

**Results of A National Proficiency Test
for 16S rRNA Gene Sequencing
AND / OR MALDI-TOF Testing**

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[Disclosures: NONE]



Objectives

- Background for dual 16S and MALDI-TOF proficiency Test;
- Survey for participation
- Criteria to select bacteria for panel
- Review number, type of participants
- Review Results
- Next Steps

Recap: 2010 - 2011

- (in 2010-2011) NMG group discussed possibility for holding a national proficiency test for 16S
- Such a test not readily available from commercial proficiency test organizations, such as CAP, CMPT etc
- We (Special Bact) were asked to conduct a test
- 2012 proficiency test sent to 13 participants/sites with panel of 2 strains

2013 Proficiency Test

- Surveyed both NMG AND CMUG memberships re: participation in 2013 test.
- Also contacted specific labs with no representation in either group.
- Again, used '**Survey Monkey**' tool to poll with 15 day deadline for response (sent on July 10 2013, response by July 26 2013)
- Encountered considerable problems with polling in this manner:

Problems with Survey Polling Method

1. Had ~30% of emails returned for reasons unknown (bad addresses? Faulty network?)
2. ~10% plus: vacation stops
3. 1 could not (**not allowed to**) open 'Survey Monkey' type polls at their workplace
4. Did 1-2 additional rounds of email if original delivery failed (time consuming!)
5. Some labs, > 1 person responded; had to try to limit to 1 contact per site
6. Some joined test late (by email)

Final List of Participants/Sites

- 25 sites with 29 participants
- Each site had choice of doing 16S rRNA gene sequencing **AND / OR** MALDI-TOF
- If doing both 16S and MALDI, requested that different people do 16S / MALDI and not to discuss results with each other
- Panel sent on Sept 4, 2013
- Results due back on Sept 23, 2013
- Option to test > 1 person at single site
- MALDI & 16S pretests [coded] by SB techs

Selecting Strains for Panel

- With mix of capacities / skill sets, selected **three** clinical isolates which were:
 - RL **1** or **2**
 - Good growers in 24-48h (did not send strict anaerobes; slow growing nocardias etc)
 - Easy to extract DNA or perform MALDI as direct or after extraction
 - Unlike 2013, included a degree of difficulty ranging from '**Easy**' (unambiguous), '**Easy to Moderate**' (some ambiguity) and '**Difficult**' (organism reportable by NML as unidentifiable)

Info Requested from participants

- Demographics of lab contact eg name, phone number, address, email etc
- Option of sending > 1 result/ site report, where > 1 participant at a single site
- This option was exercised by several labs:
 - Labs 6 & 22, 1 participant did 16S, 1 did MALDI results sent in at same time
 - Lab 20 had 4 people independently do both 16S and MALDI, results collated, sent in
 - Lab 25 had 2 people do 16S
- 25/29 (**86%**) sent results in by Sept 23/13 due date

Info Requested from participants

16S Info provided by participants:

- If method done by in-house methods or internally then sent out for sequencing
- No. bps obtained
- ID obtained; Scores /degree of identity; Comment if would **report out definitively**

MALDI: System used, version of ID software

- If strain run as 'direct' or 'extracted'
- MALDI ID; Score obtained; comment if would **report out definitively**

Results: Clinical Isolates selected for Panel

External Proficiency Test (EPT)-2013- 01:

Streptococcus suis NML 070844

- Described in case report 2009 EID 15:350-352 (Haleis et al)
- Strain recovered from blood culture of very ill patient
- had acquired isolate while handling piglets when working in an abattoir. Sequence deposited in GB ([EU 477176](#))



