Chapter 7

The Role of the Microbiology Laboratory

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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. • An important innovation in microbiological diagnostics is development of point-of-care tests. This can help overcome the issue of scarce microbiology laboratories in developing countries. • The microbiology laboratory is an essential part of effective infection prevention and control (IPC). The microbiology laboratory should be able to determine the most frequent microbes causing healthcare-associated infections, and perform at least some basic typing of microorganisms for epidemiologic evaluations. • The microbiology laboratory should produce routine reports for IPC personnel to develop incidence graphs for specific pathogens, antibiotic resistance, wards, and groups of patients. • Microbiologists, understanding the role of normal colonising flora of humans, the pathogenesis of infections, and the characteristics of specific pathogens, can interpret microbiological findings for IPC personnel.

Basics of microbiology

Microbes are living organisms that are not visible to the naked eye. They are divided into bacteria, fungi, viruses, prions, and protozoa. Macroscopic parasites are also included in the group. Microbes are ubiquitous in nature, living as free organisms in the environment or on/in plants, animals, and humans, either as normal flora (not causing harming) or as pathogenic microbes (causing diseases). While some microbes are confined to only one host, most microbes can live on/in a wide array of hosts in nature. Plant microbes do not cause disease in humans, however some animal microbes can cause disease in humans (so called zoonotic diseases).

When a microbe encounters a new host and begins 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 harm and disease, the disease is called an infectious disease (infection). Microbes that usually cause disease in a susceptible host are called primary pathogenic microbes. Microbes that live as normal flora of humans or live in the environment and do not cause disease in an otherwise healthy host are called opportunistic pathogens (can cause disease in an immunocompromised host). When we encounter unusual microbes on skin or non-living surfaces/items, it is called contamination.

Infection can be asymptomatic or symptomatic. During asymptomatic infections, as well as during the incubation period in symptomatic infections, microbes can be shed from an infected host; the host is infectious but may not realise it. After an infection, microbes can be present for some time in the host and can be further released, although the person is clinically completely healthy. This state is referred to as a "carrier state" and such persons are called "carriers".

If infection is caused by microbes that are part of normal flora, we call it endogenous infection; exogenous infection is an infection caused by microbes that are not part of the normal flora of that person. Microbes are transmitted from one host to another by a number of pathways: through air, water, food, live vectors, such as mosquitos, indirect contact with contaminated items or surfaces, or direct contact of different hosts, including hands of healthcare workers (HCW).

To cause an infectious disease, a microbe first must enter the human body (portal of entry), either through respiratory, gastrointestinal, or genitourinary tracts, or through damaged or even intact skin. The microbe usually multiplies at the site of entry, then enters through mucous membranes to tissue and sometimes to blood. When in the bloodstream, the microbe can spread throughout the body and enter any susceptible organ. After multiplication, microbes usually leave the body (portal of exit) either through respiratory, gastrointestinal, or genitourinary discharges and seeks a new host. Some microbes are transmitted with the help of insect vectors that feed on human blood. Knowing the path of disease development is essential for a clinical diagnosis. It is also important for timing and ordering the right specimen for microbiological diagnosis, as well as for using the correct measures to prevent spread of microbes. Recognition of who might get sick from a certain microbe (susceptible host) also helps with the prevention of disease transmission.

Bacteria

Bacteria are the smallest unicellular organisms with all functions of a living organism. They multiply by simple division from one mother cell to two daughter cells. When multiplying on a nutritious solid surface in the laboratory, after some time, they form so called "colonies" that are visible to the naked eye, representing offspring of the same bacteria.

The genetic material (deoxyribonucleic acid or 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 and horizontally between different bacteria. This has important consequences, especially when antibiotic resistance genes are transferred.

Most bacteria are very easily adaptable to any kind of environment. All pathogenic and most opportunistic bacteria have many virulence factors that are important in the development of infectious diseases. Some bacteria can sporulate 'form spores' – the most resistant form of life we know – if the conditions for the vegetative form is detrimental. When conditions are again favourable, the vegetative forms develop from the spore.

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

Fungi

Fungi are unicellular (yeasts) or multicellular (moulds) microorganisms that are widespread in nature. Their cell is so-called "eucaryotic", meaning they have DNA packed in the nucleus, as any other biological cell in plants and animals. Their chromosome is diploid, so the variations in genome will not be as easily expressed phenotypically as in bacteria. Some species of yeast are 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 (blastoconidia), while moulds multiply 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 a colony as for bacteria. Some pathogenic fungi can live as yeast (in the host) and as a mould form (in the environment) so they are called dimorphic fungi.

