Microbiology Overview for IP&C

CHRISTINE TURENNE, PHD, FCCM, ABMM

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Overview

- Review of laboratory testing methods
- Antimicrobial testing, interpretation
- “Walk through the lab”
- Key IP&C bugs

Clinical microbiology testing in Manitoba

Shared Health – Diagnostic Services

- Health Sciences Center (HSC)
- St-Boniface Hospital (SBH)
- Westman Lab (WL in Brandon)
- Thompson; The Pas

Cadham Provincial Laboratory (CPL)

National Microbiology Laboratory (NML)

Dynacare (Private)

Who works in these labs?

- Medical microbiologists (MDs: Fellows of the Royal College), Clinical microbiologists (CCM or ABMM board certified PhDs); PhDs
- Medical Laboratory Technologists (CSMLS certified MLTs, ~ 2 yr program minimum); BSc or other college diploma
- Medical Laboratory Assistants (MLAs)
- Admin/Support staff

Microbiological Testing in a nutshell

Preanalytical → Specimen collection and transport

Analytical
- Specimen processing
- Microscopy
- Antigen detection
- Antibody detection (serology)
- Culture, identification
- Susceptibility testing
- Molecular detection (e.g. PCR)

Postanalytical →
- Results reporting and interpretation
- Patient management/treatment

Laboratory testing methods

Microscopy: The Gram Stain

Purpose: phenotypic characterization of bacteria by brightfield microscopy; quickly provides a hint as to if/what organism may be causing infection

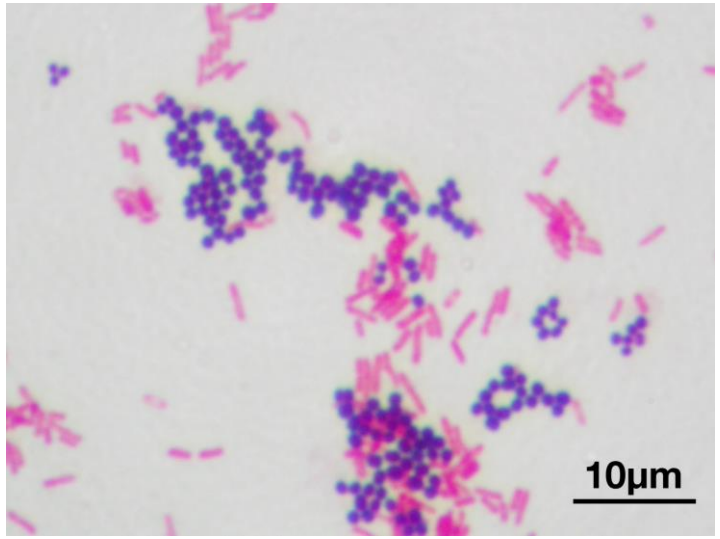
Mechanism: based on thickness of the peptidoglycan layer in the bacteria cell wall

Steps:

- Step 1: Crystal Violet (primary stain)
- Step 2: Iodine (mordant; forms complex with CV)
- Step 2: alcohol and/or acetone (rapid decolorization)
- Step 3: basic fuchsin or safranin (pink counter stain)

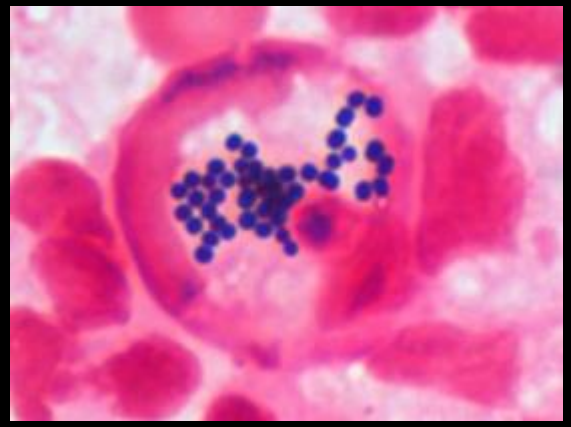
Results:

- **Gram-positive:** thick peptidoglycan layer (retains CV stain) = blue
- **Gram-negative:** thin (easily decolorized) = pink
- Shape and distribution (cocci, bacilli, clusters, chains, etc)
- Antibiotics can impact both staining and morphology

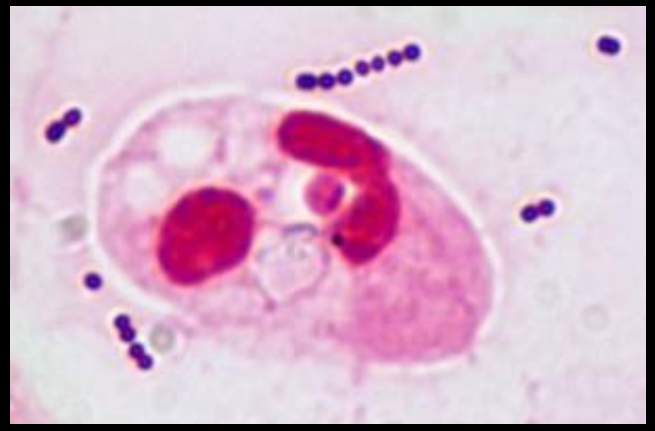


wikipedia

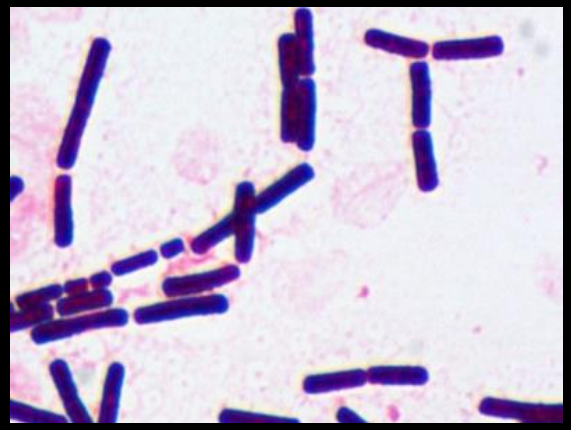
Gram-positive cocci in clumps
(e.g. *Staphylococcus*)



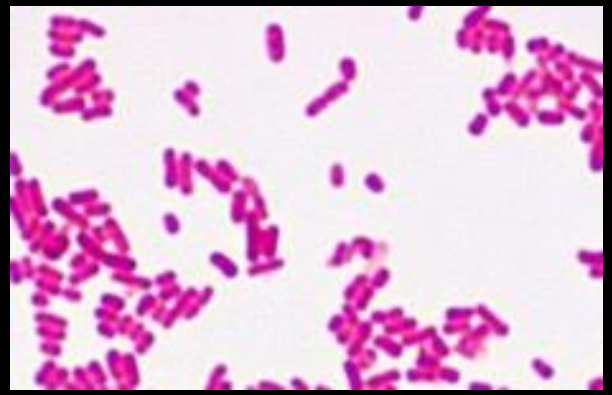
Gram-positive cocci in chains
(e.g. *Streptococcus*)



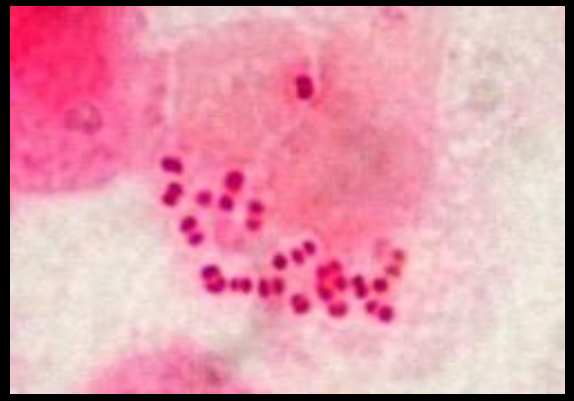
Gram-positive bacilli
(e.g. *Bacillus*)



