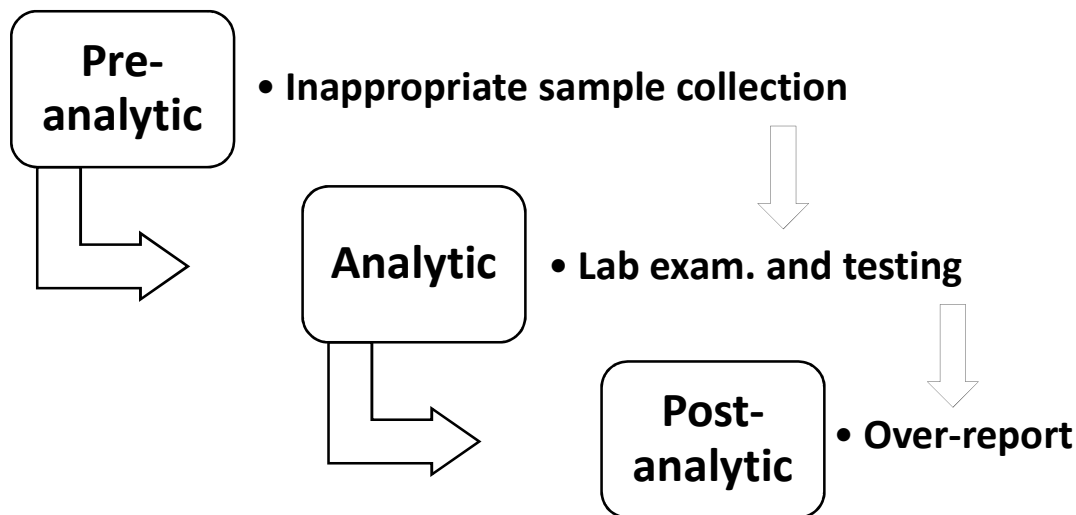
Nurses should specify source or where the urine sample is taken from

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 rd cephalosporins +/- chloramphenicol |

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

AST for AMGs, 1st and 2nd 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 |
|--------------------|---|--|---|
| Colistin | No clinical breakpoint | Breakpoint available | MIC testing by broth microdilution only |
| Tigecycline | No clinical breakpoint | Breakpoint available (For complicated- SSTI or IAI only) | MIC testing by broth microdilution |
| Fosfomycin | 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

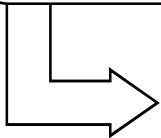
Carbapenem-resistant *Enterobacteriaceae*

- 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

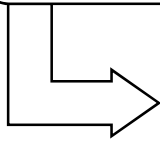
Pre-analytic

- Sample collection and transfer



Analytic

- Lab exam. and testing



Post-analytic

CRITICAL step!!!

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