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

Viruses

Viruses are the smallest particles – not cells – capable of reproducing themselves in living cells, either bacterial, plant, or animal cells. Outside a living cell viruses can survive, however they cannot multiply. They consist of one kind of nucleic acid (NA), either DNA or ribonucleic acid (RNA), and a protein coat that protects the NA. Some viruses also have a lipid envelope outside the protein coat, referred to as enveloped viruses; others do not have a lipid envelope (non-enveloped viruses).

A virus enters the host cell and the viral NA then takes over the host cell to synthesise viral proteins and NA. It assembles these into a new virus and exits the host cell to enter other host cells. During this process, host cells are damaged or even destroyed and signs and symptoms of infectious disease appear; infection can be asymptomatic in a portion of the infected population. Some viruses can incorporate their DNA into the host DNA, or can live in some host cells causing no harm – these are called latent infections that can become overt in some circumstances, depending on the virus.

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

Prions

Prions are protein particles that do not contain any NA (neither DNA nor RNA). 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, cerebrospinal fluid, or blood of a diseased person. Prion diseases are not transmitted from diseased to healthy persons by contact; transmission to a HCW has never been described.

Parasites

Parasites are either 1) microscopic protozoa, i.e., unicellular microorganisms with eucaryotic diploid nucleus that can live free in nature and/or live in some animal host including humans, some of them causing infections or 2) they are macroscopic organisms, such as helminths (worms) (endo-parasites) or lice (exo-parasites) that can cause infections – known also as infestations.

Although many parasites are widespread in the world and cause some of the most important community-acquired infections (malaria, ascariasis, etc), not many parasites cause HAIs. Table 7.4 outlines the main groups of parasites that can cause HAI with their usual habitat, survival in the environment, mode of transmission, infections they cause, and main HAI prevention methods.

Arthropods

Arthropods are a large and very diverse group of animals. They comprise insects, ticks, mites, and some other groups. Arthropods are very important as vectors of microbes (viruses, bacteria, protozoa, and helminths) both between humans and between animals and humans. Some of them can also cause a disease in humans called ectoparasitoses, as they only cause skin disease; these arthropods include *Sarcoptes scabiei* (scab-mite) causing scabies and human lice causing pediculosis.

Scabies is a highly contagious skin disease that can be spread rapidly in a health care institution unless very vigorous containment measures are instituted. The habitat of the scab-mite is only human skin; however it can survive in clothing and bedding for several days. The primary method of transmission in health care settings is by direct contact with the skin of an infested person; however transmission through clothing and bedding can also occur. The primary preventive measure is use of Contact Precautions (isolation/cohorting) in addition to simultaneous specific treatment of all cases and exposed persons in a ward. Environmental cleaning and disinfection and processing of clothing and bedding as infectious items are also necessary.

Another arthropod that can be transmitted in a health care institution is the louse. There are three types of human lice: head, pubic, and body. Head and pubic lice live on hair and body lice live on clothing (only contacting the body during feeding). Human lice survive a short time in the environment (<3 days for a body louse). Lice are transmitted from person to person by close contact, therefore preventive measures include Contact Precautions, bathing of patients, processing clothing and bedding as infectious items, and specific treatment for head and pubic pediculosis. Generally only head lice are important in health care institutions, specifically in paediatric wards.

Many arthropods can bite humans and provoke an allergic reaction to the bite. In the past decades one of them, the bed bug (*Cimex lectularius*), has resurged in the developed world, including healthcare facilities. Bed bugs do not live on humans, they live in the environment. However, it feeds on human blood; this leads to an allergic reaction on the skin. As bed bugs do not live on humans, the primary prevention method is good hygiene and pest control, including vacuuming, heat or cold treatment of the environment, trapping devices, and pesticides.

The role of the microbiology laboratory

The isolation and characterisation of microorganisms causing 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 IPC professionals, thus participating in HCW education. The microbiology laboratory also participates in the development of an institution's antibiotic policy.

Clinical role

Some infections must be quickly diagnosed clinically and treated empirically without knowledge of the causative microorganism or determination of antibiotic susceptibility (e.g., acute meningitis, sepsis, or severe pneumonia). However, if there is a clinical suspicion of infection, laboratory tests may confirm the diagnosis. Most HAIs are caused by bacteria and fungi that can be more antibiotic resistant than community-acquired pathogens or their susceptibility to antibiotics is less predictable. Etiological diagnosis of HAIs is therefore exceptionally important for targeted antimicrobial chemotherapy.

The microbiology laboratory 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 most common infectious agents, especially those causing HAIs. The laboratory should also be able to determine susceptibility to antibiotics for bacteria and fungi (See Tables 7.1.-7.4.). Targeted antimicrobial therapy will lead to better patient outcomes, and as eradication of a pathogen is achieved earlier, the danger of transmission to other patients will be decreased.