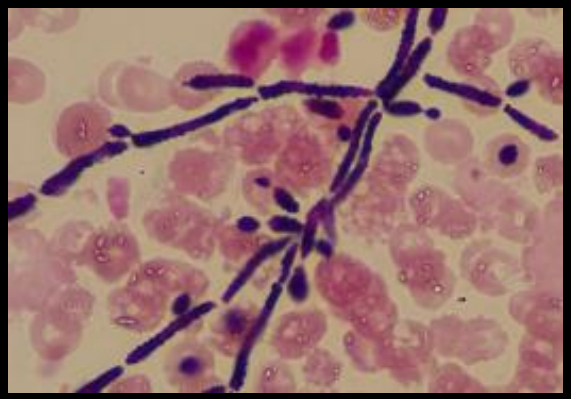
Gram-negative bacilli
(e.g. *E. coli*)



Gram-negative diplococci
(e.g. *Neisseria*)



Yeast-like organisms
(e.g. *Candida*)



Images from microbe-canvas.com & SBH micro lab

Examples of other stains

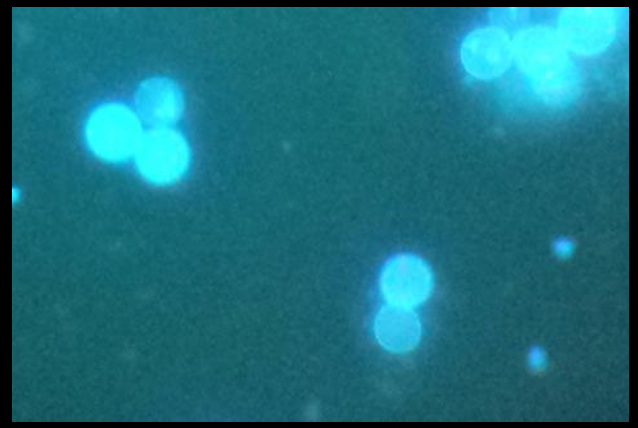
Fungal stains:

- **Calcofluor White** (fluorescent): non-specific, but binds to chitin and cellulose found in fungal cells; direct from specimen
- **Lactophenol aniline blue** (brightfield): to determine fungal morphology from culture

Acid-Fast Bacilli (**AFB stains**): contain dense, waxy cell wall that resist acid-alcohol decolorization

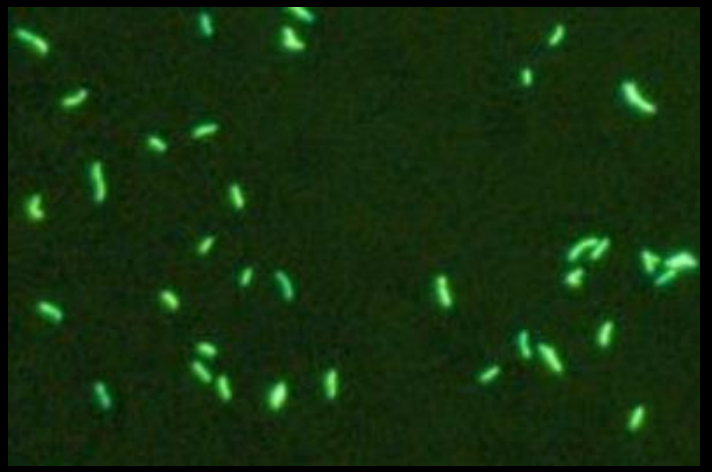
- **Auramine** (fluorescent): non-specific fluorescent stain, binds to mycolic acids of mycobacteria
- **Kinyoun** (brightfield): basic fuchsin as primary stain

Calcofluor white: e.g. "Large broad-based budding yeast-like organisms seen suggestive of *Blastomyces* species"

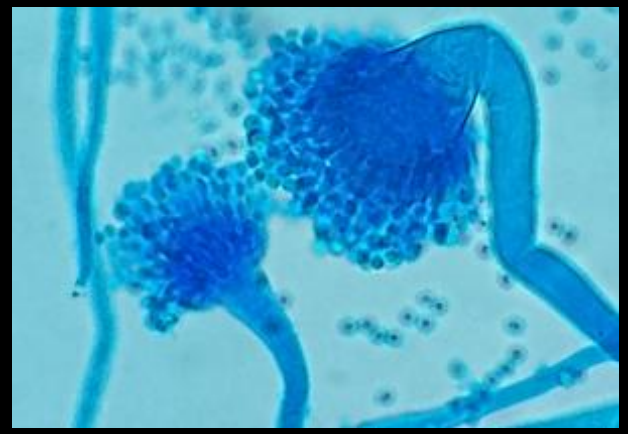


Fluorescent microscopy

Auramine: AFB (e.g. TB)

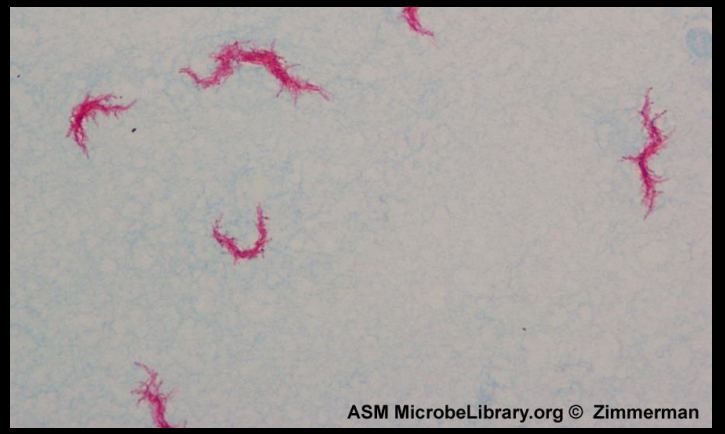


Lactophenol blue: moulds (e.g. *Aspergillus*)



Brightfield microscopy

Kinyoun: AFB (e.g. TB)



Detection by culture or NAAT

Most organisms can be cultured, but it's not always simple

Bacteria: most require nutrients, humidity and heat. Some need special media or atmospheres. Almost all clinically relevant species grow within 24-72 hours.

Fungi: Require nutrients and humidity. Many moulds require 7 – 20 days to grow, but yeast (e.g. *Candida*, *Cryptococcus*) grows quickly on routine media (like bacteria).

Mycobacteria: Rapid-growers (e.g. *M. abscessus*) within 7 days; Slow-growers (e.g. TB) require 2 – 12 weeks to grow, some in special conditions.

Viruses and chlamydia: Diagnosis primarily molecular (e.g. Nucleic acid amplification test or NAAT).

Parasites: Diagnosis primarily is by microscopy, NAAT or serology directly from specimen.

