

Research Article



Molecular Detection of β -lactamase Genes and Antibiotics Resistance for Bacterial Isolates From Pigeon's Eyes Infections

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Abstract | The inflammation of the inner surface of the eyelid and the outermost layer of the white portion of the eye is known as conjunctivitis, or pink eye. In one or both eyes, conjunctivitis can occur. A total of 89 pigeons include In this study, eye swabs were taken from pigeons suffering from eye inflammation, and then the swabs were cultured on MacConkey agar and Blood agar. The pigeons' eyes swabs taken from were cultured by a streak method and incubator with a 37C. For 24 hours. 10 samples with no growth appear, and 79 isolates diagnosed the bacterium depending on the appearance qualities of the developing colonies, pigmentation In Gram stain, as well as biochemical examinations. Different bacterial species was confirmed using the Enterosystem 18 R system and using a device. Vitek-2 compact system. Results of an antibiotic resistance test showed that the isolates showed a "significant" change in resistance to antibiotics, as the isolates showed the bacterial species *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, and *Klebsiella pneumoniae* resistance (100%) to the antibiotic Amoxycillin. While isolates of the bacterial species *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Proteus vulgaris* showed resistance (100%) to the antibiotic azithromycin. *Klebsiella pneumoniae* and *E.coli* isolates showed resistance to the antibiotic Moxifloxacin (90%), while *Staphylococcus aureus* isolates showed resistance to the antibiotic ciprofloxacin (90%). The *Bla_{TEM}* gene for seven isolates *E.coli* causing conjunctivitis infection were successfully amplified. While *Bla_{CTX-M}* gene for all *proteus vulgaris* isolates. As well as *Bla_{SIM}* gene for isolates *P. aeruginosa* and found one isolate only carry *Bla_{SIM}* gene. showed five isolates *staph. aureus* encode *PVL* gene

Keywords: *Escherichia coli*, *Staphylococcus aureus*, Pigeons, Conjunctivitis

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INTRODUCTION

Acute eye infections include lidinfection (Conjunctivitis) and dacryocystitis Ramesh et al. (2010) Bacterial adhesion and invasion of membranes and epithelial cells occur through their possession of factors that help them to adhere to and penetrate the host Okesola and Salako (2010). The most bacterial causes the show of studies have that of eye inflammation with *Staphylococcus aureus* bacteria, *Streptococcus pneumoniae*, *gonorrhoeae*, *Corynebacterium diphtheriae*, *Moraxella lacunata*, *Haemophilus influenzae* Jaloob and Jaloob (2006) Another study showed that the causes of

sickness, eyelid and conjunctivitis are by *S.aureus* *S. Pneumoniae* Sambrook and Russell (2001). *Staphylococcus* bacteria is one of the most important and common causes of eye diseases, especially the intraocular and consists of S-walls. Aureusen is a group of factors that play a role in virulence and contribute to stimulating immunity, including Lipoteichoicacidas and Peptidoglycan. The eye possesses several immunological-biochemical-physical defenses against the angioplasty: Eyelashes eyelids that operate a dirt filter and pollution molecules to prevent their entry into the ocular external and that the procedure of close and opening the involuntary eye arises when foreign objects stick to the cil-

ia, detached tears of the eye act as a means of protection for washing the eye and its parts of bacteria and residual residues, Eyelids and conjoints are made up of a tissue with many epithelial cells. The skin flakes satisfactorily. This prevents invasion by the mediation of the carpet and leads to the separation and isolation of the carpet attached to the outer surface layers Silverman (2023). Conjunctivitis leads to itching and a feeling of a foreign body in the eye, as well as tears and discharge. Conjunctivitis is either bacterial, viral, or caused by an allergy. Bacterial always occurs in one eye, unlike viral inflammation, which often begins in one eye and spreads to the last eye. Fellow et al. (2000) Conjunctivitis is also identified as red eye pink eye. Bacterial conjunctivitis is distinguished from other conjunctivitis by the incidence of the yellow mucous secretions, which often pour on the cheek, rude and complemented by inflammation of minor bloating with a smooth superficial in its center Singh et al. (2023). With iridescent redness There are two types of conjunctivitis: acute and chronic, which are caused by bacteria. The acute type is characterized by the appearance of veins, purulent secretions, and the adhesion of the mind and blindness to each other. The chronic type is less virulent and can affect the eyelids for a longer period. There is an inflammatory response in the tear organization or it might be a minor infection. The infectious bacteria endemic the conjunctiva mucous membrane region and thus become protected from the lysozyme and antibodies in the tear Mohammed et al. (2020). Treatments for bacterial infections use Sulfonamide, Erythromycin, Tetracycline or Cefotaxime and Vancomycin compounds to treat positive bacteria, adverse to a generous dye. These compounds are often used for topical treatment, which is in the form of drops or ointment. Gentamicin and Ciprofloxacin are the most effective anti-counterfeit *Pseudomonas aeruginosa* and *Enterobacteriaceae* and *Staphylococcus aureus* Norofloxacin and, Ampicillin is also used to treat corneal inflammation and ulceration but less effectively towards *Streptococcus* and *P. aeruginosa* bacteria M Al-Rawi (2005). Interest in antibiotics has increased since their first use to the present because of their great importance in the treatment of various simple and complex bacterial injuries by either killing bacteriocidal bacteria and inhibiting their growth. Bacteriostatic antibiotic work is concentrated on bacteria in two main aspects: impairment of structural integration or impairment of functional metabolism AL-Sa'ady et al. (2019). In Europe, private pharmacies have emerged to prescribe medicinal herbs as a treatment to avoid side symptoms of chemical drugs Mekonnen et al. (2022)