The right specimens from appropriate sites must be taken using proper techniques (See Tables 7.1.-7.4.). The microbiology laboratory staff can assist in ensuring good specimens by educating other staff in proper collection techniques. 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 two types:

1. Direct methods (smear from specimens, isolation of infectious agents on culture media, or proof of microbial antigens or nucleic acids in the specimen).
2. Indirect methods – proof of immune response to the infectious agents (serology).

Indirect methods are usually used for diagnosis of difficult to isolate bacteria and most viruses. It has to be recognised that serologic testing is the confirmation of a diagnosis, not the diagnosis itself. For many microorganisms antibodies take several days to develop, in many cases at least 10-14 days. Serology is mostly considered an epidemiological method, with the clear exception of some viral diseases where it is possible to make a diagnosis of an acute infection based on immunoglobulin class M (IgM), or avidity of class G (IgG), or a combination of antibodies to different viral antigens.

An important new technology in microbiology is molecular diagnostics. Diagnosis can be rapid as it is not dependent on waiting for microbial growth in cultures. These tests are sensitive, as they are based on detection of only a few microorganisms; and they are specific, detecting microbe-specific genes.

Infection control role

The microbiology laboratory is an essential part of an effective IPC program and has many roles in the control of HAIs: outbreak management, performing additional tests for epidemiologic studies, bacterial and fungal typing, HAI surveillance, and reports about new alert microbes or unusual resistance. All these functions enable the microbiological laboratory to educate not only clinical but also IPC personnel about microorganisms and their role in infection and specifically in HAI. Furthermore, daily communication between laboratory staff and the Infection Control Team (ICT) is vital, allowing for timely and rapid information sharing about causative agents of HAIs. The clinical microbiologist should ideally be a member of the Infection Control and Antibiotic Stewardship Committees and a member of the ICT.

Additional tests during 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 required. During an outbreak or in endemic situations when the causative agent is known, the microbiology laboratory may be able to use selective media for the agent in question to minimise expense and increase the sensitivity of the cultures.

To determine the cause of a single-source outbreak, the causative microorganism must be defined. A microbial species may contain subspecies and variants that differ in particular characteristics. For example, individual bacteria from the same species can differ as much as 30% in their genomes. Genetic differences are often phenotypically expressed; however this is not a rule.

Typing of bacteria and fungi

Microorganism typing determines whether two epidemiologically connected strains are really related or whether they 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 state that the patients are involved in an outbreak without epidemiological analysis. Therefore, 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 by different sites or in different times;
4. The method should be simple, unambiguous to interpret, and inexpensive.

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

Phenotyping

Using phenotyping methods we can determine physical characteristics that can distinguish different strains of the same species. These methods may be based on antigenic structure (serotyping), physiologic properties/metabolic reactions (biotyping), susceptibility to antimicrobial agents (sensitivity testing), and colicines (colicino-typing) or bacteriophages (phage typing).

Phenotyping methods are well standardised with high reproducibility. Discriminatory power is not always high if only a few types exist; however it can be very high if many types exist. These tests are simple and unambiguous to interpret. Many are inexpensive enough to be performed in every microbiology laboratory.

The main objection to phenotyping of bacteria 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 with trained staff. Furthermore, some techniques have a low reproducibility, especially in inter-laboratory comparisons. Result interpretation is not always simple or unambiguous.

Role in the surveillance of HAIs

The microbiology laboratory should produce routine periodic reports of isolates to allow the ICT to develop 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 with this incidence. 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 multi-resistant or highly pathogenic microorganisms (e.g., methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin intermediate *Staphylococcus aureus* (VISA), vancomycin resistant enterococci *(*VRE), multidrug resistant (MDR) *P.aeruginosa*, MDR *A.baumannii*, MDR *M.tuberculosis*, *C.difficile,* extended spectrum beta-lactamase (ESBL) Enterobacteria, carbapenem-resistant Enterobacteria (CRE), *E.coli* 0127, *Legionella* spp, penicillin resistant (PR) *Streptococcus pneumoniae,* 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 are required to interpret microbiological data (e.g., results of isolation, identification, susceptibility tests, serology, and typing). To interpret microbiological data for infection in an individual patient, one should first determine if the specimen was correct. The next step is to take into account if the isolated or otherwise proven microorganism is a primary or an opportunistic pathogen. Another factor is the clinical suspicion or working diagnosis. Lastly, understand the actual immune status of the patient at the time of specimen collection.

It is relatively easy to interpret the results of so-called primary sterile specimens (e.g., blood, cerebral spinal fluid, or biopsy materials). It is more difficult to interpret results of non-sterile samples (e.g., different swabs, respiratory specimens, or wound exudates) or specimens that are easy to contaminate (urine). The microbiology laboratory result often is known after antibiotic treatment has already begun, and the patient has improved or not improved in response to the antibiotic. Therefore the concordance of the microbiological result with the patient's course is another important factor in interpretation of the microbiological result if dealing with opportunistic microorganisms. It is also important to look for concordance with other laboratory and/or imaging results of the patient.

