Characterization and antimicrobial resistance of *Moraxella ovis* isolates from clinical cases of contagious ovine keratoconjunctivitis in Mexico State, Mexico

*Caracterización y resistencia antimicrobiana de aislamientos de Moraxella ovis de casos clínicos de queratoconjuntivitis contagiosa ovina en el Estado de México, México*

*Caracterização e resistência antimicrobiana de Moraxella ovis isolados de casos clínicos de ceratoconjuntivite contagiosa ovina no Estado do México, México*

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Abstract

Background: Contagious ovine keratoconjunctivitis (OKC) is a contagious disease that causes temporary or permanent blindness in sheep and goats, this disease is associated with a set of bacterial genus of which some of their species have reported antimicrobial resistance. Objective: The aim of this study was to identify the phenotypic-genotypic relationship of the antimicrobial resistance from Moraxella spp. isolates obtained of clinical cases of contagious ovine keratoconjunctivitis (OKC) in the state of Mexico. Methods: A total of 209 samples were obtained from clinical cases OKC in sheep and 60 Moraxella ovis isolates were identified by bacteriological techniques and amplification of 16s rRNA and rtxA genes by PCR. All isolates were evaluated in terms of antimicrobial resistance by the method of disk diffusion susceptibility test and the amplification of resistance genes by PCR respectively. Results: We determined 14 isolates of Moraxella ovis with antimicrobial resistance (AMR) and five multiresistant (MDR) and amplified the genes of antimicrobial resistance sul1, sul2, tetB, qnrA, qnrB, BlaTEM and not amplified the gene floR. Conclusion: Is the first report of isolation of Moraxella ovis from ocular lesions in sheep’s in the state of Mexico and the identification of six antimicrobial resistance genes. Results suggested that Moraxella ovis plays an important role in the course of the disease and provides a panorama of interest in molecular epidemiological surveillance and bacterial resistance.

Keywords: antimicrobial resistance; contagious ovine keratoconjunctivitis; epidemiological surveillance; goat; Moraxella spp.; multiresistance; resistance genes; sheep.

Resumen
**Antecedentes:** La queratoconjuntivitis contagiosa ovina (QCO) es una enfermedad infectocontagiosa que causa ceguera temporal o permanente en ovinos y caprinos, esta enfermedad está asociada a un conjunto de géneros bacterianos de los cuales algunos de estos han reportado resistencia antimicrobiana. **Objetivo:** El objetivo de este estudio fue identificar la relación fenotípica-genotípica de la resistencia antimicrobiana de aislamientos *Moraxella ovis.* obtenidos de casos clínicos de queratoconjuntivitis contagiosa ovina (QCO) en el Estado de México. **Métodos:** Se obtuvieron un total de 209 muestras de casos clínicos de QCO en ovinos y se identificaron 60 aislamientos de *Moraxella ovis* por técnicas bacteriológicas y amplificación de genes 16s rRNA y rtxA por PCR. Todos los aislamientos fueron evaluados en términos de resistencia antimicrobiana por el método de prueba de susceptibilidad de difusión en disco y la amplificación de genes de resistencia por PCR respectivamente. **Resultados:** Se determinaron 14 aislamientos de *Moraxella ovis* con resistencia antimicrobiana (AMR) y cinco multirresistentes (MDR) y se amplificaron los genes de resistencia antimicrobiana *sul1, sul2, tetB, qnrA, qnrB, BlaTEM* y el gen *floR* no fue amplificado. **Conclusión:** Es el primer reporte de aislamiento de *M. ovis* en lesiones oculares en ovinos en el Estado de México y la identificación de seis genes de resistencia antimicrobiana, se sugiere que *Moraxella ovis* juega un papel importante en el curso de la enfermedad y brinda un panorama de interés en la vigilancia epidemiológica molecular y la resistencia bacteriana. **Palabras clave:** cabra; genes de resistencia; *Moraxella* spp.; multirresistencia; oveja; queratoconjuntivitis contagiosa ovina; resistencia antimicrobiana; vigilancia epidemiológica.

