Healthcare / Hospital Associated / Nosocomial Infections & Role of Microbiology Laboratory

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1389/08/06
2010.10.28
An infection acquired in hospital by a patient who was admitted for a reason other than that infection.

This includes infections acquired in the hospital but appear after discharge, and also occupational infections among staff of the facility.

An infection occurring in a patient in a hospital or other health care facility/setting in whom the infection was not present or incubating at time of admission.
Health care worker’s ungloved hand was obtained for culture after the worker had performed an abdominal examination of the patient. Positive for MRSA (A)

After the worker’s hand had been cleaned with alcohol foam, another hand imprint was obtained, and the resulting culture was Negative for MRSA (B)
راهنماي آزمایشگاهی تشخیص عفونت‌های بیمارستانی

نویسنده(گان) (به ترتیب جوهره الفیا):

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دکتر بابک ولی‌زاده
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دکتر مهدی ونیک
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ویراستار:

دکتر بابک ولی‌زاده

وزارت بهداشت، درمان و آموزش پزشکی
مرکز مدیریت بیماری‌ها
آزمایشگاه مرکز سلامت
فصل دوم
عقونتهای ادراری
...
فصل سوم
عقونتهای تنفسی
...
فصل چهارم
عقونتهای زخم‌های جراحی
...
فصل پنجم
کشت خون، کانترهاي خونی و فرآورده‌های بانک خون
...
فصل ششم
ارزیابی میکروبی‌شناسی و غونه‌برداری از عیف‌های بیمارستانی و وسایل پزشکی
...
فصل هفتم
غونه‌برداری از هوا
...
فصل هشتم
کشت آب بیمارستان برای جداسازی لژیونلا
...
فصل نهم
کشت و اندازه‌گیری انژنوتکسین مایعات همودیالیز و دیالیز صفا قی
The most common Nosocomial infections:

- **Urinary Tract Infections (UTI)** / 33%
- **Pneumonia** / 15.5%
- **Surgical Site Infection (SSI)** / 15%
- **Bloodstream Infections (BSI)** / 13%
Nosocomial infections are an important source of morbidity and mortality in hospital settings.

A nosocomial infection is one for which there is no evidence that the infection was present or incubating at the time of hospital admission.

Viruses, fungi, and parasites are recognized as sources of nosocomial infections, *bacterial agents remain the most commonly recognized cause of hospital-acquired infections*.
Risk Factors

► Diabetes
► Renal failure
► Malignancies & Chemotherapy
► Corticosteroids
► Long hospitalizations
► Surgical procedures
► Receipt of prior antimicrobial therapy
  • Approximately 25 to 35% of hospitalized patients receive systemic antibiotics
► Presence of indwelling catheters
Nosocomial Infections

Microorganisms are spread in hospitals through several modes

► Direct contact, for example, in contaminated food or intravenous solutions

► Indirect contact, for example, from patient to patient on the hands of health care workers (MRSA)
Nosocomial Infections

- **Droplet contact**, for example, inhalation of droplets (>5 micrometer in diameter) that cannot travel more than 3 feet / 1 meter (Pertussis)

- **Airborne contact**, for example, inhalation of droplets (≤5 micrometer in diameter) that can travel large distances on air currents (Tuberculosis)

- **Vector-borne Contact**
Patients’ normal flora changes very quickly after hospitalization.

The majority of Nosocomial infections are **Endogenous** in origin, involve patient’s own flora.

The majority of Nosocomial infections are not associated with outbreaks, they are **endemic** rather than epidemic.
Nosocomial infections may never be completely eliminated, only controlled.
Within hospitals, the surgical and medical services / devices have the highest rates of infection and pediatric have the lowest.
Epidemiologic investigations of nosocomial infection are typically triggered by

► An increase in the prevalence of infection associated with a particular pathogen species

► A cluster of infected patients

► The identification of an isolate that has a distinctive antimicrobial susceptibility pattern
The laboratory can serve as an early warning system for epidemiologic surveillance.

The Microbiology Laboratory can be the source of Pseudo-outbreaks.

Quality Control problem in the laboratory that often lead to Pseudo-outbreaks.

Pseudo infections e.g. : Burkholderia cepacia

- Contaminated disinfectants & antiseptics (povidone iodine)
Specimen Collection

Accurate Identification

Accurate Antimicrobial Susceptibility Testing

Rapid Diagnostic Testing
  • latex agglutination & Real time PCR (MRSA)

Reporting of Laboratory data

Outbreak Recognition & Investigation

Molecular Typing & Organism Storage

Culture of Specimen from Hospital Personnel & the Environment
  • Only with epidemiologic evidence
Since many nosocomial pathogens are also common colonizing and culture contaminants, (e.g., coagulase negative staphylococci) specimen collection and handling can have an impact on nosocomial infection rates.
Species level Identification of Nosocomial Pathogens may be the first clue to an outbreak, and so laboratory should establish a system for sending unusual Nosocomial Pathogens to a reference laboratory for Identification.
In general, phenotypic methods have lower discriminatory power than genotypic methods.

- **Biotyping (biologic & biochemical)**
  
  - Utility of biotyping in epidemiologic studies is quite limited.
  
- **Biotyping cannot differentiate among strains where biochemical diversity is uncommon, such as the *Enterococci***
PHENOTYPIC METHODS

► Serotyping
  • Salmonella, Legionella, Shigella, Klebsiella, Streptococcus pneumoniae

► Bacteriophage typing
  • S. aureus, P. aeruginosa and Salmonella

► Antimicrobial Susceptibility Testing
Genotypic / Molecular Methods

Molecular tests is essential in many circumstances for establishing disease epidemiology.

Molecular techniques can be very effective in tracking the spread of nosocomial infections due to genetically related pathogens.
This approach has begun to change over the past 2 decades, with the development and implementation of new technologies based on DNA, or molecular analysis.

Genotyping or DNA–based typing methods have largely replaced phenotypic methods.
Establishing clonality of pathogens can aid in the identification of:

- Source of organisms (environmental or personnel)
- Determination of the mode of transmission
- Distinguish infectious from noninfectious strains
- Distinguish relapse from reinfection
Genotypic / Molecular Methods

► **Plasmid Analysis**
  - Plasmid typing was the first molecular method to be used as a bacterial typing tool

► **Pulsed-field gel electrophoresis (PFGE) (1984)**
  - Scanning more than 90% of the chromosome

► **Southern Blot Analysis-Ribotyping**
  - *E.coli*, *S.aureus*

► **PCR-based typing methods**
  - Generally survey relatively limited regions representing less than 10% of the chromosome
Reporting of Laboratory data

► Culture and AST results are important data source for Infection control and usually reviewed daily

► Some culture results warrant an early phone call
  • MRSA
  • ESBL
  • VRE
Culture of Specimen from Hospital Personnel should be performed rarely and only when epidemiologic evidence suggests personnel involvement in the transmission of a Nosocomial pathogen.