To interpret microbiological data for IPC purposes, the first issue is always to determine if the specimen was correct, either from a patient, healthy contacts, or the environment. Then the microbiologist who understands normal colonising flora of humans, the pathogenesis of infections (incubation period, inoculum size, and kind of vehicle), and the characteristics of specific pathogens (natural habitat, resistance to drying or disinfectants, and antibiotics) – can interpret the laboratory data for the ICT. In more complicated outbreaks or an endemic situation, in addition to good microbiology (especially typing) there is a need to include epidemiologic considerations for the correct interpretation of microbiological data. The microbiologist should be a clinical scientist with appropriate training; the professional background varies from country to country.

Antibiotic policy role

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 work closely with physicians and pharmacists to determine appropriate antibiotics to be included in susceptibility testing panels. Then the microbiology laboratory should provide restricted antibiotics reports to the wards, according to the formulary. The microbiology laboratory should produce periodic resistance reports for specific wards and for the entire hospital, stratified by pathogen species and infection site. These reports should be available for every physician who prescribes antibiotics; they are very important for the design of local empirical therapy.

IPC in the laboratory

In every clinical laboratory in which biological samples are investigated, the first IPC concern is usually exposure to viruses that are spread through blood and bodily fluids (human immunodeficiency virus [HIV] and hepatitis B and C viruses [HBV, HCV]). It is very important that laboratory workers take all necessary preventive measures against those viruses (see Chapter "Prevention of blood-borne virus infections in patients and personnel").

A clinical microbiology laboratory is usually classified as a biosafety level 2 laboratory. This means that it diagnoses well-characterised agents that do not cause severe or untreatable diseases in healthy adult humans and poses only a moderate potential hazard to personnel and the environment. The laboratory must have limited access. Laboratory workers must have specific training to work with microbes and take all standard precautions with all biological specimens and microbial cultures (hand hygiene, disinfection of the environment, specific precautions with sharps, working in biological safety cabinets if aerosols may be created, proper disposal of waste, and sterilisation of culture material once testing is complete).

In some clinical microbiology laboratories, pathogens such as *Mycobacterium tuberculosis* or *Legionella pneumophila* are diagnosed. These diagnostics are to be performed in a biosafety level 3 laboratory (agents which may cause serious or potentially lethal disease in healthy adult humans after inhalation, but for which vaccine or other treatment exists). Sometimes it is not possible to have a level 3 facility; therefore these microbes may be diagnosed in a level 2 laboratory. In that case, laboratory workers must be properly educated and wear appropriate personal protective equipment (PPE), the filtered exhaust air from the laboratory room is to be discharged to the outdoors, the room has negative air pressure, and all recommended practices for biosafety level 3 are rigorously followed.

Point of care microbiological tests

The main problem of microbiological diagnostics in low resource countries is the lack of nearby microbiology laboratories; these are typically not sited outside of major urban areas. Therefore, it is very important to develop point of care (POC) microbiological tests that are sensitive and specific enough, rapid, easy to perform for HCWs that have no specific education in laboratory procedures, do not require special equipment, unambiguous to interpret, and affordable. Several such tests are already in use (for the diagnosis of malaria, HIV, HCV, syphilis, measles, respiratory viruses, and tuberculosis), however much more has to be done in this field. Especially important tests from the point of view of HAI prevention and control are tests for diagnosing pathogens causing HAIs, as well as antibiotic sensitivity to identify multidrug resistant strains to rapidly stop their spread. Staff performing the POC tests must also be provided education on PPE, hazards of specimens, and good methods to dispose of specimens and tests once the testing is completed.

Minimal Requirements for Microbiology Laboratories in the Control of HAIs

1. Should be sited inside the hospital; 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.
3. Should be able to examine blood, cerebrospinal fluid, urine, stool, wound exudates or swab, and respiratory secretions, and perform serological tests (at least HIV, HBV, HCV).
4. Should be able to identify common bacteria and fungi that can cause HAIs to the 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*, *Enterobacteriaceae*, *Neisseria meningitidis, Candida albicans*, *Aspergillus* spp, 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 for relevant antibiotics using disc-diffusion

methodology.

