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EPIDEMIOLOGY AND DIAGNOSIS OF HEALTHCARE-ASSOCIATED BACTERIAL INFECTIONS:

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Abstract:
Hospital-acquired infections (HAI) contribute to the emotional stress and functional disorders of the patient and in some cases, can lead to a state of disability that reduces quality of life. Often, HAI are one of the factors that lead to death.

Keywords: hospital, infections, diagnosis; bacterial.

Introduction:

Healthcare-associated infections (HCAIs) is a localized or systemic condition resulting from an adverse reaction to the presence of an infectious agent(s) or its toxin(s) that was not present on admission to the acute care facility **(1).** According to definition given by WHO, Healthcare associated infection is an infection acquired in hospital by a patient who was admitted for a reason other than that infection. This includes infections acquired in the hospital but appearing after discharge, and also occupational infections among staff of the facility. Data included in a report given by WHO showed that pooled HAI prevalence in mixed patient populations was 7.6% in high income countries. The

European Centre for Disease Prevention and Control (ECDC) estimated that 41,31,000 patients are affected by approximately 45,44,100 episodes of HAI every year in Europe ***(2).***
HCAIs are defined as infections occurring during receiving health care, developed in a hospital or other health care services centers and appear firstly 48 hours or more after hospital admission, or occur within 30 days after receiving health care. Several studies reported that the common types of adverse events that affect hospitalized patients are: HCAIs, adverse drug events and surgical complications (1).

Incidence Rate:
Although 5–10% of hospitalized cases have HAIs in developed countries, this ratio exceeds over 25%, in developing countries. Even though ICUs have less than 10% of the beds in hospitals, more than 20% of HAIs occur in ICUs. The incidence of HAIs in pediatric ICUs (PICUs) varies between developed and developing countries (6.1 and 23.5%, respectively whereas the incidence density rate of HAIs varies between 14.1 and 27.2/1000 patient-days, respectively ***(3)***.
HAIs remain an important cause of increased morbidity, mortality, length of stay (LOS), and healthcare costs. Among the factors that make the children more susceptible to HAIs in PICUs are their immune-compromised condition, broad-spectrum antibiotic usage, and medical manipulations that disrupt the natural defenses of the host including the use of invasive devices [e.g. intravascular devices, intubation, nasogastric tubes, and urinary catheters (UC)] ***(4)***.
The United States (US) Center for Disease Control and Prevention (CDC) reported that about 1.7 million hospitalized patients acquire HCAIs every year during treatment for other health problems and more than 98,000 patients (one in 17) die from HCAIs as well ***(5).***
Pediatric intensive care units (PICU) in developing countries often admit more critically ill patients with younger age and low socioeconomic status. Rates of HCAI among pediatric patients vary according to birth weight, age, severe underlying illness, loss of skin integrity, or the presence of multiple medical devices that breech normal defense mechanisms. In developed countries, infection prevention and control programs (IPC) have been implemented as an essential element in healthcare institutes mainly focusing on device-associated infection (DAI) surveillance. In many countries of limited resources, a sustainable surveillance IPC is being developed ***(6)***.
Infections that commenced at or after 48h after admission to the PICU were included as PICU-acquired infections ***(7)***.

Urinary tract infection: the patient must have at least one of the following signs or symptoms with no other recognized cause: fever (>38°C), urgency, frequency, dysuria, and positive urine culture with counts ≥105 colony-forming units per milliliter (CFU/ml).

Pneumonia: the criteria for the definition of pneumonia were chest radiograph with new pulmonary infiltrate or progression of an existing one, accompanied by two of the following signs or symptoms: leukocytosis (>0,000/mm3) or leukopenia (<4,500/mm3), hyperthermia (>38oC) or hypothermia (<35oC), purulent sputum, tracheal aspirate bacterial count of ≥106CFU/ml.

Bacteremia*:* this was defined as the biological documentation of infection, i.e., the result of a positive blood culture.

