Lab 5: Bacteremia

Purpose

Bacteremia is the presence of bacteria in the bloodstream. The purpose of this lab is to isolate, identify, and discuss common bacteremia pathogens using common biochemical and morphological tests.

You will receive a case study patient with a simulated blood sample containing an unknown pathogen. These eight patients will be infected by one of eight possible pathogens. You will have four lab periods to diagnose and treat your patient.

Objectives

After completing this lab, you will be able to:

- Identify the common bacterial bacteremia pathogens
- Interpret the results of common biochemical and morphological tests used to diagnose bacteremia
- Use an enrichment culture to improve isolation of pathogens from dilute samples

Introduction

Normal human blood does not contain bacteria; however, bacteria can enter the bloodstream as a result of localized or systemic infections, most commonly involving infections of the urinary tract, pulmonary system, gastrointestinal system, and skin (1, 2). The presence of bacteria in the blood, or bacteremia, occurs in approximately 10 out of 1,000 adult hospital admissions. In many cases, bacteria in the blood are quickly destroyed by the body's immune system. If the bacteria are able to multiply in the blood and establish an infection, the patient may develop septicemia. Septicemia is a severe, life-threatening bloodstream infection. The mortality rates for septicemia are between 10% and 55% in adults (1, 3–5).

Bacteremia and septicemia can be caused by a wide range of bacteria (Table 1). The ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* spp.) make up approximately 2/3rds of bacteremia/septicemia cases, with *Escherichia coli* or *Staphylococcus aureus* accounting for nearly 50% of all cases (1, 2). Furthermore, concentrations less than 10 CFUs/mL blood can cause symptoms, with the severity of symptoms increasing with microbial concentration (6), although the relationship varies by pathogen and patient health.

Table 1: Common bacteremia pathogens. Modified from Brun-Buisson *et al.* 1996 (1).

Organism	Percentage of Bacteremias
Escherichia coli	28.0%
Staphylococcus aureus	20.5%
Coagulase-negative *Staphylococci*	10.2%
Streptococcus pneumoniae	8.4%
Enterococci	5.2%
Klebsiella sp.	4.1%
Beta-hemolytic *Streptococci*	3.9%
Other *Streptococci*	3.8%
Pseudomonas aeruginosa	3.6%
Enterobacter, Citrobacter, or *Serratia spp.*	3.2%
Proteus sp.	2.6%
Other Gram positives	1.8%
Other Gram negatives	1.4%
Haemophilus or *Branhamella*	1.1%
Salmonella sp.	1.0%
Acinetobacter sp.	1.0%
Neisseria meningitidis	0.2%

Pathogenesis

Bacteria most commonly enter the bloodstream through the skin, gastrointestinal tract, or the respiratory tract. As the bacteria enter the bloodstream, innate immune cells recognize and bind pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (PAMPs) using membrane bound pattern recognition receptors (PRRs). In turn, the activation of PRRs upregulates the inflammatory response, including the production of cytokines and inflammatory mediators, such interleukins, tumor necrosis factor, interferons, and histamine. As inflammatory mediators and cytokines enter the blood, they cause blood to clot, blood vessels to widen (vasodilation), and blood plasma to escape through capillary walls (capillary leak). These result in decreased blood pressure and reduce the blood supply to organs. When organs do not get enough blood, metabolic waste products accumulate, acidifying the blood pH (metabolic acidosis). If the infection continues to progress, the organs start to fail, resulting in multiple organ dysfunction syndrome.

Transmission

Bacteremias and septicemias are often the result of pulmonary, urinary tract, gastrointestinal, or skin pathogens entering the bloodstream.