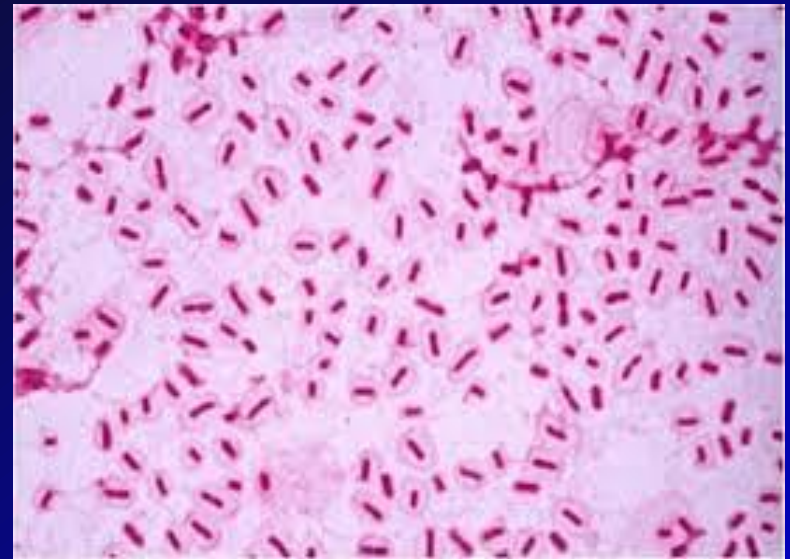
S. suis, Google Image



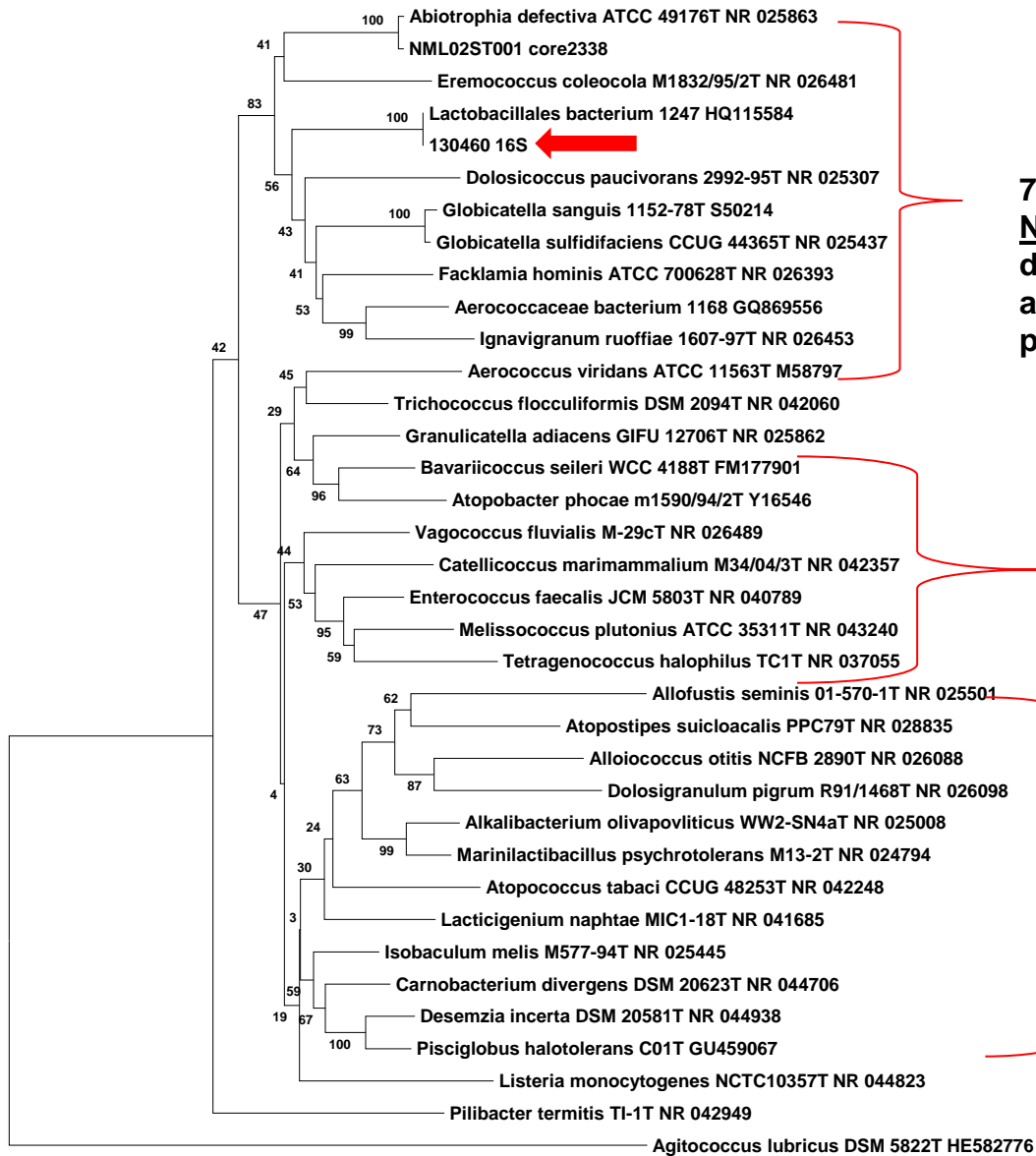
S. suis, Google Image

EPT-2013-2 *Klebsiella pneumoniae* -with caveat

- Rec'd as a catalase + oxidase – GNB for ID. [NML strain 130554] recovered from urine of patient with presumptive UTI
- As final, our lab reported out 16S result with caveat and asked client if further work would be required (would be referred to NML enterics)



- NML **130460**, 'Unidentifiable' by NML testing
- Reported: "possible genus and species novum in family *Aerococcaceae*"; Identical with seq from clinical isolate deposited by Funke (Germany) [acc. No. **HQ115584**]
- NML isolate from deep tissue wound on a wrist
- Small colony (0.5-1mm) 24h-48h, non-haemolytic
- Gram pos rod / coccobacillus; catalase -; oxidase -
- Slowly fermented few sugars; reactive in some substrates (conv., API, BIOLOG)
- AST, had high MICs to clindamycin, rest low (no CLSI interpretations available)
- CFAs ~various Gram pos cocci, eg Strept, Gemella



7 Genera in Family *Aerococcaceae*
Note: Sequence HQ115584 deposited by G. Funke lab; we agreed to write up at some future point

Genera in family *Enterococcaceae*
 Except for *Pilibacter* (below)

Genera in family *Carnobacteriaceae*,