6. Should be able to perform basic typing - serotyping (for salmonellae, shigellae, *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 who is able to communicate well 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 and Further Reading

1. Baron EJ, Miller M, Weinstein MP, et al. A guide to utilization of the microbiology laboratory for diagnosis of infectious disease: 2013 recommendations by the Infectious Diseases Society of America (IDSA) and the American society for Microbiology (ASM). *Clin Infect Dis* 2013; 57:e22-121.
2. Brooks K, *Ready Reference to microbes*, 3rd ed., Association for Professionals in Infection Control and Epidemiology, Inc., Washington, DC, 2012.
3. Brown M. Microbiology basics. In: *APIC Text of Infection Control and Epidemiology*, 4th Edition, Clinical Editor Grota P. Washington, DC, Association for Professionals in Infection Control and Epidemiology, Inc., 2014: Chapter 24.
4. Chosewood LC, Wilson DE (editors) (2009). *Biosafety in Microbiological and Biomedical Laboratories* (5th ed.). http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf [Accessed 1 September 2015]
5. Damani N. *Manual of infection prevention and control*, 3rd Ed., Oxford University Press, Oxford, UK, 2012.
6. Diekema DJ, Pfaller MA. Prevention of health care-associated infections. In *Manual of Clinical Microbiology*, 11th Ed., Jorgensen JH and Pfaller MA, Editors in Chief, ASM Press, Washington, DC, 2015:106 -119.
7. Diekema DJ, Saubolle MA. Clinical microbiology and infection prevention. *J Clin Microbiol* 2011; 49:S57-S60.
8. 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.
9. Gerner-Schmidt P, Hyytia-TreesE, Rota PA. Molecular Epidemiology. In *Manual of Clinical Microbiology*, 10th Ed., Versalovic J, Editor in Chief, ASM Press, Washington, DC, 2011:100-123.
10. Guidance for Control of Carbapenem-resistant Enterobacteriaceae (CRE) 2012 CRE Toolkit, Centers for Disease Control and Prevention, Atlanta, GA, USA. http://www.cdc.gov/hai/organisms/cre/cre-toolkit/ [Accessed 27 August 2015]
11. Iram S, Zeenat A, Yusuf NW, Aslam M. Rapid diagnosis of tuberculosis using Xpert MTB/RIF assay–Report from a developing country. *Pak J Med Sci* 2015; 31:105-110.
12. Kalenic S, Budimir A. The role of microbiology laboratory in healthcare-associated infection prevention. *Int J Infect Control* 2009; 5(2).
13. 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 1 September 2015]
14. Kulich PA, Taylor DL. *The Infection Preventionist's Guide to the Lab*. Washington DC, Association for Professionals in Infection Control and Epidemiology, Inc., 2012.
15. Memish ZA, Al-Tawfiq JA, Alhakeem RF, et al. Middle East respiratory syndrome coronavirus (MERS-CoV): A cluster analysis with implications for global management of suspected cases. *Travel Med Infect Dis* 2015; pii:S1477.
16. Moore C. Point-of-care tests for infection control: should rapid tests be in the laboratory or at the front line? *J Hosp Infect* 2013; 85(1):1-7.
17. Murray PR. The clinician and the Microbiology Laboratory. In: *Mandell, Douglas and Bennett's Princi-*

ples and Practice of Infectious Diseases, 8th Ed., Bennett JE, Dolin R, Blaser MJ, Editors, Elsevier Saunders, Philadelphia, 2015:191-223.
18. Peeling RW, Mabey D. Point-of-care tests for diagnosing infections in the developing world. *Clin Microbiol Infect* 2010; 16:1062-1069.
19. Pereira-Neves A, Benchimol M.*Trichomonas vaginalis*: in vitro survival in swimming pool water samples. *Exp Parasitol* 2008; 118:438-41.
20. 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.
21. Robertson LJ, Campbell AT, Smith HV. Survival of *Cryptosporidium parvum* oocysts under various environmental pressures. *Appl Environ Microbiol* 1992; 58:3494-3500.
22. Shonhai A, Warrener L, Mangwanya D, et al. Investigation of a measles outbreak in Zimbabwe, 2010: potential of a point of care test to replace laboratory confirmation of suspected cases. *Epidemiol Infect* 2015; 13:1-9.
23. Smyer J. Laboratory testing and diagnostic. In: *APIC Text of Infection Control and Epidemiology*, 4th Edition, Clinical Editor, Grota P. Washington DC, Association for Professionals in Infection Control and Epidemiology, Inc., 2014: Chapter 25.
24. Soun VV, Eidson M, Wallace BJ, et al. Antemortem diagnosis of New York human rabies case and review of U.S. cases. *Intl J Biomed Sci* 2006; 2: 434-45.
25. Stratton CW IV, Greene JN. Role of the Microbiology Laboratory and Molecular Epidemiology in Healthcare Epidemiology and Infection Control. In: *Hospital Epidemiology and Infection Control*, 4rd Ed., Mayhall CG, Editor, Lippincott, Williams & Wilkins, Philadelphia, 2012:1418-1431.
26. Studdiford JS, Conniff KM, Trayes KP, Tully AS. Bedbug infestation. *Am Fam Physician* 2012; 86:653-658.
27. Williams J. Bed bugs in hospitals: more than just a nuisance. *CMAJ* 2013; 185:E524.

