Chapter 7
The Role of the Microbiology Laboratory

Smilja Kalenic

Key points

- Microbes are infectious agents that are not visible to the naked eye; they are widespread in nature. Some cause human diseases. They are divided into bacteria, fungi, viruses, prions and protozoa. Macroscopic parasites are also included.
- Diagnosis of infection by the microbiology laboratory has two important functions: clinical and epidemiological.
- The microbiology laboratory should be able to determine the most frequent microbes causing healthcare-associated infections, and perform at least some basic typing.
- The microbiology laboratory should produce routine reports for infection prevention and control personnel to develop incidence graphs for specific pathogens, antibiotic resistance, wards, and groups of patients.
- Microbiologists, knowing the role of normal colonising flora of humans, the pathogenesis of infections, and the characteristics of specific pathogens can interpret microbiological findings for infection prevention and control personnel.

Basics of Microbiology[1-7]

Microbes are infectious agents that are invisible to the naked eye. They are divided into bacteria, fungi, viruses, prions and protozoa. Microbes are ubiquitous, living as free organisms in the environment or on/in plants, animals and humans, either as normal flora (not harming them) or pathogens (causing disease). While some microbes are confined to one host, most can live on/in a wide array of hosts in nature. Plant microbes are harmless to humans, however some animal microbes can cause disease (zoonotic diseases).

When a microbe finds a new host and starts to multiply, this phenomenon is usually called colonisation. The microbe can remain in balance with the host and no disease will develop. However, if the microbe causes disease, this is called an infectious disease (infection).

Microbes that usually cause disease in a susceptible host are called primary pathogens. Microbes that live as normal flora of humans or live in the environment and do not harm a healthy host – but can cause disease in an immunocompromised host – are called opportunistic pathogens. When we encounter unusual microbes on skin and non-living surfaces/items, we call it contamination.

Infection can be asymptomatic or symptomatic. After infection, microbes can remain present for some time in the host and may be passed on to others, although the person is clinically completely healthy. This state is called a “carrier state” and such persons are called “carriers”.

If infection is caused by microbes that are part of one’s normal flora, we call it endogenous; an exogenous infection is caused by microbes that are not part of the normal flora.

Microbes are transmitted from one host to another by a number of pathways including: air, water, food, live vectors, indirect contact with contaminated items or surfaces, or direct contact with different hosts. To cause an infectious disease, a microbe must first enter the human body, either through the respiratory, gastrointestinal or genitourinary tract, or through damaged or even intact skin. Microbes usually multiply at the site of entry, then enter through mucous membranes to tissue and sometimes

to blood. When in blood, they can spread throughout the body and enter any organ.

After multiplication, microbes usually leave the body, either through respiratory, gastrointestinal or genitouroinary discharges, and seek a new host. Some are transmitted by insect vectors that feed on human blood. Knowing how an infection develops is essential for clinical diagnosis and for timing and ordering the right specimen for microbiological diagnosis, as well as for taking proper measures to prevent its spread.

Bacteria

Bacteria are the smallest organisms with all the functions of life. They multiply by simple division from one mother cell to two daughter cells. When multiplying on a solid surface, they form "colonies" that are visible with the naked eye. The genetic material (DNA) is situated in one circular chromosome and several independent units called plasmids. The chromosome is haploid (only one DNA chain) so every variation can be easily expressed phenotypically.

Genetic material is transferred vertically by cell division, and also horizontally between different bacteria. The latter is especially important when antibotic resistance genes are transferred. Most bacteria are readily adaptable to any kind of environment. All pathogenic and most opportunistic bacteria have constituents that act as virulence factors, which are important in the development of infectious diseases.

Some bacteria can become dormant by forming spores, which have a strong protective coat, and are the most resistant form of life we know – if the conditions for a vegetative form is unfavourable. When conditions are once again favourable, vegetative forms of the bacteria develop.

Table 7.1 outlines the main groups of pathogenic and opportunistic bacteria that can cause healthcare-associated infections (HAI). Included are their usual habitat, survival in the environment, mode of transmission, infections they cause, and main HAI prevention methods.

Table 7.1. The most widely used chemical disinfectants in healthcare

Bacteria	Multidrug resistant strains (MDR)	Habitat	Survival in the environment (dry surfaces)*	Transmission in healthcare	Healthcare - associated infections	Specimens for diagnosis of infection/ colonisation	Main preventive measures
Acinetobacter baumannii	MDR strains	Humans: moist parts of skin, gastrointestinal tract	3 days - 5 months	Air; indirect** and direct contact	UTI, sepsis, meningitis, pneumonia	Urine, blood, CSF, sputum, aspirates	Clean environment, clean instruments, clean hands
Bordetella pertussis		Humans: nasopharyngeal mucosa (patients)	3 - 5 days	Droplets	Pertussis	Nasopharyngeal swab	Source isolation
Campylobacter jejuni, C. coli		Humans, animals: gastrointestinal tract	Up to 6 days	Faecal-oral, water, food	Diarrhoea	Stool	Safe food and water, clean hands
Clostridium difficile		Humans: gastrointestinal tract	Highly resistant (spores – 5 months)	Faecal-oral; indirect and direct contact	*Clostridium difficile* infections (CDI)	Stool	Clean environment, clean hands of healthcare workers and patients; prudent use of antibiotics