 except for *Trichococcus*,
Granulicatella,
Atopobacter (above)
 and *Agitococcus* (below)

0.02

NJ tree. Closest Genera from Order *Lactobacillales* with *Listeria monocytogenes* intended outgroup. Note genera *Pilibacter*, *Agitococcus*, less related to other taxa shown here. Other families were more distant (& so were not shown here: *Lactobacillaceae*, *Leuconostocaceae* *Streptococcaceae*)

Summary of Expected Results-sent Oct 2, 2013

EPT-2013-01 (*Streptococcus suis*):

16S: >99%-100% identity to TS of *S. suis*; unambiguous

MALDI: high (>2, Bruker; high confidence (Vitek). *S. suis*

EPT-2013-02 *Klebsiella pneumoniae*, reported with caveat:

16S: >99% to TS of *K. pneumoniae* (= *subsp pneumoniae*) but also >99% to 2 other subsp of *K. pneumoniae* (*ss ozyanae*, *ss rhinoscleromatis*) and to a validly named species, *K. variicola*

MALDI: high confidence to *K. pneumoniae*. Both species found in Bruker database, but only *K. pneumoniae* found in Vitek

EPT-2013-03 Unidentified Gram positive rod or coccobacillus:

16S: Unidentifiable; <93% to genera in family *Aerococcaceae*

MALDI: Unidentifiable (both Bruker & MALDI low confidence to all entries)

Each site given confidential Result code

Klebsiella variicola ??????