Types of culture media

Nutritive media: supports growth of most non-fastidious organisms

- E.g. Tryptic soy agar, Sabouraud's (fungi)

Enriched media: used to enhance growth of particular organisms

- E.g. blood agar (or BA)
- E.g. chocolate agar (contains lysed blood) for better growth of *H. influenzae* or N. meningitidis
- E.g. buffered charcoal-yeast extract (BCYE) for *Legionella*

Selective media: contains inhibitory agents to select for some bacteria, while inhibiting others

- E.g. phenylethyl alcohol (PEA) for selection of anaerobic Gram-positive organisms
- E.g. Thayer-Martin (also enriched) contains antibiotics for selection of *N. gonorrhoeae*

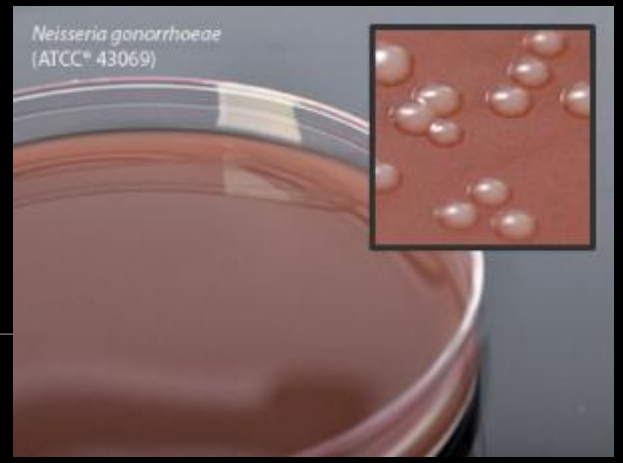
Differential media: contains factors that allow some bacteria to exhibit distinguishing characteristics from other bacteria

- E.g. MacConkey (also selective) which can differentiate lactose-fermenting (LF) Gram-negative bacteria from non-LF.

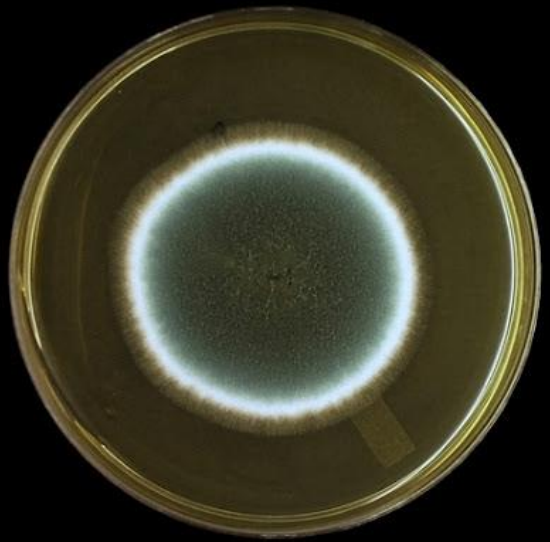
Examples of Culture Plates

E. coli on BA

Streptococci on BA:
can observe hemolysis



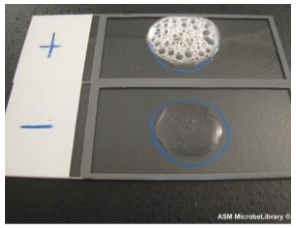
Gonococci on chocolate agar



Aspergillus on SAB

Species identification

- Microscopy & colonial morphology
- Biochemical tests
- Automated systems
- Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF)



MALDI-ToF

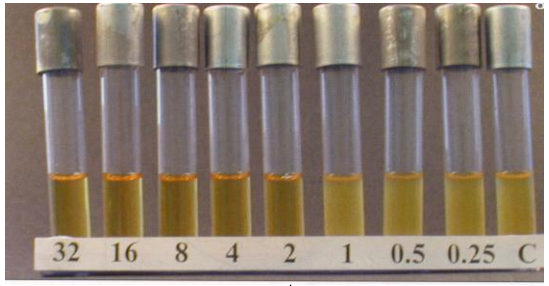
Molecular Detection

- Detection of nucleic acids of pathogens has revolutionized the microbiology laboratory.
- Virtually any organism can be identified using molecular techniques.
- Advantage: very high sensitivity, high specificity, reduced turn around time and irrelevance to viability; can be multiplexed
- Disadvantage: cost, risk of contamination, problems with validation/applicability/relevance, space requirement, expertise. Agent-specific, cannot detect unknown pathogens.
- E.g. SARS-CoV2 PCR, Influenza, RSV, TB, Pertussis

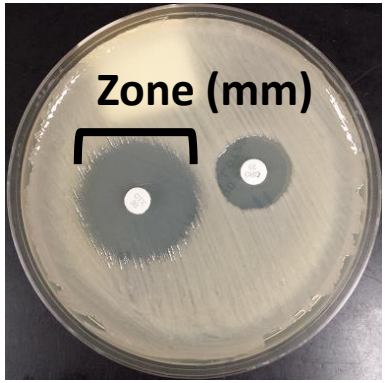
Antimicrobial testing

Antimicrobial susceptibility testing (AST)

- Purpose: to predict the likely outcome of treatment with the antimicrobial agent tested, for a specific organism.
- Results are derived from established breakpoints, which are **minimal inhibitory concentration (MIC)** or **zone diameter values** used to categorize an organism as **S, I, R**
- CLSI* Interpretive categories:
  - **Susceptible (S):** infection due to the strain may be treated with dosage of agent recommended for that infection.
  - **Intermediate (I):** isolates with MICs usually attainable in blood/tissue but response may be lower. Clinical applicability where drugs are concentrated or when high dose can be given. Also includes buffer zone for inherent variability in test methods.
  - **Resistant (R):** strains not inhibited by usually achievable systemic concentrations using normal dosages and where clinical efficacy has not been reliable.



MIC: the lowest concentration of antibiotics that inhibits bacterial growth, i.e. 2 µg/mL

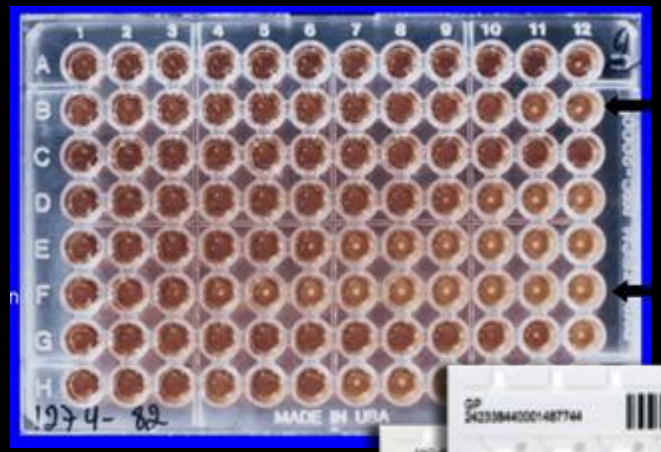


*Clinical and Laboratory Standards Institute

AST methods using in the laboratory:

MIC methods:

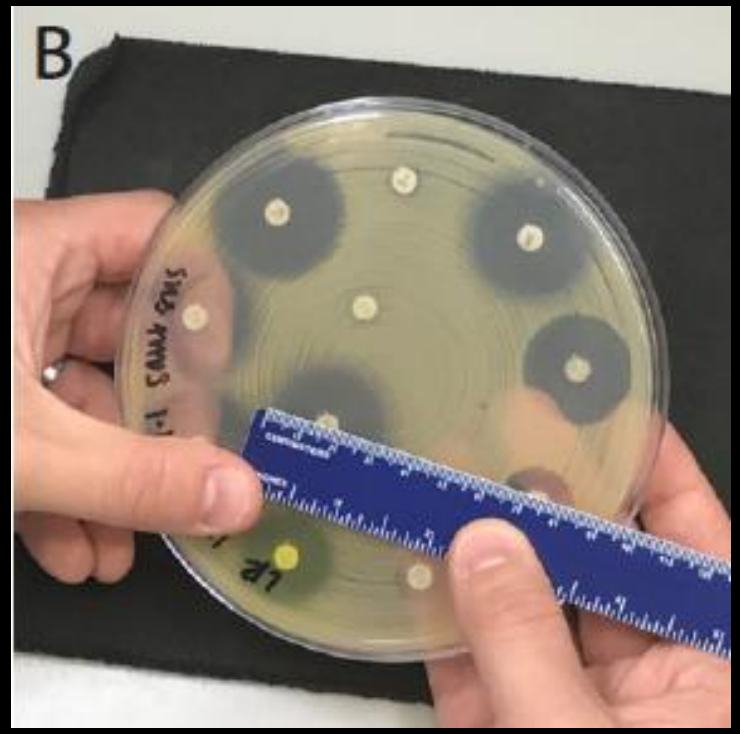
Broth microdilution panel



Gradient diffusion (e.g. Etest)