Bacteria	Multidrug resistant strains (MDR)	Habitat	Survival in the environment (dry surfaces)*	Transmission in healthcare	Healthcare - associated infections	Specimens for diagnosis of infection/ colonisation	Main preventive measures
Clostridium tetani		Environment: earth, dust	Highly resistant (spores)	Entering umbilical cord wound (on dirty instruments)	Tetanus		Sterilisation of instruments for umbilical cord
Coagulase negative staphylococci (CNS)	Methicillin-resistant *S. epidermidis*	Humans: skin, mucous membranes	ND	Contact (direct, indirect); endogenous	Different infections in immunocompromised host	Different specimens depending on infection	Clean hands, clean environment, clean equipment
Coryne-bacterium diphtheriae		Humans: nasopharyngeal mucosa (patients, carriers)	7 days – 6 months	Droplets, contact (direct, indirect)		Nasopharyngeal swab	Source isolation (vaccination)
Enterococcus species	Glycopeptide-resistant Enterococcus	Humans: gastrointestinal tract, genitourinary tract	5 days – 4 months	Indirect and direct contact; endogenous	UTI, sepsis	Urine, blood	Clean environment, clean hands; avoid the use of cephalosporins

Bacteria	Multidrug resistant strains (MDR)	Habitat	Survival in the environment (dry surfaces)*	Transmission in healthcare	Healthcare - associated infections	Specimens for diagnosis of infection/ colonisation	Main preventive measures
Enterobacter species	Extended spectrum beta lactamase, MDR	Environment, human gastrointestinal tract	5-49 days	Contact, food	UTI, sepsis, wound infection	Urine, blood, wound exudate	Clean hands, clean environment, clean equipment
Escherichia coli	Extended spectrum beta lactamase-strains	Humans: gastrointestinal and genitourinary tract	1.5 hours – 16 months	Faecal-oral, indirect and direct contact, food, water, endogenous	UTI, sepsis, pneumonia, peritonitis, neonatal meningitis	Urine, blood, sputum, aspirates, CSF, wound exudate	Clean hands, safe food and water; prudent use of antibiotics (avoiding the use of 3rd generation cephalosporins)
Helicobacter pylori		Gastric mucosa of humans	Less than 90 minutes	Contaminated gastrointestinal endoscopes	Acute and chronic gastritis	Biopsy material; urea breath test; stool for antigen detection	Properly disinfected gastrointestinal endoscopes
Klebsiella pneumoniae	ESBL strains Carbapenem resistant strains	Humans: gastrointestinal tract; humid environment	2 hours – more than 30 months	Indirect and direct contact, endogenous	UTI, sepsis (neonatal units), pneumonia	Urine, blood, sputum, aspirates	Clean hands; prudent use of antibiotics (avoiding the use of 3rd generation cephalosporins)

Bacteria	Multidrug resistant strains (MDR)	Habitat	Survival in the environment (dry surfaces)*	Transmission in healthcare	Healthcare - associated infections	Specimens for diagnosis of infection/ colonisation	Main preventive measures
Legionella pneumophila		Water (natural water, tap water, shower heads, cooling towers, hot water tanks, humidifiers, respiratory therapy equipment)	NA	Aerosols from environmental water sources (usually warm water in hospitals) no person-to-person transmission	Legionnaire's disease	Sputum, blood for serology	No patient isolation needed; hyperchlorination of water or heating to at least 55°C
Listeria monocytogenes		Soil, vegetables, human gastrointestinal tract (rarely); human birth canal	1 day - months	Contaminated food; perinatal transmission; contact with contaminated equipment in nurseries	Meningitis, bacteremia	Blood, CSF	Safe food, clean equipment in nurseries
Mycobacterium tuberculosis	MDR strains, Extremely drug resistant strains (XDR)	Respiratory tract of patients	1 day – 4 months	Airborne, droplets	Tuberculosis	Sputum	Source isolation (Vaccination)

Bacteria	Multidrug resistant strains (MDR)	Habitat	Survival in the environment (dry surfaces)*	Transmission in healthcare	Healthcare - associated infections	Specimens for diagnosis of infection/ colonisation	Main preventive measures
Neisseria meningitidis		Nasopharyngeal mucosa of humans	ND	Droplets	Acute meningitis	CSF	Source isolation, Chemoprophylaxis (Vaccination against groups A,C, Y, W135)
Proteus species	ESBL	Gastrointestinal flora of humans	1 - 2 days	Endogenous, contact (direct and indirect)	UTI, sepsis	Urine, blood	Clean hands, clean environment, clean equipment
Pseudomonas aeruginosa	MDR and poly-drug resistant (PDR) strains	Humans: gastrointestinal tract, humid skin regions; ubiquitous in humid environment, (water, soil, plants)	6 hours to 16 months	Direct and indirect contact (humid items: poorly disinfected items, ventilator circuits)	Different, usually severe infections in hospitalised, especially immunocompromised patients	Different specimens depending on infection	Clean, dry environment, disinfected/sterilised instrument and equipment; clean hands, prudent use of antibiotics