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Table 7.1. Characteristics of main groups of bacteria potentially causing HAI

Bacteria	Multidrug resistant strains (MDR)	Habitat	Survival in the environment (dry surfaces)[13*]	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 (GI) tract	3 days – 5 months	Air; indirect*** and direct contact	Urinary tract infection (UTI), sepsis, meningitis, pneumonia	Urine, blood, cerebral spinal fluid (CSF), sputum, aspirates	Contact precautions
Bordetella pertussis		Humans: nasopharyngeal mucosa (patients)	3-5 days	Droplets	Pertussis	Nasopharyngeal (NP) swab	Droplet precautions
Campylobacter jejuni, C.coli		Humans, animals: GI tract	Up to 6 days	Faecal-oral, water, food	Diarrhoea	Stool	Contact precautions Safe food and water
Clostridium difficile		Humans: GI tract	Highly resistant (spores – 5 months)	Faecal-oral; indirect and direct contact	*Clostridium difficile* infections (CDI)	Stool	Contact precautions Use of soap and water for hand hygiene rather than alcohol-based sanitizer Clean hands of patients Prudent use of antibiotics
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	Methicillin resistant CNS (MRSE)	Humans: skin, mucous membranes	ND	Contact (direct, indirect) endogenous	Different infections in immunocompromised host	Different specimens depending on infection	Standard precautions
Corynebacterium diphtheriae		Humans: NP mucosa (patients, carriers)	7 days – 6 months	Droplets, contact (direct, indirect)		NP swab; throat swab	Droplet precautions; single room/cohorting Contact precautions (healthcare worker [HCW] vaccination)

Table 7.1. Characteristics of main groups of bacteria potentially causing HAI

Bacteria	Multidrug resistant strains (MDR)	Habitat	Survival in the environment (dry surfaces)[13*]	Transmission in healthcare	Healthcare associated infections	Specimens for diagnosis of infection/ colonisation	Main preventive measures**
Enterococcus species	Glycopeptide resistant enterococci (GRE)	Humans: GI tract, genitourinary tract	5 days – 4 months	Indirect and direct contact; endogenous	UTI, sepsis	Urine, blood, stool	Contact precautions
Enterobacter species	Extended spectrum β lactamase strains (ESBL), multidrug resistant (MDR)	Environment, human GI tract	ND	Contact, food	UTI, sepsis, wound infection	Urine, blood, wound exudate, stool	Contact precautions Prudent use of antibiotics (avoiding the use of 3rd generation cefalosporins)
Escherichia coli	ESBL strains Carbapenem resistant enterobacteria (CRE)	Humans: GI 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, stool	Contact precautions Safe food and water Prudent use of antibiotics (avoiding the use of 3rd generation cefalosporins and carbapenems)
Helicobacter pylori		Gastric mucosa of humans	Less than 90 minutes	Contaminated GI endoscopes	Acute and chronic gastritis	Biopsy material; urea breath test; stool for antigen detection	Properly disinfected GI endoscopes
Klebsiella pneumoniae	ESBL strains CRE	Humans: GI tract; humid environment	2 hours – more than 30 months	Indirect and direct contact, endogenous	UTI, sepsis (neonatal units), pneumonia	Urine, blood, sputum, aspirates, stool	Contact precautions Prudent use of antibiotics (avoiding the use of 3rd generation cefalosporins and carbapenems)
Legionella pneumophila		Water (natural waters, 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; urine	Hyperchlorination of water or heating to at least 55°C No patient isolation needed

Table 7.1. Characteristics of main groups of bacteria potentially causing HAI

Bacteria	Multidrug resistant strains (MDR)	Habitat	Survival in the environment (dry surfaces)[13*]	Transmission in healthcare	Healthcare associated infections	Specimens for diagnosis of infection/ colonisation	Main preventive measures**
Listeria monocytogenes		Soil, vegetables, human GI tract (rarely); human birth canal	1 day - months	Contaminated food; perinatal transmission; contact with contaminated equipment in nurseries	Meningitis, bacteraemia	Blood, CSF	Safe food Standard precautions in nurseries
Mycobacterium tuberculosis	MDR strains Extremely DR strains (XDR)	Respiratory tract of patients	1 day – 4 months	Airborne, droplets	Tuberculosis	sputum	Airborne precautions (if not possible, then droplet isolation) (HCW vaccination)
Neisseria meningitidis		NP mucosa of humans	ND	Droplets	Acute meningitis	CSF	Droplet precautions Chemoprophylaxis
Proteus species	ESBL	GI flora of humans	1-2 days	Endogenous, contact (direct and indirect)	UTI, sepsis	Urine, blood, stool	Contact precautions
Pseudomonas aeruginosa	MDR and pan -drug resistant (PDR) strains	Humans: GI 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	Standard precautions MDR/PDR strains: contact precautions Prudent use of antibiotics
Salmonella spp		GI tract of humans and animals	NA	Faecal-oral, water, food	Diarrhoea, sepsis	Stool, blood	Safe food and water Standard precautions
Salmonella typhi		GI tract of humans	6 hours – 4 weeks	Faecal-oral, water, food	Typhoid fever	Stool, blood	Safe food and water Standard precautions
Salmonella typhimurium		GI tract of humans and animals	10 months – 4.2 years	Faecal-oral, water, food	Diarrhoea, sepsis	Stool, blood	Safe food and water Standard precautions

