Microbiology

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Bacteriology C/S

- Plate sample on blood agar plate ± others
- Incubate 35^0 – 37^0C overnight (~ 18 hours)
- Read plate for pathogens
- Do work-up to identify pathogens
- Do sensitivity testing if available

Gram Stain

- Differential staining process used in microscopy of specimens and bacterial colonies
- Bacteria divided into 2 groups of Gram positive (deep blue or purple) and Gram negative (red or pink)
- Can be used to differentiate epithelial and inflammatory cells

Skin Flora

- **Transient**
  - **Microbes that happen to be deposited on the skin but do not multiply there.**
  - **Maybe removed by a thorough wash with soap and water**
- **Resident**
  - **Colonise the skin.**
  - **Cannot be removed by washing with soap and water**
  - **Can be reduced to small numbers by disinfectants**

Transient Bacteria

- **Exogenous in origin**
- **Located on the skin surface**
- **Examples are *S.aureus, E.coli***

Resident Bacteria

- **Endogenous in origin**
- **Located in the ducts of glands, hair follicles and between squamous cells**
- **Bacteria are mainly CNS, *S.aureus*, *Corynebacterium spp.*, *Propionibacterium acnes***

Staphylococcus

- They are Gram-positive cocci that appear microscopically like a bunch of grapes
- *S.aureus* is coagulase positive
- Coagulase negative staphylococci are many in number eg. *S. saprophyticus*

Epidemiology of Staphylococcus

- ***S.aureus*** **is ubiquitous.**
  - **Colonise (nares, axillae, perineum,etc) about 30% of humans.**
  - **Person-to-person spread by hands (common), nasal discharges and aerosol (rare)**
- **CNS are ubiquitous on skin and mucosal surfaces.**
  - **Infection usually endogenous and is common cause of nosocomial line infections**
  - **Bacteria has low pathogenicity but cause infection in patients with foreign bodies**

Pathogenesis of *S.aureus* infection

- Colonisation usually precedes infection
- Major reservoir varies eg. nose for dialysis patients, oropharynx for intubated patients
- Some factors that promote colonisation :
  - Prolonged hospitalisation
  - Diabetics
  - Eczema
  - Haemodialysis patients
  - IV drug abusers
  - Elderly
  - HIV patients
- To initiate infection, the strain must adhere to tissue/surface mediated by receptors that involve host protein interactions

Pathogenesis of CNS

- Seldom occur among healthy individuals
- Important host factors are defects in mucosal membrane/skin, immunosupression and presence of foreign body
- Patients with systemic or localised defects in opsonophagocytic activity are at increased risk of infection
- In neonates administration of IV lipid emulsion is an independent risk factor
- Preceeding antibiotic therapy

CNS Infections

- **Single most important factor associated with CNS infections is the presence of a foreign body eg. indwelling catheters, intravascular catheters, CSF shunts, prosthetic joints etc.**

Staphylococci produce disease in 2 ways :

- Directly by invasion and subsequent : tissue destruction whether locally or by haematogeneous spread
- Through the effects of toxins

Infections produced by *S. aureus*

- **Direct invasion**
- **Dissemination (haematogenous)**
- **Toxin mediated**

Direct Invasion by *S. aureus*

1. Superficial
   - Pyoderma including impetigo, paronychia
   - Skin and soft tissue infections eg. boils, cellulitis

2. Deep
   - Septic arthritis
   - Osteomyelitis
   - Pyomyositis

Haematogenous Dissemination

- **Bacteremia with or without septic shock or multiple organ system failure**
- **Metastatic abscess formation (brain, lung, liver, spleen, kidney, genital tract, retroperitoneum)**

Toxin Mediated *S.aureus* Infection

- Skin disease eg. staphylococcal scalded-skin syndrome
- Gastrointestinal disease eg. gastroenteritis
- Multisystem disease eg. toxic shock syndrome

Virulence Factors of *S.aureus*

- Enzymes eg. catalase, hyaluronidase, lipase
- Toxins eg. β-toxins, leukocidin, enterotoxin, TSS toxin 1
- Immune system factors eg. antigen-antibody complexes, superantigens
- Cell wall components eg. activated C, tnf, other cytokines & mediator systems

Sequence of Events in Serious *S.aureus* Infections

- Colonization → carriage → toxin production
- Barrier breach or break
- Invasion
- Bacteremia or septicaemia
- Syndrome of severe sepsis (activated C, cytokines, toxin, other mediators)
- Complications (suppurative and inflammatory)
- Death