Bacteria	Multidrug resistant strains (MDR)	Habitat	Survival in the environment (dry surfaces)*	Transmission in healthcare	Healthcare - associated infections	Specimens for diagnosis of infection/ colonisation	Main preventive measures
Salmonella species		Gastrointestinal tract of humans and animals	1 day	Faecal-oral, water, food	Diarrhoea, sepsis	Stool, blood	Safe food and water, clean hands
Salmonella typhi		Gastrointestinal tract of humans	6 hours – 4 weeks	Faecal-oral, water, food	Typhoid fever	Stool, blood	Safe food and water, clean hands
Salmonella typhimurium		Gastrointestinal tract of humans and animals	10 months – 4.2 years	Faecal-oral, water, food	Diarrhoea, sepsis	Stool, blood	Safe food and water, clean hands
Serratia marcescens		Humans: gastrointestinal tract; humid environment	3 days – 2 months; on dry floors 5 weeks	Indirect and direct contact, contaminated intravenous fluids (especially heparin solutions)	Sepsis, wound infection	Blood, wound exudate	Clean hands, clean environment, clean equipment
Shigella species		Gastrointestinal tract of humans	2 days – 5 months	Faecal-oral, water, food	Diarrhoea	Stool	Safe food and water, clean hands

Bacteria	Multidrug resistant strains (MDR)	Habitat	Survival in the environment (dry surfaces)*	Transmission in healthcare	Healthcare - associated infections	Specimens for diagnosis of infection/ colonisation	Main preventive measures
Staphylococcus aureus	MRSA	Humans: skin, mucous membranes	7 days - 7 months	Droplets, direct and indirect contact, medical equipment, endogenous	Skin infections, pneumonia, sepsis, osteomyelitis	Swabs, sputum, blood, aspirates, biopsy, wound exudate	Clean hands, clean environment; prudent use of antibiotics (ciprofloxacin)
Streptococcus agalactiae ***(Group B streptococcus)***		Humans: birth canal	ND	Intrapartum; direct and indirect contact in delivery room and nurseries	Sepsis and meningitis of newborn	Blood, CSF	Antibiotic prophylaxis during delivery when indicated; clean hands
Streptococcus pyogenes ***(Group A streptococcus)***		Humans: oropharyngeal mucosa	3 days - 6.5 months	Droplets, contact, endogenous	Pharyngitis ("strep throat"), surgical wound infection	Oropharyngeal swab, wound exudate	Surgical masks in the operating room

Bacteria	Multidrug resistant strains (MDR)	Habitat	Survival in the environment (dry surfaces)*	Transmission in healthcare	Healthcare - associated infections	Specimens for diagnosis of infection/ colonisation	Main preventive measures
Vibrio cholerae		Gastrointestinal tract of humans; water	1 – 7 days	Faecal-oral, water, raw seafood	Cholera	Stool	Safe water and food
Yersinia enterocolitica		Gastrointestinal flora of many animals, causes diarrhoea in young animals; rarely - humans as carriers	ND	Blood transfusion in hospitals; faecal-oral in the community	Bacteremia connected to blood transfusion; (diarrhoea in the community)	Blood, stool	Safe blood products

* Survival is better if conditions are humid for most organisms (exception being Staphylococcus aureus), if microorganism is in biological material (blood, faeces, wound exudate), if the temperature is lower, and if bacteria are in higher numbers

** whenever indirect contact is involved, it is most frequently by hands of healthcare workers

ND = not done
NA = not applicable
UTI = urinary tract infection
CSF = cerebrospinal fluid

Fungi

Fungi are unicellular (yeasts) or multicellular (moulds) microorganisms that are widespread in nature. Their cell is so-called "eukaryotic" that means they have DNA packed in the nucleus, like plants and animals. Their chromosome is diploid, so the variations in genome are not as easily expressed phenotypically as in bacteria. Some yeast is part of the normal flora in humans, while moulds are usually living free in nature.

Yeasts multiply by budding a new cell from the mother cell (blast conidia), while moulds multiply both asexually (conidia) and sexually (spores). It is important to remember that fungal spores are not as resistant as bacterial spores. Growth on a solid surface will lead to the formation of colonies. Some pathogenic fungi can live as a yeast (in the host) and as a mould (in the environment); they are called dimorphic fungi.

