”Optimal” microbiological procedures

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Topics

- Optimal sample
- What happens in the Clinical Microbiological Lab?
- Tissue samples >< Fluid samples
- Interpretation
- Culture-negative infections
- Effects of optimal samples
- Future in Microbiological Diagnostics

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Optimal sample

- In general
  - Avoid antibiotic (AB) treatment prior to sampling
  - Reduce risk of contamination (instruments, sampling containers, laboratory precausion eg.)

  Preferred antibiotic-free window: 2 weeks - sampling including perioperative AB

- Intra-operative tissue samples (Osteomylitis, PJI and FRI)
  - 4-6 deep-tissue samples
    - Infection-suspected tissue
    - Implant-bone interface
    - Different locations
  - No samples from a sinus tract or open wound

- Aspiration fluid sample (PJI)
  - Joint puncture

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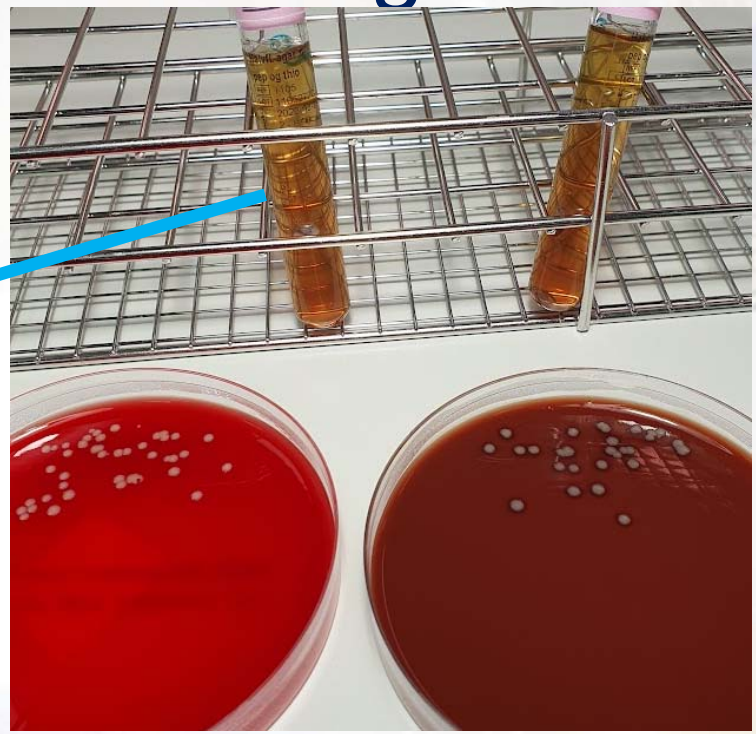
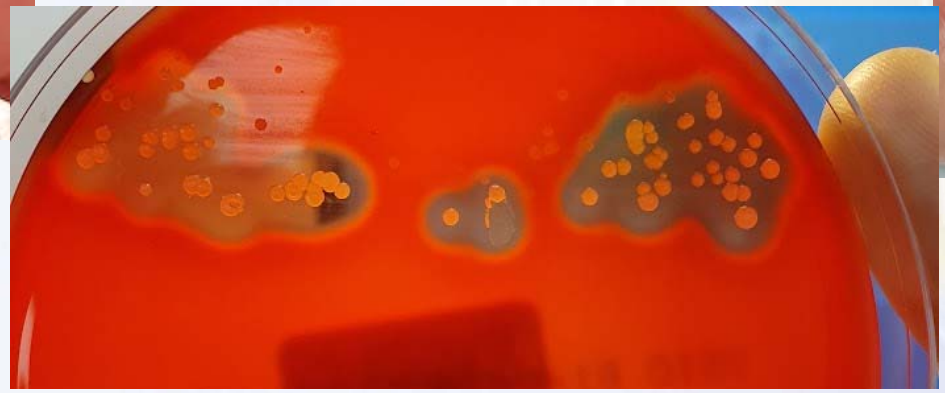
What happens in the Clinical Microbiological Lab?

- Intra-operative tissue samples
  - Received in separate containers
  - Tissue samples are divided into pieces
  - A freshly cut side of tissue is applied to 3 types of plates: Blood agar plate, Chocolate agar plate, and Anaerobic agar plate (K-vitamin + Cysteine)
  - A separate piece of tissue is applied to a thioglycolate enrichment broth (thio)
  - Incubation 14 days
  - Checked daily till day 4
  - Small samples are not divided, only put into thio
  - (If aspirate are received simultaneously it is treated as joint fluid)

  - Tissue beat-beating (homogenisation)
  - Sonication (Biofilm disruption)

What happens in the Clinical Microbiological Lab?