- Hand cultures
- Nare culture for MRSA
- Surveillance Cultures for VRE & MRSA using rectal and oropharyngeal swabs
Finding the outbreak strain on hands or in the nares of health care worker does not establish the direction of transmission or definitively implicate the health care worker as reservoir for the outbreak.
Surveillance Cultures

- Culturing cooling towers or hot water sources for Legionella
- Culture of water & dialysis fluids for hemodialysis & endotoxin testing
- All steam & dry-heat sterilizers should be checked at least once each weekly with a liquid spore suspension
Definitions of MDR/XDR/PDR

► **MDR** : Multidrug Resistant Bacteria

► **XDR** : Extensively Drug Resistant Bacteria

► **PDR** : Pan Drug Resistant Bacteria
Multidrug Resistant Organisms (MDRO)

Antibiotic-Resistant Gram-Negative rods (1970s)

- Klebsiella spp.
- Enterobacter spp.
- Citrobacter freundii
- Morganella morganii
- Providencia spp.
- Serratia spp.
- Proteus vulgaris
Major antimicrobial resistance problems are typically associated with *Gram-positive nosocomial pathogens* include:

- **Methicillin-resistant Staphylococcus aureus (MRSA)** / (1980s)

- **Glycopeptide (vancomycin)-resistant enterococci (VRE)** / (1990s)

- **Glycopeptide (vancomycin)-intermediate and -resistant S.aureus (VISA)/1990s & (VRSA)/2002**
Multidrug Resistant Organisms (MDRO) 

Gram-negative bacilli

- Extended-spectrum-beta-lactamase-producing (ESBLs) strains of Escherichia coli and Klebsiella pneumoniae (1983)

- AmpC beta-lactamases / AmpC (1988)

- Klebsiella pneumoniae Carbapenemase / KPC (1996)
MRSA
Don’t open the door to infection.

Any Opening In Your Skin Increases The Risk Of Infection.

Clean Dry and Covered!

www.cdc.gov/mrsa

Things to remember about living with MRSA:
1. Wash your hands often.
2. Take care of yourself: Eat right, exercise, quit smoking, and avoid stress.
3. Take good care of your skin.
4. Keep skin infections covered to avoid spreading MRSA to others.
5. Talk with your health care provider if you have questions or concerns.

Living with MRSA

This is really serious. I need to do something about this now!

Learning how to control the spread of Methicillin-Resistant Staphylococcus Aureus (MRSA)
Methicillin-resistant *Staphylococcus aureus* for Athletes
What YOU Need to Know
A CHILD’S FIRST LINE OF DEFENSE AGAINST MRSA: A WELL-INFORMED MOM.

What are the signs and symptoms of an MRSA skin infection?

MRSA and other staph skin infections appear as a bump or infected area on the skin that may be:
> Red
> Swollen
> Painful
> Warm to the touch
> Full of pus or other drainage
> Accompanied by a fever

If you or someone in your family experiences these signs and symptoms, cover the area with a bandage and contact your healthcare professional. This is especially important if MRSA signs and symptoms are accompanied by a fever.

MRSA is methicillin-resistant Staphylococcus aureus, a potentially dangerous type of staph bacteria that is resistant to certain antibiotics and may cause skin and other infections. Staph, including MRSA, is usually spread by having direct contact with someone else’s infected area. When treated early, MRSA skin infections usually get better.

To protect yourself and your family from MRSA, keep hands and skin clean, cover cuts and scrapes, and avoid sharing personal items such as towels or razors.

For more information, please call 1-800-CDC-INFO or visit www.cdc.gov/MRSA.
MRSA
The proportion of Staphylococcus aureus isolates that were methicillin resistant (MRSA) increased from 35.9% in 1992 to 64.4% in 2003 for hospitals / NNIS.

While 25% to 30% of people are colonized in the nose with staph.

Less than 2% are colonized with MRSA.
- Journal of Infectious Diseases. 2008
Hands may become contaminated with MRSA by contact with

Colonized or infected patients

Colonized or infected body sites of the personnel themselves; or

Devices, items, or environmental surfaces contaminated with body fluids containing MRSA
The estimated number of people developing a serious MRSA infection (i.e., invasive) in 2005 was about 94,360.

Approximately 18,650 persons died during a hospital stay related to these serious MRSA infections.

About 85% of all invasive MRSA infections were associated with healthcare, and of those, about two-thirds occurred outside of the hospital, while about one third occurred during hospitalization.

About 14% of all the infections occurred in persons without obvious exposures to healthcare.
Abscess after Intramuscular Steroid Injection in BB
### Options for empiric outpatient antimicrobial treatment of SSTIs when MRSA is a consideration*

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Considerations</th>
<th>Precautions**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clindamycin</td>
<td>FDA-approved to treat serious infections due to <em>S. aureus</em></td>
<td><em>Clostridium difficile</em>-associated disease, while uncommon, may occur more</td>
</tr>
<tr>
<td></td>
<td>D-zone test should be performed to identify inducible clindamycin resistance in erythromycin-resistant isolates</td>
<td>frequently in association with clindamycin compared to other agents.</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>Doxycycline is FDA-approved to treat <em>S. aureus</em> skin infections.</td>
<td>Not recommended during pregnancy.</td>
</tr>
<tr>
<td></td>
<td>Minocycline</td>
<td>Not recommended for children under the age of 8.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Activity against group A streptococcus, a common cause of cellulitis, unknown.</td>
</tr>
<tr>
<td>Trimethoprim-Sulfamethoxazole</td>
<td>Not FDA-approved to treat any staphylococcal infection</td>
<td>May not provide coverage for group A streptococcus, a common cause of cellulitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not recommended for women in the third trimester of pregnancy.</td>
</tr>
<tr>
<td>Rifampin</td>
<td>Use only in combination with other agents.</td>
<td>Not recommended for infants less than 2 months.</td>
</tr>
<tr>
<td>Linezolid</td>
<td>Consultation with an infectious disease specialist is suggested.</td>
<td>Drug-drug interactions are common.</td>
</tr>
<tr>
<td></td>
<td>FDA-approved to treat complicated skin infections, including those caused by MRSA.</td>
<td>Has been associated with myelosuppression, neuropathy and lactic acidosis during prolonged therapy.</td>
</tr>
</tbody>
</table>

- MRSA is resistant to all currently available beta-lactam agents (penicillins and cephalosporins)
- Fluoroquinolones (e.g., ciprofloxacin, levofloxacin) and macrolides (erythromycin, clarithromycin, azithromycin) are not optimal for treatment of MRSA SSTIs because resistance is common or may develop rapidly.

