16S rRNA Gene Sequencing on Clinical Materials: Experience of the NML Special Bacteriology Lab

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Government of Canada / Gouvernement du Canada

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Methods 2006-2014

- First requests for 16S detection in clinical materials rec'd > 10 years ago
- 'Last resort' test: attempts to recover live bacteria from sample failed at hospital or PHL level.
- Targets ='non-culturables'/non-viable bacteria (esp endocarditis patients) – bacterial infection suspected, but no growth by culture
- Initially targeted nearly full sequence (=very poor sensitivity) or tried very short fragments (200-300 bp=too sensitive, lots of contamination etc)
- ~4 yrs ago, began to target ~500 bps on 5' end; sensitivity~10x5e-10x6e CFU/ml

NML Methods

- Specimen acceptability as per NML Guide to Services (https://www.nml-lnm.gc.ca/guide2/index-eng.htm)
- From GTS, 'prefer' specimens:
  - Derived from sterile body sites; rejected if potentially non-sterile source (eg respiratory) or target not 16S actually required
  - 0.5ml / sterile fluid
  - Description of clinical findings, Gram result
  - Serum rejected in recent years
- Referrals tested for 16S and human beta globin, to assess for inhibition / integrity of extraction

NML Methods, continued

- Extraction in SB done manually
- Extraction dependant on specimen type
  - Fluid: Qiagen kit (if not bloody or too viscid)
  - Blood: Qiagen kit (bloody fluids)
  - Tissue/ more solid materials: Epicentre kit. Test 'neat' and 1:10 (after estim. conc. DNA)
  - Pathology tissue in paraffin: Qiagen paraffin kit; remove paraffin, then extract (process often degrades DNA)
- 2006-2011- used traditional gels ➡ band
- > 2011, used QIAxcel™ ➡ band

NML Methods Cont'd/ Report Criteria

- Controls: NTC, HELA cells, *Staphylococcus aureus* ATCC 25923 run as seq control
- If b-globin detected AND appropriate size band detected, product sequenced
- Weak positive often difficult to sequence
- b-globin detected but 16S band not detected = 'Inconclusive', with usual caveats [='negative result']
- b-globin not detected, is re-extracted/retested
- If 2nd b-globin negative but blood culture specimen pos, then bacterium reported w/caveat
- If 2nd b-globin not detected and specimen neg, = 'inconclusive' with extra caveat re: b-globin

~355 requests for 16S testing 2006- June 2014

- Few referrals / year til ~2010
- 2010-2014 ~ 313 (~88%) were:
  - not rejected for some reason
  - Volume acceptable for testing
  - had results logged in
  - Did not have >1 bacterium detected
- Samples rec'd by region 2006-2014:
  - AB: 2; BC: 1; MB: 151; ON: 18; QC: 103; NB: 73; NL: 1; NS: 1 (SK, PEI, NWT, YT, NU: 0)
- Testing for RUO; clinician decides relevancy

If Detected by 16S, can do additional Testing..

- If *S. aureus, N. meningitidis*, or *H. influenzae*, can send to NML lab for genotyping / resistance markers etc;
- If *Legionella*, do *mip* gene testing for species;
- If *Corynebacterium diphtheriae*, *C. ulcerans*, can go *tox* gene testing
- If *Nocardia*, other related genera, can do *sec*A1 testing
- If *Streptococcus*, can do partial *rpo*B etc

Overall Results: 50/313 positive (~16%)

CSF (N= 10/56 [18%] positive, 10 patients

No.*	**Bacteria ID**	**Clinically relevance**
1	*Ralstonia pickettii*	possible
2	*Aggregatibacter aphrophilus*	probable
3.	*Porphyromonas endodontalis*	Probable
4, 6, 9	*Streptococcus pneumoniae*	Probable
5, 7, 11	*Neisseria meningitidis*	Probable
8.	*Acinetobacter junii*	Possible

*Shown in order by date rec'd

Overview Results

Pleural Fluid (N=5/24 [21%] pos, 4 patients) (in order by date)

No.	**Bacteria ID**	**Clinical Relevance**
1.	*Fusobacterium necrophorum*	Possible/probable
2, 3.	*Haemophilus influenzae*	Possible/probable
4, 5	*Streptococcus pneumoniae*	Possible/probable

Heart* (N=8/29 [28%] pos, 8 patients) (in order by date)