Risk Factors

Individuals that fall into the following categories are at higher risk of developing a bacteremia or suffering severe symptoms:

- Immunocompromised patients
- Patients over 65
- Patients under 1
- Diabetics
- Cancer patients
- Patients with autoimmune disorders
- HIV/AIDS positive patients

Clinical Symptoms

The signs and symptoms of septicemia are correlated to the bacterial concentration in blood, but patient age and health also contribute to severity. The most common symptoms of septicemia include:

- Fever
- Chills
- Rapid heart rate
- Rapid breathing
- Confusion
- Nausea and vomiting
- Low blood pressure
- Decreased urination
- Organ failure
- Death

The symptoms of septicemia are primarily caused by the immune system's response to bacteria in the blood, and are broadly described as systemic inflammatory response syndrome, or SIRS . SIRS may or may not be related to a blood infection, and is defined as including at least two of the following (7):

- Temperature greater than 38 °C
- Temperature lower than 36 °C
- Rapid breathing (more than 20 breaths per minute)
- Low partial pressure of carbon dioxide (less than 32 mm Hg)
- White blood cell count greater than 12,000 per cubic millimeter
- White blood cell count less than 4,000 per cubic millimeter
- Greater than 10% band forms in blood

If SIRS is the result of a blood infection, the patient is diagnosed with sepsis. Severe sepsis occurs when the infection affects organ function, while septic shock is a severe drop in blood pressure leading to heart failure, stroke, organ failure, and death.

Post-sepsis Syndrome

Approximately half of sepsis survivors experience declines in cognitive functioning (8–10), increase in physical limitation (9, 10), greater risk of cardiovascular disease (11), and more hospital readmissions than non sepsis patients (8, 10). Many of these long-term symptoms occur due to organ damage, exacerbations of chronic conditions, amputations, and secondary infections (8–12).

Diagnostic Tests

Tests and procedures used to diagnose bacteremia and/or septicemia typically include:

- Delirium monitoring of adult ICU patients to detect early signs
- A physical examination to identify clinical symptoms, including high or low body temperature, rapid heart rate, and rapid breathing
- X-ray, MRI, CT scans, or ultrasounds to check for abscesses, masses, fluid collection, or necrotizing tissues
- Blood tests, including arterial blood gas testing and white blood cell counts
- Blood cultures to grow and identify pathogens in blood

Blood Collection

Aseptic blood collection is critical to identifying bacteremia pathogens because they are at low concentrations in the blood. 40-60 mL of blood should be collected by venipuncture from two different sites prior to the administration of antimicrobial therapy. The blood draw site should be prepared by cleaning it with 70% ethanol or isopropanol and swabbing concentric circles of iodine and allowing the site to dry. The collection vial should be cleaned with 70% ethanol or isopropanol prior to collection. Samples should be held at room temperature for no more than four hours prior to processing.

Blood sample processing

After blood is collected, it is typically diluted 1:10 in aerobic and anaerobic culture media (13), such as tryptic soy broth, anaerobic brain heart infusion broth, and fluid thioglycollate medium. The culture bottles are monitored daily for signs of microbial activity, such as hemolysis, clotting, turbidity, gas production, pellicle formation, or metabolism (see automated blood culture systems, below). When positive growth is detected, the culture is Gram stained and subcultured onto selective and differential media. Biochemical tests are used to confirm the identity of the pathogen.

Automated Blood Culture Systems

Automated blood culture systems detect bacteremia by measuring microbial metabolism in the aerobic and anaerobic media. As the microorganisms grow in the blood culture medium, they oxidize organic carbon to carbon dioxide. Because automated blood culture systems

continuously monitor carbon dioxide production, they are able to detect positive results in a third the time of manual systems.

Treatment and Prevention

Early intervention with intravenous antibiotics is critical for improving patient outcomes (10). The most frequently used antibiotics include levofloxacin, ceftazidime, ciprofloxacin, cefotaxime, ceftriaxone, and erythromycin (2); however, antibiotic resistance to these drugs continues to create challenges in treatment (2, 5). In addition to antibiotics, vasopressor medications, such norepinephrine, vasopressin, phenylephrine, dopamine, and epinephrine, are commonly used to increase blood pressure during septic shock (10, 14). Fluid resuscitation in patients with low blood pressure or elevated lactate can improve patient survival (14). Low dose corticosteroid therapy for patients in septic shock may reduce short-term patient mortality rates (15). Surgery may be an option to remove the source of infection. Early mobility following sepsis can reduce physical therapy time and duration of delirium during hospitalization (10, 17).