The research aims to study the most communal bacteria in causing infection in the bath's eyes, recognize the most effective antibiotics in the management of injury and isolates bacteria and compare them with antibiotics and carry its β -lactam genes.

SAMPLING COLLECTION

Collected 89 samples in different containers of pigeons' eyes with conjunctivitis and Blepharitis from different private veterinary clinics in Basra governorate, as shown in the Figure 1.

ISOLATION AND IDENTIFICATION

Swabs of pigeon eye seal were collected by a single-use sterile tanker swab (Disposable serial eye swabs). Samples were placed at Steuart's Transport Media when transported to the laboratory and used MacConkey agar and Blood agar. Swabs taken from the pigeons' eyes were cultured by a streak method and incubator with a 37°C. For 24 hours, the isolates diagnosed the bacterium depending on the appearance qualities of the developing colonies, pigmentation In Gram stain, direct microscopy and partial circles as well as biochemical examinations included catalysts, oxidase fermentation of manitol and blood degradation depending on Atiq et al. (2022) diagnosis of different bacterial species was confirmed using the Enterosystem 18 R system and using a device. Vitek-2 compact system

VITEK 2 COMPACT SYSTEM

Use this device to confirm the knowledge of isolates, as it is used to diagnose most types Bacteria through the device's own diagnostic kit, the number of diagnoses contains 47 holes inseminated with a 24-hour-old bacterial depth. It incubates for 24 hours and records the monumental changes resulting from the growth of the bacteria.

SENSITIVITY TO ANTIBIOTICS

Adopting the Kirby-Bauer et al., 1966 method, improved with Lyngdoh, et al. (2020). And using the next antibiotics equipped by Oxoid, Moxifloxacin, Amoxycillin, ciprofloxacin, Cephalexin, Erythromycin, Ampicillin, Doxycycline azithromycin, Polymyxin, Trimethoprim. The medium of Muller-Hinton has been inoculated with suspension of cells using a sterile cotton, the dishes left 5 minutes to dry after which antibiotic tablets were stabilized using a sterile tongs and incubated 37°C for 48.24 hours.

MOLECULAR ANALYSIS

Molecular analysis for BlaTEM, BlaCTX-M, BlaSIM, and VLP gene production was performed according to the instructions of the Genomic DNA Mini-Kit Geneaid, and then DNA was extracted. The BlaTEM gene of *E. coli*, the BlaCTX-M gene of *Proteus vulgaris*, the BlaSIM gene of *Pseudomonas aeruginosa*, and the VLP gene of *Staphylococcus aureus*. The final volume of the PCR reaction was 20 μ l, which included 12.5 μ l of the mixture, and 5 μ l of the template for the isolate, and 0.5 μ l of primers. In order to

Table 1: The genes and specific primer sequences

Target gene	Nucleotides sequences (5-3)	Base pair	Reference
Bla _{TEM}	F: CATTTCCGTGTCGCCCTTATTC R: CGTTCATCCATAGTTGCCTGAC	800	Patel et al., (2022)
Bla _{CTX-M}	F: CGCTTTGCGATGTGCAC R: ACCGCGATATCGTTGGT	550	Khalid Ibrahim (2023)
BlaSIM	F: ACATTATCCGCTGGAACAGG R: TACAAGGGATTTCGGCATCG	570	Alkhudhairy et al.,(2020)
PVL	F:ATCATTAGGTAAAATGTCTGCACATGATCCA R:GCATCAACTGTATTGGATAGCCAAAAGC	433	Al-Shwani et al.,(2021)

Table 2: Shows the percentage of isolation of bacteria isolated from Conjunctivitis and Blepharitis.