Sepsis*:* this was defined as a systemic response to an infection, followed by one or more of these conditions: a) temperature >38oC or <36oC; b) heart rate >90 beats/min; c) respiratory rate >20 breaths/min or PaCO2 <32mmHg; d) leukocyte count >12,000cells/mm3, or >10% of juveniles; e) sepsis with hypotension, associated with the presence of perfusion abnormalities that may include lactic acidosis, oliguria, or acute alteration in mental status defined as septic shock; total patients per day was defined as the somatory of total time of hospitalization by the patients in PICU; epidemiological investigations' incidence rate was defined as the number of each infection as the numerator and the number of days those patients were at risk as the denominator per 1,00013; device-

associated incidence rate was defined by dividing the number of each infection by the number of days those patients were exposed to risk factors per 1,000 ***(4)***.

Epidemiology of Bacterial infections:
The epidemiology of an infection consists of the reservoirs, modes of transmission of pathogens and risk factors associated with an increased risk for this infection.

Risk factors:
Risk factors for the development of infections are classified into the following categories

1. Host factors:
a) Age:
10% of childhood deaths under the age of 5 years in high-income countries were attributable to infections, with the majority of acute infection-related deaths occurring in PICUs ***(8).*** **b) Sex:**
Some studies suggest that urinary tract infections are most common in females because of the short urethra and its close proximity to the anus ***(8).***
c) Underlying disease:
Many patients who develop infections have underlying diseases or conditions which interfere with local or systemic host defense mechanisms. These include diabetes mellitus, liver cirrhosis, chronic renal failure, cardiac and pulmonary diseases. Underlying malignancies are also a risk factor ***(3*****).**
d) Immune status:
Impaired host defenses make patients more susceptible to infection. Conditions that may weaken a person's defenses include malnutrition, extremes of age, inherited and acquired immune deficiencies, immunosuppressive therapy, surgery and inadequate immunization ***(3***).
e) Malnutrition:
Poor nutritional status may predispose to some nosocomial infections e.g., pneumonia, urinary tract infection, postoperative wound infection and bloodstream infection because under nutrition is associated with increased length of hospital stay and delayed wound healing ***(3).***

2. Therapeutic factors:
a) Invasive procedures and instrumentation:
High technology medicine is synonymous with mechanical ventilatory support, hemodynamic monitoring, total parenteral nutrition, peritoneal dialysis, hemodialysis, intracranial pressure monitoring. All of these necessitate the use of invasive devices and procedures including central venous and arterial catheters, urinary catheters, endotracheal tubes, and prosthetic devices ***(6).***
In general, invasive devices of all types are far more important in determining susceptibility to nosocomial infection than underlying diseases because they facilitate invasion by colonizing microorganisms, bypassing or further impairing host defenses**.** It was found that two thirds of nosocomial bacteremias in the PICU can be attributed to colonized intravascular catheters which act as a focus for infection **(*6*).**

b) Antibiotics:
One of the factors that contribute to initiation of infection is the widespread use of broad- spectrum antibiotics which suppress the normal flora and allow the emergence of resistant strains of bacteria ***(6).***
More than 70% of the bacteria that causes hospital acquired infections are resistant to at least one of the drugs most commonly used to treat them. Persons infected with drug resistant organisms are more likely to have longer hospital stays and require treatment with second or third- choice drugs that may be less effective, more toxic, and, or more expensive ***(9).***

c) Other therapies:

Some drugs [e.g., narcotics, sedatives, antacids or H2 histamine receptor antagonists] may increase susceptibility to infection by indirect methods**.** Even transfusion therapy increases the risk of infection (***9).***

3. Pathogen factors:

Nosocomial infections are characterized by enormous microbial diversity, large infecting inocula and polymicrobial pattern which make the patient at a greater risk for complications e.g. bloodstream infections and shock ***(10).***

Most nosocomial pathogens exhibit some resistance to antibiotics, and many are also more virulent than others because of (a) their capacity to multiply in aqueous reservoirs for prolonged periods e.g., *Pseudomonas*, (b) elaboration of endotoxins (e.g., all of the gram-negative bacilli) or exotoxins *(P. aeruginosa*, or *S. aureus*) or (c) the production of adhesins or exoglycocalyx (e.g., *Coagulase-negative staphylococci*), conferring the capacity to adhere and form biofilms on biologic and prosthetic surfaces resistant to host defenses and even antibiotics ***(10).***

4. Environmental factors:

a) Intensive care unit:

Patients in the PICU are at a higher risk of acquiring nosocomial infections compared with patients in general wards. This is due to the high frequency of invasive procedures required for monitoring and treatment**.** Antibiotics are used more frequently and in greater quantity than in any other unit in the hospital; antimicrobial resistance assures survival of nosocomial pathogens**.** Overcrowding, understaffing in PICUs and insufficient nurse-to-patient ratio facilitate transfer of resistant organisms from patient to patient and are also associated with outbreaks of nosocomial infections ***(5)***.