Streptococcus

- **Gram-positive cocci in chains**
- **Can be beta (clear), alpha (greenish) or non-haemolytic on blood agar plate**
- **Group A beta-haemolytic streptococci or *S.pyogenes***
- **Vancomycin resistant enterococci (VRE) is an important nosocomial pathogen**

Epidemiology of *S.pyogenes* (1)

- Common cause of community-acquired pharyngitis and skin infections.
- Carriage in throat , skin, rectum (<1%) and vagina (<1%)
- Likely that besides contact transmission, there is airborne transmission in SSI

Epidemiology of *S.pyogenes* (2)

- **Implicated in burn wound infections, necrotising fasciitis, puerperal and neonatal infections**
- **Important as cause Acute glomerulonephritis and Acute Rheumatic Heart Disease**

Epidemiology of Enterococci

- Normal habitat in gastrointestinal tract
- Reservoir sites are wounds, skin (inguinal, antecubital fossa), chronic decubital ulcers, vagina and perineal or meatal areas of hospitalised males
- Most important method of spread of VRE is through transient carriage on hands of HCWs

Pathogenesis of Streptococcal Toxic Shock Syndrome (TSS)

- Initial focus is the skin and soft tissues of previously healthy individuals
- Strains have a high propensity to initiate invasive disease
- Pathogenesis not definitely elucidated. Most interest on pyrogenic exotoxins (SPEs)
- Other virulence properties involved eg. M protein, enzymes

Pathogenesis of Nosocomial Infection by Enterococci

- Generally enterococci are part of normal flora and have little pathogenic potential in normal host
- Opportunistic pathogens in the elderly, immunocompromised or when invasive procedures are done
- Little known about virulence factors
- Cause UTI, bacteremia, endocarditis, intraabdominal/pelvic infections, skin/soft tissue infections etc.

Gram-negative Bacteria

- **Fermenters eg. *Escherichia coli***
- **Non-fermenters - Gram - negative bacteria that do not utilize carbohydrates as a source of energy or degrade them through metabolic pathways other than fermentation eg. *Acinetobacter baumannii***

Enterobacteriaceae

- Family of fermentative GNB commonly isolated from clinical specimens and are therefore also important nosocomial pathogens.

- Facultative anaerobes that with few exceptions ferment glucose, reduce nitrate to nitrite and are oxidase negative.

Enterobacteriaceae examples

- *Escherichia coli*
- *Klebsiella* species (*pneumoniae*)
- *Enterobacter* species
- *Proteus mirabilis*

Pathogenesis of NI caused by Enterobacteriaceae

1. Pathogen-specific factors

- Adhesion
- Capsules
- Iron chelators
- Other factors and Tropisms

2. Host factors

- control the extent of colonisation
- determine the susceptibility of the host to disease

Adhesion

- Bacterial adhesion → attachment to surfaces → colonisation → overgrowth → tissue invasion.
- Mediated by both fimbrial and nonfimbrial adhesins that are encoded on plasmids and on the bacterial genome eg. P fimbrae - can anchor bacteria to uroepithelial cells → cause pyelonephritis in adults and children.

Capsules

- **Can partly protect the bacteria against the bactericidal effect of serum and against phagocytosis eg. *Klebsiella* spp.**
- **Can also directly suppress the host immune response.**

Iron Chelators

- Ability of some GNB to acquire iron for growth becomes an important factor in many infections.
- Almost all the iron in the human body is bound to various proteins eg. Hb → limits the availability of free iron for utilization by bacteria.
- Some bacteria have high affinity iron chelators (siderophores) that can scavenge iron for growth purposes.

Other Pathogen Factors & Tropisms

- Virulence factors eg. motility, growth in alkaline pH, colonise skin of HCWs, produce urease→hydrolyse urea in urine→increases urinary pH.
- Liberation of toxins eg. endotoxin.
- Some bacteria have increased device affinity or specific tropism eg. *Enterobacter* spp grow in infusion fluids.

Host Factors

- Control the extent of colonisation with Enterobacteriaceae eg. oropharyngeal colonisation increases with the severity of the underlying disease.
- Determine the susceptibility of the host to developing disease eg. diabetics more susceptible.

Epidemiology of NI caused by Enterobacteriaceae

- Reservoir eg. GIT and oropharyngeal
- Factors controlling colonisation.
- Gastric pH
- Fibronectin
- Hormonal modulation of colonisation
- Modes of transmission and outbreaks

Factors controlling colonisation

- Sick patients endogenous oropharyngeal flora is a common source of Enterobacteriaceae → nosocomial GN pneumonia. Also increase in number under selective pressure of antibiotic use or with increased hospitalisation.

