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Title: *Moraxella nonliquefaciens* and *M. osloensis* are Important *Moraxella* Species that Cause Ocular Infections

Running Title: Moraxella Ocular Pathogens

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Abstract

Purpose. *Moraxella* is an ocular bacterial pathogen isolated in cases of keratitis, conjunctivitis, and endophthalmitis. Gram-negative brick-shaped diplobacilli from ocular specimens, and slow growth in culture, are early indications of *Moraxell*a ocular infection; however, identifying *Moraxella* to species can be complex and inconsistent.

Methods. In this study, bacteria consistent with *Moraxella* were identified to species using: 1) DNA sequencing coupled with vancomycin susceptibility, 2) MALDI-TOF Mass Spectrometry, and 3) Biolog ID System**.** Study samples consisted of 9 ATCC *Moraxella* controls, 82 isolates from keratitis, 21 isolates from conjunctivitis, and 4 isolates from endophthalmitis.

Results. The ATCC controls were correctly identified. For keratitis, 66 (80.5%) were identified as *M. nonliquefaciens*, 7 (9.0%) as *M. lacunata*, 5 (6%) as *M. osloensis*, 2 (2.5%) as *Acinetobacter lwoffi*, 1 (1.0%) as *M. bovis/nonliquefaciens*, and 1 (1.0%) as *M. osloensis/nonliquefaciens*. For conjunctivitis, 9 (43.0%) were identified as *M. osloensis*, 6 (29.0%) as *M. nonliquefaciens*, 3 (14.3%) as *Roseomonas*, 2 (9.5%) as *Acinetobacter* (*parvus, junii*), and 1 (4.5%) as *M. catarrhalis/M. nonliquefaciens*. From endophthalmitis, 3 of 4 of the isolates were *M. nonliquefaciens*. Overall, *M. nonliquefaciens* and *M. osloensis* were identified in 70% (75 of 107) and 13% (14 of 107) of cases, respectively, totaling 83% (89 of 107).

Conclusions. *M. nonliquefaciens* and *M. osloensis* are important bacterial pathogens of the eye as determined by DNA sequencing, MALDI-TOF MS, and Biolog. Although *Moraxella catarrhalis* is a clinical pathogen, other species of *Moraxella* appear to have a prominent role in eye infections.

Introduction

As first described by Morax[1], the genus, *Moraxella*, appears to have a specific tropism as an ocular pathogen for keratitis[2-5], conjunctivitis[6], and endophthalmitis[7]. Moraxella keratitis appears to be on the rise based on recent reports.[2-5,8] Bacterial conjunctivitis is generally self-limiting, but *Moraxella* conjunctivitis can persist for weeks and presents as a follicular conjunctivitis which can be misdiagnosed as inclusion conjunctivitis due to *Chlamydia*.[6,9-11] *Moraxella* has been reported as an ocular pathogen in Japan[2,4,12], the United States[5,6,7,9,10], and Pakistan[13]. Although quite susceptible to ophthalmic topical antibiotics as highlighted on our frequently updated website (http://eyemicrobiology.upmc.com/Antibiotic.htm), *Moraxella* keratitis can persist and induce severe inflammation resulting in hypopyon formation. Like most bacteria, with entrance to the inner eye, *Moraxella* can also cause endophthalmitis.[7]

In the clinical laboratory, *Moraxella* is easily identified to genus by its classic appearance as a Gram-negative diplobacilli (brick shaped) which is easily distinguish from other Gram-negative bacteria (Figure 1), and this may be the only indication of *Moraxella* infections due to antibiotic pretreatment or the fastidious nature of *Moraxella*. *Moraxella* can retain crystal violet during Gram staining and it is classified as oxidase-positive, non-glucose fermenting bacteria. Clinically, ocular *Moraxella* isolates are generally only identified to the genus as *Moraxella*, because it is difficult and complex to classify *Moraxella* to species using phenotypic testing. There are multiple species of *Moraxella*, but the most noted, *M. catarrhalis*, formerly known as *Branhamella catarrhalis*, is not the only species implicated in ocular infection.

Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) and molecular techniques such as DNA sequencing can identify bacteria to genus and species with more consistent results.[14,15] A commercial system, Biolog GenIII

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(Hayward, CA), utilizing 96 substrates and controls, can also be used to identify bacteria to genus and species.

The objective of the present study is to more precisely identify ocular isolates of *Moraxella* to include species level differentiation. It is important to recognize these bacteria as pathogens.

Methods

Laboratory Diagnosis of Ocular *Moraxella* Infection

The ophthalmic microbiology laboratory at the University of Pittsburgh Medical Center, Pittsburgh, PA, is a fully certified clinical laboratory dedicated for the diagnosis and treatment of ocular infection. (http://eyemicrobiology.upmc.com) Samples for keratitis (cornea) are collected using soft-tipped applicators, spatula, surgical blades, and jeweler's forceps. Conjunctival samples are generally obtained with soft-tipped applicators, and samples to detect endophthalmitis are obtained by tapping the vitreous humor with a needle and syringe. The initial diagnostic test to detect infection from ocular samples are the Gram and Giemsa stain which can provide rapid and definitive identification of an etiologic pathogen.

