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Original Article

Genetic Detection of Some Bacterial Species Associated with Bovine Keratoconjunctivitis Infections in Basra Governorate

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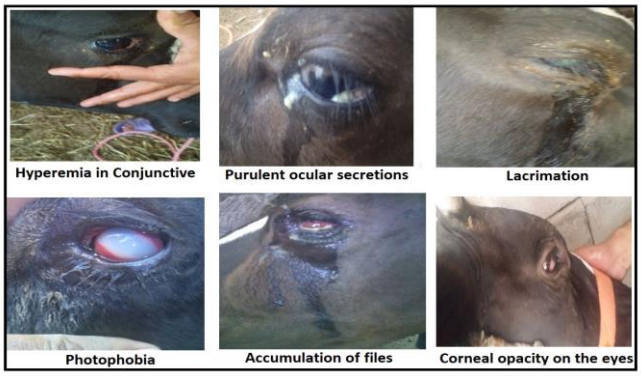
ABSTRACT

Veterinary chemicals are pharmaceuticals or treatments that are used in cattle to treat or prevent disease, injury, and pests. Livestock survival and productivity would be severely reduced without veterinary drugs, especially for intensively maintained animals like pigs and poultry. Infectious bovine keratoconjunctivitis is a bacterial eye illness of cattle, and there is limited knowledge on the study of bacterium pathogen-contaminated eyes of animals in Basra province. As a result, the purpose of this research was to isolate and classify some bacterial species associated with infection in cattle with keratoconjunctivitis. This study included examination of 120 eye swabs from cows from different regions in the Basra governorate. The current study was carried out from October 2018 to February 2019. Out of 120 cases involved in this study, 30 cases were identified to have keratoconjunctivitis. The results of the study showed that eye infections in cows were mostly unilateral, in one eye (69%), and less than bilateral infections in both eyes (30.9%). Twenty different bacterial isolates were obtained, and the isolated bacteria were identified genetically by 16S rDNA and sequencing as Escherichia coli 40%, Bacillus subtilis 10%, Enterobacter cancer genus 10%, Enterobacter hormaechei 10%, Enterobacter cloacae 15%, and Klebsiella pneumonia 15%.

GRAPHICAL ABSTRACT

Genetic Detection of Some Bacterial Species Associated with Bovine keratoconjunctivitis Infections in Basra Governorate



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Introduction

Veterinary chemicals are substances that are used to prevent, diagnose, treat, change, or relieve disease or injury in animals. Prescription drugs such as antibiotics and over-the-counter products are examples of veterinary chemicals, e.g., drenches and vaccines [1-5]. The following are some of the most commonly utilized veterinary chemicals on livestock: a) Worm drenches for the intestines; b) treatments for lice, blowflies, and ticks; c) vaccines against illnesses including pulpy kidney, tetanus, and scabby mouth; d) antibiotics as drugs that are used to treat bacterial infections; and e) anti-inflammatory drugs used to treat pain [6, 7].

Pinkeye, also known as infectious bovine keratoconjunctivitis (IBK), is a highly contagious and infectious ocular illness of cattle characterized by conjunctivitis and ulcerative keratitis that affects cattle all over the world [8-11]. The sickness is also found in other livestock [12] and wildlife [13], and it is thought to be a multifactorial disease. The ocular disease maybe is not a fatal disease, but the morbidity is relatively high and combines with conjunctivitis, keratitis, congestion cornea, and sometimes abscesses [14]. Many studies from different countries have demonstrated that keratoconjunctivitis in large and small ruminants result from various bacteria or viruses like *Rickettsia*, *Chlamydia*, Viruses, *Mycoplasma spp.*, *Neisseria catarrhalis*, and *Moraxella bovis* [15].