Table 7.2 outlines the main groups of fungi that can cause HAIs with their usual habitat, survival in the environment, mode of transmission, infections they cause, and main HAI prevention methods.

Viruses

Viruses are the smallest infectious agents, however they require living cells (bacterial, plant, or animal) for reproduction. Outside a living cell, viruses can survive, but not multiply. They consist of either DNA or RNA, protected by a protein coat; some viruses also have a lipid envelope outside the protein coat.

When a virus enters a host cell, viral nucleic acid (NA) makes the cell synthesise viral proteins and NA. It then assembles and exits the host cell to enter other host cells. During this process, host cells are damaged or destroyed and signs and symptoms of infectious disease appear. Infection can also be asymptomatic. Some viruses can incorporate their DNA into the host DNA, or can live in host cells causing no harm – these latent infections sometimes become re-activated.

Table 7.3 outlines the main groups of viruses that can cause HAIs with their usual habitat, survival in the environment, mode of transmission, infections they cause, and main HAI prevention methods.

Prions

Prions are protein particles; they do not contain any NA. They are known to be connected with some neurological diseases (Creutzfeldt-Jakob disease – familial spongiform encephalopathy; variant Creutzfeldt-Jakob disease – bovine spongiform encephalopathy, and some other diseases). Prions are highly resistant to the usual methods of disinfection and even sterilisation. There is a possibility of iatrogenic transmission of these diseases through transplantation, or contamination of instruments with brain tissue, dura mater, or cerebrospinal fluid of diseased persons.

Parasites

Parasites include protozoa, i.e., unicellular microorganisms with a eukaryotic diploid nucleus that can live free in nature, and/or in animal hosts including humans. Some of them cause infections. There are also helminths (worms) that cause infections – known as infestations. Although many parasites are widespread in the world and cause some of the most important community-acquired infections (malaria, ascaridosis, etc.), not many cause HAIs.

Table 7.4 outlines the main groups of parasites that can cause HAIs with their usual habitat, survival in the environment, mode of transmission, infections they cause, and main HAI prevention methods. There is a group of animals – insects and arthropods – that transmit microbes (viruses, bacteria, parasites) between humans or between animals and humans. Some of them can also cause disease in humans. One such arthropod is *Sarcoptes scabiei* causing scabies in humans. Scabies is a highly contagious skin disease that can be spread rapidly in a health care institution unless very vigorous containment measures are instituted.

Role of the Microbiology Laboratory

The diagnosis of infections performed by the microbiology laboratory has two important functions. The first is clinical - everyday management of infections. The second is epidemiological - knowledge of an infective microbe in a patient can lead to finding its source and route of transmission. This allows staff to stop infections from spreading. Furthermore, the microbiology laboratory interprets microbiology data for clinicians and for infection prevention and control (IPC) professionals, thus participating in healthcare worker (HCW) education and the facility's antibiotic policy.

Table 7.2. Characteristics of main groups of fungi potentially causing healthcare-associated infections

Fungi	Habitat	Survival in the environment (dry surfaces)*	Transmission in healthcare	Healthcare - associated infections	Specimens for diagnosis of infection / colonisation	Main preventive measures
Candida albicans (yeast)	Soil, animals, humans, inanimate objects	1 - 120 days	Direct and indirect contact, endogenous	Different opportunistic infections	Different specimens depending on infection	Clean hands, clean equipment
Candida glabrata (yeast)	Soil, animals, humans, inanimate objects	120 - 150 days	Direct and indirect contact, endogenous	Different opportunistic infections	Different specimens depending on infection	Clean hands, clean equipment
Candida parapsilosis (yeast)	Soil, animals, humans, inanimate objects	14 days	Direct and indirect contact, endogenous	Different opportunistic infections	Different specimens depending on infection	Clean hands, clean equipment

Fungi	Habitat	Survival in the environment (dry surfaces)*	Transmission in healthcare	Healthcare - associated infections	Specimens for diagnosis of infection / colonisation	Main preventive measures
Aspergillus species (mold)	Ubiquitous in soil, water, food, decaying material, outdoor and indoor air	Conidia and spores are resistant forms	Inhalation, (contact)	Pneumonia, disseminated infections in severely immunocompromised patients	Sputum, different other specimens depending on infection	Reverse/protective isolation of susceptible patients
Mucor (mold)	Soil, plants, fruits, animal excreta, food	Conidia and spores are resistant forms	Inhalation	Different opportunistic infections in immunocompr-omised patients (zygomycosis)	Different specimens depending on infection	Reverse/protective isolation of susceptible patients; safe food and drinks
Rhizopus (mold)	Soil, plants, fruits, animal excreta, food	Conidia and spores are resistant forms	Inhalation	Different opportunistic infections in immunocompr-omised patients (zygomycosis)	Different specimens depending on infection	Reverse/protective isolation of susceptible patient; safe food and drinks

* Survival is better at low temperature, high humidity, and presence of serum or albumin

Table 7.3. Characteristics of main groups of viruses potentially causing heathcare-associated infections