All corneal ulcers that are either central or are over 3 mm in size of infiltration are scraped for microscopic examination and isolation of pathogens. The decision to perform laboratory studies is a judgment call by the attending ophthalmologist.

Moraxella can be isolated in culture on trypticase soy agar supplemented with 5% sheep blood (5%SB) and chocolate agar. Our laboratory recommends that ocular collection be placed directly to the media. Growth is better on 5% SB, but growth is generally slow with colonies not

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appearing until 48 hours of incubation at 37^{O} C in 6% CO_2. The initial appearance can be pinpoint colonies with larger colonies (2-3 mm) appearing after 2 days. Most *Moraxella* (not *M. catarrhalis*) isolated from ocular specimens will appear as grey to white colonies, often with a clearer beach and umbonate colony morphology, giving a fried egg appearance (Figure 2).

Antibiotic Susceptibility of Ocular Moraxella

In vitro antibiotic susceptibility for ocular isolates are performed using Kirby Bauer Disk Diffusion[16] on Mueller Hinton Agar supplemented with 5% sheep blood. *Moraxella* susceptibility cannot be determined on regular Mueller Hinton agar because of the need for red blood cells to grow. It is important to note that ocular *Moraxella* infections are not treated systemically (i.e. orally or intravenously). Ocular *Moraxella* and other bacterial pathogens of keratitis are treated topically with antibiotics or intravitreally by direct injection in cases of endophthalmitis. There are no standards for susceptibility interpretation of topical or intravitreal treatment, but the CLSI (Clinical and Laboratory Standards Institute) standards can be used to guide therapy if the concentration of antibiotics in the ocular tissue is assumed to be equal or greater than in the serum.[16] A routine keratitis testing battery of antibiotics for patient topical treatment consists of bacitracin, vancomycin, gentamicin, ciprofloxacin, ofloxacin, polymyxin B, cefazolin, tobramycin, sulfacetamide, moxifloxacin, and cefoxitin. In general, *Moraxella* isolates test susceptible to these antibiotics (http://eyemicrobiology.upmc.com/AntibioticSusceptibilities/Keratitis.htm). A zone greater of 18mm denotes susceptibility to vancomycin. *Streptococcus pneumoniae* ATCC 49619 was the control strain for assuring the antibiotic disk concentrations.

116 ***Moraxella* Study Isolates**

117 Since 1993, all bacterial pathogens isolated at UPMC from keratitis, conjunctivitis, and
118 endophthalmitis have been stored at -80^{O} C with broth containing 15% glycerol for validation of
119 new testing and patient treatment. These isolates constitute an Ocular Clinical Tissue Bank in
120 which the isolates were de-identified to comply with IRB protection of patient identity. Clinical
121 presentation data, patient identity and demographics were not tabulated for any of the bacterial
122 isolates.

123 The collection was reviewed for the retrieval of *Moraxella* isolates from keratitis,
124 conjunctivitis, and endophthalmitis. No patient contact was involved in this study (University of
125 Pittsburgh, Institutional Review Board, IRB# PRO17050362). None of these isolates (except *M.*
126 *catarrhalis*) had been identified to species.

127 Our identifications also included nine American Type Culture Collection (ATCC)
128 controls: *M. bovis* ATCC^{T} 10900, *M. caviae* ATCC^{T} 14659, *M. cuniculi* ATCC^{T} 14688, *M.*
129 *nonliquefaciens* ATCC^{T} 19975, *M. osloensis* ATCC^{T} 19976, *M. atlantae* ATCC^{T} 29525, *M.*
130 *lincolnii* ATCC^{T} 51388, *M. lacunata* ATCC^{T} 17967, and *Moraxella* (*Branhamella*) *catarrhalis*
131 ATCC^{T} 24250.

132

133 **DNA Sequencing**

134 Presumptive *Moraxella* isolates were retrieved from -80^{O} C and streaked onto 5%SB.
135 and incubated at 37^{O} C in a 6% CO_2 incubator for 48 hours. The isolates were Gram stained to
136 presumptively identify as Gram-negative diplobacilli. If identified as Gram negative diplobacilli,
137 the isolates were passed onto new 5% SB and incubated at 37^{O} C in a 6% CO_2 incubator for 48
138 hours. The DNA from the isolates were extracted using Epicentre DNA Extraction solution

