Burn Infection
&
Laboratory Diagnosis
Introduction

Burns are one of the most common forms of trauma.
2 million fires each year
1.2 million people with burn injuries
100,000 hospitalizations
5,000 patients die from related complications

Infection
Burn infections must meet at least 1 of the following criteria:

1. Patient has a change in burn wound appearance or character, such as rapid eschar separation, or dark brown, black, or violaceous discoloration of the eschar, or edema at wound margin and histologic examination of burn biopsy shows invasion of organisms into adjacent viable tissue.

2. Patient has a change in burn wound appearance or character, such as rapid eschar separation, or dark brown, black, or violaceous discoloration of the eschar, or edema at wound margin and at least 1 of the following:
   a. organisms cultured from blood in the absence of other identifiable infection
   b. isolation of herpes simplex virus, histologic identification of inclusions by light or electron microscopy, or visualization of viral particles by electron microscopy in biopsies or lesion scrapings.
3. Patient with a burn has at least 2 of the following signs or symptoms with no other recognized cause: fever (>38°C) or hypothermia (<36°C), hypotension, oliguria (<20 cc/hr), hyperglycemia at previously tolerated level of dietary carbohydrate, or mental confusion
   and
   at least 1 of the following:
   a. histologic examination of burn biopsy shows invasion of organisms into adjacent viable tissue
   b. organisms cultured from blood
   c. isolation of herpes simplex virus, histologic identification of inclusions by light or electron microscopy, or visualization of viral particles by electron microscopy in biopsies or lesion scrapings.
Comments

- Purulence alone at the burn wound site is not adequate for the diagnosis of burn infection; such purulence may reflect incomplete wound care.
- Fever alone in a burn patient is not adequate for the diagnosis of a burn infection because fever may be the result of tissue trauma or the patient may have an infection at another site.
- Surgeons in Regional Burn Centers who take care of burn patients exclusively may require Criterion 1 for diagnosis of burn infection.
Risk factors in burn wound infection

- **Patient Factors:**
  - Extent of burn > 30 per cent of body surface
  - Depth of burn. full-thickness & partial thickness
  - Age of patient
  - Pre-existing disease
  - Secondary impairment of blood flow
  - Acidosis
Risk factors in burn wound infection

- **Microbial Factors:**
  - Density
  - Motility
  - Metabolic products
  - Endotoxins
  - Exotoxins
  - Permeability factors
  - Other factors
Pathogenesis

• Avascularity
• Impaired migration of host
• Toxic substance released by scar tissue
  Impaired local host immune responses
• Moist, warm and nutrition environment
Pathogenesis

• Skin is never sterile
• Colonization of resident and transient flora
• Hair follicles and sebaceous glands
• Burn wound surface are sterile immediately following thermal injury
Pathogenesis

• Bacteria of resident flora are resistant to heat injury. Bacteria in the hair follicles and sweat glands survive, quantitative counts of biopsied specimen show the same $10^3$ bacteria per gram of tissue as found in the tissue prior to burning.

• Proliferation of bacteria in glands greater than $10^5$, break out of the follicles and glands and migrate through the tissue.

• Bacterial proliferation occurs in the subscar tissue and when level exceed $10^6$ or $10^7$ invasion into blood stream.
Pathogenesis

- Heavily colonize the wound surface within the first 48 h unless topical antimicrobial agents are used.
- After an average of 5 to 7 days these wounds are subsequently colonized with other microbes.
- GPC, GNB, Yeast.
- Hosts normal GI and RT flora.
- Hospital environment.
- Health care workers hands.
- Time related changes in burn wound microbial colonization.
Microbial Etiology

- Gram Positive Bacteria
- Patients endogenous skin flora or health care workers
- Gram Negative Bacteria
- Patients GI tract and RT
- Yeast
- Use of broad spectrum antibiotic therapy
Microorganisms causing invasive burn wound infection