Automated AST (e.g. VITEK 2™)

Disc diffusion a.k.a. Kirby-Bauer (KB):



(zone diameter measured in mm)

Example of an AST profile

Specimen: Urine

Pathogen: E. coli >10^8 CFU/mL

Reported to the physician

Isolate 1	**MIC**	**Interpretation**	**Breakpoint**
Ampicillin	>16	R	>16
Pip/Tazo	<16	S	>16
Amox/Clav	<8	S	>16
Cefazolin	<4	S	>16
Gentamicin	<2	S	>8
TMP/SMX	<2	S	>2
Nitrofurantoin	<32	S	>64
Ciprofloxacin	<1	S	>2

Walk through the micro laboratory

Microbiology Lab Benches

Specimen Processing

Blood Cultures

Urines

Fluids and Tissues (sterile)

Wounds (non-sterile)

Respiratory

Gastrointestinal/GI (stool)

Genitourinary/GU (genital)

Mycology

Mycobacteriology

Surveillance

- MRSA, CPE, VRE

Molecular

- NAAT, sequencing

QC

Teaching

- i.e. MLT students, clinical microbiology fellows, Infectious Diseases & Medical Microbiology residents

Specimen collection

Technically, not a lab bench (pre-analytical) but considered by many:

THE MOST IMPORTANT PART!

Poor specimen collection can lead to:

- Specimen rejection (e.g. improper/no label, improper/non-sterile container, leaking container, improper test request, etc.)
- Poor results (garbage in, garbage out), leading to misdiagnosis and inappropriate therapy (e.g. swabs, contaminated by normal flora, insufficient volume, prolonged transport, etc)

Consult the Shared Health Lab Information Manual (or CPL Guide to Services) for instructions on specimen collection

Specimen processing

Specimen is received by the laboratory

Specimen is registered into the system

Specimen is set-up for the test requested

- Planting media (manually in a BSC or automated)
- Incubation at the right temperature and atmosphere

Appropriate culture media are selected, inoculated, and incubated under appropriate temperature and atmospheric conditions.

Direct stains are performed (microscopy)

https://www.mountsinai.on.ca/

Blood Cultures

Blood culture set: aerobic and anaerobic (10 mL blood each) x 2 sites (= 4 bottles)

Blood culture bottles are incubated for 5 days in automated system.

Positive cultures:

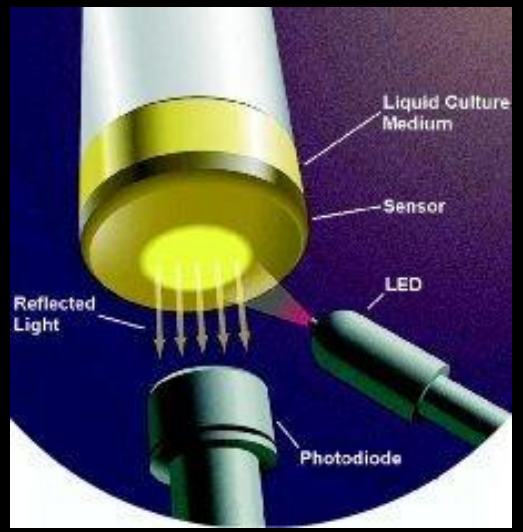
- Gram stain – result called immediately (critical result)
- Appropriate media is inoculated to isolate the organism.
- MALDI-ToF is performed on a sweep from 4hrs of bacterial growth for faster reporting of species identification (if Gram shows a pure culture).

All organisms are reported; AST performed if not a potential contaminant.

Potential contaminants (if detected in only 1 of 2 sites):

- *Bacillus* (and other aerobic large Gram-positive bacilli), *Corynebacterium*, *Cutibacterium*, Coagulase-negative Staph, “viridans group” streptococci, *Micrococcus* spp., etc.

Blood Cultures



Urine

Specimen processed only if clinically justified (as indicated on the requisition)

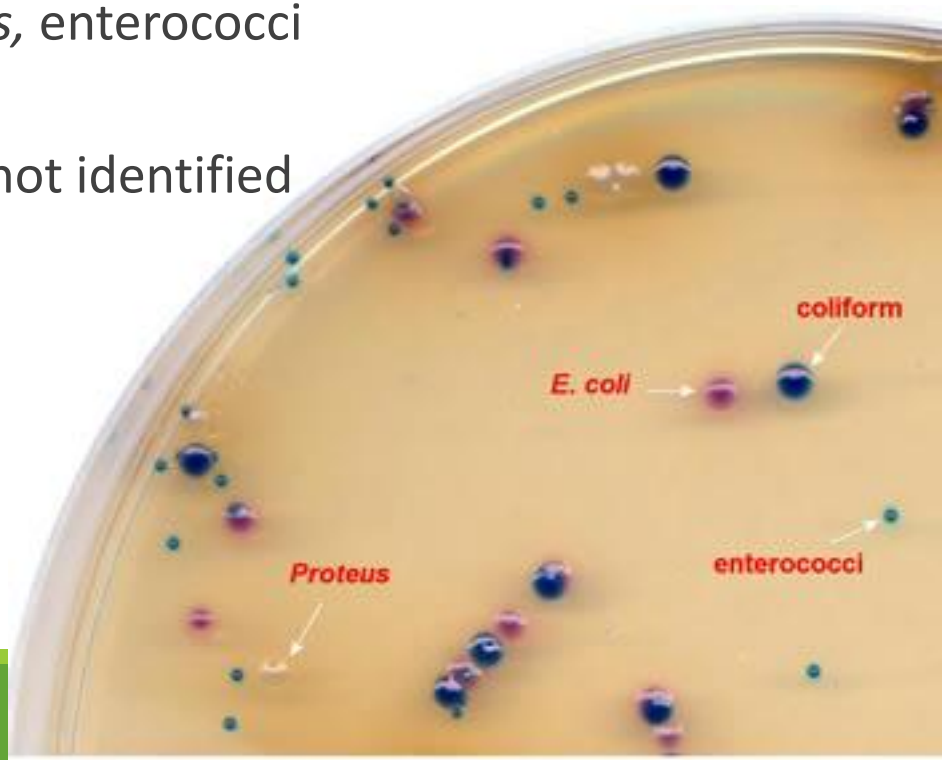
Only typical uropathogens will be identified, e.g.

- *Enterobacterales*, *P. aeruginosa*, GBS, *S. aureus*, *S. saprophyticus,* enterococci
- Workup of atypical organisms requires lab consult
- Mixed/contaminated urine, non-pathogens (skin/vaginal flora) not identified

New chromogenic media reduces TAT

All urines will have a quantitative culture done

- Report will give pathogen and quantity per litre
  - Eg. >1x10E8/L of *E. coli*



Sterile fluids / Tissues

CSF, aseptically obtained tissues, biopsies, fluids, bone, aspirates, prosthetic devices

Gram stain – result reported ASAP

~ All organisms that grow on routine media are reported

If an unusual organism is anticipated or suspected, extra testing and/or precautions may be required and must be requested

- e.g. slow growing organism, AFB, fungus, anaerobes

~Most organisms will get AST if truly sterile specimen.