- 1st described in 2004 System. Appl. Microbiol 27:27-35 by Rosenblueth et al
- Validated in 2004 IJSEM 54:631-632, List no. 97
- Species differed from *S pneumoniae* by DNA-DNA hybrid+ 5 other gene targets; recommended *rpoB* as a possible secondary gene target.
- Species differed by (at least) **adonital negative**
- Recovered from plants but also blood cultures etc

In 2013: **J. Med Microbiol. 62:801-803** by Seki et al:

- *K. variicola* cause of fatal sepsis; MisID by Microscan as *K. pneumoniae*; ID as *K variicola* based on 16S, *rpoB*, next Gen Sequencing.
- Unknown if treatment options differ

Vitek MS uses % similarity of test strain to an entry, eg 99.9% *S. suis*

Meaning of Consistency Categories (A - C)

Category	Description
A	Species Consistency: The best match was classified as 'green' (see above). Further 'green' matches are of the same species as the first one. Further 'yellow' matches are at least of the same genus as the first one.
B	Genus Consistency: The best match was classified as 'green' or 'yellow' (see above). Further 'green' or 'yellow' matches have at least the same genus as the first one. The conditions of species consistency are not fulfilled.
C	No Consistency: Neither species nor genus consistency (Please check for synonyms of names or microbial mixture).

Meaning of Score Values

Range	Description	Symbols	Color
2.300 ... 3.000	highly probable species identification	(+++)	green
2.000 ... 2.299	secure genus identification, probable species identification	(++)	green
1.700 ... 1.999	probable genus identification	(+)	yellow
0.000 ... 1.699	not reliable identification	(-)	red

Biotyper results include:

- a) Summary report contains run scores for all run samples
- b) List of top matches for individual samples
 - One can also click on sample identifier and jump to specific list of top matches for 1 sample
 - Interpretation done by comparing score with Bruker score values (shown above)
 - Some papers: scores too stringent, could be lowered for some taxa

<u>EPT 2013 1 direct plating-1</u> (++) (A)	Streptococcus suis	<u>2.196</u>	Streptococcus suis	<u>2.137</u>
<u>EPT 2013 1 direct plating-1</u> (++) (A)	Streptococcus suis	<u>2.311</u>	Streptococcus suis	<u>2.109</u>
<u>EPT 2013 1 direct plating-1</u> (++) (A)	Streptococcus suis	<u>2.173</u>	Streptococcus suis	<u>2.173</u>
<u>EPT 2013 1 formic acid extract</u> -1 (+++)(A)	Streptococcus suis	<u>2.401</u>	Streptococcus suis	<u>2.273</u>
<u>EPT 2013 1 formic acid extract</u> -1 (+++)(A)	Streptococcus suis	<u>2.332</u>	Streptococcus suis	<u>2.23</u>
<u>EPT 2013 1 formic acid extract</u> -1 (+++)(A)	Streptococcus suis	<u>2.333</u>	Streptococcus suis	<u>2.236</u>
<u>EPT 2013 2 direct plating-1</u> (++) (A)	Klebsiella pneumoniae	<u>2.019</u>	Klebsiella pneumoniae	<u>1.941</u>
<u>EPT 2013 2 direct plating-1</u> (+) (B)	Klebsiella pneumoniae	<u>1.867</u>	Klebsiella pneumoniae	<u>1.85</u>
<u>EPT 2013 2 direct plating-1</u> (+) (B)	Klebsiella pneumoniae	<u>1.914</u>	Klebsiella pneumoniae	<u>1.889</u>
<u>EPT 2013 2 direct plating-1</u> (+) (B)	Klebsiella pneumoniae	<u>1.85</u>	Klebsiella pneumoniae	<u>1.701</u>
<u>EPT 2013 2 formic acid extract</u> -1 (++) (A)	Klebsiella pneumoniae	<u>2.112</u>	Klebsiella pneumoniae	<u>2.022</u>
<u>EPT 2013 2 formic acid extract</u> -1 (++) (A)	Klebsiella pneumoniae	<u>2.161</u>	Klebsiella pneumoniae	<u>2.073</u>
<u>EPT 2013 2 formic acid extract</u> -1 (++) (A)	Klebsiella pneumoniae	<u>2.151</u>	Klebsiella pneumoniae	<u>2.071</u>
<u>EPT 2013 3 direct plating-1</u> (-) (C)	not reliable identification	<u>1.461</u>	not reliable identification	<u>1.324</u>
<u>EPT 2013 3 direct plating-1</u> (-) (C)	not reliable identification	<u>1.531</u>	not reliable identification	<u>1.519</u>
<u>EPT 2013 3 direct plating-1</u> (-) (C)	not reliable identification	<u>1.52</u>	not reliable identification	<u>1.477</u>
<u>EPT 2013 3 formic acid extract</u>				