* Data from controlled clinical trials are needed to establish the comparative efficacy of these agents in treating MRSA SSTIs. Patients with signs and symptoms of severe illness should be treated as inpatients.

** Consult product labeling for a complete list of potential adverse effects associated with each agent.

### Role of decolonization

Regimens intended to eliminate MRSA colonization should not be used in patients with active infections. Decolonization regimens may have a role in preventing recurrent infections, but more data are needed to establish their efficacy and to identify optimal regimens for use in community settings. After treating active infections and reinforcing hygiene and appropriate wound care, consider consultation with an infectious disease specialist regarding use of decolonization when there are recurrent infections in an individual patient or members of a household.
Nasal screening for MRSA colonization upon hospital admission is becoming a common practice.

Hospitalized MRSA-colonized patients are known to be at increased risk for subsequent MRSA infections compared with patients who are not colonized.

Patients with MRSA colonization can become a source of MRSA transmission to other patients, who can later develop MRSA colonization and infection.
Nasal screening for MRSA colonization

Two methods are commonly used

- **First is based on standard microbiologic techniques**
  - Swabs are typically plated onto MRSA-specific agar plates containing antibiotics that prevent growth of all bacteria except MRSA / At least 24 hours
  - Approximately $5 to $10

- **Second method relies on rapid molecular tests for MRSA / 75 minutes to 6 hours**
  - Approximately $200 per test
**Screening Specimens for MRSA**

**BBL™ CHROMagar™ MRSA**

Nasal Specimen Collection Protocol

**one**

Use a transport device approved for the collection of anterior nares specimens. For example, BBL CultureSwab™ Liquid Stuart (cat# 220090) or BBL CultureSwab™ Liquid Almes (cat# 220093) would be acceptable swabs for nasal collection.

**two**

Carefully insert the swab into the patient’s nostril (the swab tip must be inserted up to 2.5 cm [1 inch] from the edge of the nares), or until resistance is met at the level of the turbinates (which might be slightly less than that in some neonates and infants). Roll the swab 5 times.

**three**

Insert the same swab into the second nostril and repeat sampling as in the preceding step.

**four**

Return swab to its container and send to the laboratory immediately.
MRSA colonies; CHROMagar Staph aureus : 6 μg of cefoxitin / ml
Accuracy of BBL CHROMagar™ MRSA Medium

(Flayhart, et al. JCM 2005)

- Sensitivity=95.2% (~ mecA PCR)
- Specificity=99.7%
- 86% isolates identified at 24 hr.
Screening for MRSA colonization

► Pharyngeal sampling has been shown to increase the MRSA detection rate by 26%

► Inguinal screening has been shown to increase the MRSA detection rate by 44%

► If nasal decolonization is performed, a topical antibiotic such as mupirocin is recommended
Eradication of MRSA colonization / decolonization

- **Oral antimicrobials** (usually rifampin and trimethoprim-sulfamethoxazole, or rifampin and doxycycline, or rifampin and minocycline) and/or
  - **Rifampin should never be used as a single agent to treat infection or colonization with MRSA**

- **Nasal decolonization with intranasal topical mupirocin** (bid for 5 days)

- **Skin antisepsis** (e.g. chlorhexidine baths) has been used in addition to the above regimens
Topical agent

Nasal colonization of Staphylococcus aureus

Treat skin infections (e.g., impetigo)

No need for routine testing; test on request only
## Tests for Mupirocin High-Level Resistance in *S. aureus*

<table>
<thead>
<tr>
<th>Disk diffusion Screen</th>
<th>MIC Screen</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 µg mupirocin disk</td>
<td>Single well - 256 µg/ml</td>
</tr>
<tr>
<td>MHA; ambient air; 35°C; 24 h</td>
<td>Standard broth microdilution method</td>
</tr>
<tr>
<td><strong>No zone</strong> = high-level mupirocin resistance</td>
<td><strong>Growth</strong> = high-level mupirocin resistance</td>
</tr>
<tr>
<td><strong>Any zone</strong> = absence of high-level mupirocin resistance</td>
<td><strong>No growth</strong> = absence of high-level mupirocin resistance</td>
</tr>
</tbody>
</table>

**Staphylococcus aureus**, are important health-care-associated pathogens, are resistant to inactivation by *drying* and can persist in the environment and on environmental surfaces for extended periods. (26-27 days)
These organisms can be shed from heavily colonized persons and discharged into the air, and directly associated with the concentration of the bacterium in the anterior nares.

Approximately 10% of healthy carriers will disseminate S. aureus into the air.

The dispersal of S. aureus into air can be exacerbated by concurrent viral upper respiratory infection.
People represent the primary reservoir of S. aureus.

Although S. aureus has been isolated from a variety of environmental surfaces:

- Stethoscopes
- Floors
- Charts
- Furniture
- Dry mops

The role of environmental contamination in transmission of this organism in health care appears to be minimal.
S. aureus contamination of surfaces and tanks within burn therapy units, however, may be a major factor in the transmission of infection among burn patients.
Vancomycin-Resistant-Enterococci / VRE

*E. faecium is the most frequently isolated species of VRE in hospitals*
Colonized patients are the principal reservoir of VRE

► Immunosuppressed
► ICU patients
► Patients previously hospitalized for extended periods
► Received multi-antimicrobial or vancomycin therapy

Detection of vancomycin-resistant enterococci on rectal swabs from colonized patients
VRE: Stool Swab on Enterococcus Plate

Infectious Diseases Society of America, 2006
Many of the environmental surfaces found to be contaminated with VRE

In outbreak investigations have been those that are touched frequently by the patient or the health-care worker.

- Bedrails
- Doorknobs
- Bed linens
- Gowns
- Overbed tables
- Blood pressure cuffs
- Computer table
- Bedside tables
Contamination of environmental surfaces with VRE occurs in clinical laboratories.

Areas where colonized patients are present, but the potential for contamination increases when such patients have diarrhea.
Importance of environmental reservoirs of VRE

Enterococci can persist in a viable state on dry environmental surfaces for extended periods of time (7 days to 4 months)
Although routine environmental sampling is not recommended.