Extent of work-up may depend on likelihood of contamination

Wound Cultures

Skin/subcutaneous tissue swabs, ulcer swabs, fluids/tissue from non-sterile sites, iv tips, bile

The lab will identify all routine pathogens, with AST if appropriate

- E.g. B-hemolytic streptococci, *S. aureus*, *P. aeruginosa*

Other organisms identified (+/- AST) if

- Pure culture
- Mixed but predominant

Anaerobic work-up only from sites where anaerobes are not normal flora (e.g. deep wounds)

Respiratory Specimens - Bacterial

Lab screens specimen with gram stain to determine acceptability for culture (specimen quality is important) and to give rapid information.

Exceptions: no screening for CF or pediatric patients

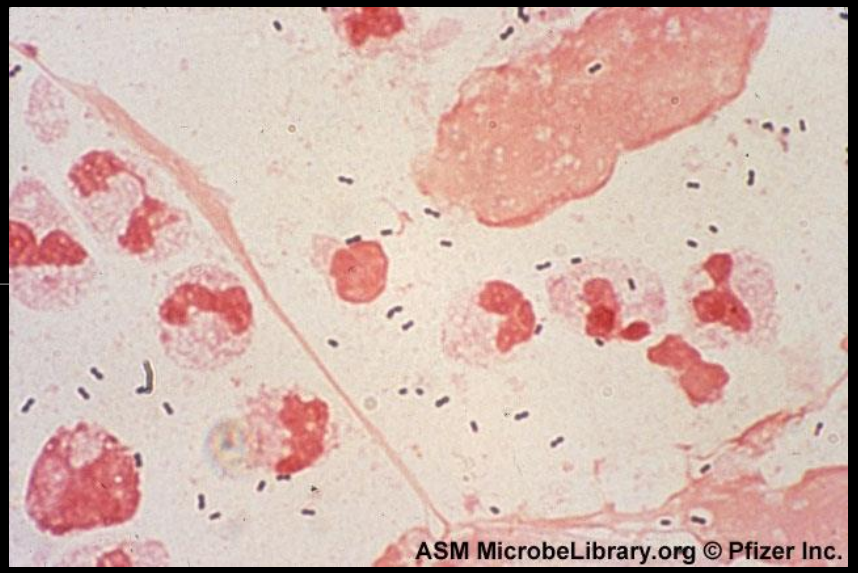
Lab will culture for routine bacterial pathogens only, e.g.:

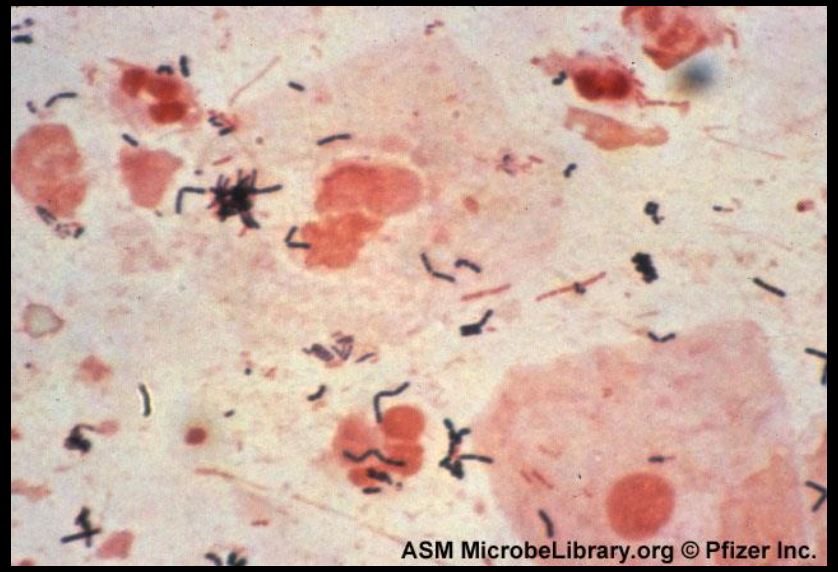
- *S. pneumoniae*
- *H. influenzae*
- *Enterobacterales*, *Pseudomonas*, Gram-negative rods
- *S. aureus*
- GAS

Specific request and specimens needed for TB (or AFB), *Legionella*, *B. pertussis* (PCR), fungi, etc.

Sputum Gram Stains

Culture = *S. pneumoniae:*

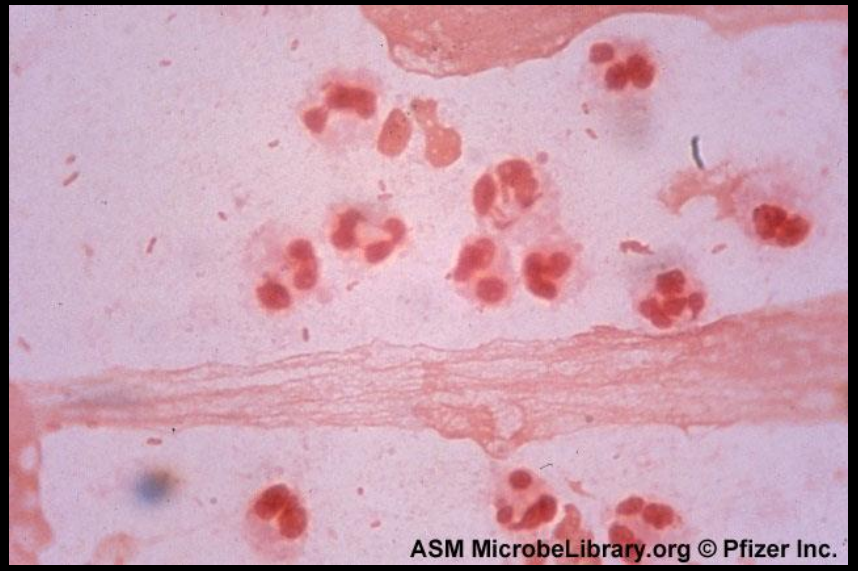




Acceptable for culture: few epi cells

Unacceptable for culture: mixed oral flora, high number of epithelial cells

Culture = *E. coli:*



Respiratory Specimens - Viral

- 3 testing approaches:
  - Single target assay: SARS-CoV2 (COVID-19)
  - Quadplex assay: SARS-CoV2, Flu A, Flu B, RSV
  - Multiplex assay (CPL only): as above, plus rhinovirus, enterovirus, adenovirus, parainfluenza x4, endemic coronaviruses x3, human metapneumovirus, bocavirus
    - Requests are prioritized for ICU patients with ILI, outbreak investigations, transplant patients
- Which testing is performed depends on circulating viruses (seasonality, pandemicity), availability of testing (equipment and staffing), patient management needs, etc.
- **Hospital labs** focus on rapid testing for patient management needs and are limited to Influenza (A and B), RSV and SARS-CoV2.
- **CPL labs** have a slightly longer TAT but have higher throughput capacity and ability to test for an expanded panel of respiratory viruses under specific circumstances.

Stool Culture

Stool culture for Enteric pathogens

- Now done only at CPL by NAAT, followed by culture if positive
- Pathogens sought are: *E. coli* O157:H7, other Shiga-toxin producing strains, *Campylobacter*, *Shigella*, *Salmonella*
- Cultures for *Vibrio cholera*, *Aeromonas spp,* are on request only

C. difficile testing (2 steps):

- **Unformed stool** from patient suspected of having *C. diff* disease
- Step 1 (screen): detection of *C. diff* antigen glutamate dehydrogenase (GD) by rapid antigen test. If positive, must do step 2.
- Step 2: Alethia® (DNA amplification assay) to detect **toxigenic** *C. diff*.