Summary: 4 reps / strain by 2 methods [done [coded] during pretest] (some rows removed to fit slide)

- 1. S. suis (FA/direct) 2.137-2.196; 1. S. suis (extracted) 2.332-2.401**
- 2. K. pneumoniae (FA/direct) 1.85-2.019 ; 2. K. pneumoniae (extracted) 2.117-2.161**
- 3. No reliable ID (FA/direct) 1.372-1.531 3. No reliable ID (extracted) 1.314-1.440**

NOTE: EXTRACTED SCORES > DIRECT PREP IN EACH CASE

EPT-2013-1. *Streptococcus suis* -Result Code* [bps]

Identified as	Result code, bp/Method	Summary
<p><i>S. suis</i> 16S -10/23 (44%) nearly full (>1445 bps) sequence -13/23 (56%) partial (<900 bps) - 2 sent prep out for seq</p>	<p>1* [1425 bp]; 5 [459bp]; 6 [350]; 7 [498]; 8[1528]; 9 [870]; 10[704]; 11 [744]; 12[n-full]; 13 [803]; 14 [500]; 15 [500]; 16[725]; 17 [419]; 20- 1[1447]; 20-2[1534]; 20-3[1512]; 20- 4[1518]; 22[440]; 23[1517]; 24[750]; 25-1[1451]; 25-2[1480]</p>	<p>16S only= 14 Both 16S and MALDI = 9 Total 16S= 23</p>
<p><i>S. suis</i>, MALDI</p>	<p>2V; 3Bd; 4Be; 6V; 10 Be; 15V; 17Bd; 18V; 19V; 20-1Be; 20-2Be; 20-3Be; 20-4Be; 21V; 22Be</p>	<p>B:9 [2d/7e] V:6 MALDI= 15</p>
<p>In Summary</p>	<p>100 % concurrence 16S & MALDI -all high confidence scores: <u>16S</u>: >99%; <u>MALDI (V)</u>>99%; (B) >2</p>	<p>Bruker & Vitek concurred</p>

*16S ID as Strept sp closest to (100%) *S. suis*; V, Vitek MS-manufacturer method is direct only; Bd, Bruker, direct method or Be, extracted method; **yellow**, did both 16S and MALDI; a few labs did many replicates, reporting same result.

EPT-2013-2. *Klebsiella pneumoniae* / *K. variicola*

Identified	Result code, bp/Method	Summary
16S <i>K. pneumo</i> or <i>K pneumo ss</i> <i>pneumo</i> or <i>K.</i> <i>pneumo</i> 'group'	1; 6; 7; 8; 9; 10; 11; 13 ; 14; 15*; 16; 17; 20-1; 20-2; 20-3; 20-4; 22; 23; 24	19/23 (83%)
<i>K pneumoniae</i> OR <i>K variicola</i>	5; 25-1; 25-2**	3/23 (13%)
16S <i>Klebsiella</i> sp	12 [enteric; usually done biochemically]	1/23 (4%)
MALDI <i>K. pneumoniae</i> ; <i>Kp</i> 'group' or 'complex'	2V; 3Bd; 4Be; 6V; 10 Be; 15V*; 17Bd; 18V; 19V; 20-1Be; 20- 2Be; 20-3Be; 20-4Be; 21V; 22Be	15/15 (100%)

* Atypical colony noted, would do biochems & sent out to ref lab if required;