Virus	Habitat	Survival in the environment (dry surfaces)*	Transmission in healthcare	Healthcare - associated infections	Specimens for diagnosis of infection **	Main preventive measures
Adenovirus Several types	Humans, water, fomites (e.g., ophthalmological equipment and solutions), environment	7 days – 3 months	Direct and indirect contact	Eye infections, respiratory infections	Serum sample	Individual eye drops
Coronavirus, including severe acute respiratory syndrome (SARS) virus	Humans	3 hours SARS virus: 72-96 hours	Droplets	Respiratory infections	Serum sample	Source isolation, clean environment, clean hands
Coxackie B virus	Humans	>2 weeks	Faecal-oral; direct and indirect contact	Generalised disease of newborn	Serum sample	Clean hands, clean environment
Cytomegalovirus	Humans	8 hours	Blood products, tissue and organs for transplantation; mucosal contact with secretions and excretions	Huge range of different diseases	Serum sample	Safe blood products and tissues/organs for transplantation

Virus	Habitat	Survival in the environment (dry surfaces)*	Transmission in healthcare	Healthcare - associated infections	Specimens for diagnosis of infection **	Main preventive measures
Hepatitis A virus	Humans	2 hours – 60 days	Faecal-oral	Hepatitis A	Serum sample	Clean hands, clean environment, safe food and water
Hepatitis B virus	Humans	>1 week	Blood, bodily fluids, tissue and organs for transplantation	Hepatitis B	Serum sample	Safe blood products and tissues/organs for transplantation
Hepatitis C virus	Humans	NA	Blood, bodily fluids, tissue and organs for transplantation	Hepatitis C	Serum sample	Safe blood products and tissues/organs for transplantation
Herpes simplex virus	Humans	4.5 hours – 8 weeks	Droplets, close contact	Different mucosal and skin infections	Serum sample	If infected, HCW should not care for susceptible persons (newborn, immunocompromised)
Human immuno-deficiency virus	Humans	>7 days	Blood, bodily fluids, tissue and organs for transplantation	Acquired immune deficiency syndrome	Serum sample	Safe blood products and tissues/organs for transplantation

Virus	Habitat	Survival in the environment (dry surfaces)*	Transmission in healthcare	Healthcare - associated infections	Specimens for diagnosis of infection **	Main preventive measures
Influenza virus	Humans	1-2 days	Droplets, direct and indirect contact, HCW as symptomatic or asymptomatic	Influenza	Serum sample	Source isolation; HCW vaccination
Norovirus	Humans	8 hours – 7 days	Faecal-oral, direct and indirect contact, aerosols from vomitus	Diarrhoea	Stool	Clean hands, clean environment, safe food
Respiratory syncytial virus	Humans	Up to 6 hours	Droplets, direct and indirect contact	Acute respiratory infections in young children	Nasopharyngeal exudate	Source isolation, clean hands, clean environment
Rotavirus		6-60 days	Faecal-oral, direct and indirect contact	Diarrhoea	Stool	Clean hands, clean environment
Rubula virus (mumps)	Humans	ND	Droplets	Mumps (parotitis)	Serum sample	Source isolation, vaccination

Virus	Habitat	Survival in the environment (dry surfaces)*	Transmission in healthcare	Healthcare - associated infections	Specimens for diagnosis of infection **	Main preventive measures
Rubivirus (rubella)	Humans	ND	Droplets	Rubella (German measles)	Serum sample	Source isolation, vaccination
Morbillivirus (measles)	Humans	ND	Droplets	Measles	Serum sample	Source isolation, vaccination
Varicella-zoster virus	Humans	ND	Droplets, close contact	Varicella	Serum sample	Source isolation, HCW vaccination

* Survival is better at low temperature, presence of biological material and if viruses are in higher numbers

** Diagnosis is mostly done by serology. If laboratory can perform direct diagnostics, it will be by antigen detection or nucleic acid detection in the sample from infectious site

NA = not applicable
ND = not done
HCW = healthcare worker

Clinical role

Some infections must be diagnosed clinically and treated empirically (acute meningitis, sepsis, or severe pneumonia), without previous isolation of causative microorganisms or determination of antibiotic susceptibility. However, if there is a clinical suspicion of infection, laboratory tests may confirm the diagnosis and suggest the correct treatment (especially as most HAIs are caused by bacteria and fungi that are more antibiotic resistant than community-acquired pathogens). Targeted antimicrobial therapy leads to better patient outcomes, and as eradication of a pathogen is achieved earlier, the danger of transmission to other patients will be decreased.

Microbiology is becoming more important in clinical medicine and in the prevention of HAIs, especially as new or antibiotic-resistant pathogens emerge, and new diagnostic technologies are developed. The microbiology laboratory should be able to diagnose the commonest infectious agents, especially those causing HAIs, and determine susceptibility to antibiotics for bacteria and fungi (See Tables 7.1 and 7.2).