Genital Specimens

Specimens for sexually transmitted infections or STI (GC and chlamydia) are generally by molecular assays (at CPL)

Culture of GC (cervical swab in female or urethral swabs in males) can be done; requires special media. Useful for AST.

"Vaginal cultures" are **not** typically done

- Exceptions: GBS in pregnancy, vaginitis/STD in children
- Gram stains provide diagnosis of vaginosis and candidiasis. Wet mounts or antigen for trichomonas

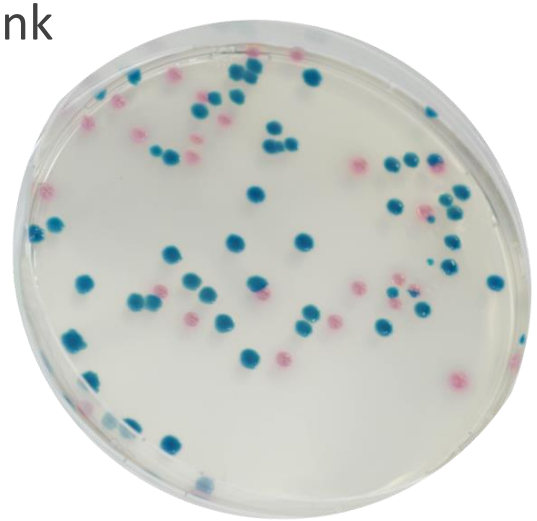
Surveillance Specimens

Chromogenic media that includes antibiotics (selective) and other components for colony differentiation

MRSA *S. aureus* = pink

VRE *E. faecalis* = blue or *E. faecium* = pink

Carbapenemase-producing Enterobacterales (CPE) *Klebsiella* = blue or *E. coli* = pink



Key IP&C Bugs

i.e. organisms reported to IP&C

MRSA

Methicillin-Resistant *S. aureus*

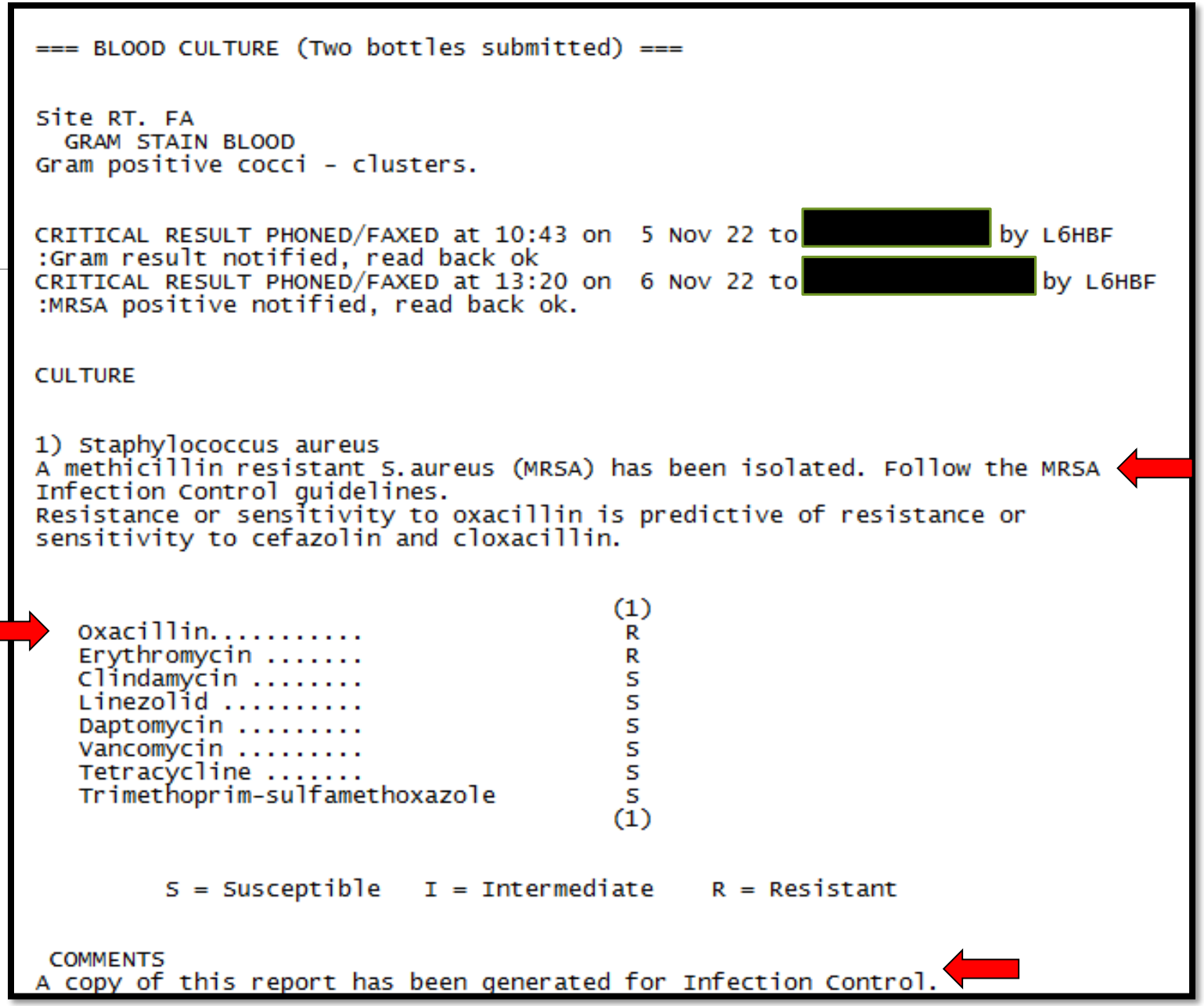
Gram-positive cocci in clusters, catalase+, coagulase+, "methicillin" R, *mecA*+

Similar infection spectrum to MSSA

- MRSA: 1.5 – 2x increased risk of mortality (Cosgrove 2003)

Resistant to all β-lactams

Acquisition of altered PBP (PBP2a, encoded by ***mecA*** gene)



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=== BLOOD CULTURE (Two bottles submitted) ===


Site RT. FA
  GRAM STAIN BLOOD
Gram positive cocci - clusters.


CRITICAL RESULT PHONED/FAXED at 10:43 on  5 Nov 22 to [redacted] by L6HBF
:Gram result notified, read back ok
CRITICAL RESULT PHONED/FAXED at 13:20 on  6 Nov 22 to [redacted] by L6HBF
:MRSA positive notified, read back ok.


CULTURE


1) Staphylococcus aureus
A methicillin resistant S.aureus (MRSA) has been isolated. Follow the MRSA
Infection Control guidelines.
Resistance or sensitivity to oxacillin is predictive of resistance or
sensitivity to cefazolin and cloxacillin.