Type Bacteria	No. Type of isolation	Conjunctivitis	Blepharitis
<i>Staphylococcus aureus</i>	23 ((29.11%	16 (69.5)	7((30.4%
<i>Streptococcus epidermidis</i>	20((25.31%	8 (40%)	12((60%
<i>Escherichia coli</i>	12(15.18%)	7 ((58.3%	5((41.6%
<i>Pseudomonas aeruginosa</i>	9(7.90%)	5((55.5%	4(44.4%)
<i>Streptococcus pneumonia</i>	5((6.32%	4(%80)	1((20%
<i>Klebsiella pneumoniae</i>	4((5.06%	1((25%	3((75%
<i>Moraxilla catarrhalis</i>	4((5.06%	4((100%	0
<i>Proteus vulgaris</i>	2((2.53%	2((100%	0
Total number	79	47(59.49%)	32((40.50%
X ² : 4.93 P<.05			

measure the reaction conditions, we used a thermal cycler (A&B Singapore). Specific primer sequences were used in PCR experiments as in (Table 1). The reaction volume for BlaTEM was 20 µl. Khalid Ibrahim (2023). While BlaSIM and VPL genes as in Alkhudhairy et al. (2020).

RESULTS

The study found 89 samples cultured were ten samples with no growth, as shown in the Figure 1, including 79 gram-negative bacterial isolates and gram-positive bacterial isolates, as shown in the Figure 1

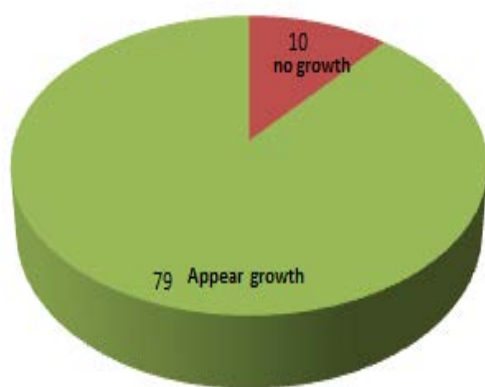


Figure 1: The number of negative growth and positive samples for bacterial culture.

The results of bacterial culture on different culture media showed a difference in the growing isolates, which was confirmed by various diagnostic tests, including Gram stain, oxidase, catalase, and a test using the Enterosystem 18 R. As shown in the Figure 2.



Figure 2: Diagnosis of different bacterial species was using the Enterosystem 18 R system

Depending on the insulation source used Vitek-2 compact, system. The results showed a difference in the percentages of microbial species within one group. In the example of conjunctiva infection, the maximum percentage of

isolation was for the bacteria *Staphylococcus aureus*, which was (34.04%), while in the case of Blepharitis infection, the highest percentage was for the bacteria *S.epidermidis*, which was (37.5%). The result is not significant at $P<.05$, as shown in Table (1).

The prevalence of antibiotics in treating many pathogens and their widespread and indiscriminate use has directed toward the emergence of many bacterial strains resistant to them and the reduction of sensitive strains.

Results of an antibiotic resistance test showed that the isolates showed a “significant” change in resistance to antibiotics, as the isolates showed the bacterial species *Staphulococcus aureu*, *Pseudomonas aeruginosa*, *Streptococcus pneumonia*, *Moraxilla catarrhalis*, and *Klebsiella pneumoni-ae* resistance (100%) to the antibiotic Amoxycillin. While isolates of the bacterial species *Staphulococcus aureu*, *Escher-ichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Proteus vulgaris* showed resistance (100%) to the anti-biotic azithromycin. *Klebsiellae pneumonia* and *E.coli* isolates showed resistance to the antibiotic Moxifloxacin (90%), while *Staphylococcus aureus* isolates showed resistance to the antibiotic ciprofloxacin (90%) Figure 3.

