A practical approach to the microbiological diagnosis of infectious keratitis

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INTRODUCTION

Microbiology
MATERIAL
SPECIMEN
INTERPRETATION
KEY POINTS

Infectious keratitis

- Inflammation of underlying corneal stroma caused by replicating organisms
  - Bacteria, Viruses, Fungi, Protozoa
- Acute presentation
  - Significant pain and distress
- Rapid initiation of aggressive treatment needed
  - To improve poor natural outcome
  - To halt disease process
  - To limit extent of corneal scarring and loss of vision

Sight-threatening condition - urgency

Helpful contribution of microbiology ? Id & AST

Infectious keratitis: primary pathogens

- Corneal trauma / ulcer
  - P. aeruginosa
  - S. aureus
  - S. pneumoniae
  - Viridans group streptococci
  - Moraxella spp
  - AFB-rapid growers (M. chelonae, M. fortuitum)
  - Nocardia spp
- Contact lens associated
  - Gram negative bacilli including P. aeruginosa, Serratia spp
  - Bacillus spp
- Acanthamoeba spp
- Fusarium spp

Microbiological diagnosis

Improve strategies to increase likelihood of detection of aetiologial agents of OCULAR INFECTIONS

Keys of success:
The best laboratory is not enough!! Essential close collaboration with micro lab
Pathway to microbiological diagnosis of ocular infections

- Urgent notification
- Alarming notification

MICROBIOLOGY

Pathway to microbiological diagnosis

Garbage IN = Garbage OUT

MICROBIOLOGY

Microbiological diagnosis of ocular infections

- Cultures
  - Bacteria (aerobic, anaerobic & mycobacteria), fungi
  - Viruses, Acanthamoeba spp
- Direct microscopy
  - Gram, Giemsa, ...
  - White calcofluor, Immunofluorescence
- Molecular Biology
  - Various PCR methods and targets
    - Minute or scant quantity of specimens
    - Limited viability

MICROBIOLOGY

Communication between microbiologist and ophthalmologist

From laboratory to physician
- Notification of insufficient quantity of specimen
- Reduction of ordered tests to target specific organisms
- Alarming results (= medical emergencies)

To facilitate physicians’ immediate clinical decisions

From physician to laboratory

Communication between microbiologist and ophthalmologist

From laboratory to physician
- Notification of insufficient quantity of specimen
- Reduction of ordered tests to target specific organisms
- Gram negative cocci (N.gonorrhoeae) in conjunctival specimen
- Paeruginosa in a corneal culture
- Bacillus spp in inner eye cultures
- Yeast or mold structural elements (Direct microscopy)

To facilitate choice of the more appropriate procedures and interpretation
**Communication between microbiologist and ophtalmologist**

**From laboratory to physician**
- Notification of insufficient quantity of specimen
- Reduction of ordered tests to target specific organisms
- Gram negative cocci (N.gonorrhoeae) in conjonctival specimen
- P.aeruginosa in a corneal culture
- Bacillus spp in inner eye cultures
- Yeast or mold structural elements (Direct microscopy)

**From physician to laboratory**
- Very small amount of material, warning
- Corneal specimens related to lens, LASIK associated, trauma with foreign object, etc.
- 6-12 months postoperative infection; to perform AST even on Staphylococcus epidermidis

**Material for physician**
- Instructions (+ training !)
- Primary fresh culture media
  - Schedule to replace expiring media
  - Blood agar, chocolate agar
  - Thio Broth or TSB
  - Media for anaerobic, fungal and mycobacterial cultures
- Slides
- Specimen collection & Transport devices
- Topical anesthetic (proparacaine hydrochloride 0.5%)

**Specimen collection**

1. Instillation of 1 or 2 drops of proparacaine HCL
   - Some topical anesthetics and topical dyes: inhibitory to a variety of microorganisms (culture, PCR)

2. Specimens from the conjonctiva
   - From both eyes
   - Comparison of microbiological growth from unaffected eye with affected eye
   - Lower tarsal conjonctiva
   - Gentle scraping with a Kimura spatula
   - Or Dacron/Floaked swabs (moistened with Thio or TSB)
   - Not cotton or calcium alginate swabs
   - To avoid touching eyelashes

3. Corneal scrapings
   - From the advancing edge of ulcer
     - By scraping multiple areas of ulceration and suppuration
     - With a Kimura spatula (short firm strokes in one direction)
     - To avoid touching eyelashes
     - 3 to 5 scrapings per cornea

**Specimen processing**

- Identification of plates
- Inoculation of each set of scrapings onto appropriate media
  - By successive « C » imprints
  - (Or Zig-zag with swab)
- Preparation of smears
  - By applying scrapings in a gentle circular motion over clean identified glass slides
  - Immersion for 5'-10' in methanol (fixing)
    - Gram, Giemsa, Calcofluor, Immunofluorescence, ...
Specimen handling, transportation

To identify and transfer to the microbiology lab without any delay! (< 30’ – 2h)
- Inoculated identified plates
- Collection device with transport media
- if specimens not inoculated at bedside
- Specific transport media for PCR tests
- Slides for smear staining
- For research of *Acanthamoeba spp.*
- Call the lab

**Interpretation**

- **Smears**
  - Gram, Giemsa
  - Presence of PMN ➔ bacterial infection?
  - Presence of mononuclear cells ➔ viral infection
  - Bacteria
  - High positive predictive value
  - Low sensitivity
- Calcofluor white
  - Fungi; *Acanthamoeba*
- Immunofluorescence
  - Viruses

**Interpretation of cultures**

<table>
<thead>
<tr>
<th>Ocular specimen group</th>
<th>Microorganisms</th>
<th>Normally present bacteria: % healthy adults with</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Coagulase negative staphylococci</td>
<td>75-90</td>
</tr>
<tr>
<td></td>
<td>Propionibacterium spp.</td>
<td>50-70</td>
</tr>
<tr>
<td></td>
<td>Corynebacterium spp.</td>
<td>10-75</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus aureus</em></td>
<td>10-40</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus epidermidis</em></td>
<td>2-17</td>
</tr>
<tr>
<td></td>
<td><em>Streptococcus pyogenes</em></td>
<td>0-5</td>
</tr>
<tr>
<td></td>
<td><em>Hemophilus influenzae</em></td>
<td>0-5</td>
</tr>
<tr>
<td></td>
<td>Gram-negative bacilli</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Fungi</td>
<td>0.5</td>
</tr>
<tr>
<td>Group 2</td>
<td>none</td>
<td>none</td>
</tr>
</tbody>
</table>

Group 1: all sites that can be touched with finger or swab
- lid, conjunctiva, component and products of lacrymal system
- NOT corneal swabs!

Groupe 2:
- ALL cornea specimens (including swabs)
- All other specimens: inner eye, lens, iris, retina, sclera, ocular fluids, etc.

**Acanthamoeba sp**

Calcofluor white (cysts)

Culture track left behind by amebae in the lawn of the culture media
Microbiological diagnosis of keratitis

Key points

- Wide range of infectious agents
- Wide range of microbiological methods
- Minute quantity of specimen
- To target (priority) analysis to perform
- Essential close working relationships between physicians and microbiologists
- Development and implementation of optimal protocol
- Increase chances of detecting ocular pathogens
- Crucial quality of pre-analytic issue
- Short time from collection to inoculation
- Direct inoculation by ophthalmologist
- To organize training and setting up

More timely intervention
Less patient morbidity
Improved chances of salvaging eye and preserving sight
Cost savings

Useful references