**Resumo**

**Antecedentes:** A ceratoconjuntivite contagiosa ovina (OKC) é uma doença infeccioso contagioso que causa cegueira temporária ou permanente em ovinos e caprinos, esta doença está associada a um conjunto de gêneros bacterianos dos quais alguns deles relataram resistência antimicrobiana. **Objetivo:** O objetivo deste estudo foi identificar a relação fenotípica-genotípica da resistência antimicrobiana de *Moraxella* spp. isolados obtidos de casos clínicos de ceratoconjuntivite contagiosa ovina (OKC) no estado do México. **Métodos:** Um total de 209 amostras foram obtidas de casos clínicos de OKC em ovinos e obtidos e 60 isolados de *Moraxella ovis* foram identificados por técnicas bacteriológicas e amplificação dos genes 16s rRNA e rtxA por PCR. Todos os isolados
foram avaliados quanto à resistência antimicrobiana pelo método de teste de suscetibilidade à difusão em disco e pela amplificação de genes de resistência por PCR respectivamente. **Resultados:** Determinamos 14 isolados de *Moraxella ovis* com resistência antimicrobiana (AMR) e cinco multirresistentes (MDR) e amplificou os genes de resistência antimicrobiana *sul1, sul2, tetB, qnrA, qnrB, BlaTEM* e não amplificou o gene *floR*. **Conclusão:** É o primeiro relato de isolamento de *Moraxella ovis* em lesões oculares em ovinos no estado do México e a identificação de seis genes de resistência antimicrobiana. Sugere-se que *Moraxella ovis* desempenha um papel importante no curso da doença e fornece um panorama de interesse em vigilância epidemiológica molecular e resistência bacteriana.

**Palavras-chave:** cabra; genes de resistência; *Moraxella spp.*; multirresistência; ovelha; ceratoconjuntivite contagiosa ovina; resistência antimicrobiana; vigilância epidemiológica

**Introduction**

Contagious ovine keratoconjunctivitis (OKC) is a disease that causes temporary or permanent blindness in sheep and goats, the clinical signs range from conjunctivitis to the formation of a corneal ulcers. Several bacterial pathogens have been isolated of the OKC such as *Moraxella, Mycoplasma* and *Chlamydia* (Akerstedt and Hofshagen, 2004; Gupta et al., 2014; Jelenčik et al., 2019). However, three species of the genus *Moraxella* have been associated with infectious keratoconjunctivitis in large and small ruminants, such as *Mor. ovis* (Dagnall, 1994a), *Mor. bovis* (Karthik et al., 2017) and *Mor. bovoculi* (Farias et al., 2015). The differential biochemical tests of *Moraxella* species are phenylalaninedeaminase and gelatinase tests although they may be inconsistent. The molecular tests are based on the amplification of the *16s-23S rRNA* genes ISR (Shen et al., 2011; O´Connor et al., 2012; Sosa and Zunino, 2013) and the *rxtA* gene that encodes cytotoxin A (Farias et al., 2015).

In the treatment of keratoconjunctivitis, it’s based on the use of antimicrobials such as tetracyclines, sulfonamides, chloramphenicol, tulathromycin systemically or locally (Alexander, 2010; Maboni et al., 2015), it has been observed that some *Moraxella* species have shown resistance to these antimicrobials (Catry et al., 2007; Loy and Brodersen,
Antimicrobial resistance is associated with the presence of genes that are within the bacterial genome such as tet and otr that confer resistance to tetracyclines (Martí et al., 2006; Mosquito et al., 2011) sul and dfr that confer resistance to sulfonamides and trimethoprim (Kerrn et al., 2002; Ho et al., 2009), cat, cmlA and floR that confer resistance to phenicols (Schwarz et al., 2004; Chiu et al., 2006), mph and mrr that confer resistance to macrolides and lincosamides (Lüthje and Schwarz, 2006), BlaTEM, BlaRob and BlaCARB that confer resistance to betalactamases (Bush and Jacoby, 2010; Dallenne et al., 2010) qnr, gyr and par that confer resistance to quinolones (Cattoir et al., 2007; Jacoby et al., 2008). Some of these genes such as tetH, sul2, floR, blaRob-1, srt, (3´)-Ic, mph and mrr have been described in the genome within a pathogenicity island in Mor. bovoculi (Dickey et al., 2016) although there is no other study reported. Hence, the aim of the present study was to isolate Moraxella spp. from ocular lesions in sheep in the state Mexico and the phenotypic-genotypic characterization of the bacterial antimicrobial resistance in order to elucidate and understand the pathogenesis of these microorganisms in the disease.