                                          (1)
  Oxacillin.....                           R
  Erythromycin .....                       R
  Clindamycin .....                        S
  Linezolid .....                          S
  Daptomycin .....                         S
  Vancomycin .....                         S
  Tetracycline .....                       S
  Trimethoprim-sulfamethoxazole            S
                                          (1)


        S = Susceptible    I = Intermediate     R = Resistant


 COMMENTS
A copy of this report has been generated for Infection Control.
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VRE

Vancomycin-resistant enterococci

Gram-positive cocci in chains, catalase-, PYR+, BE+

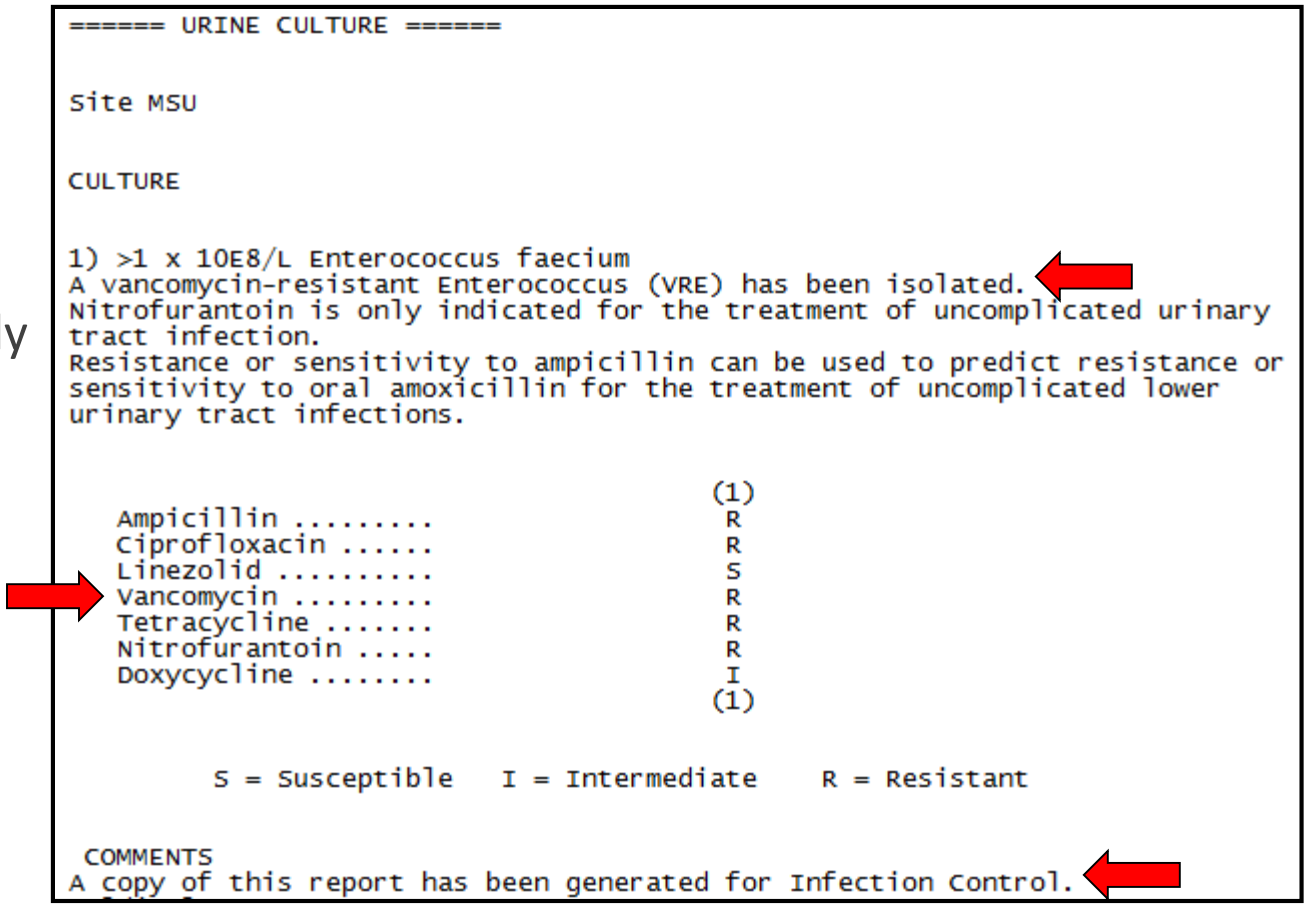
Vancomycin resistance: altered cell wall precursors prohibits vancomycin binding; **plasmid-mediated**, *vanA* or *vanB* genes, typically in *E. faecium* & *E. faecalis*

Mostly colonization, but can cause UTI, wound infections, sepsis

E. faecalis causes 90% of infections, but most VRE are *E. faecium*

E. casseliflavus & *E. gallinarum* (not VRE)

- vanC: low-level R to vanco; chromosomal



====== URINE CULTURE ======

Site MSU

CULTURE

1) >1 x 10E8/L Enterococcus faecium
A vancomycin-resistant Enterococcus (VRE) has been isolated.
Nitrofurantoin is only indicated for the treatment of uncomplicated urinary tract infection.
Resistance or sensitivity to ampicillin can be used to predict resistance or sensitivity to oral amoxicillin for the treatment of uncomplicated lower urinary tract infections.

	(1)
Ampicillin	R
Ciprofloxacin	R
Linezolid	S
Vancomycin	R
Tetracycline	R
Nitrofurantoin	R
Doxycycline	I
	(1)

S = Susceptible I = Intermediate R = Resistant

COMMENTS
A copy of this report has been generated for Infection Control.

CPE, CPO

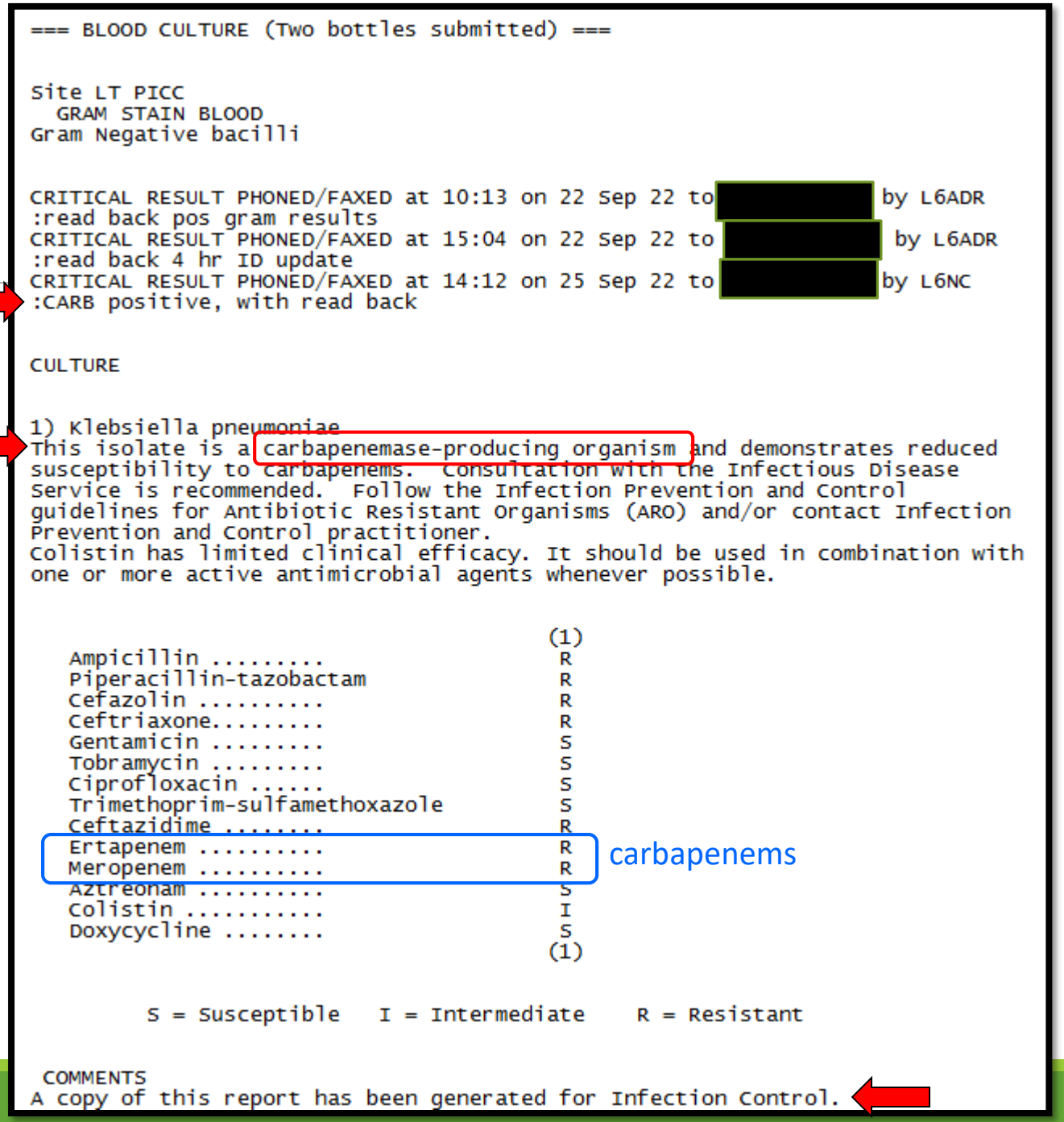
Carbapenemase Producing *Enterobacterales*
Carbapenemase Producing Organisms