Laboratory surveillance of environmental surfaces during episodes when VRE contamination is suspected can help determine the effectiveness of the cleaning and disinfecting procedures.
 ► Careful cleaning of patient rooms and medical equipment contributes substantially to the overall control of MRSA, VISA, or VRE transmission

 ► The use of stronger solutions of disinfectants for inactivation of either VRE, MRSA, or VISA is not recommended based on the organisms’ resistance to antibiotics
Carbapenemases
<table>
<thead>
<tr>
<th>Drug</th>
<th>Route of Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem</td>
<td>IV</td>
</tr>
<tr>
<td>Meropenem</td>
<td>IV</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>IM, IV</td>
</tr>
<tr>
<td>Doripenem</td>
<td>IV</td>
</tr>
</tbody>
</table>
Mechanisms of Carbapenem Resistance

- **Carbapenemase hydrolyzing enzymes**
- **Porin loss “OprD”**
- **ESBL or AmpC + porin loss**
<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Mechanism</th>
<th>Enzyme(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Enterobacteriaceae</em></td>
<td>Cephalosporinase + porin loss</td>
<td>Carbapenemase</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>Porin loss</td>
<td>Carbapenemase</td>
</tr>
<tr>
<td></td>
<td>Up-regulated efflux</td>
<td></td>
</tr>
<tr>
<td><em>Acinetobacter spp.</em></td>
<td>Cephalosporinase + porin loss</td>
<td>Carbapenemase</td>
</tr>
</tbody>
</table>
Carbapenemases

- All carbapenemases hydrolyze penicillins, extended spectrum cephalosporins, and carbapenems.

- Two major groups based on the hydrolytic mechanism at the active site:
  - Serine at the active site: class A and class D
  - Zinc at the active site: class B, (METALLO-B-LACTAMASES)
## Carbapenemase Classification

<table>
<thead>
<tr>
<th>Molecular Class</th>
<th>A</th>
<th>B</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aztreonam Hydrolysis</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>EDTA Inhibition</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
## Carbapenemase Classification

<table>
<thead>
<tr>
<th>Classification</th>
<th>Enzyme</th>
<th>Most Common Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class A</td>
<td>KPC, SME, IMI, NMC, GES</td>
<td>Enterobacteriaceae (rare reports in P. aeruginosa)</td>
</tr>
<tr>
<td>Class B</td>
<td>IMP, VIM, GIM, SPM</td>
<td>P. aeruginosa Enterobacteriaceae Acinetobacter spp.</td>
</tr>
<tr>
<td>Class D</td>
<td>OXA</td>
<td>Acinetobacter spp.</td>
</tr>
</tbody>
</table>
Carbapenemases Class A

First identified 1982 in UK

Four major families

Chromosomally encoded
- Serratia marcescens enzyme (SME)
- Not metalloenzyme carbapenemases (NMC)
- Imipenem-hydrolyzing β-lactamases (IMI)

Plasmid encoded
- Klebsiella pneumoniae carabapenemases (KPC)
- Guiana Extended-Spectrum (GES)
Klebsiella pneumoniae Carbapenemase

KPC
► Predominantly in K. pneumoniae (KP)

► Reported in Enterobacter spp., Salmonella spp., E. coli, P. aeruginosa, and Citrobacter spp.

► First identified in KP clinical isolate in 1996 (KPC-1)

► KPC 1-8 have been reported.
When to Suspect a KPC Producer

► **Enterobacteriaceae**

► **Resistance to extended spectrum cephalosporins** (cefotaxime, ceftazidime, and ceftriaxone)

► **Variable susceptibility to cephemycins** (cefoxitin, cefotetan)

► **Disk zone of < 22 mm** for Ertapenem or Meropenem
For disk diffusion greater than 90% sensitivity achieved only when **Ertapenem** tested

**Meropenem** and **Imipenem** susceptibility demonstrated poor sensitivity

**Specificity** of **meropenem** and **imipenem** susceptibility testing (I or R) are higher than that for **Ertapenem**

**Ertapenem** resistance does not necessarily predict resistance to other carbapenems
Phenotypic test for “carbapenemase” activity

The modified Hodge test can detect both KPC and metallo-β-lactamases (MBLs), but does not differentiate between them.

>90% sensitivity/specificity in detecting KPC
Modified Hodge test

- Variable sensitivity/specificity in detecting other carbapenemases (e.g., low level metallo-β-lactamases)

- Differentiation of MBLs from other carbapenemases is not important for patient management, but these tests can be useful for epidemiological studies
Carbapenem “S” and Carbapenemase Screening Breakpoints for *Enterobacteriaceae*

<table>
<thead>
<tr>
<th>Agent</th>
<th>Standard CLSI “S” breakpoints</th>
<th>Screening values in “S” range suggesting carbapenemase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC (µg/ml)</td>
<td>Disk (mm)</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>≤2</td>
<td>≥19</td>
</tr>
<tr>
<td>Imipenem*</td>
<td>≤4</td>
<td>≥16</td>
</tr>
<tr>
<td>Meropenem</td>
<td>≤4</td>
<td>≥16</td>
</tr>
</tbody>
</table>

* Imipenem does not work as a screen for *Proteus/Providencia/Morganella*
** NA, not applicable (poor test performance)
When should we do Modified Hodge Test?

If “R” to cefotaxime, ceftazidime, and/or ceftriaxone
Note: cefepime gives variable results in carbapenemase producers

<table>
<thead>
<tr>
<th>MIC</th>
<th>Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>(µg/ml)</td>
<td>(mm)</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>2</td>
</tr>
<tr>
<td>and/or</td>
<td></td>
</tr>
<tr>
<td>Imipenem*</td>
<td>2-4</td>
</tr>
<tr>
<td>and/or</td>
<td></td>
</tr>
<tr>
<td>Meropenem</td>
<td>2-4</td>
</tr>
</tbody>
</table>

Perform Modified Hodge Test (Confirmatory Test)

* Imipenem does not work as a screen for Proteus/Providencia/Morganella
** NA, not applicable (poor test performance)

**Modified Hodge Test**

1. Prepare a 1:10 dilution of McF 0.5 suspension of *E. coli* ATCC 25922.

2. Swab onto MHA plate to create lawn as for disk diffusion test; Place ertapenem or meropenem (best) disk on lawn.

3. Streak test isolates (#1 - #3) from edge of disk outward (use 1 mcl loop).

4. Incubate overnight.

5. Look for growth of *E. coli* around test isolate streak - indicates carbapenem-hydrolyzing enzyme.

**Enhanced growth of *E. coli* ATCC 25922**

Modified Hodge Test for Carbapenemase Detection in Enterobacteriaceae

Background
The Modified Hodge Test (MHT) detects carbapenemase production in isolates of Enterobacteriaceae. In the United States, the most common carbapenemase found in Enterobacteriaceae is the *Klebsiella pneumoniae* carbapenemase (KPC). Other carbapenemase, like the metallo β lactamase (MBL) and the SME-1 in *Serratia marcescens*, can also produce a positive MHT, but are found infrequently in the United States.