Materials and methods

The experimental protocol was approved by the Review Commission of the Internal Committee for the Care of Laboratory Animals - Teaching, Research, Service and Production of the FMVZ (CICUAL-DISP FMVZ).

Bacteriological sampling and isolation

Sample size was calculated considering the clinical cases of OKC through the finite population sampling formula (Jaramillo, 2009). A pilot sampling was carried out for the estimation of the p indicator, obtaining a prevalence of .23 (107/454), estimating an = 209 samples. Samples (n=209) were collected from a total of 845 clinically healthy sheep with ocular lesions (e.g. ulcers, blindness) suggestive of OKC recognized by and general physical examination and ophthalmological tests of 15 production units distributed in six municipalities in the state of Mexico between February and June, 2017. Specimens were obtained by conjunctival swabs in the lesion area without touching the palpebral edge, the samples were placed in Stuart transport medium (STM, Cat. 1058-A, Dibico. Cuautitlán Izcalli, Mexico) and maintained in refrigeration at 4°C processed before 24h (Akerstedt and Hofshagen, 2004).
Regarding the isolation of the collected samples, the inoculations were performed on 5% ovine blood agar plates (ABS, BLL, Cat BD211037. Becton-Dickinson. CDMX, Mexico) and were incubated in aerobic conditions at 37 °C for 24-48 hrs. Colony growth criteria and classical biochemical tests described in the literature were used for the identification of Moraxella species involved in keratoconjunctivitis (Angelos et al., 2007; Angelos and Ball, 2007; Shen et al., 2011).

Genotypic identification through the 16s rRNA and rtxA genes of Moraxella spp.

DNA extraction using a heated colony in a total volume of 100 µl of sterile distilled water, which was heated to 95 °C for 10 minutes followed by a centrifugation stage of the cell suspension for 5 minutes at 9279 G (Eppendorf® Microcentrifuge 5415, , Merck KGaA, Darmstadt, Germany) and subsequent DNA collection (Dallenne et al., 2010).

The genes 16s rRNA and rtxA were amplified by a multiplex PCR with a final reaction volume of 25 µl for each one containing: 12.5 µl Master Mix (Gotaq Green Master mix, Cat M7122. Promega Corporation, WI, USA), 1 µl of each primer (Ovi16S1F/Ovis1849R, Bviv16S1F/Bovi1541R and Bovo1915R) for the 16s rRNA gene and for the rtxA gene (MbxAF/MbxAR, MbvAF and MovAR), 4 µl of bacterial DNA and 7.5 µl nuclease free water (Nuclease-Free Water Cat. P1195. Promega Corporation, WI, USA). The DNA sequences and the PCR product sizes are described in the (Table 1).