Table 7.1. Characteristics of main groups of bacteria potentially causing HAI

Bacteria	Multidrug resistant strains (MDR)	Habitat	Survival in the environment (dry surfaces)[13*]	Transmission in healthcare	Healthcare associated infections	Specimens for diagnosis of infection/ colonisation	Main preventive measures**
Serratia marcescens		Humans: GI 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	Standard precautions
Shigella species		GI tract of humans	2 days – 5 months	Faecal-oral, water, food	Diarrhoea	stool	Safe food and water Contact precautions
Staphylococcus aureus	Methicillin resistant *S. 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, nares, blood, aspirates, biopsy, wound exudate, perianal swab, stool	Contact precautions Prudent use of antibiotics (avoid ciprofloxacin)
Streptococcus agalactiae Group B streptococcus (GBS)		Humans: birth canal	ND	Intrapartal; direct and indirect contact in delivery room and nurseries	Sepsis and meningitis of newborn	Blood, CSF, vaginal swab	Antibiotic prophylaxis during delivery when indicated Standard precautions
Streptococcus pyogenes Group A streptococcus (GAS)		Humans: oropharyngeal mucosa	3 days-6.5 months	Droplets, contact, endogenous	Pharyngitis ("strep throat"), surgical wound infection	Oropharyngeal swab, wound exudate	Staff with GAS infections or carriers of GAS should not work in the operating theatre
Vibrio cholerae		GI tract of humans; water	1 – 7 days	Faecal-oral, water, raw seafood	Cholera	stool	Safe water and food Standard precautions
Yersinia enterocolitica		GI flora of many animals, causes diarrhoea in young animals; rarely humans as carriers	ND	Blood transfusion in hospitals; faecal-oral in the community	Bacteraemia connected to blood transfusion; diarrhoea in the community	Blood, stool	Safe blood products Standard precautions

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

** See "Isolation precautions"

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

ND = not done; NA = not applicable

Table 7.2. Characteristics of main groups of fungi potentially causing HAI

Fungi	Habitat	Survival in the environment (dry surfaces)[13*]	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	Standard precautions
Candida glabrata (yeast)	Soil, animals, humans, inanimate objects	102-150 days	Direct and indirect contact, endogenous	Different opportunistic infections	Different specimens depending on infection	Standard precautions
Candida parapsilosis (yeast)	Soil, animals, humans, inanimate objects	14 days	Direct and indirect contact, endogenous	Different opportunistic infections	Different specimens depending on infection	Standard precautions
Aspergillus species (mould)	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	Protective isolation of susceptible patients Containment of areas where construction/ renovation is in process to reduce exposure**** Safe tap water, food and drinks
Mucor (mould)	Soil, plants, fruits, animal excreta, food	Conidia and spores are resistant forms	Inhalation	Different opportunistic infections in immunocompromised patients (zygomycosis)	Different specimens depending on infection	Protective isolation of susceptible patients Containment of areas where construction/ renovation is in process to reduce exposure**** Safe food and drinks
Rhizopus (mould)	Soil, plants, fruits, animal excreta, food	Conidia and spores are resistant forms	Inhalation	Different opportunistic infections in immunocompromised patients (zygomycosis)	Different specimens depending on infection	Protective isolation of susceptible patient Containment of areas where construction/ renovation is in process to reduce exposure**** Safe food and drinks

*Survival is better in low temperature, high humidity and presence of serum or albumin;
** See “Isolation precautions”
*** Whenever indirect contact is involved, it is most frequently by hands of healthcare workers
**** See “Health care facility design, construction and renovation”