The ocular infection may be causing temporary blindness/or permanent blindness, and the consequences could be extremally worse by turning down grazing behavior, which affects animal welfare and productivity [16]. So, ocular infections in livestock have obviously an important role in economic deflection [17]. However, multiple potential factors are associated with inflammation of the eye, including direct contact with lacrimal and nasal secretions contaminated with bacteria that have a significant role in spreading infections and producing corneal and conjunctival irritation. In moderate and acute infections and severe and persistent infections, ocular infections can affect one eye [unilateral] or both eyes [bilateral]. Conjunctivitis, excessive tears, photosensitivity, ocular pain, squinting of the eyelid, corneal edema, and corneal ulceration are all symptoms of ocular infection, as are corneal rupture and blindness [18, 19].

An outbreak probably occurs, especially when there is an implication of bad housing/animal management such as animal overcrowding and ignorance in maintaining of sanitation environment. Additionally, many seasonal insects such as domestic flies, insects, and stable flies have mechanical and dynamic important roles in conveying pathogenic bacteria [20, 21]. Little information is available regarding the study of bacteria pathogen-infected eyes of the livestock in Basra province. Therefore, the object of this study was to isolate and identify the occurrence of bacterial infection from cattle clinically associated with keratoconjunctivitis.

Material and methods

Sampling of animals

The current study was carried out from October 2019 to February 2020. Four regions (Al-Qurna, Al-Dayer, Al-Shenana, and Al-Nashwa) in Basrash province, selected purposively for this study. Clinical examination, including history and visual and physical examinations, has been previously done [22, 23]. According to the owner, the main complaint was eye problems in adult and young animals observed some weeks ago. A total of one hundred and twenty conjunctival swabs specimen were obtained from the cattle that showed an ocular infection symptom of infection. The age, breed, and sex were recorded from all examined animals. Swab sticks wet with sterile normal saline were used to collect the samples. The eyelid membrane was opened widely, allowing a spinning swab stick to be used on the corneal surface and conjunctiva, after which it was transferred to sterile test tubes containing 5 ml of sterile nutrition broth. Following that, all samples sent to the bacteriological laboratory were owned by Veterinary Medicine.

Bacterial identification

For 24 hours, the tubes were incubated aerobically in the incubator at 37°C. After that, it was cultivated on blood agar containing 7%

sheep blood, MacConkey agar, Eosin Methylene blue, and incubated aerobically for 24 hours at 37°C. Pure colonies were saved on brain heart infusion agar for the purpose of conducting genetic identification of bacterial strains, and colonies were further sub-cultured to investigate the morphology of forming colonies and germ interactions that were taken from it to Gram stain.

Extraction of genomic DNA

The isolates' DNA was isolated using a genomic DNA purification kit. Electrophoresis on 0.8 percent Agarose revealed the result, which was visible under UV light as bands of DNA [24].

Identification by Polymerase Chain Reaction (PCR)

Primers for 16SrDNA F- 5' AGAGTTTGATCCTGGCTCAG-3' and R- 5' GGTTACCTTGTTACGACTT-3" were used [25], as well as a PCR software developed earlier [26] from [27]. With minor tweaks, the amplification stages were abstracted from [28].

Sequencing for 16S rDNA and identification of bacterial species

The national instrumentation center for environmental management (nicem) sequenced the gene (http://nicem.snu.ac.kr/main/?enskin=index.html). For complete identification of bacterial isolates, a homology search was conducted using the Basic Local Alignment Search Tool (BLAST) program, available at the National Center Biotechnology Information (NCBI) online at (http://www.ncbi.nlm.nih.gov), and the Bio Edit program, available at the National Center Biotechnology Information (NCBI) online at (http://www.ncbi.nlm.nih.gov).

Result and Disscution

Out of 120 samples involved in this study, 30 were identified as having keratoconjunctivitis with an estimated prevalence of (25%). The results of the study showed that eye infections in cows were mostly unilateral, in one eye (69%), and less than bilateral infections in both eyes (30.9%), as showed in Table 1. The highest ocular infection in the examined animals was found during the winter (from January to February) comparing with other times (Table 2).