The PCR protocol was established as follows: Regarding 16s rRNA gene; initial denaturation of 5 min at 95°C followed by 35 cycles: denaturation of 40 s at 95°C, alignment of 40 s at 55°C, extension 1 min at 72 °C; and a final extension of 7 min at 72 °C and for the gene rtxA 35 Cycles denaturation of 50 s at 95 °C , alignment of 50 s at 65 °C, extension 1 min at 72 °C and a final extension 4 min at 72 °C carried out in a ThermoCycler (MultiGeneTM Mini, TC 020-24, Labnet International Inc. CA, USA). All the amplification products were identified through horizontal electrophoresis in 1% agarose gels stained with 0.5 µg/mL of ethidium bromide and visualized with a UV transilluminator (Mini-Bis 16mm, DNr Bio-Imaging Systems. Neve Yamin Israel; Shen et al., 2011; Farias et al., 2015). A Staphylococcus aureus ATCC 25923 strain was used as negative control.

Table 1. Primer designing for Moraxella spp. identification using PCR.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Species</th>
<th>Primers</th>
<th>Sequences 5’- 3’</th>
<th>Fragment size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>16s rRNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rtxA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Antimicrobial susceptibility tests

The susceptibility tests were carried out using disk diffusion method on agar Mueller Hinton (AMH, BD Bioxon. Becton-Dickinson. CDMX, Mexico) supplemented to 5% with ovine blood defibrinated according to the guidelines of the Institute of Clinical and Laboratory standards (CLSI, 2016). The bacterial solution turbidity suspended Mueller-Hinton was adjusted (MH, BBL TM. Becton-Dickinson. CDMX, Mexico) at a scale of 0.5 Mc Farland equivalent to a concentration of 1-2 x10^8 CFU/mL, the following antimicrobials were used; ampicillin (10 µg), carbencillin (100 µg), cephalothin (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), nitrofurantoin (30 µg) netilmicin (30 µg), gentamicin (10 µg), amikacin (30 µg), sulfamethoxazole/trimethoprim (25 µg), norfloxacin (10 µg), tetracycline (30 µg) and nalidixic acid (30 µg) (BBL ™ Sensi-Disc™. Becton-Dickinson. CDMX, Mexico), the plates were incubated at 37 °C for 18-24 hours. Regarding the interpretation of the results the following profiles were established: sensitive (S), intermediate (I) and resistant (R). An Escherichia coli ATCC 25922 strain was used as control. As no standardized criteria for the interpretation of sensitivity exist for Moraxella spp., breakpoints established for Gram-negative pathogens related with cattle respiratory disease were used (Maboni et al., 2015). For instance, the critical breakpoints for determining against respiratory pathogens of Pasteurella multocida, Moraxella catarrhalis, Mannheimia haemolytica, Pseudomon a aeruginosa and Haemophilus somnus (CLSI, 2013; 2016).

Antimicrobial resistance genes

Polymerase chain reaction (PCR) was used for the identification of antimicrobial resistance genes, seven primers were used, sequences and the amplification products sizes are described in (Table 2), for the detection of genes sul1 and sul2 (sulfonamides), Blatem
(β-Lactams), tetB (tetracyclines), floR (florfenicol/chloramphenicol), as well as, a multiplex PCR for qnrA and qnrB genes (quinolones), following the same reaction condition previously published (Kern et al., 2002; Chiu et al., 2006; Martí et al., 2006; Cattoir et al., 2007; Dallenne et al., 2010). As positive controls, were used Escherichia coli ATCC 25922 and other E. coli isolates from sewage characterized phenotypically resistant for AM (ampicillin), CB (carbencillin), CF (cephalothin), CL (chloramphenicol), NA (nalidixic acid), TE (tetracycline), NET (netilmicin), NF nitrofurantoin, CPF (ciprofloxacin) and genotypically possess qnrA+, qnrB+ and BlaTEM+ genes (Talavera-González et al., 2021).