Table 7.3. Characteristics of main groups of viruses potentially causing HAI

Virus	Habitat	Survival in the environment (dry surfaces)[13*]	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	Conjunctival swab NP swab	Standard precautions Separate eye drops for every patient
Coronavirus	Humans	3 hours	Droplets Direct and indirect contact	Respiratory infections Diarrhoea	Serum sample Respiratory sample Stool sample	Droplet precautions Contact precautions
Coronavirus: SARS (Severe Acute Respiratory Syndrome)-CoV	Animals Humans	SARS virus: 72-96 hours	Droplets Direct and indirect contact Faecal-oral ?	Severe respira-tory infections	Serum sample Respiratory sample	Airborne precautions (if not possible then droplet precautions) plus contact precautions
Coronavirus: MERS (Middle East Respiratory Syndrome)-CoV	Animals Humans	ND	Droplets Direct and indirect contact	Severe respiratory infections	Respiratory sample	Airborne precautions (if not possible then droplet precautions) plus contact precautions
Coxackie B virus	Humans	>2 weeks	Faecal-oral; direct and indirect contact	Generalized disease of newborn	Serum sample Blood CSF	Standard precautions
Cytomegalovirus	Humans	8 hours	Blood products, tissue and organs for transplan-tation; mucosal contact with secretions and ex-cretions	Huge range of different dis-eases	Serum sample	Safe blood products and tissues/organs for transplantation
Ebola (Marburg)	Humans Animals	ND	Direct contact Blood, secretions, respiratory droplets, semen, contaminated syringes and needles, aerosols	Haemorrhagic fever	Serum sample, blood	Airborne precautions (if not possible then droplet precautions) plus contact precautions

Table 7.3. Characteristics of main groups of viruses potentially causing HAI

Virus	Habitat	Survival in the environment (dry surfaces)[13*]	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	Safe food and water Standard precautions
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	Lesion swab CSF	If infected, HCW should not care for susceptible persons (newborn, immunocompromised)
HIV	Humans	>7 days	Blood, bodily fluids, tissue and organs for transplantation	AIDS	Serum sample	Safe blood products and tissues/organs for transplantation
Influenza virus	Humans	1-2 days	Droplets, directs and indirect contact, HCW as symptomatic or asymptomatic diseased persons	Influenza	NP swab	Droplet precautions HCW vaccination
Norovirus	Humans	8 hours – 7 days	Faecal-oral, direct and indirect contact, aerosols from vomitus	Diarrhoea	Stool	Contact precautions
Rabies virus	Dogs (and other animals); humans	Inactivated soon by drying[24]	Saliva and other body fluids and secretions of patient through contact with open wounds, skin abrasions or mucous membranes Human bite/scratch Aerosol transmission?	Rabies	Corneal impression Skin biopsy Saliva CNS tissue at autopsy	Droplet precautions and contact precautions Post-exposure prophylaxis in persons who were exposed to saliva and other body fluids and secretions of patient or were bitten/ scratched by patient

Table 7.3. Characteristics of main groups of viruses potentially causing HAI

Virus	Habitat	Survival in the environment (dry surfaces)[13*]	Transmission in healthcare	Healthcare associated infections	Specimens for diagnosis of infection**	Main preventive measures***
Respiratory syncycial virus	Humans	Up to 6 hours	Droplets, direct and indirect contact	Acute respiratory infections in young children	NP exudate	Droplet precautions
Rotavirus	Humans	6-60 days	Faecal-oral, direct and indirect contact	Diarrhoea	Stool	Contact precautions
Rubula virus	Humans	ND	Droplets	Mumps (parotitis)	Serum sample	Droplet precautions HCW vaccination
Rubivirus	Humans	ND	Droplets	Rubella (German measles)	Serum sample	Droplet precautions HCW vaccination
Morbillivirus	Humans	ND	Droplets	Measles	Serum sample	Droplet precautions HCW vaccination
Varicella-zoster virus	Humans	ND	Droplets, close contact	Varicella	Serum sample	Droplet/airborne precautions HCW vaccination

* Survival is better in low temperature, presence of biological material and if viruses are in higher numbers
** Diagnosis is performed using serology, if applicable and if laboratory can perform direct diagnostics, it will be mostly antigen detection or nucleic acid detection in the sample from infectious site, rarely virus isolation
*** See “Isolation precautions”
**** whenever indirect contact is involved, it is most frequently by hands of healthcare workers
NA = not applicable; ND = not done; HCW = healthcare worker

Table 7.4. Characteristics of main groups of parasites potentially causing HAI

Parasite	Habitat	Survival in the environment	Transmission in healthcare	Healthcare associated infections	Specimens for diagnosis of infection	Main prevention methods*
Cryptosporidium (protozoa)	Humans	2 hours on dry surface[13] longevity in water[21]	Faecal-oral, water, food	Diarrhoea	Stool	Safe water and food Standard precautions
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[19]	Sexually transmitted in the community; contaminated medical equipment in gynaecology	Vaginal infection in women	Vaginal discharge	Disinfected/sterilised medical equipment in gynaecology
Enterobius vermicularis (helminth)	Intestinal tract of diseased person		Faecal-oral, ingestion of para-site eggs that can contaminate environment; airborne	Enterobiasis	Perianal tape	Standard precautions Changing linen and patient clothes without creating aerosols

* See “Isolation precautions”