No.	**Bacteria ID**	**Clinical Relevance**
1.	*Streptococcus equis ss ruminatum*	possible
2., 7.	*Staphylococus aureus*	Possible/probable
3.	*Haemophilus parainfluenzae*	Possible
4.	*Neisseria meningitidis*	Possible/probable
5.	*Haemophilus influenzae*	Possible/probable
6.	*Streptococcus gordonii*	Possible/probable
8.	*Corynebacterium diphtheriae***	Endocarditis (*tox* gene neg)

***Mitral, tricuspid, aortic, pericardial tissue, material from pacemaker; ** RARE**

Aspirates/Tissue [no. positive/no. of site, %]

- Liver [2/8, 25%]: *Streptococcus intermedius, Pretovella stercorea;*
- Psoa/muscle [3/6, 50%]: *St. intermedius; Staph. epidermidis; Streptobacillus moniliformis* {?}
- Lymph [2/6, 33%]: *Staph lugdunensis; P. acnes*
- Wound [1/3, 33%]: *Staph schleiferi* {?}
- Thigh [1/3, 33%]: *Strept intermedius*
- Lung [1/5, 20%]: *Fusobacterium nucleatum*
- Bone, Marrow [1/8, 12%]: *Ralstonia pickettii*

Yellow: commonly found to be contaminant; {?}: unusual agent for site

Other Aspirate/Tissue [no. positive/no. of site, %]

- Eye [2/4, 50%]: *Prop acnes; Staph epidermidis*
- Peritoneal [1/3, 33%]: *Ureaplasma parvum* {?}
- Brain [2/13, 15%]: *Staph aureus; Strept intermedius*
- 'Swab' [1/2, 50%, breast abscess]: unidentified, like NML 120705 (represents family, genus and species novum)
- Misc. 'Tissue' [0/17, 0%] eg: breast, hip, dural, tumour, spinal, femur, other
- Misc. 'Biopsy' [1/8, 12%]: *Strept pneumoniae*; (vertebral, breast, other)
- Misc. 'Aspirate' [4/15, 27%]: *B. cereus, Actinomyces odontolyticus, Fusobacterium nucleatum* (chest empyema), *Strept pyogenes*

Yellow: commonly found to be contaminant; {?}: unusual agent for site?

Fluids not CSF

- Synovial/ Knee Fluid: [0/34, 0%]
- Various other Fluids [4/22, 18%] including: hip, substernal, paraspinal, subhepatic, peritoneal, ankle, calf, pulmonary, joint, subdural, 'fluid', 'broth', abscess, joint, 'chest tube', ankle, other:
  - *Cardiobacterium hominis*
  - *Granulicatella adiacens*
  - *Streptococcus intermedius*
  - *Streptococcus pneumoniae*
- Blood (blood, serum, plasma) [1/47, 2%]: *B. circulans*

Yellow: commonly found to be contaminant

NML- Top Five Bugs Detected by 16S

- *Streptococcus pneumoniae* (N=7) [CSF x3; pleural x2; bx]
- *Neisseria meningitidis* (N=5) [CSF x4; mitral]
- *Streptococcus intermedius* (N=4) [tissue/fluid aspirates 4x]
- *Haemophilus influenzae* (N=4) [pleural 2x, mitral, pericardial]
- *Fusobacterium* spp (N=3) [pleural 2x, lung bx]

Top candidates for being Contaminants:

- *P. acnes* (N=2) [lymph tissue; eye fluid]
- *Staph epidermidis* (N=2) [psoas muscle, eye fluid]

Least Frequently Positive by Specimen Type:

- Synovial fluid, blood (blood w/ or w/o EDTA; plasma, serum)

Advantages of 16S Testing-all 3 talks

- If specimen positive & deemed to be relevant, may provide closure to otherwise difficult cases
- Testing is relatively rapid (results ~1-3 days, compared with 5-7d for culture; >21 d for TB)
- Testing very rapid & precise if RT established for various gene targets for ID/resistance (*Mycobacteria*)
- Testing may raise possibility of extremely rare or unusual causes of infection, eg
  - *Fusobacterium* spp from pulmonary sources
  - *C. diphtheriae* from endocarditis
- If bacterium 'relevant', clinician can more confidently modify treatment

Challenges-from all 3 talks

- Value of results: is for 'RUO'
- Protocols not optimized for specimen types
- "Insensitivity" Issues: esp synovial (too viscid?); blood; other; LOD unknown
- Samples with multiple/double peaks/ ambiguous bps) suggestive of >1 bacterium present ('Sepsitest'?)
- Weak positives difficult to sequence
- Human B-globin not detected: esp CSF, blood culture bottle = longer TAT
- Detection ≠ pathogen…bacterium may be contaminant; expert interpretation required
- Resolution: can't speciate some by 16S alone

Questions?

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