Table 1: Categorized cattle based on ocular infection condition (n=120)

Ocular clinical signs	Number of infected animals	Percentage %
Unilateral infections	83	69.1 %
Bilateral infections	37	30.9 %
Total	120	100%

Table 2: Distribution of sampling by months

Months	Positive samples	Percentage %
October	14	11.60%
November	13	10.80%
December	27	22.50%
January	30	25%
February	36	30%

Infected animals showed conjunctival hyperemia (15.8%), (Figure 1), serious, mucoid and/or purulent ocular secretions (20.8%) (Figure 2) photophobia (24.1%) (Figure 3), lacrimation (15%) (Figure 4), congregation of flies on the eyes (12.5%) (Figure 5), and corneal opacity (11.6%) (Figure 6), Table 3.

Table 3: Clinical signs of infected cows with keratoconjunctivitis

Clinical signs	Number of animals	Percentage%
conjunctival hyperemia	19	15.8%
purulent ocular secretions	25	20.8%
Photophobia	29	24.1%
Lacrimation	18	15%
congregation of flies on the eyes	15	12.5%
corneal opacity	14	11.6%

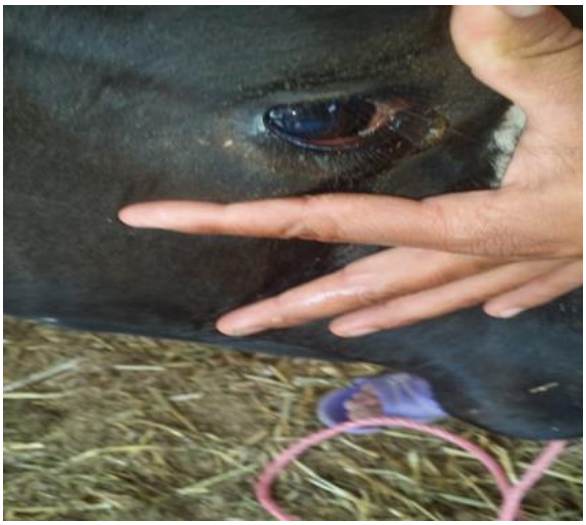

Figure 1: Hyperemia in Conjunctiva

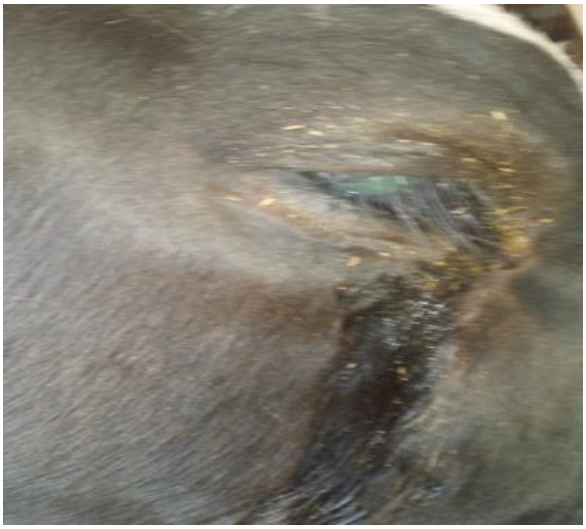

Figure 4: Lacrimation