Quick Extract (Madison, WI). PCR was performed with Taq DNA polymerase (New England Biolabs, Ipswich, MA) and the 16S rRNA gene sequence was amplified (95^{O} C for 5 minutes, 33 cycles of 95^{O} C for 30 seconds, 50^{O} C for 15 seconds, 72^{O} C for 1 minute, followed by a 10 minute extension at 72^{O} C) using Primers 27F and 1492R.[18] If a single band was visualized by gel electrophoresis of the PCR products, the amplicons were purified using Qiagen QIAquick PCR Purification kit (Hilden, Germany). The PCR products were each analyzed with a single Sanger sequencing reaction using Primer 330F at the University of Pittsburgh Genomics Research Core.[17] DNA sequences were initially compared to the NCBI non-redundant nucleotide database using BLASTN.[18-20] After initial screening, sequences were aligned with type strain sequences using ClustalW and a neighbor joining tree[21] was constructed using the Kimura 2 parameter model[22] and 1000 bootstrap replicates.[23] Due to differing lengths of sequence obtained, all positions with less than 50% site coverage were eliminated. Evolutionary analyses were conducted in MEGA7.[24]

MALDI-TOF MS

As with DNA sequencing, presumptive *Moraxella* isolates were retrieved from -80^{O} C and streaked onto 5%SB. The isolates were incubated at 37 ^{O} C in a CO_2 incubator for 48 hours. The isolates were Gram stained to presumptively identify as Gram-negative diplobacilli, passed to another 5% SB for 24 hours, and delivered for MALDI-TOF MS testing (UPMC, Clinical Microbiology).

Colony material from each isolate was transferred to a polished steel target (Bruker Daltonik, Bremen, Germany) using a clean toothpick. One microliter of Matrix (Bruker HCCA [α-cyano-4-hydroxycinnamic acid in 50% acetonitrile with 2.5% trifluoroacetic acid]) was

applied within an hour and air dried for 10 minutes. The target was analyzed using the Bruker Microflex LT/SH MALDI-TOF instrument and Bruker Biotyper software version 4.1 with the MBT BDAL library containing 7854 mass spectra. The library contained 22 species of *Moraxella*. Spectra were obtained after 240 laser shots yielding spectra with mass/charge (m/z) ratios between 2k and 20k Da. Measurements meeting the quality criteria (log score ≥1.8) and a log scopre > 0.2 between different species were deemed acceptable identifications. Samples with scores below this cutoff or with < 0.2 log between different species were retested using the formic acid tube extraction method as previously described.[25] A control organism (*E. coli* ATCC 25922 or *P. aeruginosa* ATCC 27853) was extracted and analyzed once each day to ensure the extraction procedure yielded successful identification.

Biolog Gen III Plate

The Biolog identification system was performed according to manufacturer's protocol. (Biolog, GEN III MicroPlate ™, Instructions for Use, www.biolog.com). As with MALDI-TOF MS and DNA sequencing, presumptive *Moraxella* isolates were retrieved from -80^{O} C and streaked onto 5%SB. The streaks were passed to 5%SB and testing was performed on 24 hour growth. Biolog testing was performed on a GEN III MicroPlate™ which contained 94 biochemicals consisting of 71 carbon source utilization assays, 23 chemical sensitivity assays, a negative control, and a positive control. These provide a phenotypic fingerprint for species identification by utilizing tetrazolium redox dyes to colorimetrically indicate carbon utilization or resistance to inhibitory chemicals.

In brief, a medium for fastidious bacteria (IF-C, Biolog) was inoculated with a *Moraxella* isolate to a turbidity of 65% transmittance measured by a Turbidimeter (Biolog). The inoculum

was aliquoted to the microplate using a reservoir and multipipetor to 96 wells at a volume of 0.1ml per well. The plate was incubated at 34^{O} C and read manually for color changes at 4 hours, 8 hours, and 20 hours. The tabulated data at each time point was entered into the Biolog's Identification Systems Software (OOP 188rG Gen III Database v2.8). The database contained 14 species of *Moraxella*. Species identification was determined as the most probable as indicated by the software. It must be noted that there is an automated system for reading the plates for color changes. Costs dictated the manual approach for this study.

Identification of *Moraxella* to Species

Isolates of *M. catarrhalis* were not of prime interest in this study because these isolates can be identified to genus and species using standard laboratory methods. *M. catarrhalis* are Gram-negative diplococci (not diplobacilli) closely resembling *Neisseria* but are oxidase-positive, fast growing, form friable movable tan-like colonies, and are resistant to vancomycin.[26,27] The API NH (bioMérieux, La Balme-les-Grottes, France) system can accurately confirm identification.[28]

In this study, all presumptive *Moraxella* isolates were observed to be Gram-negative diplobacilli. There was some variability in the size of the bacilli, whereas most were brick-shaped, boxcar bacilli, but some were thinner bacilli and diplococcobacilli. Some isolates retained crystal violet staining which is a characteristic of *Moraxella*. As noted previously, the colonies initially appeared as pinpoint colonies with larger colonies (2-3 mm) appearing after 2 days. These colonies appeared as grey to white, often with a clearer beach that give a fried egg appearance (Figure 2). Susceptibility to vancomycin indicated a *Moraxella* species other than *M. catarrhalis*.