<table>
<thead>
<tr>
<th>Group</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-positive organisms</td>
<td><em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td></td>
<td>Methicillin-resistant <em>S. aureus</em></td>
</tr>
<tr>
<td></td>
<td>Coagulase-negative <em>Staphylococci</em></td>
</tr>
<tr>
<td></td>
<td><em>Enterococcus</em> spp.</td>
</tr>
<tr>
<td></td>
<td>Vancomycin-resistant <em>Enterococci</em></td>
</tr>
<tr>
<td>Gram-negative organisms</td>
<td><em>Pseudomonas aeruginosa</em></td>
</tr>
<tr>
<td></td>
<td><em>Escherichia coli</em></td>
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<tr>
<td></td>
<td><em>Klebsiella pneumoniae</em></td>
</tr>
<tr>
<td></td>
<td><em>Enterobacter</em> spp.</td>
</tr>
<tr>
<td></td>
<td><em>Proteus</em> spp.</td>
</tr>
<tr>
<td></td>
<td><em>Acinetobacter</em> spp.</td>
</tr>
<tr>
<td></td>
<td><em>Bacillus</em> spp.</td>
</tr>
<tr>
<td>Fungi</td>
<td><em>Candida</em> spp.</td>
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<tr>
<td></td>
<td><em>Aspergillus</em> spp.</td>
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<tr>
<td></td>
<td><em>Fusarium</em> spp.</td>
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<tr>
<td></td>
<td><em>Alternaria</em> spp.</td>
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<tr>
<td></td>
<td><em>Rhizopus</em> spp.</td>
</tr>
<tr>
<td></td>
<td><em>Mucor</em> spp.</td>
</tr>
<tr>
<td>Viruses</td>
<td><em>Herpes simplex virus</em></td>
</tr>
<tr>
<td></td>
<td><em>Cytomegalovirus</em></td>
</tr>
<tr>
<td></td>
<td><em>Varicella-zoster virus</em></td>
</tr>
</tbody>
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## IRAN

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. aeruginosa</td>
<td>37.5%</td>
</tr>
<tr>
<td>S. aureus</td>
<td>20.2%</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>10.4%</td>
</tr>
</tbody>
</table>

*Indian J Med Res 2007*

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. aeruginosa</td>
<td>73.1%</td>
</tr>
<tr>
<td>S. aureus</td>
<td>10.3%</td>
</tr>
<tr>
<td>Others</td>
<td>16.6%</td>
</tr>
</tbody>
</table>

*Annals of Burns and Fire Disasters 2005*
• Infections due to anaerobic bacteria typically occur to electrical burns
Microbiological Analysis of Burn Wound Infections

- Diagnosis of burn wound infection based on clinical sign and symptom alone is difficult
- **Regular sampling** by surface swab or tissue biopsy for culture
- Quantitative culture of tissue biopsy samples and histological verification of microbial invasion is **gold standard**
Burn wound sampling Techniques

- Regular sampling
- Multiple samples from several areas
- First few days to weeks following injury
- Daily or every 48 h during dressing changes
- Frequency may be decreased to weekly once when the burn has been excised and clinical sign of infection are not present
Sample Type

- Superficial wound sample
- Tissue Biopsy
Superficial wound sample

• Clinical microbiology laboratory routinely provide semiquantitative or qualitative results from cultures of superficial wound samples.

• Surface swab
• Capillarity Gauze
• Direct plate

• Collection of surface sample after the removal of dressings and topical antimicrobial agents and cleansing of the wound surface with 70% alcohol.
Surface swabs

- Surface swabs are an effective method for routinely collecting multiple superficial wound samples.
- In order to obtain enough cellular material for culture the end of a sterile swab is moved over a minimum 1 cm area of the open wound.
- Sufficient pressure should be applied to the tip of the swab to cause minimal bleeding in the underlying tissue.
- **Dry or moistened swab?**
- Moist swab technique provide better reproducibility.
Capillarity Gauze

- Gauze squares moistened in non bacteriologic saline for several minute, inoculate agar culture plates.
- Relatively time consuming and expensive
- Superior to swab culture
- Quantitative culture results is more reproducible.
Tissue Biopsy

- Serial multiple samples from beneath the scar
- Incision
- punch
Specimen Transport

• There are no published standards for transport of burn wound specimens
• Superficial swab and tissue biopsy samples should be received by the laboratory as soon after collection as possible
• Transport media
• Inoculates sample onto culture media within 1-2 h after collection
Analysis of Burn Wound Specimen

- Gram stain
- Surface swab culture
- Quantitative tissue culture
- Histological analysis
Gram stain