Purpose
Carbapenemase production is detected by the MHT when the test isolate produces the enzyme and allows growth of a carbapenem susceptible strain (*E.coli ATCC 25922*) towards a carbapenem disk. The result is a characteristic cloverleaf-like indentation. See Figure 1.

Reagents
1. 5 ml Mueller Hinton broth (MHB) or 0.85% physiological saline
2. Mueller Hinton agar (MHA)
3. 10 μg meropenem or ertapenem susceptibility disk
4. *E. coli ATCC 25922*: 18–24hr subculture

Equipment
1. Turbidity meter
2. 35°C ± 2°C ambient air incubator
Modified Hodge Test for Carbapenemase Detection in Enterobacteriaceae

Supplies
1. Sterile cotton-tipped swabs
2. 1 ml sterile pipette
3. Sterile loop

Specimen
Test organisms: 18–24 hr subculture

Special safety precautions
Biosafety Level 2

Quality control
Perform quality control of the carbapenem disks according to CLSI guidelines.
Perform quality control with each run.
- MHT Positive *Klebsiella pneumoniae* ATCC BAA-1705
- MHT Negative *Klebsiella pneumoniae* ATCC BAA-1706
Modified Hodge Test for Carbapenemase Detection in Enterobacteriaceae

**Procedure**

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1</td>
<td>Prepare a 0.5 McFarland dilution of the <em>E. coli</em> ATCC 25922 in 5 ml of broth or saline.</td>
</tr>
<tr>
<td>Step 2</td>
<td>Dilute 1:10 by adding 0.5 ml of the 0.5 McFarland to 4.5 ml of MHB or saline.</td>
</tr>
<tr>
<td>Step 3</td>
<td>Streak a lawn of the 1:10 dilution of <em>E. coli</em> ATCC 25922 to a Mueller Hinton agar plate and allow to dry 3–5 minutes.</td>
</tr>
<tr>
<td>Step 4</td>
<td>Place a 10 µg meropenem or ertapenem susceptibility disk in the center of the test area.</td>
</tr>
<tr>
<td>Step 5</td>
<td>In a straight line, streak test organism from the edge of the disk to the edge of the plate. Up to four organisms can be tested on the same plate with one drug.</td>
</tr>
<tr>
<td>Step 6</td>
<td>Incubate overnight at 35°C ± 2°C in ambient air for 16–24 hours</td>
</tr>
</tbody>
</table>
Interpretation/Results

- After 16–24 hours of incubation, examine the plate for a clover leaf-type indentation at the intersection of the test organism and the *E. coli* 25922, within the zone of inhibition of the carbapenem susceptibility disk.

- **MHT Positive** test has a clover leaf-like indentation of the *E. coli* 25922 growing along the test organism growth streak within the disk diffusion zone.

- **MHT Negative** test has no growth of the *E. coli* 25922 along the test organism growth streak within the disc diffusion.

See the CLSI guidelines (M100) for recommendations on detection of carbapenemase production in Enterobacteriaceae that test susceptible to carbapenem.

**Expected values**

A positive MHT indicates that this isolate is producing a carbapenemase.  
A negative MHT indicates that this isolate is not producing a carbapenemase.

**Method limitations**

The class of carbapenemase cannot be determined by the results of the MHT.  
Some isolates show a slight indentation but do not produce carbapenemase.
Carbapenem breakpoints for the Enterobacteriaceae /CLSI 2010

<table>
<thead>
<tr>
<th>Carbapenem</th>
<th>MIC (µg/ml)</th>
<th>Zone Size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>Doripenem</td>
<td>≤ 1</td>
<td>2</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>≤ 0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>Imipenem</td>
<td>≤ 1</td>
<td>2</td>
</tr>
<tr>
<td>Meropenem</td>
<td>≤ 1</td>
<td>2</td>
</tr>
</tbody>
</table>
If it is carbapenemase negative, the strain could be susceptible to both imipenem and meropenem, even if it is resistant to ertapenem

PCR : The method of choice to confirm KPC - Gene blaKPC
Metallo-B-lactamase (MBL) / Carbapenemase B

- **Acinetobacter baumannii complex**

- **Imipenem-EDTA double-disk synergy test (DDST) (200 mg EDTA to imipenem disks)**

- **EDTA chelates the metal required for classB B-lactamase activity and has been used in screening tests for metallo-B-lactamase production**
Pseudomonas aeruginosa (PA) is a common cause of hospital-acquired infections worldwide.

Multi-drug resistant PA can possess a variety of resistance mechanisms, including B-lactamases, efflux pumps, and the ability to alter membrane permeability.

Presence of metallo-beta lactamase and carbapenemase.

Phenotypic testing for Class A and B carbapenemase production, as well as AmpC production, was performed using disk diffusion techniques.
Gram-negative bacteria rarely are associated with episodes of airborne transmission because they generally require moist environments for persistence and growth. The main exception is Acinetobacter spp., which can withstand the inactivating effects of drying. Acinetobacter spp. were cultured from the patients, air, and room air conditioners.
Infections caused by Acinetobacter spp. represent a significant clinical problem.

Mortality rates associated with Acinetobacter bacteremia are 17%–52%, and rates as high as 71% have been reported for pneumonia.

Average infection rates are higher from July through October compared with rates from November through June.
Acinetobacter spp. have also been detected on dry environmental surfaces:

- Bed rails
- Counters
- Sinks
- Bed cupboards
- Bedding
- Floors
- Telephones
- Medical charts

and in the colonized or infected patients.