### Table 2. Antimicrobial resistant used for the identification of Moraxella spp.

<table>
<thead>
<tr>
<th>Resistance</th>
<th>Primers</th>
<th>Sequences 5’-3’</th>
<th>Fragment size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfonamides</td>
<td>sul1 F</td>
<td>CGG CGT GGG CTA CCT GAA CG</td>
<td>433 bp</td>
<td>Kern et al. (2002)</td>
</tr>
<tr>
<td></td>
<td>sul1 R</td>
<td>GCC GAT GCG GTG AAG TTC CG</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>sul2 F</td>
<td>CGC CTC AAG GCA GAT GGC ATT</td>
<td>293 bp</td>
<td>Kern et al. (2002)</td>
</tr>
<tr>
<td></td>
<td>sul2 R</td>
<td>GCG TTT GAT ACC GGC ACC CGT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>tet B F</td>
<td>TTG GTT AGG GGC AAG TTT TG</td>
<td>650 bp</td>
<td>Martí et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>tet B R</td>
<td>GTA ATG GGC CAA TAA CAC CG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quinolones</td>
<td>qnrA F</td>
<td>AGA GGA TTT TCT ACG CCA GG</td>
<td>580 bp</td>
<td>Cattoir et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>qnrA R</td>
<td>TGC CAG GCA CAG ATC TTG AC</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>qnrB F</td>
<td>GGM ATH GAA ATT CGC CAC TG</td>
<td>264 pb</td>
<td>Cattoir et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>qnrB R</td>
<td>TTT GGY GY CGC CAG TCG AA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-lactamases</td>
<td>MultiTSO-T BlaTEM F</td>
<td>CAT TTC CGT GTC GCC CTT ATT C</td>
<td>800 bp</td>
<td>Dallenne et al. (2010)</td>
</tr>
<tr>
<td></td>
<td>MultiTSO-T BlaTEM R</td>
<td>CGT TCA TCC ATA GTT GCC TGA C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Florfenicol/Chloramphenicol</td>
<td>floR F</td>
<td>CTT TGG CTA TAC TGG CGA TG</td>
<td>266 bp</td>
<td>Chiu et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>floR R</td>
<td>GAT CAT TAC AAG CGC GAC AG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Y=T o C; R=A or G, S=G or C, D=A or G or T, H=A or C or T; M=A o C.

### Results

A total of 209/861 examined sheep’s (24.27% prevalence) in six municipalities in the state of Mexico, showed lesions compatible with OKC. With respect to the total number of the studied samples (209), we identified 60 isolates of Moraxella spp. by biochemical tests. Through the 16s rRNA gene, 54 strains of Mor. ovis were correctly identified from the 60 isolates (90%). The 1541 and 1959 pb amplicons, corresponding to Mor. bovis and
Mor. bovoculi respectively not amplified by any isolate. For the rtxA gene, 57 strains of Mor. ovis were identified of the 60 isolates (95%) (Table 3) and no isolates amplified a band of 943 pb corresponding to Mor bovis.

Table 3. Moraxella spp. isolates obtained of clinical cases OKC in sheep in the Mexico State.

<table>
<thead>
<tr>
<th>UPP</th>
<th>Municipal</th>
<th>Animals</th>
<th>Ocular injuries*</th>
<th>Samples</th>
<th>Bacteriology</th>
<th>Molecular</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>C</td>
<td>KC</td>
<td>K</td>
<td>U</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mor. ovis</td>
<td>Mor. ovis</td>
<td>1949 pb</td>
<td>Mor. ovis</td>
</tr>
<tr>
<td>3</td>
<td>Tenango</td>
<td>282</td>
<td>51</td>
<td>14</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Toluca</td>
<td>189</td>
<td>48</td>
<td>7</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Xonacatlán</td>
<td>117</td>
<td>23</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Lerma</td>
<td>80</td>
<td>20</td>
<td>10</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>Capulhuac</td>
<td>43</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>Calimaya</td>
<td>150</td>
<td>8</td>
<td>8</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Total/</td>
<td>861</td>
<td>16</td>
<td>39</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

Prevalence | 24.27% | 28.70% | 90% | 95% |

*The ocular lesions correspond to the number of animals sampled, (UPP) Number of Production Units, (C) conjunctivitis, (KC) keratoconjunctivitis (K) keratitis (U) corneal ulcers.