The right specimens from appropriate sites must be taken using proper techniques (See Tables 7.1-7.4). Specimens should be sent to the laboratory as quickly as possible. Microbiology laboratory staff can assist in ensuring good specimens by educating other staff. Identification of the microorganism and its antibiotic susceptibility should be as precise as possible (identification to the species level).

Microbiological diagnostic methods can be divided into direct methods (smear from specimens, isolation of infectious agents on culture media, or evaluation of microbial antigens or NAs in a specimen) and indirect methods – evaluation of an immune response by the patient to the infectious agent (serology). The latter is usually used for diagnosis of difficult to isolate bacteria and most viruses; however antibodies take at least 10-14 days to develop. Therefore, serology is mostly an epidemiological method, with the clear exception for some viral diseases where a diagnosis of acute infection can be based on immunoglobulin class M, or avidity of class G, or a combination of antibodies to different viral antigens.

An important new technology in microbiology is molecular diagnostics. Diagnosis can be rapid because it does not require microbial growth in

cultures. It is sensitive, as it can detect a small number of microorganisms. It is specific, as it detects microbe-specific genes. However expensive machines and reagents are required – beyond the reach of many laboratories.

Infection prevention and control role[8-12]

The microbiology laboratory has many roles in the control of HAIs: outbreak management, performing additional epidemiological tests, bacterial and fungal typing, HAI surveillance, and reports about new alert microbes or unusual resistance to antimicrobials. In some countries, the microbiology laboratory is responsible for reporting infections to public health departments.

The laboratory can educate both clinical and IPC personnel about microorganisms and their role in infections, especially in HAIs. Furthermore, daily communication of laboratory staff with the Infection Control Team (ICT) is vital, allowing for timely and rapid information transmission about causative agents of HAIs. The clinical microbiologist should ideally be a member of the Infection Control and Antibiotic Committees and a member of the ICT.

Outbreak investigation

Sometimes the ICT requires additional data to clarify endemic or epidemic situations. Microbiological tests of blood products, environmental surfaces, disinfectants and antiseptics, air, water, hands of personnel, anterior nares of personnel, etc., may be necessary. During an outbreak or in endemic situations when the causative agent is known, the microbiology laboratory can use selective media for the agent in question to minimise expense. To determine the cause of a single-source outbreak, the causative microorganism must be defined.

Typing of bacteria and fungi

Microorganism typing determines whether two epidemiologically connected strains are really related or differ from strains that are not epidemiologically connected. If strains are unrelated, the patients do not belong to the same outbreak. If strains are related it is impossible to say that the patients are involved in an outbreak without epidemiological analysis. So, epidemiology and typing are complementary.

Typing methods differ in several important points:

1. Typability, i.e., the method can type most or even all strains of the same species;
2. Discriminatory power, i.e., the method can differentiate well between different types;
3. Inter-laboratory and intra-laboratory reproducibility, i.e., the method can provide the same typing results in repeated testing on different sites or in different times; and
4. The method should be simple, unambiguous to interpret, and inexpensive.

There are two groups of typing methods: phenotyping and genotyping.

Phenotyping

Phenotyping methods can determine characteristics that differ between different strains of the same species. These methods may be based on antigenic structure (serotyping), physiologic properties/metabolic reactions (biotyping), susceptibility to antimicrobial agents (resistotyping), colicines (colicinotyping), or bacteriophages (phage typing).

Phenotyping methods are well standardised with high reproducibility. Discriminatory power is not always high (if only a few types exist), but can be very high (if many types exist). They are simple and unambiguous to interpret. Many are cheap enough to be performed in every microbiology laboratory.

The main objection to phenotyping is that bacterial genes are not always expressed. Two phenotypically different strains can actually have the same genetic background or two phenotypically identical strains can actually differ genetically. Sometimes the emergence of a particular phenotype is specific enough to explain an outbreak. However, if a phenotype is widespread and frequent, genotyping will be required for outbreak management.

Genotyping

Molecular techniques have revolutionised the potential of the microbiology laboratory because they have very high typability and discriminatory power. Genotyping can demonstrate definitively the relatedness or difference between two isolates of the same species. However, genotyping

methods require sophisticated and expensive equipment and materials and trained staff. Furthermore, some tests have a low reproducibility, especially in inter-laboratory comparisons. Result interpretation is neither always simple nor unambiguous.

Role in the surveillance of HAIs

The microbiology laboratory should produce routine reports of bacterial isolates to allow the ICT to make incidence graphs for specific pathogens, wards, and groups of patients. These data can be made available immediately if the laboratory is computerised. A 'baseline incidence' can be established and any new isolates can then be compared to it. Graphs enable the ICT to discover the beginning of an outbreak earlier than it can be discovered clinically. Periodic reports are also important because they demonstrate trends of specific pathogens, and can be very useful in planning preventive measures.