208 **Results**

209 The laboratory records (1993-2017) indicated that there were 9 cases of keratitis, 18 cases
210 of conjunctivitis, and 0 cases of endophthalmitis caused by *M. catarrhalis*. These *Moraxella*
211 isolates and ATCC controls of *M. caviae, M. cuniculi, M. catarrhalis* (all once part of
212 *Branhamella*), and most *M osloensis* were vancomycin resistant.

213 All control ATCC isolates were identified correctly by DNA sequencing coupled with
214 vancomycin susceptibility, MALDI-TOF MS, and Biolog Gen III plates. The vancomycin zone
215 of inhibition for *M atlantae* was 16mm, and zones of inhibition were not clear for *M. lincolnii*
216 and *M. lacunata*.

217 Table 1 summarizes the identification of *Moraxella* from keratitis, conjunctivitis, and
218 endophthalmitis using DNA sequencing with vancomycin susceptibility, MALDI-TOF MS, and
219 Biolog Gen III plates. Identification was reported for 82 cases of keratitis, 21 cases for
220 conjunctivitis, and 4 cases of endophthalmitis. The identification of DNA sequencing was more
221 closely associated with MALDI-TOF MS (106 of 116) than with the identification of DNA
222 sequencing with Biolog (78 of 116) (p=0.001, Fisher's Exact) and MALDI-TOF MS with Biolog
223 (87 of 116) (p=0.005, Fisher's Exact).

224 Table 2 details the incidence of *Moraxella* species for keratitis, conjunctivitis, and
225 endophthalmitis. Identification of *Moraxella* to species was based on DNA sequencing coupled
226 with vancomycin susceptibility and MALDI-TOF MS.

227 Many isolates could not be identified by 16S rRNA sequencing alone. In the segment of
228 the 16S rRNA gene sequenced in this study, the *M. catarrhalis* and *M. nonliquefaciens* type
229 strains are identical (Figure 3), but can be distinguished based on vancomycin resistance in *M.*
230 *catarrhalis* and susceptibility in *M. nonliquefaciens*. The sequences from strains K127, K1630,

K1664, K2450, K2695, and K2757 cluster together, but are essentially equidistant from *M. bovis, M. bovoculi, M. caprae, M. equi,,* and *M. lacunata,* suggesting that they may be members of a new species. Sequence analysis could differentiate *M. atlantae, M. caviae, M. lincolnii*, and *M. osloensis.* DNA sequencing was complemented with vancomycin susceptibility to identify most *Moraxella* species. Vancomycin resistant isolates were associated with *M. catarrhalis and M. osloensis*.

For keratitis, 66 (80.5%) were identified as *M. nonliquefaciens*, 7(9.0) as *M. lacunata,* 5 (6.0%) as *M. osloensis*, 2 (2.5%) as *Acinetobacter lwoffi*, 1 (1.0%) as *M. bovis/ nonliquefaciens*, and 1 (1.0%) as *M.osloensis/ nonliquefaciens*. All of the *M. nonliquefaciens* were susceptible to vancomycin, while 3 of the *M. osloensis* were susceptible to vancomycin and 2 were resistant. Although *M. osloensis* can be resistant to vancomycin, our study indicates that this may not be a consistent characteristic. On the closer laboratory examination of the two *Acinetobacter* isolates, both were initially classified as *Moraxella* based on the observation of diplococcobacilli on Gram stain.

For conjunctivitis, 9 (43.0%) were identified as *M. osloensis*, 6 (29%) as *M. nonliquefaciens*, 3 (14.0%) as *Roseomonas mucosa*, 2 (9.5%) as *Acinetobacter*, and 1 (4.5%) as *M. catarrhalis/ nonliquefaciens*. Seven of the *M. osloensis* isolates were resistant to vancomycin and two were susceptible, while vancomycin resistance was noted for one *M. nonliquefaciens* isolates with 5 susceptible. The three *Roseomonas mucosa* isolates were re-examined and all three were Gram-negative diplobacilli consistent with *Moraxella*, but all three presented as pinkish highly mucoid colonies slightly different from colonies observed with *Moraxella*. Once again, on closer examination, both *Acinetobacter* isolates were diplococcobacilli on Gram stain.

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From endophthalmitis, 3 of 4 (75%) of the isolates were *M. nonliquefaciens* with all three isolates susceptible to vancomycin. The lone *Neisseria shayeganii* isolate (identified by DNA sequencing only) from endophthalmitis was observed to be Gram-negative diplococcoid, oxidase-positive, and vancomycin resistant.

Overall, *M. nonliquefaciens* and M. *osloensis* were identified in 70% (75 of 107) and 13% (14 of 107) of cases, respectively, totaling 83% (89 of 107).