232 Antimicrobial susceptibility tests

The 60 isolates of Mor. ovis were sensitive to gentamicin and norfloxacin 100% (60/60).

Antimicrobial resistance of 18.3% (11/60) to nalidixic acid was observed, 11.6% (7/60) to nitrofurantoin, 10.0% (6/60) to ampicillin, 6.6% (4/60) to chloramphenicol 3.3% (2/60) to cephalothin, 3.3% (2/60) to tetracycline and 1.6% (1/60) to cefotaxime, to netilmicin, to amikacin and sulfamethoxazole/trimetropim (Table 4).

A total of 68.33% (41/60) of the isolates showed sensitivity to all the antimicrobial used, and 31.66% (19/60) were resistant to one or more antimicrobial. It was determined that 23.33% (14/60) of the isolates were resistant to one to two antimicrobial and 8.33% (5/60) were multi-resistant strains (Table 4).

Table 4. Susceptibility test of Moraxella spp. causing OKC.

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Mor. ovis (n= 60) (#) prevalence</th>
<th>Zone diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>I</td>
</tr>
<tr>
<td>NA</td>
<td>11(18.3)</td>
<td>8(13.3)</td>
</tr>
<tr>
<td>NF</td>
<td>7(11.6)</td>
<td>1(1.6)</td>
</tr>
<tr>
<td>AM</td>
<td>6(10.0)</td>
<td>7(11.6)</td>
</tr>
<tr>
<td>CL</td>
<td>4(6.6)</td>
<td>2(3.3)</td>
</tr>
</tbody>
</table>
# isolates, (#) percentage, (S) susceptible, (I) intermediate, (R) resistance. AM ampicillin; CB carbenicillin; CF cephalothin; CFX cefotaxime; CP ciprofloxacin; CL chloramphenicol; NF nitrofurantoin; NET netilmicin; GE gentamicin; AK amikacin; STX sulfamethoxazole/trimethoprim; NOF norfloxacin; TE tetracycline; NA nalidixic acid (CLSI 2013; 2016).

**Phenotypic and genotypic profiles**

*Mor. ovis* presented 36.66% (22/60) and 33.33% (20/60) amplification of *sul1* and *sul2* gene respectively. Isolates of *Mor. ovis* 46.66% (28/60) amplified the gene *Bla<sub>TEM</sub>*. The gene that confers resistance to tetracyclines, 8.33% (5/60) of the isolates of *Mor. ovis* amplified a band 650 pb corresponding to *tetB*. *Mor. ovis* isolates (23/60) 38.33% amplified the *qnrA* gene and (22/60) 36.66% the *qnrB* gene (Table 5).

**Table 5.** Phenotypic and genotypic profile of antimicrobial resistance in *Moraxella*.

<table>
<thead>
<tr>
<th>Phenotypical Resistance</th>
<th>Resistance Genes</th>
<th>Mor. Ovis (n=60)</th>
<th>Total</th>
<th>Prevalence of resistance genes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM</td>
<td><em>Bla&lt;sub&gt;TEM&lt;/sub&gt;</em></td>
<td>5(6) 6(7) 17(48)</td>
<td>28(60)</td>
<td>46.66</td>
</tr>
<tr>
<td>NA</td>
<td><em>qnrA</em></td>
<td>8(11) 1(8) 14(41)</td>
<td>23(60)</td>
<td>38.33</td>
</tr>
<tr>
<td>STX</td>
<td><em>sul1</em></td>
<td>1(1) 1(1) 20(58)</td>
<td>22(60)</td>
<td>36.66</td>
</tr>
<tr>
<td></td>
<td><em>sul2</em></td>
<td>1(1) 1(1) 18(58)</td>
<td>20(60)</td>
<td>33.33</td>
</tr>
<tr>
<td>TE</td>
<td><em>tetB</em></td>
<td>1(2) 0(1) 4(57)</td>
<td>5(60)</td>
<td>8.33</td>
</tr>
<tr>
<td>CL</td>
<td><em>floR</em></td>
<td>0(4) 0(2) 0(54)</td>
<td>0(60)</td>
<td>0.00</td>
</tr>
</tbody>
</table>

# gene amplification, (#) all isolates, (R) resistance, (I) intermediate, (S) susceptible, (AM) ampicillin, (TE) tetracycline; (CL) chloramphenicol, (STX) sulfamethoxazole/trimethoprim (NA) nalidixic acid.