** would do biochemicals to discern among closely related species & subspecies

EPT-2013-3: UNIDENTIFIED, Order Lactobacillales. 16S

Identified	Result code, Comment	Summary
Unidentified, Lactobacillales	1; <u>6</u> ; 7*; 10**; <u>11</u> ; 12*; 13***; 15*; 16*; <u>20-1</u> ; <u>20-2</u> ; <u>22</u> ****; 24; 25-1	14/23 (60.8%)
<i>Facklamia tabacinasalis</i> <i>Globicatella sp</i> <i>Globicatella sp</i> <i>Facklamia sp</i> <i>Globicatella sp or Abiotrophia</i> <i>Lactobacillus bacterium</i> <i>Klebsiella pneumoniae</i> <i>Globicatella genus</i> <i>Globicatella genus</i> <i>Oral Canine Taxon</i> <i>Resembles Globicatella</i> <i>Globicatella sp</i> <i>Globicatella sp</i> <i>Globicatella sp</i>	5 (91%, single ID); <u>6</u> (97% report Indeterminate); 8 (<94% to type species in Genus) 9 (unable to ID; ml Facklamia sp) <u>11</u> (97% & 92% respectively) 14 (would report definitive ID) 17 (no definitive ID... oops ??) <u>20-1</u> (would study biochem, FAME etc) <u>20-2</u> (would report ID) <u>20-3</u> * <u>20-4</u> ; (would ID to Genus only) 22 (98%) 23 (97%, with Abiotrophia at 92) Would ID as genus <i>Globicatella</i> 25-2 (<93% Unable to Identify definitively)	Many provided >1 answers. Several would report out an ID of some sort to genus (pink)

Some labs provided > 1 answer (underlined). All were captured here

* Would forward to reference centre for ID to genus and species and not report out

** Unable to ID, no further report ;

*** would report UNID catalase neg Gram pos coccus

**** called taxon the genus Lactobacillales

EPT-2013-3. Unidentified, Order Lactobacillales. MALDI

Identified	Result code, bp/Method	Summary
MALDI 'No Reliable Identification'	2V; <u>3Bd</u> ; 4Be*; 6V**; <u>10</u> ; 15V**; <u>17Bd</u> ; 18V; 20-1Be; 20-3Be; 20-4Be; 22Be	12/15
<i>Agromyces cerinus</i> <i>Acinetobacter lwoffii</i> <i>Rhizobacterium radiobacter</i> ; <i>Lactobacillus saerimneri</i> [direct] "P150" [Extracted] <i>Micrococcus luteus/lylae</i> <i>?mixed Abiotrophia or Strept. mitis/oralis</i> <i>Arthrobacter ramosus</i>	<u>3Bd</u> (score 1.245, No reliable ID) <u>10</u> (score 1.301; not reporting) <u>17</u> (1.393, not reliable ID) <u>17</u> (1.294, not reliable ID) <u>19V</u> (no ID***) <u>19V</u> (75%; no ID***) 21V (**) <u>20-2Be</u> ; (1.8; would report <i>Arthrobacter sp</i>)	Note that Vitek does not have an extraction method

Some labs provided > 1 answer (underlined).

* Also provided Bruker category C

** would send out as NO ID then send out for sequencing or refer out to reference centre

***would do biochemical confirmation before reporting

Info Inferred from Results. If Bruker, scores slightly higher if extracted

- **EPT-2013-1:** all labs identified *S. suis* correctly with high confidence, whether by 16S (either nearly full or partial sequence) or MALDI (whether Bruker or Vitek)
- **EPT-2013-2:** by 16S, 83% identified bacterium as *K. pneumoniae*, *K. pneumoniae* 'group' or 'complex'
- No lab described what group or complex contained;
- only **3** participants from **2** sites explicitly mentioned *K. variicola* and that it could not be resolved from *K. pneumoniae*
- All MALDI users, whether Bruker or Vitek ID bacterium as *K. pneumoniae*; remember, *K variicola* not included as a Vitek entry but that response did not come up for Bruker labs?validation of 16S or poor Bruker entry?