Alert organism reports

The early isolation of a new or unusual microorganism, without any further typing, enables the ICT to take appropriate measures to stop it from spreading. The ICT should identify, together with laboratory personnel, possible 'alert' microorganisms, such as multiresistant or highly pathogenic microorganisms (methicillin-resistant *S. aureus*, vancomycin-resistant *S. aureus*, vancomycin-resistant Enterococcus, multidrug-resistant [MDR] *P.aeruginosa*, MDR *A.baumannii*, MDR *M.tuberculosis*, *C.difficile*, etc.). Any new isolates should be reported immediately to the wards and the ICT. Alert organism surveillance may be all that can be performed if the facility is understaffed. In addition, laboratory staff may report clustering of infections (two related isolates in different patients in the same time frame).

Interpreting microbiology data

Microbiologists must interpret microbiological data (results of isolation, identification, susceptibility tests, serology, typing). To interpret microbiological data for an individual patient, one should first make sure the specimen was correct. Is the microorganism concerned a primary or opportunistic pathogen? What is the clinical diagnosis? And lastly, what was the immune state of the patient at the time of specimen collection?

It is relatively easy to interpret the results of specimens from normally sterile sites (blood, cerebrospinal fluid, biopsy materials, and urine); however it is

harder to interpret results from non-sterile samples (respiratory specimens, wound exudates, etc.). As the result comes often after antibiotic treatment has already begun, has the patient reacted or not reacted to the antibiotic? Do other laboratory and/or imaging results affect the diagnosis?

To interpret microbiological data for IPC purposes, the relevant specimens are needed, either from the patient, healthy contacts, or the environment. A microbiologist who knows the normal colonising flora of humans, pathogenesis of infections (incubation period, inoculum size, kind of vehicle), and the characteristics of specific pathogens (natural habitat, resistance to drying, to disinfectants, and to antibiotics) – can then interpret laboratory data for the ICT. In a more complicated outbreak or endemic situation, besides good microbiology (especially typing), there is a clear need for an epidemiologist to interpret microbiological data.

Ideally the microbiologist should be a medical doctor specialising in clinical microbiology. If this is not possible, then a properly educated scientist is required.

Antibiotic policy

Determining antibiotic susceptibility patterns for microorganisms causing HAIs is vital for individual patient care. It can also help in planning antibiotic policy and designing the local antibiotic formulary. The microbiology laboratory should only report antibiotics contained in the formulary. Periodic resistance reports should be provided for specific wards and for the whole institution broken down by pathogen species and infection site. These reports should be available for every physician who prescribes antibiotics. These reports are very important for the design of empirical therapy.

Infection Prevention and Control in the Laboratory

All laboratory staff may be exposed to viruses that are spread through blood and bodily fluids (human immunodeficiency virus [HIV], hepatitis B virus [HBV], hepatitis C virus [HCV]). Laboratory workers must take preventive measures against those viruses.

The clinical microbiology laboratory is usually at biosafety level 2. This means that staff work with well-characterised agents that only pose a

moderate potential hazard to personnel and the environment. Laboratory access should be limited to the people working in it; staff should take precautions for handling biological specimens and microbial cultures (hand hygiene, disinfection of the environment, specific precautions with sharps, and use of biological safety cabinets if aerosols are a risk).

If *Mycobacterium tuberculosis* or *Legionella pneumophila* are expected, diagnostic tests should be performed in a biosafety level 3 facilities (for agents which may cause serious or potentially lethal disease in healthy adults after inhalation, but for which vaccine or other treatment exists). If this is not possible, and a level 2 laboratory is used, it should be secured; the room should have negative air pressure and the exhaust air should be filtered and discharged outdoors. The laboratory workers should be properly educated and follow all recommended practices for biosafety level 3 rigorously.

Microbiology Diagnostics in Low Resource Settings

The main problem for microbiological diagnostics in low resource countries is the lack of microbiology laboratories; they are usually sited in major urban areas. Therefore, it is very important to have point-of-care microbiological tests that are sensitive and specific, rapid, easy for HCWs that have no specific education in laboratory procedures to perform without special equipment, unambiguous to interpret, and affordable. Several such tests are already in use (for malaria, HIV serology), however more are needed. Especially important tests from the point of view of HAI prevention and control would be tests for diagnosing tuberculosis and for identifying multidrug resistant strains to stop their spread.

Minimal Requirements for Microbiology Laboratories in the Control of HAIs

1. Should be sited inside the health care facility; if this is not possible, then negotiate a contract for diagnostic microbiology with the nearest laboratory.
2. Should be available every day, including Sundays and holidays, ideally on a 24-hour basis. Gram stain should be available on a 24-hour basis.
3. Should be able to examine blood, cerebrospinal fluid, urine, stool, wound exudates or swab, respiratory secretions, and perform serological tests (HIV, HBV, HCV).

