Clinical Utility of 16S rRNA Gene Sequencing

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DO THE WELSH AND SCOTS GET ON?

OH YES... THEY BOTH HATE THE ENGLISH
PCR and sequencing of the 16S rRNA gene has been successfully used for bacterial identification in culture negative samples where there is a clinical suspicion of microbial involvement.
Why use the 16S rRNA gene for bacterial detection and identification?

- Found in all bacteria and in multiple copies in most bacteria
- Able to identify most bacteria to species level
- Useful when bacteria fastidious or slow growing
- Bacteria detected alive or dead
- Results not affected by antibiotic therapy
- Well populated database aids identification

Prokaryotic ribosome
16S rRNA - a *de facto* gold standard for clinical molecular identification

**Biology**
- Universal
- Highly conserved
- Long enough (~1500 bp) to provide significant discrimination between many species
- Bonus: structural information can guide alignment and phylogenetic reconstruction

**History**
- Early work described the usefulness of this gene in taxonomy
- Many species now represented in databases
16S gene as a PCR target

- Peaks represent hypervariable regions while troughs represent conserved regions [5’ portion commonly used for speciation of bacteria]
- PCR amplifies 5 variable regions using 27F and 797R primers
Method

- Samples were processed in DNA-free tubes with mechanical disruption or sonication followed by heating at 95°C.
- Automated DNA extraction was performed on the Magnapure compact instrument (Roche Diagnostics).
- Bacterial DNA was amplified using DNA-free reagents (Molzym Life Science, Germany) and 16S PCR primers 27F and 797R.
- Positive and negative extraction controls and a no template control were included in every run.
- The expected amplicon size was 750-770bp.
Sequencing was performed using an ABI3130 Genetic Analyser and the consensus sequence analysed using the NCBI BLAST tool.

Bacterial identifications were made according to the Clinical and Laboratory Standards Institute guideline MM18-A4.
Limitations and confounders

- Compared to traditional methods for routine organisms
  - Expensive
  - Time consuming
- Sampling: organism not homogenously distributed
- Quantity/quality of genetic material not sufficient
- Mixture of organisms in specimen
  - in situ
  - introduced
- 16S not sufficiently divergent for discrimination of some species
Case 1

Derek thought the paper cut he got on his finger the week before might be infected...
Mr TK

- 50
- Caucasian
- Ex smoker 7 years
- Previous heavy alcohol use but none now
- Strong FH of ischaemic heart disease
Mr TK

- Fit and well till February 2010
- Developed significant arthritis/arthralgia and was investigated by rheumatologists at Clatterbridge
- ***Pseudogout***
Over next 12 months significant night sweats and fevers

March 2011 – SOB on exertion

CXR normal

CRP 43

WCC N
Differential Diagnosis

- Infection
- Malignancy esp haematological
- Connective tissue disease
- Hyperthyroidism
- HIV
- Inflammatory bowel disease
Mr TK

- April 2011
- GP heard murmur
- Recent travel to Portugal
- Admitted to Countess of Chester
- ECG ST-elevation
- 3 VF cardiac arrests
- Immediate transfer to the regional cardiothoracic centre
- Cardiogenic shock requiring inotropic support
- Normal angiogram.

- Transthoracic echocardiography revealed vegetations on the mitral and aortic valves

- A diagnosis of infective endocarditis was made.
Treatment

- Empirical IV benzylpenicillin, flucloxacillin and gentamicin
- Urgent mitral and aortic valve replacement
- Post-operatively, changed to ciprofloxacin and teicoplanin
- Initial blood cultures were negative.
Possible Aetiology?

- Any ideas?
Microbiological Investigations

- 6 sets of blood cultures were negative.
- Serology for brucellosis negative
- Serology for bartonellosis negative
- Serology for Q fever negative
- Autoimmune screen negative
Diagnosis

- 16S rRNA PCR sequencing at RLUH of the aortic valve
- Identified *Tropheryma whippelii*
- Result achieved within 48hrs
- Result confirmed by reference lab 14 days later
- Diagnosis of Whipple’s endocarditis made
Treatment

- Antibiotic treatment was rationalised to intravenous ceftriaxone.
- Upon discharge, switched to long-term co-trimoxazole prophylaxis.
- Patient remains well five months later.
Whipple’s Disease

- Tropheryma whippelii
- Typically affects middle aged caucasian men
- Source of transmission not well established
- Prodromal arthralgia and arthritides
- Commonly followed by weight loss, chronic diarrhoea and abdominal pain.
- Can affect any system.
- Usually diagnosed by intestinal biopsy.
- Only 1000 cases described
- Relapse rate up to 15% post treatment
Clinical Manifestations

- There are four cardinal clinical manifestations of Whipple's disease
  - Arthralgias
  - Weight loss
  - Diarrhea
  - Abdominal pain
CNS
- Supranuclear opthalmoplegia
- Cognitive changes
- Cranial nerve palsies
- Myoclonus
- Seizures
- Oculomasticatory/facial myorrhythmias

Ocular
- Uveitis

Resp
- Pleural effusions
- Pulmonary infiltration

CVS
- Myocarditis/Pericarditis
- Endocarditis

GI
- Malabsorption
- Chronic diarrhoea

Skin
- Melanoderma

MSK
- Migratory arthralgias
- Arthritis
- Hypertrophic Osteoarthropathy
- Myalgias

General
- Adenopathy
- Lethargy
Clinical lessons

- In culture-negative endocarditis, 16S rRNA PCR sequencing can contribute significantly to microbiological diagnosis.

- Whipple’s disease is a destructive and potentially fatal condition without appropriate treatment, so early diagnosis needed.