Discussion

DNA sequencing coupled with vancomycin susceptibility, MALDI-TOF MS, and Biolog GenIII plates have given us more diagnostic options to identify *Moraxella* to species when combined with established laboratory tests such as Gram stain, culture isolation, and susceptibility testing. Gram stain and culture provided us with classical laboratory characteristics. Vancomycin provided us an additional test for separation and identification of *Moraxella* to species. *M. liquefaciens* was generally found to be susceptible to vancomycin and *M. osloensis* was found to be more resistant.

Biolog using GenIII plates was more problematic in confidently identifying *Moraxella* to species based on manual interpretation. We were required to repeat testing for many isolates, because the controls were not positive or negative as expected and identification was not conclusive. It may be that the automated Biolog system without human interpretation would be more definitive for identification. We had more confidence with DNA sequencing coupled with vancomycin susceptibility and MALDI-TOF MS for identifying the *Moraxella* isolates to species.

It may be unusual to some that we are testing the susceptibility of Gram-negative bacteria to vancomycin, but vancomycin is part of a broad-spectrum battery of antibiotics used in our laboratory to 'guide' the topical treatment of bacterial keratitis. In general, the topical empiric treatment of *Moraxella* keratitis is fortified tobramycin (14 mg/ml) and cefazolin (25 mg/ml). Some ophthalmologists may use topical vancomycin (25 mg/ml) instead of cefazolin or use topical monotherapy with a commercially available fluoroquinolone (moxifloxacin, ciprofloxacin). From a previous study, we suggested topical tobramycin (3mg/ml) to be used as treatment for conjunctivitis, but other commercial topical antibiotics can be used (i.e. fluoroquinolones).[6] Endophthalmitis is treated by direct intravitreal injection with vancomycin (1 mg) and amikacin (0.4 mg) or ceftazidime (2 mg).

Our study determined that *M nonliquefaciens* is the predominant species isolated from keratitis and *M osloensis* was a frequent species implicated in conjunctivitis. A small sample (3 of 4) demonstrated that *M nonliqufaciens* was implicated in endophthalmitis, and this has been reported previously.[7] Our study also indicated *Roseomonas mucosa* has characteristics similar to *Moraxella* and there needs to be close examination of cultures and diagnostic testing to definitively distinguish these two genera. We have designated *Roseomonas mucosa* as a conjunctivitis pathogen, and it has been previously reported to be a causative agent in keratitis[29] and endophthalmitis.[30,31]

The literature has sparse reports of ocular *Moraxella* infections, but there is no definitive consistent method to precisely identify *Moraxella* ocular isolates to genus and species. A recent report of 17 cases of keratitis accurately identified 4 cases of *M. catarrhalis*, 1 case of *M. osloensis*, and 12 cases of *Moraxella* species with the VITEK 2 system using Gram-negative cards (SYSMEX bioMèrieux, Tokyo, Japan) with ID-test HN20 Rapid Kit (Nissui

Pharmaceutical, Tokyo, Japan).[2] Public Health England has provided an algorithm for identification of Moraxella, not specifically for ocular isolates, that included a battery of laboratory tests and the possible introduction of mass spectrometry and nucleic acid amplification testing.[32] Automated systems and test kits can be accurate methods to identify ocular isolates of *Moraxella* to genus for expedient patient care. Advances in technology now allow for a more precise and consistent methods to correlate specific ocular clinical presentations to species of *Moraxella*.

In conclusion, our study has identified *M. liquefaciens*, *M. osloensis*, and other *Moraxella* species as ocular pathogens. DNA sequencing coupled with vancomycin susceptibility and MALDI-TOF MS are reliable methods for the identification of *Moraxella* to species, but added investigation with automation may be required to validate Biolog.