Antimicrobial Resistance

Antimicrobial resistance to the most commonly used drugs to treat septicemia is alarmingly high. More than half of pathogens tested by Pradipta *et al* were resistant to at least one of levofloxacin, ceftazidime, ciprofloxacin, cefotaxime, ceftriaxone, or erythromycin (2). Furthermore, multi-drug resistance continues to develop in the ESKAPE pathogens (2, 5, 16), which account for nearly 2/3rds of bacteremias (1, 2).

Prevention

Bacteremia can be prevented by preventing infections and proper treatment of existing infections. Vaccinations, proper wound cleaning, and good hygiene can prevent an infection from starting. Early treatment of infections can reduce the chances of developing bacteremia, while early intervention following sepsis can reduce the severity of symptoms.

Pre-Lab Preparation

You will not be expected to identify the bacteria based on biochemical results by memory, nor will you be expected to memorize the treatments; a guide will be provided on any assessment as needed.

Day 1

- Read the introduction material, special safety notes, and procedures section

Day 2

- Skim the introduction material, special safety notes, and procedures section
- Bring your worksheet to lab

- Make sure at least one member of your team brings the lab manual to lab

Day 3
- Skim the introduction material, special safety notes, and procedures section
- Bring your worksheet to lab
- Make sure at least one member of your team brings the lab manual to lab

Day 4
- Skim the introduction material, special safety notes, and procedures section
- Bring your worksheet to lab
- Make sure at least one member of your team brings the lab manual to lab

Special Safety Notes

In addition to the regular safety rules, please keep the following in mind:
- We will be working with live cultures of pathogenic bacteria. Wear PPE (including gloves) at all times
- If you spill, immediately soak the area in disinfectant, alert your neighbors, the instructor, and TA
- Do not ingest or allow blood to come into contact with skin. The blood is an animal product and should be treated as potentially infectious.

Procedure

Day 1

1. Review your patient file and discuss it with your team
2. Inoculate one tube of BHI with 1 mL each of your blood sample.
3. Incubate at 37 °C until the next class period
4. Answer all Day 1 questions on the worksheet

Day 2

1. Remove your tubes from the incubator and record your observations
2. Run any biochemical tests you need to complete your diagnosis
3. Answer the *Day 2* questions on your worksheet

Day 3

1. Remove your plates from the incubator and record your observations
2. Run any biochemical tests you need to complete your diagnosis
3. Answer the Day 3 questions on your worksheet

Day 4

1. Record the results from your biochemical tests
2. Using the morphological and biochemical data gathered, identify the pathogen infecting your patient, and plan a treatment using the provided resources
3. Arrange your biochemical tests with identifying information on your bench
4. Visit at least four other team's stations. Using their results, diagnose their patients.
    a. You are welcome to diagnose as many extra patients as you would like, but at minimum, you must diagnose four other patients
    b. You do not have to plan treatments for their patients.
    c. If you are looking for a letter of recommendation from me, I highly recommend you put in the extra work
5. Complete the worksheet. It is due at the end of class
6. Do not discard any of your media. We will take care of it after everybody has had the chance to look at them

Post-Lab Checklist

Day 1

- Dispose of the artificial urine samples in the autoclave bag
- Keep the worksheet, lab manual, and patient files. Bring them with you each day.
- The worksheet is due at the end of Day 4

Day 2

- Do not leave until you have answered all *Day 2* questions on the worksheet.
- Keep the worksheet, lab manual, and patient files. Bring them with you each day.
- Do not discard your tubes.
- The worksheet is due at the end of Day 4

Day 3

- Do not leave until you have answered all *Day 3* questions on the worksheet. There will be a lot going on in the next lab period, and this exercise will help you get through the next lab with minimal frustration
- Keep the worksheet, lab manual, and patient files. Bring them with you each day.
- Do not discard any of your plates. The TAs will tell you where to leave them
- The worksheet is due at the end of Day 4

Day 4

- Be sure your biochemical tests are labeled clearly. You will need to identify which tests belong to your group and what those tests you ran
- Turn in your worksheet
- Do not dispose of your biochemical tests. Leave them at your station. The TAs will clean up after class

References

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