4. Should be able to identify common bacteria and fungi that can cause HAI to species level (*Staphylococcus aureus, Escherichia coli*, *Salmonella*, *Shigella*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus pyogenes* [Group A *streptococci*], *Streptococcus agalactiae* [Group B *streptococci*], *enterococci*, *Campylobacter jejuni/coli*, other enterobacteria, *Neisseria meningitidis*, *Candida albicans*, aspergilli, etc.), besides other common microorganisms that cause severe community-acquired infections (*Streptococcus pneumoniae*, *Neisseria gonorrhoeae*, *Vibrio cholerae*, *Corynebacterium diphtheriae*).
5. Should be able to perform susceptibility testing to relevant antibiotics using disc-diffusion methodology.
6. Should be able to perform basic typing - serotyping (for salmonella, shigella, *P. aeruginosa*, *N. meningitidis*) and biotyping (e.g., for *S. typhi*).
7. Should have quality assurance procedures (both internal quality control and external quality control [national or international]).
8. Should have a clinical microbiologist (if possible a medical doctor) who has good skills of communication with clinical and ICT staff.
9. May have the ability to perform simpler genotyping methods or access to genotyping methods centrally at state or regional laboratories. The central laboratory can then assist with epidemiological investigations of HAIs.

References

1. K. Brooks, *Ready Reference to microbes*, 2nd edn, Association for Professionals in Infection Control and Epidemiology, Washington, DC, 2007.
2. Diekema DJ, Pfaller MA. Infection Control Epidemiology and Microbiology Laboratory. In *Manual of Clinical Microbiology*, 9th Ed., Murray PR, Editor in Chief, ASM Press, Washington, DC, 2007:118-128.
3. Gastmeier P, Schwab F, Baerwolff S, Rueden H, Grundmann H. Correlation between the genetic diversity of nosocomial pathogens and their survival time in intensive care units. *J Hosp Infect* 2006;62:181-186
4. Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infect Dis* 2006; 6:130. http://www.biomedcentral.com/1471-2334/6/130 [Accessed July 19, 2011]

5. Murray PR, Witebsky FG. The clinician and the Microbiology Laboratory. In: *Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases*, 7th Ed., Mandell GL, Bennett JE, Dolin R, editors, Elsevier, Philadelphia, PA, 2010:233-265.
6. Peeling RW, Mabey D. Point-of-care tests for diagnosing infections in the developing world. *CMI* 2010; 16(8):1062-1069.
7. Pereira-Neves A, Benchimol *M.Trichomonas vaginalis*: in vitro survival in swimming pool water samples. Exp Parasitol. 2008; 118(3):438-41.
8. Peterson LR, Hamilton JD, Baron EJ, et al. Role of clinical microbiology laboratory in the management and control of infectious diseases and the delivery of health care. *Clin Infect Dis* 2001; 32:605-611.
9. Poutanen SM, Tompkins LS. Molecular Methods in Nosocomial Epidemiology. In: *Prevention and Control of Nosocomial Infections*, 4th Ed., Wenzel RP, Editor, Lippincott, Williams & Wilkins, Philadelphia, 2003: 481-499.
10. *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed. ed. 2009. http://www.cdc.gov/biosafety/publications/bmbl5/index.htm [Accessed July 19, 2011]
11. Soll DR, Pujol C, Lockhart SR. Laboratory procedures for the epidemiological analysis of microorganisms. In: *Manual of Clinical Microbiology*, 9th Ed., Murray PR, Editor in Chief, ASM Press, Washington, DC, 2007:129-151.
12. Stratton CW IV, Greene JN. Role of the Microbiology Laboratory in Hospital Epidemiology and Infection Control. In: *Hospital Epidemiology and Infection Control*, 3rd Ed., Mayhall CG, editor, Lippincott, Williams & Wilkins, Philadelphia, PA. 2004:1809-1825.

Further Reading

Monica Cheesbrough. *District Laboratory Practise in Tropical Countries*. Part 2, 2nd edition. Cambridge University Press, 2006.

Table 7.4. Characteristics of main groups of parasites potentially causing healthcare-associated infections

Parasite	Habitat	Survival in the environment	Transmission in healthcare	Healthcare - associated infections	Specimens for diagnosis of infection	Main prevention methods
Cryptosporidium (protozoa)		2 hours on dry surface				
Plasmodium species (protozoa)	Liver, erythrocytes of diseased person	NA	Mosquito-borne in the community; infected blood	Malaria	Blood	Safe blood products
Trichomonas vaginalis (protozoa)	Vaginal mucosa	Several hours in humid environment	Sexualy transmitted in the community; contaminated medical equipment in gynecology	Vaginal infection in women	Vaginal discharge	Disinfected/sterilised medical equipment in gynecology
Enterobius vermicularis (helminth)	Intestinal tract of diseased person	Eggs: at least 1 year	Faecal-oral, ingestion of parasite eggs that can contaminate environment	Enterobiasis	Perianal tape	Clean environment (changing linen without creating aerosols), clean hands

*NA = not applicable