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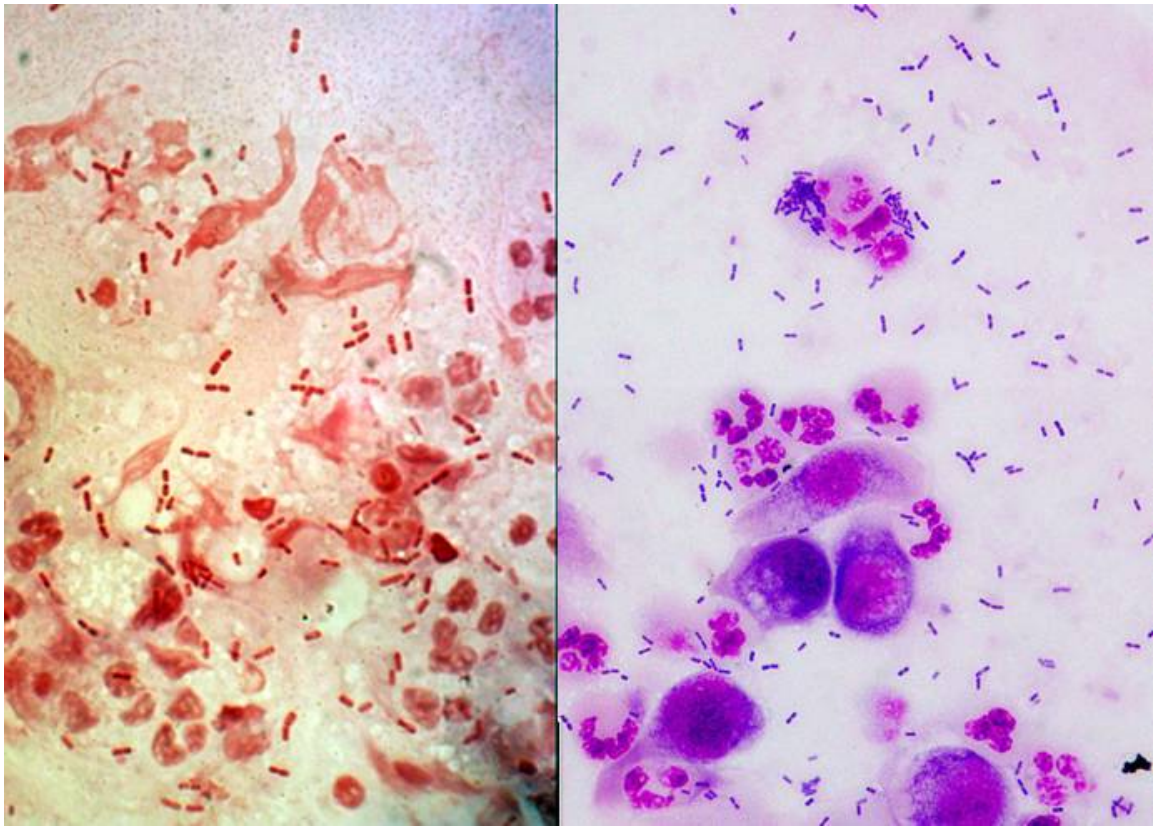
409 **Legend**

410 Figure 1. The presence of *Moraxella* diplobacilli from corneal scrapings using Gram stain

411 (left picture) and Giemsa (right picture).



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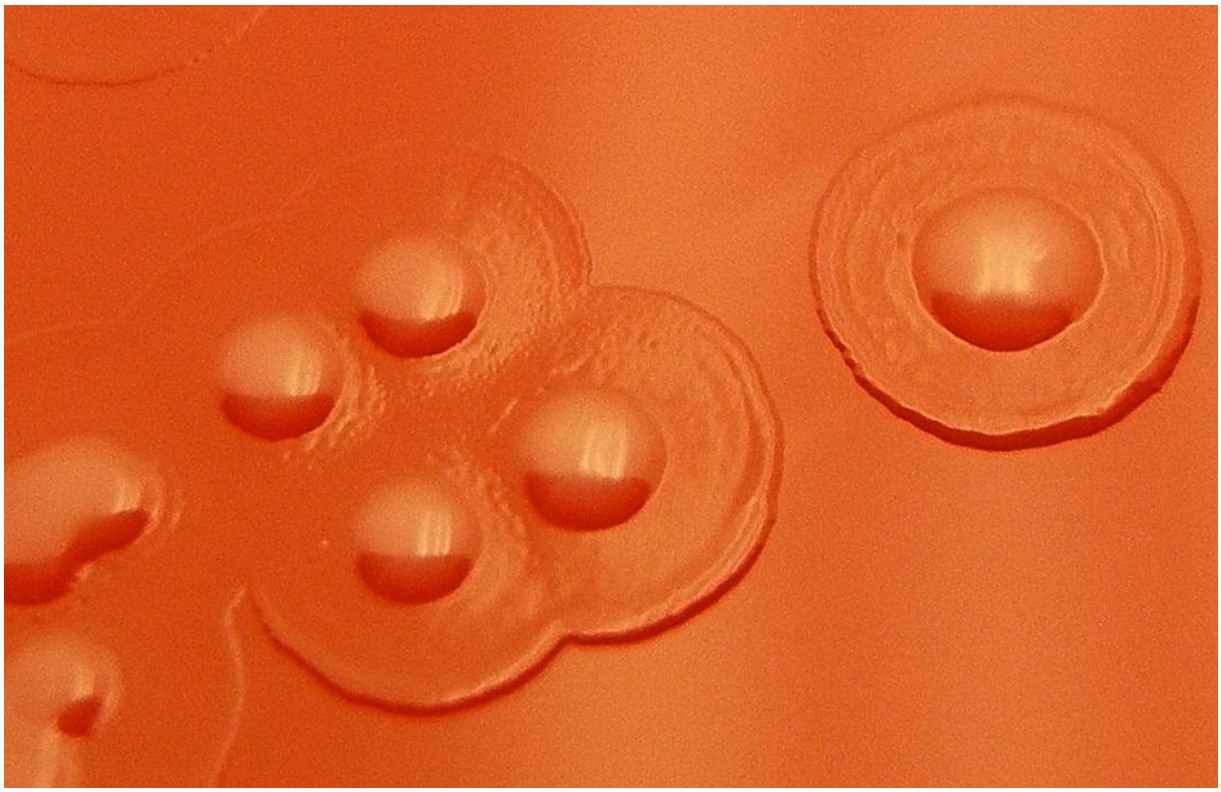
414 Figure 2. Fried egg appearance of *Moraxella* growing on trypticase soy agar supplemented
415 with 5% sheep blood.