The incidence of nosocomial infections in the PICU is also increasing as a result of the ability to sustain life of critically ill patients through better organ support techniques while at the same time most of these patients are immunocompromised ***(5).***

b) Length of hospital stay:

Prolonged hospitalization acts as a risk factor for the development of nosocomial infections ***(11).***

Reservoirs of infection:

Defined as object in or on which a microorganism can survive and in some cases, multiply. Inanimate objects, human beings, and animals can all serve as reservoirs, providing the essential requirements for a microorganism to survive at specific stages in its life cycle. *Pseudomonas spp*. survive and multiply in nebulizers and *hepatitis B virus* survives on the surface of haemodialysis machines. ***(12)***.

Infectious reservoirs present in health care settings, may include everything from patients, visitors, and staff members to furniture, medical equipment, medications, food, water, and blood ***(13).***

Human reservoir may be either a case or a carrier. Case is a patient with an acute clinical infection while carrier is a person who is colonized with a specific patho- genic microorganism, but shows no signs or symptoms of infection. Carrier may have a sub clinical or asymptomatic infection, e.g. *Hepatitis B virus* ***(13)***.

Carriers fall into four categories:

- Incubatory carrier: a person who has acquired the infection and incubating the illness but does not yet show symptoms. Incubation periods vary from one infectious disease to other.
- Convalescent carrier: a patient in the recovery stage of an illness but continues to shed the pathogenic microorganism for an indefinite period, e.g. a patient who has had a *Salmonella* infection commonly sheds the organism in his feces even after symptoms disappear.
- Intermittent carrier: a patient occasionally sheds the pathogenic microorganism from time to time, e.g. some people are intermittent carriers of *Staphylococcus aureus*.
- Chronic carrier: a patient always has the infectious organism in his body, e.g. chronic carriers of hepatitis B virus ***(14).***

Portal of entry:
The portal of entry is the path by which an infectious agent invades a susceptible host. Usually, this path is the same as the portal of exit. For example, the portal of entry for tuberculosis and diphtheria is through the respiratory tract, In addition, each invasive device e.g. intravenous line, creates an additional portal of entry into patient's body thus increasing the chance of developing an infection ***(15).***

Portal of exit:
The portal of exit is the path by which an infectious agent leaves its reservoir. Usually, this portal is the site where the microorganism grows. Common portals of exit associated with human reservoirs include the respiratory system, genitourinary tract, and gastrointestinal tract, skin, mucous membranes and placentas (transmission from mother to fetus) ***(15***).

Mode of transmission:
The microorganism can be acquired by inhalation (through respiratory tract), ingestion (through gastrointestinal tract), and inoculation (through accidental sharp injury). It is important to remember that some microorganisms have more than one transmission route to get from the reservoir to a new host ***(16).***

Contact transmission: Contact is the most common mode of transmission of infection in the health care settings. Contact transmission, may be subdivided into direct contact, indirect contact, and contact with droplets that enter the environment.

Droplet transmission: Droplet transmission results from contact with contaminated respiratory secretions. A person with a droplet-spread infection coughs, sneezes, or talks, releasing infected secretions that spread through the air to the oral or nasal mucous membranes of a person nearby. Microbes in droplet nuclei (mucus droplets) can travel up to about 3 feet (1m). Droplet transmission differs from airborne transmission in that the droplets don't remain suspended in the air but tie on surfaces. Examples of diseases spread by droplets include influenza, whooping cough, etc *(17).*

Airborne transmission: Airborne transmission occurs when fine microbial particles or dust particles containing pathogens remain suspended in the air for a prolonged period, and then are spread widely by air currents and inhaled. The tiny particles remain suspended in the air for several hours and may cause infection when a susceptible person inhales them. Examples of diseases spread by the airborne include pulmonary tuberculosis, varicella, and measles ***(17).***

Causative organisms of infection
Information on the isolated organism is useful as it helps to determine the suitable antibiotic. It is believed that patients given appropriate therapy are more likely to survive than those given inadequate or inappropriate treatment.