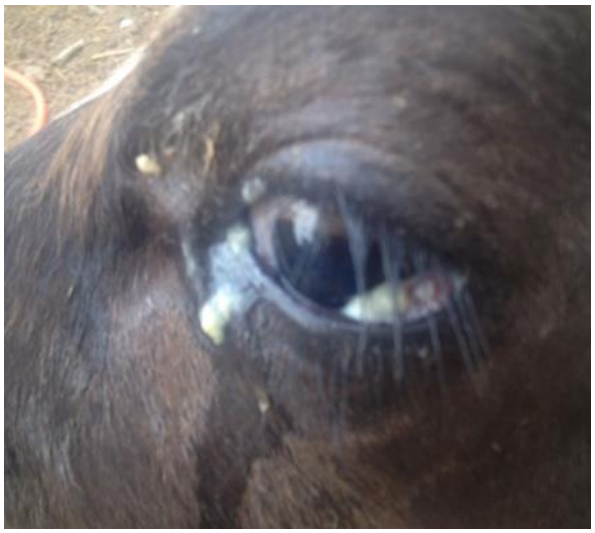

Figure 2: Purulent ocular secretions

Figure 5: Accumulation of flies

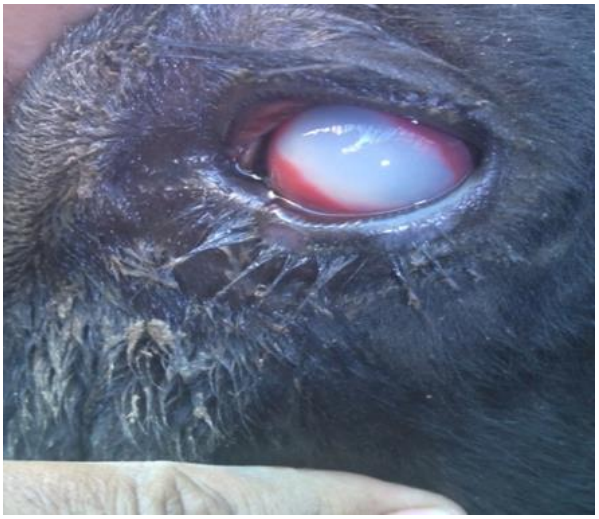

Figure 3: Photophobia

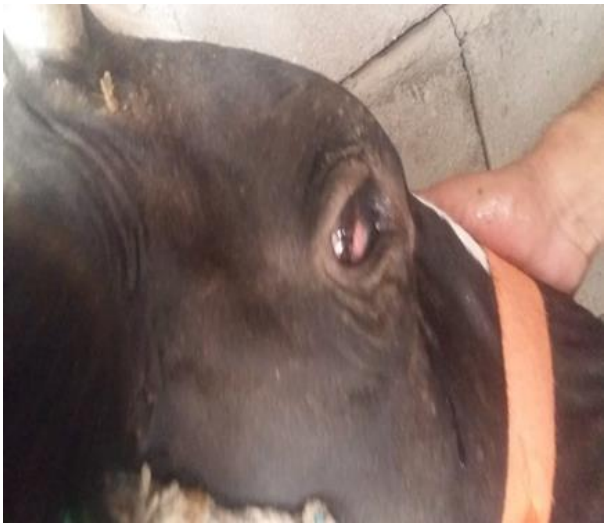

Figure 6: Corneal opacity on the eyes

Bacterial identification by 16S rDNA

A genomic DNA purification kit was used to recover DNA from twenty different strains. The result revealed under UV light after electrophoresis on 0.8 percent Agarose, and the isolated DNA from each isolate was submitted to PCR for amplifying 16S rDNA (figure 7). By comparing the gene's unique band to the typical molecular DNA ladder, 1250 bp was determined (100bp).

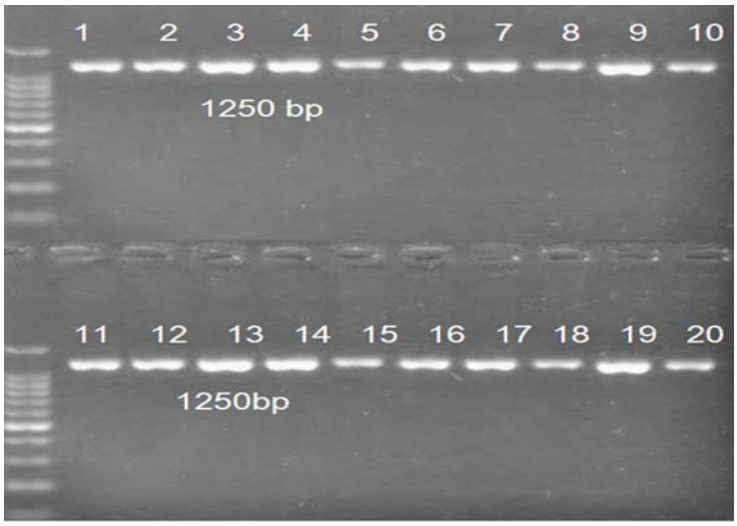

Figure 7: Amplification of 16S rDNA

Sequencing for 16S rDNA and identification of bacterial species

Table 4 shows the results of 16S rDNA nucleotide sequencing for 20 isolates. Isolates were identified to the strain level. Table 5 shows the number and proportion of bacteria recovered from inflammatory smears, while Figures 8 and 9 illustrate the phylogenetic tree of some of the isolates.