416

417

418 Figure 3. A diagram of the neighbor joining tree of 16S rRNA sequences for the
419 *Moraxella* isolates in the study. Panel A details the neighbor joining tree (25) constructed with
420 all sequences from this study and relevant type strains. Clades corresponding to *M.*
421 *nonliquefaciens/M.catarrhalis* and *M. osloensis* are condensed. Panel B depicts clade
422 corresponding to *M. nonliquefaciens/M.catarrhalis*. Panel C corresponds to *M. osloensis*. Red
423 indicates strains from our clinical collection and black indicates select type strains. The
424 vancomycin susceptibility status is indicated as S for susceptible and R for resistant.

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Table 1 - Identification of Moraxella species from Keratitis, Conjunctivitis, and Endophthalmitis using DNA Sequencing, Biolog, and MALDI-TOF MS.

Isolate	Van S/R	Identification based on: (DNA Sequencing with vancomycin susceptibility)	Biolog ID	MALDI-TOF MS
ATCC controls				
Moraxella bovis 10900	S	M bovis	M bovis	M bovis
M caviae 14659	R	M caviae	M caviae	No ID
M cuniculi 14688	R	M cuniculi	M cuniculi	M catarrhalis
M nonliquefaciens 19975	S	M nonliquefaciens	No ID	M nonliquefaciens
M osloensis 19976	R	M osloensis	M osloensis	M osloensis
M atlantae 29525	I	M atlantae	No ID	M atlantae
M lincolnii 51388	Q	M lincolnii	No ID	M lincolnii
M lacunata 17967	Q	M lacunata	No ID	M lacunata
M catarrhalis 24250	R	M catarrhalis	M catarrhalis	M catarrhalis
Keratitis Isolates				
1. K10	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
2. K11	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
3. K21	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
4. K48	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
5. K63	S	M nonliquefaciens	M equi	M nonliquefaciens
6. K77	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
7. K127	S	M equi, M lacunata, M bovoculi	M nonliquefaciens	M lacunata, M bovis
8. K195	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
9. K225	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
10. K237	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
11. K290	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
12. K441	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
13. K484	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
14. K639	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens

Keratitis Isolate	Van S/R	Identification based on: (DNA Sequencing with vancomycin susceptibility)	Biolog	MALDI-TOF MS
15. K660	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
16. K678	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
17. K720	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
18. K840	S	M nonliquefaciens	M equi	M nonliquefaciens
19. K885	S	M osloensis	M canis	M osloensis
20. K892	S	M osloensis	M osloensis	M osloensis
21. K913	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
22. K942	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
23. K1038	S	M nonliquefaciens	M equi	M nonliquefaciens
24. K1059	S	M nonliquefaciens	M bovis	M nonliquefaciens
25. K1109	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
26. K1124	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
27. K1128	S	M osloensis	M osloensis	M osloensis
28. K1135	R	M osloensis	M osloensis	M osloensis
29. K1184	S	M osloensis	M nonliquefaciens	M nonliquefaciens
30. K1211	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
31. K1219	S	M nonliquefaciens	No ID	No ID
32. K1244	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
33. K1248	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
34. K1250	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
35. K1259	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
36. K1350	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
37. K1361B	R	Acinetobacter lwoffi	M osloensis	Acinetobacter lwoffi
38. K1373	S	M nonliquefaciens	M catarrhalis	M nonliquefaciens
39. K1377	S	M nonliquefaciens	No ID	No ID
40. K1442	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
41. K1449	S	M nonliquefaciens	No ID	No ID
42. K1454	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
43. K1524	S	M nonliquefaciens	M equi	M nonliquefaciens
44. K1586	S	M nonliquefaciens	M equi	M nonliquefaciens