The survival periods of Acinetobacter spp. on dry surfaces approximated that for S. aureus (26–27 days).
Clinically important, opportunistic organisms in tap water include

- *Pseudomonas aeruginosa*
- *Pseudomonas spp.*
- *Burkholderia cepacia*
- *Stenotrophomonas maltophilia*
Air Culture for Fungi
Air Culture for Fungi

- Environmental sampling of hospital air for fungi is not recommended on routine basis

- Hospitalized patients with extreme Immune Suppression are susceptible to infections

- Most highly filtered areas:
  - Total fungal colony counts < 15 CFU/m³ at 25 C
  - Total pathogen colony counts < 1 CFU/m³ at 25 C
    - A.fumigatus, A.flavus, A.terreus, Fusarium spp
<table>
<thead>
<tr>
<th>Sampler type</th>
<th>Principle</th>
<th>Rate (liters/min)</th>
<th>Comment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Sieve impactor²</td>
<td>Impaction on agar plate</td>
<td>28</td>
<td>Low volume precludes short-term collections that might relate to specific activities; effective for high concentrations of spores.</td>
</tr>
<tr>
<td>(2) Slit impactor³</td>
<td>Impaction on rotating agar plate</td>
<td>30-700</td>
<td>High volume allows short-term collections that might relate spore aerosols to specific activities; effective for low concentrations of spores. Quantification may be compromised at high levels of spores.</td>
</tr>
<tr>
<td>(3) Centrifugal impactor⁴</td>
<td>Impaction on plastic strips containing agar media</td>
<td>40</td>
<td>Calibration difficult, thus limited to relative determinations.</td>
</tr>
<tr>
<td>(4) Impingers (glass)</td>
<td>Impingement into liquids</td>
<td>12.5</td>
<td>Low-volume sampling rates and tendency to disrupt clumps limits application to nonclinical sampling.</td>
</tr>
<tr>
<td>(5) Filters (cassette)</td>
<td>Filtration of air through 0.2-um-pore-size filters</td>
<td>1-2</td>
<td>Not practical for viable microbes.</td>
</tr>
<tr>
<td>(6) Settling plates</td>
<td>Gravity</td>
<td></td>
<td>Most significant spores are too small and buoyant to settle. Lack of quantification severely limits utility.</td>
</tr>
</tbody>
</table>
Settling Plates have often been utilized in the past.

The smallest fungal particles are often the pathogens of greatest concern.

Aspergillus fumigatus spores are 2 to 3.5 micM in diameter.

Only volumetric methods of sampling provide reliable data.
Microbiological Assay of the Environmental & Medical Device Surfaces
Before 1970, hospitals conducted regularly scheduled culturing of the air and environmental surfaces.

By 1970, CDC were advocating the discontinuation of routine environmental culturing because
Rates of healthcare-associated infection had not been associated with levels of general microbial contamination of air or environmental surfaces, and

Because meaningful standards for permissible levels of microbial contamination of environmental surfaces or air did not exist
Microbiologic sampling of air, water, and inanimate surfaces (i.e., environmental sampling) is an expensive and time-consuming process that is complicated by many variables in protocol, analysis, and interpretation.
Environmental surfaces can be divided into:

- Medical equipment surfaces
- Housekeeping surfaces (e.g., floors, walls, and tabletops).
Surface sampling has been used to determine

Investigation of an outbreak of disease or infections when environmental reservoirs are implicated epidemiologically in disease transmission.

- Linking microorganisms from environmental samples with clinical isolates by molecular epidemiology

Mode of spread
Surfaces without residual disinfectant

Swab/ Swab rinse Methods

- Swabbing is the oldest and most widely used method for the microbiological examination (1917)
- The swab rinse method remains a rapid, simple, and inexpensive
- The swab method to be best suited for flexible, uneven, and heavily contaminated surfaces
Swab/ Swab rinse Methods

- **Cotton or calcium alginate swabs**

- **Size of the area to be swabbed e.g.** : $1cm^2$

- **Slowly rotate the moisten swab on surface**

- **Place swab head in a tube of TSB or Saline (5 or 10 ml)**

- **Cap tube and shake end to end 50 times or process for 30 s on a vortex mixer at high speed.**
Direct Immersion

-One of the most efficient methods for recovering surface microorganisms from items small enough to be placed into culture tube or flask and thoroughly exposed to rinse solution

-Place a suitable volume of rinse fluid (e.g.: 10 to 50 ml) in bottle.

-Shake the container vigorously for 1 min or end to end approximately 50 times.
Quantitative Culture

► Make serial 10-fold dilutions

► Pipette 0.1 ml of each dilution (undiluted, 1:10, 1:100) onto agar plate surface

► Count colonies after 48 h of incubation

► Count colonies on plates having between 30 and 300 colonies
Culture Media

- Nutrient-rich, nonselective agar e.g.; TSA
  - Nutrient-rich, nonselective agar; TSA + sRBC

- Avoid using high-nutrient-content media, e.g. Blood agar for culturing environmental microorganism damaged (Tap water)

- MAC agar

- Sabouraud agar

- Cetrimide agar (P. aeruginosa)
RODAC; Replicate Organism Direct Agar Contact method (1945-1964)

The RODAC plate has been shown to be the method of choice when the surfaces to be examined are smooth, firm, and nonporous.

When the plate is inverted, the hardened agar makes direct contact with the surface.
The surface area covered by a RODAC plate (55 mm) is approximately 25 cm²

RODAC method employs special Petri plates, which are poured with 15.5-16.5 ml of an appropriate plating medium, resulting in a raised agar surface
Highest quality BBL and Difco Media, including D/E Neutralizing Agar, Trypticase™ Soy Agar and more!

60 mm RODAC plates feature media surface area that conforms to IES standards.

Grid sits inside RODAC dish — so it won’t get in the way of your marker.

Excellent stackability — a RODAC exclusive!

Convex dish base results in unique RODAC surface for better contact between agar bed and surface area tested.

User-friendly, wide sides are easy to grasp and apply to surfaces.

Compatible with air sampling equipment.
Surfaces with residual disinfectant

- Nonspecific neutralization of disinfectants by incorporation of protein supplements in rinse media or Double–strength broth media (e.g.; 2x TSB or BHI)

- Membrane filter assay method
Surfaces with residual disinfectant

Specific neutralization of disinfectants

- Sodium thiosulfate to neutralize chlorine & iodine
- Lecithin + Polysorbate (Tween) 80 to neutralize quaternary ammonium compounds
- Polysorbate (Tween) 80 to neutralize phenolic disinfectants
- Sodium bisulfite or Glycine to neutralize aldehydes
- Sodium thioglycollate to neutralize mercurials
BD / BBL™

➢ Trypticase™ Soy Agar with Lecithin and Polysorbate 80 Contains lecithin to neutralize quaternary ammonium compounds and polysorbate 80 to neutralize phenolic disinfectants

➢ D/E Neutralizing Agar contains lecithin, polysorbate 80, sodium bisulfite to neutralize aldehydes, sodium thioglycollate to neutralize mercurials and sodium thiosulfate to neutralize chlorine
Counts under *five* colonies per plate are achievable in such *critical areas* as operating rooms.

But there is no evidence that these levels must be maintained in order to prevent infections or that any particular level of contamination in such areas is directly correlated with an increased risk of infection.