Snapshot of Bruker report for *K. pneumoniae*

Bruker Daltonik MALDI Biotyper Classification Results

file:///C:/Documents and Settings/TOF-User/Application Data/Bruker Dal...

Analyte19



Analyte Name:

Analyte Description:

Analyte ID:

Analyte Creation Date/Time:

9/12/2013 3:19:33 PM

Applied MSP Library(ies):

Applied Taxonomy Tree:

Bruker Taxonomy

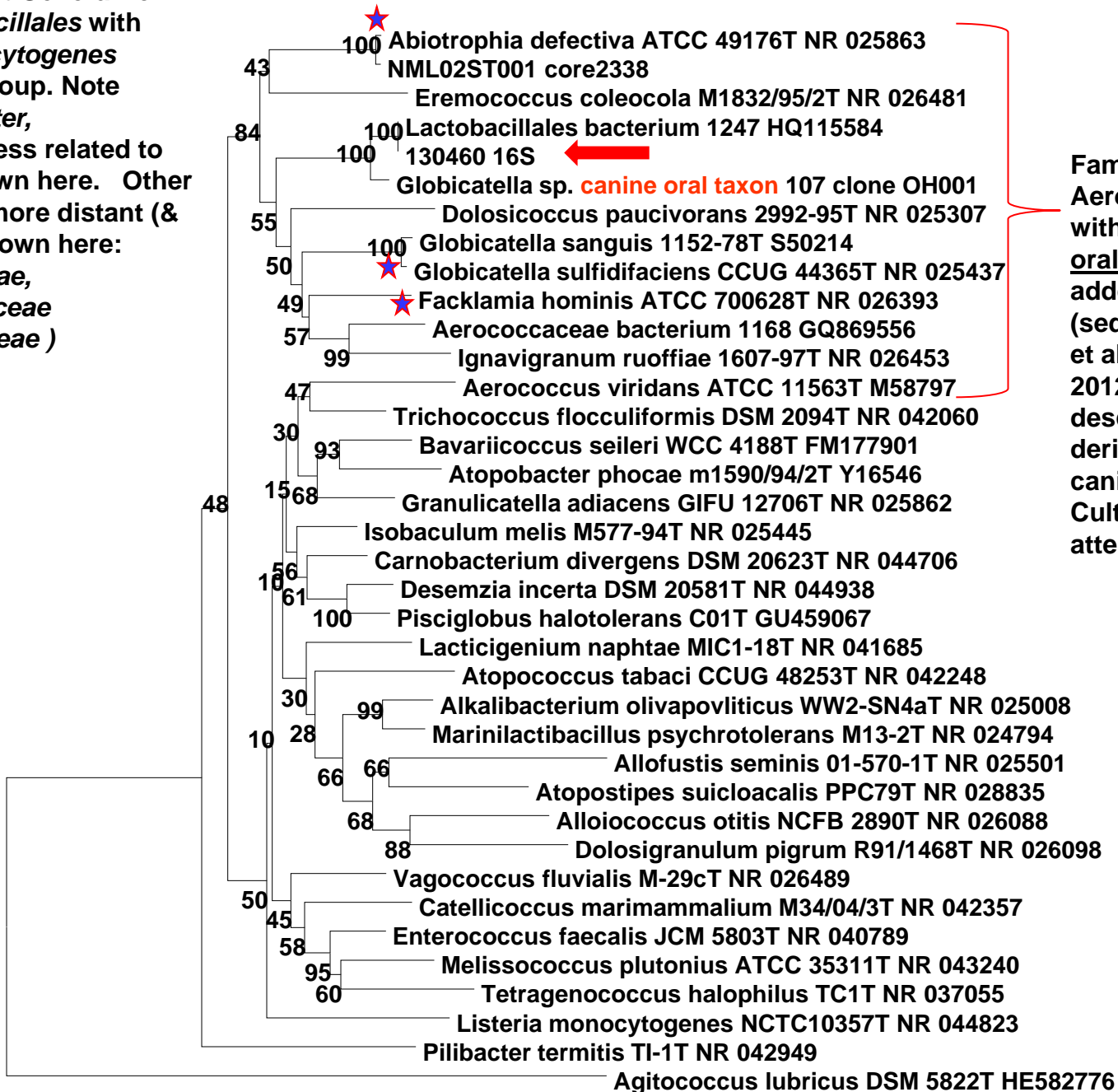
Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (++)	Klebsiella pneumoniae ssp pneumoniae 9295_1 CHB	2.075	72407
2 (+)	Klebsiella pneumoniae 37585 PFM	1.899	573
3 (+)	Klebsiella pneumoniae 37595 PFM	1.898	573
4 (+)	Klebsiella pneumoniae ssp ozaenae DSM 16358T HAM	1.827	574
5 (+)	Klebsiella pneumoniae RV_BA_03_B LBK	1.8	573
6 (+)	Klebsiella pneumoniae ssp pneumoniae DSM 30104T HAM	1.796	72407
7 (-)	Shigella sonnei NML BADD Reference	1.687	124493614
8 (-)	Klebsiella pneumoniae ssp ozaenae CCM 5792T CCM	1.641	574
9 (-)	Klebsiella pneumoniae ssp rhinoscleromatis DSM 16231T HAM	1.613	39831
10 (-)	Escherichia coli NML ATCC25922	1.611	124493614