1. Bacteria:
- **Commensal bacteria** found in normal flora of healthy humans. These have a significant protective role by preventing colonization by pathogenic microorganisms. Some commensal bacteria may cause infection if the natural host is compromised. For example, cutaneous *coagulase negative staphylococci* cause intravascular line infection and intestinal *Escherichia coli* is the most common cause of urinary infection ***(5).***
- **Pathogenic bacteria** have greater virulence, and cause infections (sporadic or epidemic) regardless of host status.

a) **Gram-positive bacteria:** *Staphylococcus aureus* (cutaneous bacteria that colonize the skin and nose of both hospital staff and patients) cause a wide variety of lung, bone, heart and bloodstream infections and are frequently resistant to antibiotics; *beta-hemolytic streptococci* are also important.
b) **Anaerobic Gram-positive spore forming rods** (e.g. *Clostridium*) cause gas gangrene.
c) **Gram-negative bacteria:** *Enterobacteriacae* (e.g. *Escherichia coli, Proteus, Klebsiella, Enterobacter* and *Serratia* marcescens), may colonize sites when the host defenses are compromised (catheter insertion, cannula insertion) and cause serious infections (surgical site, lung, bacteraemia and peritoneum infection)**.** Gram- negative organisms such as *Pseudomonas spp.* are often isolated in water and damp areas. They may colonize the digestive tract of hospitalized patients. Other bacteria selected are a unique risk in hospitals. For instance, *Legionella* species may cause pneumonia (sporadic or endemic) through inhalation of aerosols containing contaminated water (air conditioning, showers, and therapeutic aerosols) ***(5).***
In a study conducted at Assuit University Hospitals, Egypt with special reference to extended spectum B- lactamase producing Organisms from January 2006 to December 2006. Analyses of organisms causing nosocomial infections, especially blood Stream infections, showed that gram positive organisms were reported in 69.1% of cases (MRSA was the most prevalent (18.9%) followed by methicillin resistant coagulase negative *Staphylococci* (16%)). Gram Negative bacilli represented 29.1% of cases with *klebisiella pneumoniae* being the most common (10.3%) followed *E. coli* (8.6%). Candida Spp. was only reported in (1.7%) of isolates (***5***).

2. Viruses
Cytomegalovirus (CMV) infections lead to significant disease in immunocompromised hosts .Researches have shown that critically ill patients who are considered to be immunocompetent traditionally may also be at risk of having CMV infection. Sepsis as a result of bacterial or fungal infections has the ability to promote the release of immunomodulatory cytokines and lead to the reactivation of CMV. Moreover, respiratory syncytial virus (RSV) infections are the major cause of lower respiratory tract infections such as bronchopneumonia and bronchiolitis. Infection by RSV leads to the intermediate or intensive care admission of approximately 1–2% of each birth cohort annualy in Switzerland ***(18).***
Viruses are also important cause of nosocomial infection besides bacteria. Usual detection showed that 5% of all nosocomial infections are due to viruses. They are transmitted by hand-mouth, respiratory route and faeco-oral route. Viruses cause hepatitis and healthcare services can transmit hepatitis viruses to both patients and workers. Unsafe injection practices commonly transmit Hepatitis B and C. Other viruses include HIV, influenza, rotavirus, and herpes-simplex virus ***(10).***
Viral–bacterial co-infection develops in up to 23% of cases of severe pneumonia, resulting in a higher incidence of respiratory failure and septic shock. The viral infection is believed to occur first then predispose children to bacterial invasion. Methicillin resistant staph areus (MRSA) is shown recently to be associated with mortality in healthy children infected with influenza; this was noted especially in the 2009 influenza pandemic .This dangerous coinfection is a vigorous mortality predictor causing strict destruction of lung despite proper antibiotics. Despite the mechanism explaining viral–bacterial coinfection is unknown, the highest risk subgroup of children with influenza–S. aureus co-infection were reported in one study to differ from those with influenza alone in having cytokine storm that is associated with a diminshed monocyte response to ex vivo stimulation with lipopolysaccharide ***(19).***
There is possibility of transmission of many viruses including the *hepatitis B and C viruses* through transfusions, dialysis, injections or endoscopy, *respiratory syncytial virus (RSV), rotavirus, and enteroviruses* (transmitted by hand- to-mouth contact and via the fecal-oral route). Other viruses such as *cytomegalovirus, HIV, influenza viruses*, *herpes simplex virus*, and *varicellazoster* virus, may also be transmitted ***(20).***