<table>
<thead>
<tr>
<th>Colonies per Rodac® plate</th>
<th>0 = excellent</th>
<th>0–25 = good</th>
<th>26–50 = fair</th>
<th>50 and over = poor</th>
</tr>
</thead>
</table>

*Table: Colonies per Rodac® plate*
RODAC: Use a minimum of 15 plates for an average hospital room, with sampling sites chosen randomly to prevent biasing of results.
Presence of gram negative rods is undesirable because *E. coli* and *Salmonella* are gram negative rods.

The presence of gram positive cocci is also of concern because this may indicate that the sanitation process is unable to eliminate staph and strep.
Critical medical instrument (e.g.; Surgical instrument): Absence of all viable microbial forms, including bacterial spores

Semicritical medical instrument (e.g.; flexible fiber-optic endoscopes): Absence of all microorganism except sporeformer not recognized as pathogen

Noncritical medical instrument (e.g.; Stethoscopes): Absence of recognized pathogen

Noncritical environmental surfaces: At least minimal levels of hygiene for medical devices such as X-ray and housekeeping surfaces such as floors, walls
Nosocomial Bloodstream Infection

Catheter-related bloodstream infection
Nosocomial Bloodstream Infection

Intravascular Device–Related Bloodstream Infection

► Central venous catheters are the most frequent cause of nosocomial bloodstream infection

► Estimated 250,000 to 500,000 episodes of IVD-related bloodstream infection occur in the U.S annually

► These episodes are associated with an attributable mortality rate of 12% to 25%
<table>
<thead>
<tr>
<th>Microbiology of Device-Associated Bacteremia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase-negative staphylococci including <em>Staphylococcus epidermidis</em>†</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td><em>Enterococcus</em> spp.</td>
</tr>
<tr>
<td><em>Serratia marcescens</em> ‡</td>
</tr>
<tr>
<td><em>Candida albicans</em> ‡</td>
</tr>
<tr>
<td><em>Candida tropicalis</em> ‡</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> §</td>
</tr>
<tr>
<td><em>Klebsiella</em> spp. †</td>
</tr>
<tr>
<td><em>Enterobacter</em> spp. †</td>
</tr>
<tr>
<td><em>Citrobacter freundii</em> †</td>
</tr>
<tr>
<td><em>Corynebacterium</em> (especially <em>C. jeikeium</em>) ‡</td>
</tr>
<tr>
<td><em>Burkholderia cepacia</em> complex §</td>
</tr>
</tbody>
</table>

*Most common pathogen for long-term lines; also associated with lipid infusions in neonates.*
†Frequently associated with contaminated infusate.
‡Most often associated with total parenteral nutrition; usually along the catheter path, but occasionally as a result of contaminated infusate.
§May arise from a water source (e.g., infusate) or may reflect cutaneous colonization.
‖*C. jeikeium* bacteremia occurs almost exclusively in severely immunosuppressed patients who are or have been receiving broad-spectrum antibiotics and who have indwelling intravascular devices.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase-negative staphylococci</td>
<td>27</td>
<td>37</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>Gram-negative rods</td>
<td>19</td>
<td>14</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Candida spp.</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>
Table 2. Etiology of 28 cases of catheter-related bloodstream infection.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>No. (%) of isolates (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase-negative staphylococci(^a)</td>
<td>11 (37.9)</td>
</tr>
<tr>
<td>Enterobacteriaceae(^b)</td>
<td>7 (24.1)</td>
</tr>
<tr>
<td>Yeast(^c)</td>
<td>6 (20.7)</td>
</tr>
<tr>
<td>Staphylococcus aureus(^d)</td>
<td>3 (10.3)</td>
</tr>
<tr>
<td>Enterococcus faecium(^e)</td>
<td>2 (6.9)</td>
</tr>
</tbody>
</table>

\(^a\) Includes 9 *Staphylococcus epidermidis* isolates (7 were methicillin-resistant strains) and 2 *Staphylococcus* species isolates (all were methicillin-resistant strains).

\(^b\) Includes 5 *Serratia marcescens* isolates, 1 *Enterobacter aerogenes* isolate, and 1 *Enterobacter cloacae* isolate. There were no multidrug-resistant strains.

\(^c\) Includes 3 *Candida parapsilosis* isolates, 2 *Candida albicans* isolates, and 1 *Saccharomyces cerevisiae* isolate (all yeasts were susceptible to amphotericin B, fluconazole, itraconazole, and voriconazole, except for the *S. cerevisiae* isolate, which was resistant to itraconazole).

\(^d\) All isolates were methicillin resistant.

\(^e\) Both isolates were ampicillin resistant.
Coagulase-negative staphylococci

- Coagulase-negative staphylococci are the most frequent causes of catheter-related infections.

- They can produce extracellular slime that facilitates adherence and may limit the access of antibiotics, and may reduce the host's inflammatory response.

- There may be difficulty in interpretation of the significance of these isolates as coagulase-negative staphylococci are commonly isolated from contaminated blood cultures.
Scanning electron micrograph of a Staphylococcus biofilm
Yeasts

► 8 % of all Nosocomial bloodstream infections

► Candida albicans and nonalbicans

► Malassezia furfur (in patients receiving intralipid infusions)
Catheter-related bloodstream infection

- **Peripheral venous catheter**: Usually inserted into the veins of the forearm or the hand; most commonly used short-term intravascular device; rarely associated with bloodstream infection.

- **Nontunneled CVC (Central venous catheters)**: Most commonly used CVC; accounts for an estimated 90% of all catheter-related bloodstream infections; increased risk of infection with internal jugular vein site of insertion.
Triple-Lumen Catheter/ CVC
Contamination may reach system through defects in containers

Contamination during manufacture

Contamination due to malfunctioning air inlet filter

Contamination may also enter the system through
1. Pressure measuring devices, transducers
2. Heparinized flush solutions
3. Stopcocks
4. I.V. piggyback
5. Y. junctions
6. Administration of blood products or medications
7. CVP manometers

In-line filter may trap bacteria but shed endotoxin

Contamination may reach circulation at the catheter insertion site

Contamination may enter system at catheter/administration set junction
Collection from IV catheter

- Using 2 separate 70% alcohol preps, scrub catheter hub connection for 15 s. Air dry (30-60 s)

- Collect and discard blood; 3 ml for adult & 0.2 ml for pediatric

- Using new syringe, collect blood for culture
The catheter-tip sample was taken after scrubbing the skin surrounding the insertion site with 2% chlorhexidine and cutting off the tip (distal 5-cm segment) using sterile scissors.
The most widely used laboratory technique for the clinical diagnosis of catheter-related infection is the *semiquantitative method*, in which the catheter segment is rolled across the surface of an agar plate, *roll plate technique*

*Semiquantitative* (≥15 cfu per catheter segment)
For short-term catheter tip cultures, the roll plate technique is recommended for routine clinical microbiological analysis.