Table 4: Identified bacterial strains by gene sequencing

Bacterial species	Identical to strain	Percentage of identity (%)	Length (bp)
Escherichia coli	E21	99%	1192
Escherichia coli	wid15	99%	723
Escherichia coli	CMC357	98%	476
Escherichia coli	UCCB108	99%	1383
Bacillus subtilis	subsp. inaquosorum	99%	1149
Enterobacter cancerogenus	pca7	99%	1171
Enterobacter hormaechei	subsp. xiangfangensBUFF38	99%	1493
Enterobacter cloacae	109	99%	514
Klebsiella pneumoniae	DSM 30104	100%	1130
Enterobacter hormaechei	FG MZN 14 TR	100%	1146
Pseudomonas aeruginosa	ST11	100%	1410

Table 5: The percentage of bacterial species from twenty isolates

Bacterial species	The percentage from 20 isolates
Escherichia coli	20%
Bacillus subtilis	10%
Enterobacter cancerogenus	10%
Enterobacter hormaechei	10%
Enterobacter cloacae	15%
Klebsiella pneumoniae	15%
Pseudomonas aeruginosa	20%

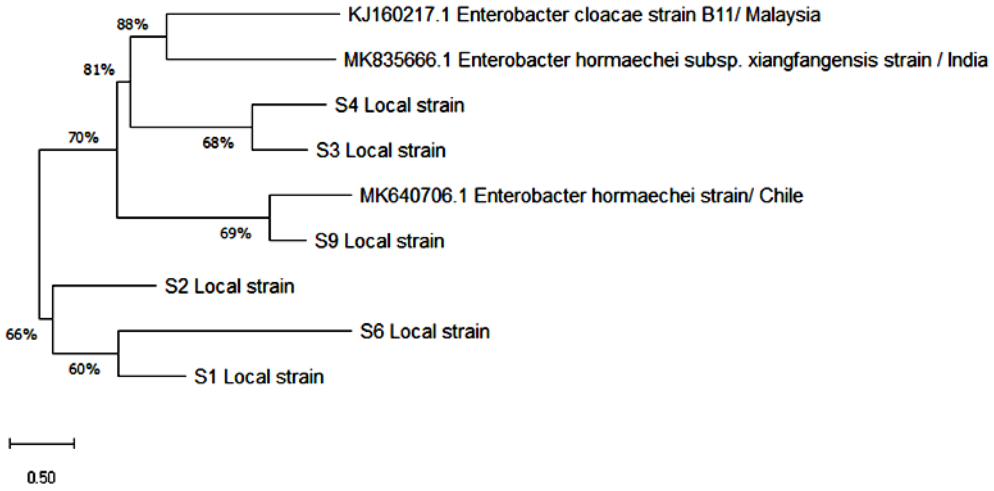

Figure 8: Phylogenetic tree of *Enterobacter*

Phylogenetic tree analysis of Enterobacter showed five distinct groups of local strains. S1, S2, S3 & S4, S6, and S9. S3 & S4 have a similarity with those previously isolated from Malaysia and India. S9 has a similarity with a strain isolated from Chile. The other three strains are far away from previously compared strains.