3. Fungi
Fungal parasites are opportunistic pathogens causing nosocomial infections to immunecompromised individuals. Aspergillus spp. cause infections by environmental contamination. Candida albicans and

Cryptococcus neoformans are responsible for infection during hospital stay. Candida infections come from endogenous microflora of the patient while Aspergillus cause infections by inhalation of its fungal spores from contaminated air during construction or restoration of health care facility ***(10)***.
Candida is considered a part of the normal skin, gastrointestinal and vaginal flora. The majority of candidal infections are endogeneous. Given it its opportunistic nature, Candida will not cause infection unless the normal flora or the host antifungal defense response has been ecologically altered. Antibacterial agents modification of the endogenous microbial flora is a major risk factor allowing for fungal overgrowth on mucosal and skin surfaces. A change in the physical benignity of the skin and mucous membranes barriers by intravascular access devices, surgery, wounds, chemotherapy, or of host defenses are other leader pathogenic elements facilitating spread of infection into the systemic circulation. A critical step in the pathogenesis of invasive candidiasis is colonization of mucous membranes and skin. It has been used for suspecting the development of infections in critically ill patients. Differentiation between colonization and infection is difficult ***(21)***.
Many risk factors may favor or contribute to patient colonization. Investigators have showed that use of antibiotics, presence of central venous catheter, and prolonged hospital stay represent important risk factors for the development of fungal infections. The use of antibiotic play a critical role in the pathogenesis of fungal infection which emerges during therapy or immediately after it. By suppressing susceptible endogenous bacterial flora in the body, antibiotic favor fungal colonization ***(21).***

Many fungi are opportunistic organisms and cause infections during extended antibiotic treatment and severe immunosuppression (*Candida albicans*, *Aspergillus spp., Cryptococcus neoformans, Cryptosporidium*).These are a major cause of systemic infections among immunocompromised patients. Environmental contamination by airborne organisms such as Aspergillus spp. which originate in dust and soil is also a concern, especially during hospital construction ***(21).***
Yeasts belonging to the genus *Candida* are the most common fungal organisms causing infections in critically ill hospitalized patients. *Candida albicans* has been the most frequently isolated species in nosocomial fungal infections; however, during the last years, other closely related *Candida spp*. became involved in hospital-acquired infections ***(18).***

Candida species capable of causing human infections are: *C.albicans* (50% to 70% of infections), *C.tropicalis* or *C.glabrata* (second most common), *C.krusei*, *C. parapsilosis*, *C.guillermondi*, *C.pseudotropicalis*, *C.stellatoidea*, *C. lusitaniae*, and *C. rugosa.* Fluconazoleresistant *C.krusei* and *C.glabrata* have become more common pathogens in nosocomial fungal infections ***(21).***

Clinical Manifestation of Infections:
Manifestations may be local (eg, cellulitis, abscess) or systemic (most often fever). Manifestations may develop in multiple organ systems. Severe, generalized infections may have life-threatening manifestations (eg, sepsis and septic shock). Most manifestations resolve with successful treatment of the underlying infection ***(18)***.

Clinical
Most infections increase the pulse rate and body temperature, but others (eg, typhoid fever, tularemia, brucellosis, dengue) may not elevate the pulse rate commensurate with the degree of fever (relative bradycardia). Hypotension can result from hypovolemia, septic shock, or toxic shock. Hyperventilation and respiratory alkalosis are common. Alterations in sensorium (encephalopathy) may occur in severe infection regardless of whether CNS infection is present ***(18)***.