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What happens in the Clinical Microbiological Lab?

- Aspiration fluid samples (joint fluid)
  - Microscopy
  - 10 µl fluid spread on a Chocolate agar plate
  - 3-4 ml fluid is incubated for 5.6 day in a blood culture bottle (automated blood culture system)

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Tissue samples >< Fluid samples

- 4-6 tissue samples (time to positivity* ≈ 1-4 days):
  - Multiple samples
  - Compare samples when polymicrobial findings
  - Ability to estimate the degree of positivity (e.g. 1 colony vs. 200 colonies)
  - Different specialised media including absorption of antibiotics in the media

- Fluid samples (time to positivity* ≈ hours - 3 days):
  - Typically a diagnostic puncture = 1 sample
  - Microscopy find samples with 10^4-10^5 copies/mL
  - Blood culture systems, high sensitivity and specificity
  - Hard to estimate the degree of positivity

*Including susceptibility pattern

Interpretation

Guidelines, Deep tissue samples

Infection confirmed:

- Major criteria 2 distinct species / 4-6 samples

Infection likely:

- Combined clinical features + 1 distinct species / 4-6 samples

Guidelines, Aspiration fluid samples

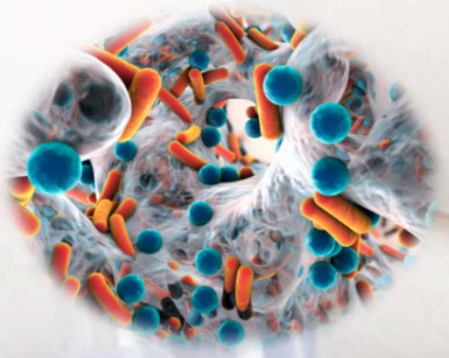
Infection likely:

- Positive culture 1 distinct species

Distinct species are not clearly defined, maybe:

- Phenotypically identical (colony size, colour, smell etc)
- Identical susceptibility pattern
- Species identification -> MALDI-TOF MS

Interpretation?



"Everyday" interpretation (suggestion):

- Infection confirmed despite AB treatment during sampling:
  - *S. aureus, S. lugdunensis, P. aeruginosa, Enterobacterales* (only one species), *S. pyogenes, S. dysgalactiae*
  - ≥ 3 samples with *S. epidermidis*, other KNS, *Corynebacterium spp, Enterococci, S. agalactiae, Cutibacterium acnes* (skin flora)
- Infection possible, no AB treatment during sampling:
  - The above
  - 2 samples with *S. epidermidis*, other KNS, *Corynebacterium spp, Enterococci, S. agalactiae, Cutibacterium acnes* (skin flora)
- Microbiological diagnostics not helpful – sinus tract or open wound? :
  - ≥ 3 different species in different amounts (e.g. 2/5; 3/5; 4/5)
  - Combination of faecal flora and skin flora

Culture-negative infections

- AB treatment during sampling?
  - Yes,
    - Option to perform multiple specific PCR
    - 16S rDNA PCR and sequencing
    - Microbiome
  - No,
    - Maybe fastidious microorganisms
    - Option Microbiome
- Question the primary diagnosis?

Effects of optimal samples

- Patient
  - Effective treatment
  - Precise susceptibility pattern
  - AB with bactericidal effect (if possible)
  - Orally administered AB
  - Avoid unnecessary side-effects
  - Reduced antibiotic pressure on normal microbial flora
- Organisation
  - Reduces risk of antimicrobial resistance
  - Change to orally administered AB
  - In some cases continuous IV-infusion
  - Knowledge of pathogens and resistance patterns in Denmark

Future in Microbiological Diagnostics

Today, not implemented

- PCR panels with 20-40 pathogens designed for platforms that can be operated 24-7 (≈ 200 € / sample vs. 20 € / sample)

Near future

- Optimised separation/extraction of pathogens from human tissue
- Sequencing directly on sample material without prior culture or PCR
- PCR detection of pathogen components from blood

Future

- Many of the diagnostic methods used today still valid
- Sequencing of pathogens from blood

Thank you for your attention

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