1 example of EPT-2013-2: *K. pneumoniae* matches with various entries, note entry for *K. variicola* did not come up even with poorer score [examined a number of replicates, all the same]

EPT-2013-3 : Info Inferred from 16S Results

- Unidentifiable, order Lactobacillales
- Majority (61%) found organism **unidentifiable**, and used 'Lactobacillales' to describe bacterium
- Many participants provided > 1 response
- A number of participants cited matches to sequences of genera in the family *Aerococcaceae* (*Globicatella*, *Abiotrophia*, *Facklamia*)
- Some cited an 'oral canine derived sequence' as a possible identification, without providing context.
- **Four** participants would have reported out a genus name as a definitive ID, incorrectly.
- One participant would try biochemical testing to further characterize, rather than forward to reference centre

NJ tree. Closest Genera from Order *Lactobacillales* with *Listeria monocytogenes* intended outgroup. Note genera *Pilibacter*, *Agitococcus*, less related to other taxa shown here. Other families were more distant (& so were not shown here: *Lactobacillaceae*, *Leuconostocaceae* *Streptococcaceae*)



Family Aerococcaceae, with the canine oral taxon seq added (seq from Dewhurst et al PLOS One 2012 7(4)e36067, describing seq derived from canine oral biome Culture not attempted)

EPT-2013-3 : Info Inferred-MALDI Results

- Expected “No Reliable Identification” type wording.
- Many labs provided > 1 response
- Most alluded to fact that Identification was unsatisfactory; expected more to say that this would be sent to reference centre for sequencing/ characterization if relevant.....
- Other MALDI responses were derived from a **variety** of families/ orders (not related to phylogenetic relationships)
- One participant would have reported out a **genus name** as a definitive ID, incorrectly.
- One participant would try **biochemical testing** to further characterize, rather than forward to reference centre (expect limited new info from that approach)

Next Steps

Several possibilities:

1. Similar test next year to same group or to slightly extended group [as there will be many more MALDIs in place across Canada by 2014]
2. NMG recommend that labs subscribe to external proficiency testing done by traditional groups [CAP, CMPT, QMPLS, Quebec] and stop NMG effort
3. **Caveats**-if using test results locally to document participation in an external proficiency test:
 - No formal followup possible if lab performed poorly
 - No link to accreditation done by provincial or national bodies
4. Test itself: too easy? Too difficult? Add more difficult to grow bacteria (eg anaerobes) [some feedback, participants enjoyed test]
5. May write up exercise for publication