Hematologic
Infectious diseases commonly increase the numbers of mature and immature circulating neutrophils. Mechanisms include demargination and release of immature granulocytes from bone marrow, IL-1- and IL-6-mediated release of neutrophils from bone marrow, and colonystimulating factors elaborated

by macrophages, lymphocytes, and other tissues. Exaggeration of these phenomena (eg, in trauma, inflammation, and similar stresses) can result in release of excessive numbers of immature leukocytes into the circulation (leukemoid reaction), with leukocyte counts up to 25 to 30×109/L. Conversely, some infections (eg, typhoid fever, brucellosis) commonly cause leukopenia. In overwhelming, severe infections, profound leukopenia is often a poor prognostic sign. Characteristic morphologic changes in the neutrophils of septic patients include Döhle bodies, toxic granulations, and vacuolization. Anemia can develop despite adequate tissue iron stores. If anemia is chronic, it is a normochromic, normocytic anemia characterized by low serum iron, low total iron-binding capacity, and normal to increased serum ferritin. Serious infection may cause thrombocytopenia and disseminated intravascular coagulation (DIC) ***(5)***.

Other organ systems

Pulmonary compliance may decrease, progressing to acute respiratory distress syndrome (ARDS) and respiratory muscle failure.

Renal manifestations range from minimal proteinuria to acute renal failure, which can result from shock and acute tubular necrosis, glomerulonephritis, or tubulointerstitial disease.

Hepatic dysfunction, including cholestatic jaundice (often a poor prognostic sign) or hepatocellular dysfunction, occurs with many infections, even though the infection does not localize to the liver. Upper GI bleeding due to stress ulceration may occur during sepsis.

Endocrinologic dysfunctions include:

- Increased production of thyroid-stimulating hormone, vasopressin, insulin, and glucagon
- Breakdown of skeletal muscle proteins and muscle wasting secondary to increased metabolic demands
- Bone demineralization

Hypoglycemia occurs infrequently in sepsis, but adrenal insufficiency should be considered in patients with hypoglycemia and sepsis. Hyperglycemia may be an early sign of infection in diabetics.

Diagnosis of Infections:

The diagnostic process begins with a full clinical evaluation (history and examination), followed by bedside or laboratory-based investigations. Each has advantages and disadvantages, but they are rarely employed in isolation. The typical process begins with clinical tests and uses this information to guide bedside tests, followed by laboratory tests - with the probability of the diagnosis shifting with each piece of added information. Bacterial infection can occur in any part of the body with multiple different organisms and therefore the number of available tests is vast. This review discusses broad categories of tests, with specific examples to illustrate the process ***(22).***

Depending upon the type of infection present, an appropriate specimen is obtained accordingly and sent to the laboratory for definitive identification by using biochemical or enzyme-based tests. A Gram stain is first performed to guide the way, which should show typical gram-positive bacteria, cocci, in clusters. Second, the isolate is cultured on mannitol salt agar, which is a selective medium with 7–9% NaCl that allows *S. aureus* to grow, producing yellow-colored colonies as a result of mannitol fermentation and subsequent drop in the medium's pH. Furthermore, for differentiation on the species level, catalase (positive for all *Staphylococcus* species), coagulase (fibrin clot formation, positive for *S. aureus*), DNAse (zone of clearance on nutrient agar), lipase (a yellow color and rancid odor smell), and phosphatase (a pink color) tests are all done. For staphylococcal food poisoning, phage typing can be performed to determine if the staphylococci recovered from the food to determine the source of infection ***(19).***

Diagnostic microbiology laboratories and reference laboratories are key for identifying outbreaks and new strains of *S. aureus*. Recent genetic advances have enabled reliable and rapid techniques for the identification and characterization of clinical isolates of *S. aureus* in real- time. These tools support infection control strategies to limit bacterial spread and ensure the appropriate use of antibiotics.

These techniques include real-time PCR and quantitative PCR and are increasingly being employed in clinical laboratories ***(23).***

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