**Discussion**

In the present study, an OKC prevalence of 24.27% (209/861) was obtained. This was lower than reported in the United Kingdom (Dagnall, 1994a; 1994b) 72.38% (97/134). Severity of eye lesions were observed, which could be related to the period taken from...
the samples, to the type of sheep raising systems and predisposing factors. An increase in cases of OKC in Norway during autumn and winter seasons, this is due to the way in which sheep farming is practiced in this country where in summer the animals take them to graze without human intervention and in winter they are collected and confined in barns (Akerstedt and Hofshagen, 2004). In Mexico, the sheep breeding systems are of intensive form, semi-intensive to extensive, mixed and grazing. In the present study, sampling was carried out in the spring-winter period, which is characterized by the presence of flies, dust, direct sunlight and the variety of microorganisms as well as other factor involved in the evolution of the disease (Egwu et al., 1989). Dagnall (1994b) reported a prevalence of 28.86% (28/97) for Mor. ovis from ovine isolates, similarly, Akerstedt and Hofshagen, (2004) obtained a prevalence of 28.23% (24/85) in sheep herds with the disease, similar data were obtained in this work reporting 27.75% (58/209) of Mor. ovis isolates from sheep with OKC.

The first isolation of Mor. bovoculi was reported in the United States (Angelos et al., 2007) and later in different countries as Uruguay (Sosa and Zunino, 2013), Argentina, Norway and Brazil (Libardoni et al., 2012; Farias et al., 2015) obtained in cases of IBK. The first report of Mor. bovoculi was made in sheep’s by Farias et al. (2015) in Brazil, while on the other side, Karthik et al. (2017) was the first author to identify to Mor. bovis of ocular injures of sheep in India, in our study it was not possible to isolate Mor. bovis and Mor. bovoculi of ocular injures samples in sheep.

Shen et al. (2011) amplified the 16s rRNA gene in 89.5% (51/57) of the isolates identifying correctly as Mor. bovoculi (44/51) and Mor. bovis (7/51), in this study were amplified 90.0% (54/60) of the isolates correctly identified as Mor. ovis.

Farias et al. (2015) amplified the gene rtxA in 100% (33/33) of the isolates of bovine and ovine with keratoconjunctivitis, identifying Mor. bovis (15/33), Mor. bovoculi (11/33) and Mor. ovis (7/33), in this study we amplified the rtxA gene 95% (57/60) of all isolates identifying; Mor. ovis. Respecting to the six Mor. ovis isolates that did not amplify the 16s rRNA and the three Mor. ovis isolates did not show any amplification for rtxA gene, which possibly could be related to the variations in the reading frame of the sequences by deletions or absence of repeated sequence regions surrounding the RTX operon which has been reported in non-hemolytic M. bovis strains (Angelos et al., 2003) in recent studies by (Dickey et al., 2018) detected a recombination in the nucleotide sequences within the non-coding regions in the rRNA and RTX genes in strains of Mor. bovoculi.
The first study of antimicrobial sensitivity in vitro *Mor. ovis* strains performed by (Elad et al., 1988) reported resistance to penicillin, ampicillin, streptomycin and neomycin were reported previously, in another study conducted by Catry et al. (2007) reported strains resistant to erythromycin. The most recent work by Maboni et al. (2015), showed strains sensitive to gentamicin, chloramphenicol, florfenicol and sulfonamides, in this study we reported strains of *Mor. ovis* susceptible to gentamicin, as well as resistant strains to ampicillin, chloramphenicol, tetracycline and sulfamethoxazole/trimethoprim.