***Plasmid-mediated** carbapenemase: e.g. KPC, NDM, VIM, IMP, OXA-48-like

Serious threat, usually resistant to many (or all) first-line antibiotics

Occurs mostly in *Enterobacterales* (CPE), e.g. *E. coli, K. pneumoniae, E. cloacae cplx*

Can also occur in non-*Enterobacterales* (CPO) such as *P. aeruginosa* and *Acinetobacter* spp.



=== BLOOD CULTURE (Two bottles submitted) ===

Site LT PICC
GRAM STAIN BLOOD
Gram Negative bacilli

CRITICAL RESULT PHONED/FAXED at 10:13 on 22 Sep 22 to [redacted] by L6ADR
:read back pos gram results
CRITICAL RESULT PHONED/FAXED at 15:04 on 22 Sep 22 to [redacted] by L6ADR
:read back 4 hr ID update
CRITICAL RESULT PHONED/FAXED at 14:12 on 25 Sep 22 to [redacted] by L6NC
:CARB positive, with read back

CULTURE

1) Klebsiella pneumoniae
This isolate is a carbapenemase-producing organism and demonstrates reduced susceptibility to carbapenems. Consultation with the Infectious Disease Service is recommended. Follow the Infection Prevention and Control guidelines for Antibiotic Resistant Organisms (ARO) and/or contact Infection Prevention and Control practitioner.
Colistin has limited clinical efficacy. It should be used in combination with one or more active antimicrobial agents whenever possible.

	(1)
Ampicillin	R
Piperacillin-tazobactam	R
Cefazolin	R
Ceftriaxone.....	R
Gentamicin	S
Tobramycin	S
Ciprofloxacin	S
Trimethoprim-sulfamethoxazole	S
Ceftazidime	R
Ertapenem	R
Meropenem	R
Aztreonam	S
Colistin	I
Doxycycline	S
	(1)

carbapenems

S = Susceptible I = Intermediate R = Resistant

COMMENTS
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AMR-GNB:

Antimicrobial resistant Gram-negative bacilli

1. >1 x 10E8/L of Klebsiella pneumoniae/variicola
Cephalexin is only indicated for the treatment of uncomplicated urinary tract infection. Nitrofurantoin is only indicated for the treatment of uncomplicated urinary tract infection. This isolate contains an extended spectrum beta lactamase (ESBL). This isolate should be considered clinically resistant to penicillins and aztreonam; susceptibility to specific cephalosporins and beta-lactam/inhibitor combinations should be based upon their in vitro susceptibility testing results. Consultation with the Infectious Disease Service is recommended.

2. >1 x 10E8/L of Escherichia coli
Nitrofurantoin is only indicated for the treatment of uncomplicated urinary tract infection. Cephalexin is only indicated for the treatment of uncomplicated urinary tract infection.

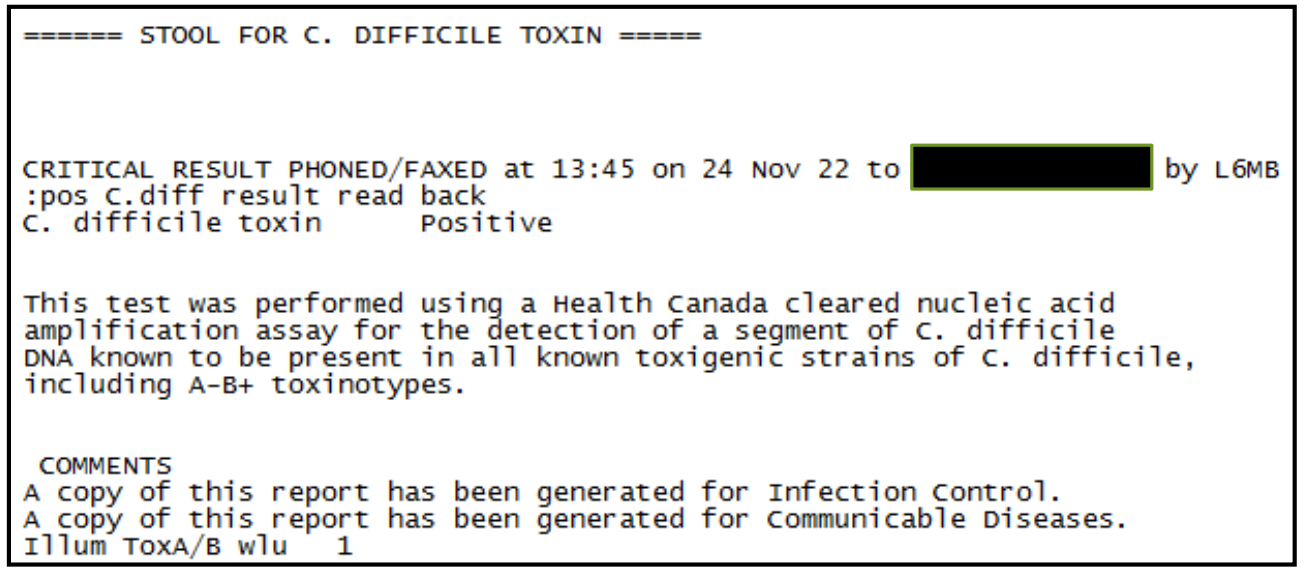
	1	2
Ampicillin	R	I
Amoxicillin-clavulanate	R	S
Piperacillin-tazobactam	R	S
Cefazolin	R	S
Ceftriaxone.....	R	
Ertapenem	S	
Meropenem	S	
Gentamicin	S	S
Ciprofloxacin	R	R
Nitrofurantoin	I	S
Trimethoprim-sulfamethoxazole	R	S
Cephalexin	R	S
Tobramycin	R	S
Ceftazidime	R	

C. difficile

Anaerobic Gram-positive bacilli, spore-former, GDH+, fluoresces under UV, “horse stable” smell

Toxigenic *C. difficile*

- Toxin A and/or toxin B
- Disease spectrum: asymptomatic colonization – mild, self-limiting diarrhea – pseudomembranous colitis - toxic megacolon – sepsis – death
- Can cause outbreaks in health care facilities



====== STOOL FOR C. DIFFICILE TOXIN =====

CRITICAL RESULT PHONED/FAXED at 13:45 on 24 Nov 22 to [redacted] by L6MB
:pos C.diff result read back
C. difficile toxin Positive

This test was performed using a Health Canada cleared nucleic acid amplification assay for the detection of a segment of C. difficile DNA known to be present in all known toxigenic strains of C. difficile, including A-B+ toxinotypes.

COMMENTS
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A copy of this report has been generated for Communicable Diseases.
Illum ToxA/B wlu 1


WHAT
WHERE
WHEN
WHY