- Thought to be rare but new techniques may increase number of cases diagnosed.
Clinical lessons

- Tropheryma whipplei was initially described by Nobel Prize winner George Hoyt Whipple in 1907.

- Important consideration in culture-negative endocarditis, even in the absence of abdominal symptoms.
Case 2

"The virus is that bad, huh?"
Ms CC

- 23 female
- Caucasian
- Cord blood stem cell transplantation for relapsed ALL in CR2
Ms CC

- Complicated by GVHD requiring increased immunosuppression
- Pre-emptive Rx of reactivated CMV with valganciclovir
Ms CC

- Day +140 post transplant
- Fever
- Left pleural effusion
- Two gluteal abscesses
Ms CC

- Respiratory function worsened
- Ventilated
Ms CC

- BAL
- CMV 81000 (serum 480000)
- Adenovirus PCR POS in serum  BAL NEG
- Pleural/gluteal abscess culture negative
- 16 rRNA gene sequencing
Ureaplasma parvum
Mycoplasma and Ureaplasma Species

- General Characteristics
  - Once thought to be viruses because of size
  - Mycoplasmas are the smallest free-living organism in nature
  - Four human pathogens
    - *Mycoplasma pneumoniae* - respiratory
    - *Mycoplasma hominis* - urogenital
    - *Ureaplasma urealyticum* – urogenital
    - *Ureaplasma parvum* - urogenital
Mycoplasma and Ureaplasma Species (cont’d)

- Pleomorphic organisms – do not have a cell wall (resistant to cell-wall-active antibiotics)
- Slow growing, highly fastidious, facultative anaerobes
- Require complex media for growth
- Susceptible to heat and drying
- Transmitted via direct sexual contact, mother-child exposure during delivery or respiratory secretions
M. hominis & Ureaplasma species

- Most often associated with urogenital tract infections
- May be isolated from asymptomatic individuals
- Can be transmitted to the fetus at delivery
- Opportunistic pathogens
Laboratory Diagnosis

- Cultures must be delivered immediately to the lab, because the organisms are very susceptible to drying.
- Should be placed in transport media.
- Swabs should be of Dacron, polyester or calcium alginate with a plastic or aluminum shaft.
- If not plated immediately, should be frozen at -70°C.
- Most infections detected via serologic evaluation.
Ms CC

- Rx azithromycin, ganciclovir and cidofovir
- Died after 6 weeks on ITU
Is that chicken cooked to 170 degrees? Are you going to eat those sprouts? They're a haven for bacteria!

Why microbiologists hardly ever get a second dinner date.
Mr JL

- 42 male
- Caucasian
- IVDU
- Presented with femoral DVT and multiple septic pulmonary emboli
Blood culture on admission
Gram positive bacillus
Unable to ID by phenotypic methods
Authorised as skin contaminant
Mr JL

- Empyema drained
- GPB isolated
Mr JL

- Blood culture and empyema isolate subjected to 16S rRNA gene sequencing
Actinomyces turicensis
**Actinomyces**

**PHYSIOLOGY AND STRUCTURE**

- facultatively anaerobic or strictly anaerobic,
- gram-positive rods.
- no acid-fast
- grow slowly in culture
- filamentous forms or hyphae
  (resembling fungi)
- true bacteria: lack mitochondria,
  : nuclear membrane,
  : reproduce by fission,
  : inhibited by penicillin but not antifungal antibiotics.
-Human infection: *Actinomyces israelii, Actinomyces naeslundii,*
  *Actinomyces radingae, Actinomyces turicensis*
- upper respiratory, gastrointestinal, and female genital tracts.
  not normally present on the skin surface.
- low virulence potential, cause disease only when the normal mucosal barriers are disrupted by trauma, surgery, or infection

**Actinomycosis**
- in keeping with the original idea that these organisms were fungi or "mycoses"
- development of chronic granulomatous lesions → suppurative, form abscesses connected by sinus tracts.
- **sulfur granules** in the abscesses and sinus tracts: yellow or orange, are masses of filamentous organisms bound together by calcium phosphate
- The areas of suppuration are surrounded by fibrosing granulation tissue
EPIDEMIOLOGY

- Actinomycosis - endogenous infection
- Disease is classified according to the organ systems involved.
  - Cervicofacial infections: have poor oral hygiene or have undergone an invasive dental procedure or oral trauma. In the mouth, invade into the diseased tissue and initiate the infectious process.
  - Thoracic infections: generally have a history of aspiration, in the lungs and then spreading to adjoining tissues.
  - Abdominal infections: gastrointestinal surgery or have suffered trauma to the bowel
  - Pelvic infection: secondary manifestation of abdominal actinomycosis or primary infection in a woman with an intrauterine device
  - Central nervous system infections: represent hematogenous spread from another infected tissue, such as the lungs.
**CLINICAL DISEASES**

- **cervicofacial type**: most cases of actinomycosis
  - acute, pyogenic infection, slowly evolving, relatively painless process
  - tissue swelling with fibrosis and scarring, as well as draining sinus tracts along the angle of the jaw and neck
- **thoracic actinomycosis**: nonspecific. Abscesses form in the lung tissue early in the disease and then spread into adjoining tissues as the disease progresses.
- **Abdominal actinomycosis**: abdomen, potentially involving virtually every organ system.
- **Pelvic actinomycosis**: benign form of vaginitis or, more commonly, there can be extensive tissue destruction, including the development of tuboovarian abscesses or ureteral obstruction.
- **central nervous system actinomycosis**: solitary brain abscess, but meningitis, subdural empyema, and epidural abscess are also seen.
Conclusions

- 16S rRNA sequencing can be a useful tool for direct bacterial identification from tissue and fluids
- May have direct clinical impact
- May help better define emerging epidemiology and clinical syndromes