- Intestinal colonisation is found in both healthy and hospitalised patients.

Gastric pH

- Stomach normally sterile.
- Colonisation occurs in critically ill patients and it increases with time.
- Increased pH increases gastric colonization with gram negative bacteria.
- However whether gastric colonisation influences oropharyngeal colonisation and the bacteria then be aspirated into the lower respiratory tract remains unclear.

Fibronectin

- A glycoprotein present on oral epithelial cells which facilitates adherence of Gram-positive bacteria.
- Loss of fibronectin uncovers cellular binding sites and leads to increased rate of oropharyngeal colonisation by GNB.
- Loss of fibronectin when salivary protease secretion is increased in critically ill patients and which then degrades the fibronectin.

Hormonal modulation of colonisation

- Very little known.
- Recurrent UTIs occur in postmenopausal females. Hypothesized that the lack of oestrogen leads to diminished colonisation of the vagina by lactobacilli → colonisation by *E. coli* especially because of increased vaginal pH(>4).

Modes of Transmission and Outbreaks of Enterobacteriaceae

- Primarily spread from person to person via unwashed hands of HCWs or from environmental reservoirs to patients.

- Extensive use of 3rd generation cephalosporins mostly as monotherapy is a risk factor for ESBLs (extended spectrum β lactamases) in ICUs and oncology wards.

Non fermenters

- *Pseudomonas aeruginosa, Acinetobacter* spp., *Stenotrophomonas maltophilia*
- Have minimal growth requirements.
- Differ substantially in virulence.
- Important nosocomial pathogens because of the many reservoirs they inhabit in the hospital and their resistance to commonly used antibiotics.

Rate of Nosocomial Infection for non-fermentative GNB

- NNIS reported during 1987-1997 that 13% of nosocomial infections were due to nonfermentative gram-negative bacilli.
- Isolated mainly from the respiratory and urinary tracts.

Pathogenicity of Non fermenters

- Attachment to host cells.
- Production of extracellular polysaccharides (glycocalyx or alginate).
- Production of extracellular toxins (exotoxins).
- Resistance to serum bactericidal factors.
- Presence of lipopolysaccharide cell wall (endotoxin).

Pseudomonas aeruginosa

- **Adheres to tracheal and corneal epithelium which is enhanced by cell injury due to viral infection or trauma.**
- **Glycocalyx facilitates adherence→chronic infection in cystic fibrosis. Inhibits phagocytosis, i/c killing by leukocytes and penetration of aminoglycosides.**
- **Exotoxins and other enzymes increases virulence.**
- **Cell wall produces endotoxins.**

Epidemiology of *P. aeruginosa* infections

- Conveyed into hospitals via food (vegetables) and tap water. Reservoir in hospitals are moist areas, fluids etc.
- Patients especially haematology-oncology patients are colonised in the rectum and pharynx.
- Nosocomial transmission via contact with environmental sources or from patient-to-patient spread via personnel.

Stenotrophomonas maltophilia

- **Not particularly virulent but can cause serious infection in compromised host.**
- **Majority of strains produce proteases including hyaluronidase and elastase which are potential virulence factors.**
- **Selected for in patients treated with imipenem.**
- **Transmission via hands of personnel.**

Acinetobacter spp.

- Widely distributed in nature.
- Part of normal flora of humans (one-fourth of normal and one-third of hospitalised individuals) and many animals.
- Usually infects immunocompromised or debilitated hosts.
- Multiple modes of transmission involving environment and hands of HCWs.
- Sometimes cause outbreaks in ICUs and Burns.

Resistance of Non fermenters

- **Commonly used antibiotics. Need antibiotic susceptibility testing because sometimes hard to predict.**

- **Disinfectants eg. *P. aeruginosa*, *B. cepacia* found in providone iodine, chlorhexidine, benzalkonium chloride, hexachlorophene.**

Replication and Survival of Non fermenters

- ***Pseudomonas*** **spp. and** ***Burkholderia cepacia*** **replicate in a wide range of moist environments eg. tap & deionised water.**

- **Capable of prolonged survival in moist or dry environments eg.** ***A. calcoaceticus*** **survived 9 days on dry Formica surface cf. 1 day for** ***P. aeruginosa*****.**

Conclusion

Prevention and control of hospital acquired infections by various types of bacteria require attention to many aspects of patient care and the hospital environment.


Thank You