Keratitis Isolate	Van S/R	Identification based on: (DNA Sequencing with vancomycin susceptibility)	Biolog	MALDI-TOF MS
45. K1630	S	M equi, M bovoculi, M lacunata	M equi	M lacunata
46. K1643	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
47. K1650	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
48. K1661	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
49. K1664	S	M equi, M bovoculi, M lacunata	No ID	No ID
50. K1773	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
51. K1784	S	M equi, M bovoculi, M lacunata	M ovis	No ID
52. K1661	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
53. K1854	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
54. K1855	R	M osloensis	M osloensis	M osloensis
55. K1916	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
56. K1932	S	M nonliquefaciens	M caprae	M nonliquefaciens
57. K1947	R	Acinetobacter lwoffi	Acinetobacter lwoffi	Acinetobacter lwoffi
58. K2169	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
59. K2231	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
60. K2265	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
61. K2275	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
62. K2294	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
63. K2301	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
64. K2359	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
65. K2380	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
66. K2419	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
67. K2436	S	M nonliquefaciens	M equi	M nonliquefaciens
68. K2450	S	M equi, M bovoculi, M lacunata	M nonliquefaciens	M lacunata
69. K2451	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
70. K2519	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
71. K2565	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
72. K2622	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
73. K2625	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
74. K2695	S	M equi, M bovoculi, M lacunata	M nonliquefaciens	M lacunata

Keratitis Isolate	Van S/R	Identification based on: (DNA Sequencing with vancomycin susceptibility)	Biolog	MALDI-TOF MS
75. K2711	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
76. K2717	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
77. K2718	S	M nonliquefaciens	M bovis	M bovis
78. K2734	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
79. K2757	S	M equi, M bovoculi, M lacunata	No ID	M lacunata
80. K2774	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
81. K2880	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
82. K2891	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
Conjunctivitis Isolates				
1. B186	R	M osloensis	M osloensis	M osloensis
2. B353	R	M osloensis	M osloensis	M osloensis
3. B364	R	Acinetobacter parvus	No ID	Acinetobacter parvus
4. B455	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
5. B470	R	M osloensis	M osloensis	M osloensis
6. B590	R	M osloensis	M osloensis	No ID
7. B601	R	Acinetobacter junii	Acinetobacter junii	Acinetobacter junii
8. B662	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
9. B694	R	Roseomonas mucosa	No ID	Roseomonas mucosa
10. B695	S	M osloensis	M osloensis	M osloensis
11. B795	R	M osloensis	M canis	M osloensis
12. B803	R	M osloensis	No ID	M osloensis
13. B825	S	M nonliquefaciens	No ID	No ID
14. B841	R	Roseomonas mucosa	No ID	Roseomonas mucosa
15. B911	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
16. B971	R	Roseomonas mucosa	No ID	Roseomonas mucosa
17. B1221	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
18. B1225	S	M osloensis	M bovis	M osloensis

Conjunctivitis Isolates	Van S/R	Identification based on: (DNA Sequencing with vancomycin susceptibility)	Biolog	MALDI-TOF MS
19. B1418	R	M nonliquefaciens	M equi	M nonliquefaciens
20. B1431	R	M osloensis	M osloensis	M osloensis
21. B1659	R	M nonliquefaciens	M catarrhalis	M catarrhalis
Endophthalmitis Isolates				
1. E141	R	Neisseria shayeganii	No ID	No ID
2. E542	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens
3. E614	S	M nonliquefaciens	M bovis	M nonliquefaciens
4. E812	S	M nonliquefaciens	M nonliquefaciens	M nonliquefaciens

Vancomycin susceptibility was determined using the standard Kirby-Bauer disk diffusion method. Zones greater than 18mm were interpreted as susceptible (S) and less were resistant (R). M atlantae had a zone of 16mm. “I” is intermediate and “Q” is questionable. “No ID” indicated that no identification was made. Biolog is an identification system that uses 94 biochemicals to phenotypically fingerprint bacterial isolates. MALDI-TOF MS is matrix-assisted laser desorption ionization time-of-flight mass spectrometry

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Table 2 - The Identification of Moraxella to species as Isolated from Keratitis, Conjunctivitis, and Endophthalmitis (1993-2016)

	Incidence (Percent)
Keratitis (n=82)	
Moraxella nonliquefaciens	66 (80.5%)
Moraxella lacunata	7 (9.0%)
Moraxella osloensis	5 (6.0%)
Acinetobacter lwoffi	2 (2.5%)
Moraxella bovis/ nonliquefaciens	1 (1.0%)
Moraxella osloensis/ nonliquefaciens	1 (1.0%)
Conjunctivitis (n=21)	
Moraxella osloensis	9 (43.0%)
Moraxella nonliquefaciens	6 (29.0%)
Roseomonas mucosa	3 (14.0%)
Acinetobacter (parvus, junii)	2 (9.5%)
Moraxella catarrhalis/nonliquefaciens	1 (4.5%)
Endophthalmitis (n=4)	
Moraxella nonliquefaciens	3 (75%)
Neisseria shayeganii	1 (25%)
All Infections	
Moraxella nonliquefaciens	70% (75 of 107)
Moraxella osloensis	13% (14 of 107)