- Degree of correlation between surface swab gram stain and culture
- Gram stain may provide an index of the degree of microbial colonization of the burn wound
- Not suitable for diagnosing burn wound infection
- Not provide information on the antimicrobial susceptibility profiles
Surface swab culture

- Provide qualitative or semiquantitative results
- Not suitable for quantitative results
- Blood agar plate and MacConkey agar
- Four quadrant method
- Inspected plate after 24 h aerobic incubation at 37
• A qualitative microbiology report provides the identification of all potential pathogen regardless of amount and report antimicrobial susceptibility test

• **Semiquantitative microbiology report**

• Estimation of the relative predominant of all potential pathogens according to growth in each of the four plated quadrant

  1+,2+,3+,4+

• Identification of each pathogen to the genus and / or species level and antimicrobial susceptibility test
• Quantitative count may be reported from surface swab culture provided a standard area was swabed (e.g., 4 cm²)
• A bacterial suspension made by vortexing the swab in 1 ml of tween 80
• Bacterial suspension plated onto blood and MacConkey agar in 0.1 and 0.01 ml quantities by spreading the sample using a sterile rod
• Incubation aerobically for 24 h at 37
• Colony count and report per cm² for all potential pathogen
• Identification of each pathogen to the genus and / or species level and antimicrobial susceptibility test
Quantitative tissue culture

- The burn wound tissue biopsy sample is first weighed and homogenized in 1 ml of 1% tween 80 using a disposable tissue grinder.
- Bacterial suspensions in 0.1 ml and 0.01 ml quantities from undiluted sample spread over the surface of blood and MC agar.
- If high counts are suspected, original homogenized diluted 1:10 to 1:1,000.
- Incubate plates for 24 h at 37.
- Report colony count per gram tissue.
Histological analysis

<table>
<thead>
<tr>
<th>Histological analysis</th>
<th>Quantitative methods</th>
<th>colonization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0</td>
<td>No growth</td>
<td></td>
</tr>
<tr>
<td>Grade Ib,II,III</td>
<td>$10^3$ To $10^6$ org/gr</td>
<td>Increasing colonization and early invasion to superficial dermis</td>
</tr>
<tr>
<td>Grade IV</td>
<td>$&gt;10^4$ org/gr</td>
<td>Wound infection and need for more aggressive therapy</td>
</tr>
</tbody>
</table>
• Quantitative microbiology is not a diagnostic substitute for histological examination

• High tissue counts may be found during colonization that do not correlate with microscopic tissue invasion
Distinguishing burn wound colonization from infection

- Redness, pain, edema, fever
- Leukocytosis
- Polymorphonuclear leukocyte
- Presence of a moderate to heavy amount of one or more pathogenic bacteria morphotype
- Colonization is present when bacteria are cultured from the burn wound surface in the absence of clinical or microscopic evidence of infection
- Gram stain of a sample taken from a colonized wound normally shows little or no purulence
- Note: secondary inflammatory response to injury
- Gram stain typically show a mixture of normal skin flora and potential pathogen, with lack of predominance of any potential pathogen
- Skin normal flora
- Staphylococcus spp (CNS), Micrococcus spp, Corynebacterium spp, Propionibacterium acnes, Streptococcus viridance group, Neisseria spp, Peptococcus spp
Antimicrobial Susceptibility Testing

- Systematic administration
- Topical administration
- No published standard method for topical antimicrobial susceptibility testing
- **Agar Diffusion**
  - Lake of reproducibility
  - Standardization
Antimicrobial Resistance and burn units

- MRSA
- VRE
- ESBL
- Multiple resistant P. aeruginosa and Acinetobacter spp

100% P. aeruginosa resistance to:
Amikacin, Gentamycin, carbencillin, Ciprofloxacin, Toberamycin, Ceftazidime

58% S. aureus and 60% CNS resistance to:
Methicillin

*Indian J Med Res 2007*

85% P. aeruginosa resistance to:
Amikacin, Gentamycin, Ciprofloxacin

90% S. aureus resistance to:
Cloxacillin and Cephalexin

*Annals of Burns and Fire Disasters 2005*
Burn Units Antibiogram

• Determine Specific pattern of burn wound microbial colonization
• Time related changes in the predominant microbial flora
• Anti microbial susceptibility profile
• In time periods
• Trend in nosocomial spread of these pathogens