Oxytetracycline is usually the first choice for the antimicrobial treatment of keratoconjunctivitis (Alexander, 2010), however *Moraxella* spp. showed resistance over time to this antimicrobials (Maboni et al., 2015). Likewise, florfenicol was reported as an effective therapeutic option to combat keratoconjunctivitis (Gokce et al., 2002; Angelos et al., 2011). The use of antimicrobial is essential in the controlling of OKC by *Mor. ovis*, in order to avoid exacerbation of the lesions associated with other bacterial infections (Dagnall, 1994b).

This is the first study to amplify the antimicrobial resistance genes *sul1*, *sul2*, *tetB*, *qnrA qnrB* y *Bla TEM* in *Mor. ovis*. The unique study associated with antimicrobial resistance determinants (ARD) in *Mor. bovoculi* was performed by Dickey et al. (2016) they described ten ARD that were located on a genomic island greater than 27 kb in the sequences of *Mor. bovoculi* and Mb58069 isolates that were resistant to florfenicol, oxytetracycline, sulfonamides and showed intermediate resistance to macrolides.

In a similar study conducted by Roberts et al. (1991) they described in tetracycline-resistant strains of *Mor. catarrhalis* that carry the *tetB* gene on its chromosome. The *tetB* gene has the widest range of Gram-negative bacteria such as *E. coli* (Medina et al., 2011; Mirzaagha et al., 2011), *Acinetobacter baumannii* (Martí et al., 2006), *Actinobacillus actinomycetemcomitans* (Roe et al., 1995), *Haemophilus influenzae* (Robert and Smith, 1980) and *Treponema denticolaum* (Roberts, 1996).

Bacteria carrying genes of resistance *sul1*, *sul2*, *sul3* and *Bla TEM* such as *E. coli* (Kerrn et al., 2002; Infante et al., 2005; Ho et al., 2009; Medina et al., 2011; Gnida et al., 2014; Memariani et al., 2015), *Klebsiella pneumoniae*, *Pseudoma aeruginosa* (Peymani et al., 2017), *Proteus mirabilis* (Feizabadi et al., 2010; Gong et al., 2018), *Salmonella* spp. (Maka et al., 2015), *Stenotrophomonas maltophilia* (Hu et al., 2011). Bacteria carriers of
genes qnrA and qnrB as K. pneumoniae (Rodríguez-Martínez et al., 2003), E coli (Wang et al., 2003; Jiang et al., 2008; Aguilar-Montes de Oca et al., 2015), P. aeruginosa, Enterobacter cloacae (Wu et al., 2007), Actinobacter baumanii (Touati et al., 2008), Salmonella enterica (Murray et al., 2008), Enterobacter aerogenes, Citrobacter freundii (Park et al., 2007), Kluyvera (Kraychete et al., 2016), among others.

In conclusion, the present study, Mor. ovis was identified by using the 16s rRNA and RtxA genes by PCR, confirming PCR as the most sensitive tests for the diagnosis of bacterial agents involved in keratoconjunctivitis. It will be possible to establish new criteria for the choice of antimicrobial treatments based on the phenotypic and genotypic characteristics of antimicrobial resistance of the results obtained, which will also allow the surveillance molecular epidemiology of antimicrobial resistance genes in bacterial populations of Moraxella spp.

Declarations

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Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

Author contributions
Acosta-Dibarrat J, Talavera Rojas M, Soriano Vargas E and Ortiz Arana G designed the experiment. Acosta-Dibarrat J and Ortiz Arana G administered the project. Ortiz Arana G, Palomares-Resendiz EG, Salgado-Miranda C and Enriquez-Gomez E worked on the aspects involved in the methodology. Ortiz Arana G and Acosta Dibarrat J wrote and prepared the manuscript. All authors provided critical feedback of the writing and editing.

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