For suspected pulmonary artery catheter infection, culture the introducer tip.
Semiquantitative method

► The terminal 4cm segment of the catheter tip is rolled over the entire surface of the blood agar plate (and optionally, either MAC or EMB), 5 times and incubate for 72-96 hrs at 35°C in candle jar then the number of colonies counted.

► Do not accept catheter tip in saline or transport medium.
Semiquantitative method / Roll Plate

Skin surface-catheter interface

Sterile scissors
Approximately 5 cm (2 in)
Dry, sterile, screw-capped tube or sterile urine cup

Sterile forceps

Blood agar plate

Catheter tip
Roll 4 or 5 times across surface of agar

Venous catheter tip
Semiquantitative method

Identify to at least the genus level any of the following:

► Each organism with count > 15 CFU

► For > 15 CFU of gram-positive rods perform only Gram stain and catalase & check hemolysis

► For < 15 CFU, identify only significant pathogens (e.g.: Candida albicans, Group A Streptococci, and Gram-negative rods)

► Do not perform AST on isolates unless the blood culture is positive and comparative results are desirable
For cultures of an anti-infective catheter tip, use specific inhibitors in the culture media.

Chlorhexidine–Silver Sulfadiazine-Impregnated Central Venous Catheters

The neutralizing medium consisted of sodium oleate, sodium thiosulfate, Tween 80, lecithin.
Quantitative culture of the catheter segment requires either flushing the segment with broth, or vortexing, or sonicating it in broth, followed by serial dilutions and surface plating on blood agar.

Quantitative / vortex or sonication methods (>10^2 cfu per catheter segment)

Sensitivities of the 3 methods are as follows: sonication, 80%; roll plate method, 60%; and flush culture, 40%–50%
Quantitative cultures of peripheral and CVC blood samples

This technique relies on quantitative culture of paired blood samples, one of which is obtained through the central catheter hub and the other from a peripheral venipuncture site. Isolator

Simultaneous quantitative cultures of blood samples with a ratio of >5:1 (CVC vs. peripheral)

>3:1 (CVC vs. peripheral); Significant IDSA, 2009 / NEW
Differential time to positivity (positive result of culture from a CVC is obtained at least 2 h earlier than is a positive result of culture from peripheral blood) - Significant Automated Blood Culture

Differential time to positivity for CVC versus peripheral blood cultures sensitivity was 91% and specificity was 94%
Table 3. Comparison of the validity values (95% CI) of 3 techniques for the detection of catheter-related bloodstream infection.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Semiquantitative superficial cultures&lt;sup&gt;a,b&lt;/sup&gt;</th>
<th>Differential quantitative blood cultures&lt;sup&gt;a,c&lt;/sup&gt;</th>
<th>Differential time to positivity&lt;sup&gt;b,c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>78.6 (59.0–91.7)</td>
<td>71.4 (51.3–86.8)</td>
<td>96.4 (81.7–99.9)</td>
</tr>
<tr>
<td>Specificity</td>
<td>92.0 (87.0–95.6)</td>
<td>97.7 (94.3–99.4)</td>
<td>90.3 (85.0–94.3)</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>61.1 (43.5–76.9)</td>
<td>83.3 (62.6–95.3)</td>
<td>61.4 (45.5–75.6)</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>96.4 (92.4–98.7)</td>
<td>95.6 (91.4–98.1)</td>
<td>99.4 (96.6–99.9)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>90.2 (85.3–93.9)</td>
<td>94.1 (90.0–96.9)</td>
<td>91.2 (86.4–94.7)</td>
</tr>
</tbody>
</table>

**NOTE.**  
<sup>a</sup> P values for the comparison of validity values between semiquantitative superficial cultures and differential quantitative blood cultures were: sensitivity, .75; specificity, .01; positive predictive value, .09; negative predictive value, .79; and accuracy, .15.  
<sup>b</sup> P values for the comparison of validity values between semiquantitative superficial cultures and differential time to positivity were: sensitivity, .13; specificity, .61; positive predictive value, .99; negative predictive value, .12; and accuracy, .83.  
<sup>c</sup> P values for the comparison of validity values between differential quantitative blood cultures and differential time to positivity were: sensitivity, .04; specificity, <.001; positive predictive value, .10; negative predictive value, .04; and accuracy, .29.
Only 15%–25% of the central venous catheter tips that reach the microbiology laboratory turn out to be culture-positive, thereby confirming CR-BSI.

Furthermore, not all CR-BSIs require the catheter to be withdrawn; therefore, diagnosis of CR-BSI by conservative methods (without catheter withdrawal) is highly convenient.
Infusate-related bloodstream infection
Infusate-related bloodstream infection is uncommon and is defined as the isolation of the same organism from both infusate and separate percutaneous blood cultures, with no other source of infection.

The sudden onset of symptoms of bloodstream infection soon after the initiation of an infusion resulting from the administration of contaminated iv fluid, is often diagnostic.
Gram Negative Bacilli

Pseudomonas fluorescens Bloodstream Infections Associated with a Heparin/Saline IV Flush CDC, 2005

P. fluorescens is a member of the fluorescent pseudomonad, Optimal temperature range for growing the organism is 25°C–30°C

Serratia marcescens blood stream infections associated with contaminated magnesium sulfate solutions
Culture of Blood Bank Products
Bacterial Contamination of blood products is rare

Predominance of Gram-Negative bacilli, ability to proliferate at 0 to 6 C and produce endotoxin

Gram-positive bacteria (except Listeria) grow poorly in cold

Platelets stored at RT (22 C) are now the most common cause of transfusion-related sepsis caused by Staphylococci, Pseudomonas, Enterobacter cloacae
Culture of Blood Bank Products

► Remove 20 ml of blood or blood product

► Inoculate 3-5 ml into 4 blood culture bottles aerobic & anaerobic

► Incubate at 35 C & RT for 7 days

► Continue as like as Blood culture procedure
Culture of Blood Bank Products

► Patient’s blood should be cultured simultaneously

► Positive culture of blood bank product and patient’s blood samples yield the same organism is significant

► But does not establish the source of contamination (blood collection, blood bank, water bath in which frozen blood product are thawed, ward, etc)
The US does not allow the use of blood stored for longer than 42 days - in the UK this is lower, at 35 days

The risk of blood poisoning or pneumonia doubled once the 29-day mark passed

After two weeks in storage, red blood cells start to undergo changes which lead to the release of cytokines

These are known to hinder immune function, and in high levels could possibly make patients more susceptible to infection