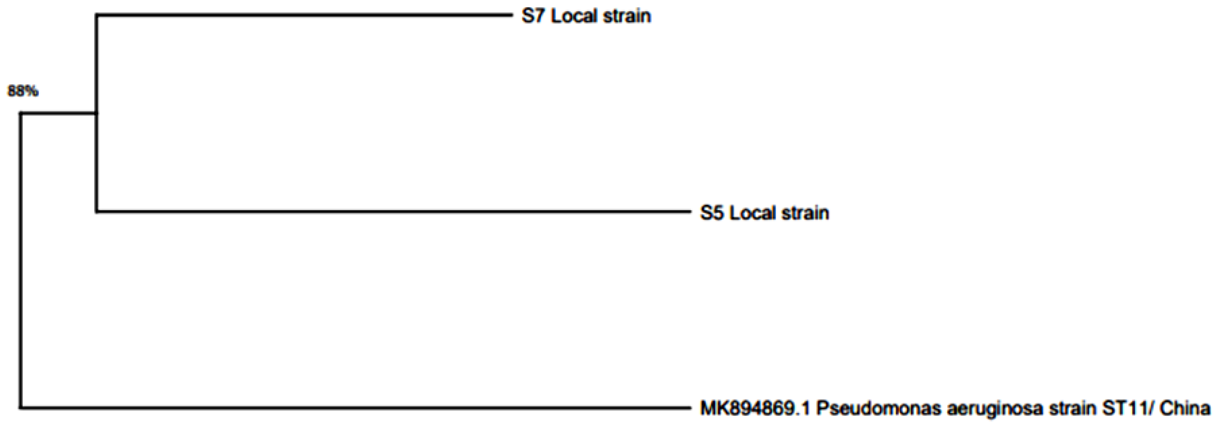

Figure 9: phylogenetic tree of Pseudomonas aeruginosa

Phylogenetic tree analysis of Pseudomonas aeroginosa showed two related strains S5 and S7. Those strains have a high similarity with Pseudomonas aeroginosa isolated in China.

Infectious bovine keratoconjunctivitis (IBK) is an infectious ocular illness that affects cattle, particularly those of the dairy breeds. Gram-negative bacteria, primarily Moraxella Bovis, cause the disease, and it is treated by parenteral or systemic antibiotic therapy. In most cases, M. Bovis infections are treated topically with antibiotic-containing formulations, especially when clinical symptoms are present. The antibiotic benzathine cloxacillin is known to be effective in the treatment of M. Bovis infections, and it has been frequently suggested for topical use. Due to an extremely painful ailment affecting beef and dairy cattle worldwide [29, 30], infectious bovine keratoconjunctivitis generates major economic losses resulting from lower weight gain in beef breeds and loss of milk production, short-term disruption of breeding plans, and treatment expenditures [31].

IBK is a set of organisms and predisposing factors that cause ocular alterations that favor bacterial colonization of the eye, similar to many other disorders. When dealing with less aggressive gram-negative and positive bacteria strains, predisposing factors may be an essential component of the IBK ocular illness complex [32, 34]. In our study, out of 120 samples, 24 were identified to have keratoconjunctivitis with an estimated prevalence by 25%. The results of the study showed that eye infections in cows were

mostly unilateral, in one eye (69%), and less than bilateral infections in both eyes (30.9%). Infected animals showed conjunctival hyperemia (15.8%), serious, mucoid and/or purulent ocular secretions (20.8%), photophobia (24.1%), lacrimation (15%), the congregation of flies on the eyes (12.5%), and corneal opacity (11.6%). Because the 16S rDNA exists in this region in all bacteria, an amplified region of around 1250bp was found using the PCR technique, which agreed with the finding of previous studies [26, 35]. The isolates identified to the level of strain with the percentage of bacteria isolated from inflammatory smears were Escherichia coli by 20%, Bacillus subtilis by 10%, Enterobacter cancerogenus by 10%, Enterobacter hormaechei by 10%, Enterobacter cloacae by 15%, Klebsiella pneumonia by 15% and Pseudomonas aeruginosa by 20%, which corresponded to past research [36–39].

Conclusion

Phylogenetic tree analysis of Enterobacter showed five distinct groups of local strains. Some have a similarity with those previously isolated from Malaysia and India. Other has a similarity with a strain isolated from Chile. Furthermore, three strains are far away from previously compared strains. On the other hand, Phylogenetic tree analysis of Pseudomonas aeroginosa showed a high similarity with Pseudomonas aeroginosa isolated in China.

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Authors' contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

The authors declare no conflicts of interest.

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