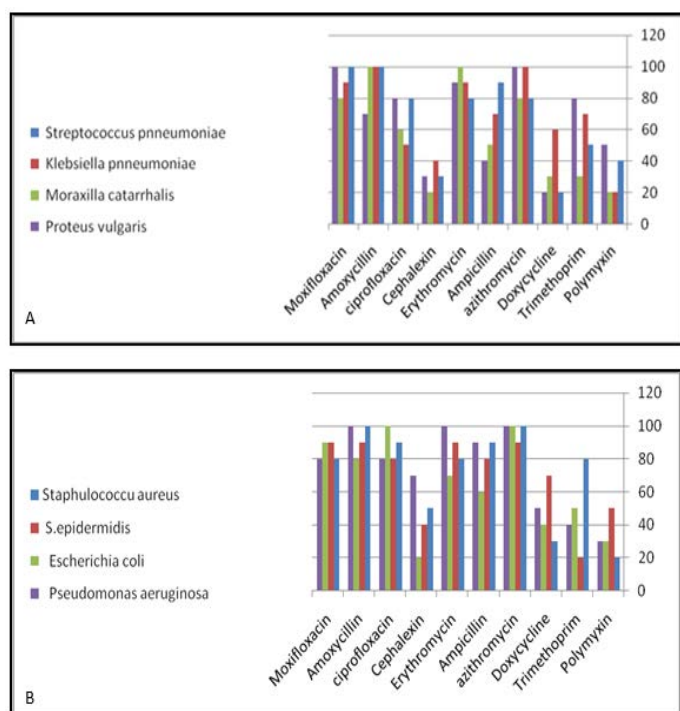


Figure 3: (A and B) shows the percentage of bacterial resistance to antibiotics

MOLECULAR ANALYSIS

Out of 79 isolates, bacterial isolates were selected for PCR amplification, and *Bla_{TEM}* gene for seven isolates *E. coli* causing conjunctivitis infection were successfully amplified as shown in Figure 4. While *Bla_{CTX-M}* gene for all *proteus vulgaris* isolates as Figure 5. As well as *Bla_{SIM}* gene for 9

isolates of *P. aeruginosa* and found one only carry *Bla_{SIM}* gene Figure 6. in this study showed five isolates *staph. au-reus* encode *PVL* gene as Figure 7.

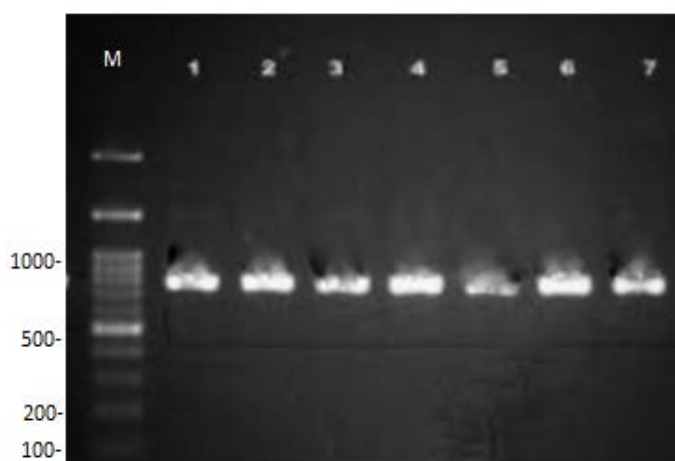


Figure 4: PCR product for Gel electrophoresis of *Bla_{TEM}* gene 800 bp. Lane (M : ladder 2000 bp.), Lanes (1-7) show results of *Bla_{TEM}* gene

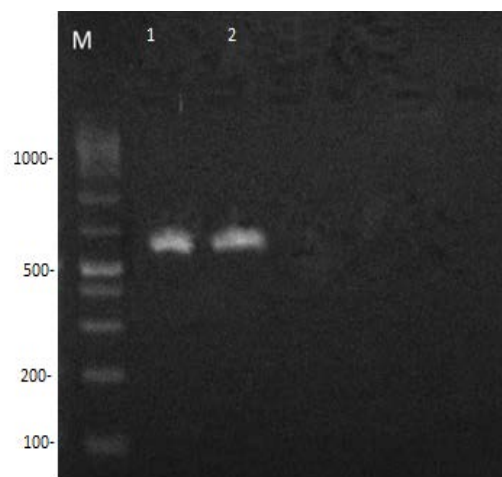


Figure 5: PCR product for Gel electrophoresis of *Bla_{CTX-M}* gene 550 bp. Lane (M : ladder 1500 bp.), Lanes (1-2) show results of *Bla_{CTX-M}* gene



Figure 6: PCR product for Gel electrophoresis of *Bla_{SIM}* gene 570 bp. Lane (M : ladder 1500 bp.), Lanes (6) show positive results of *Bla_{SIM}* gene

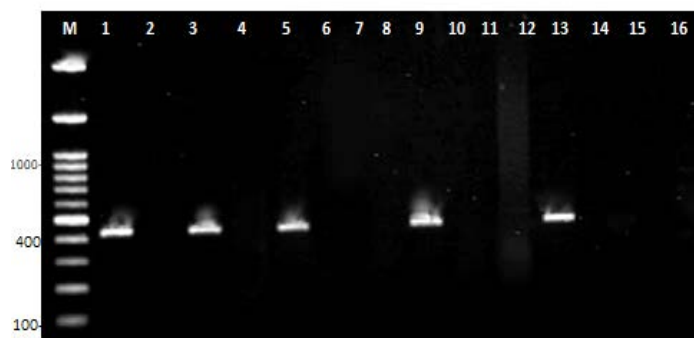


Figure 7: PCR product for Gel electrophoresis of *VPL* gene 433 bp. Lane (M : ladder 1500 bp.), Lanes (1,3,5,9,13) show results of *VPL* gene.

DISCUSSION

Many microorganisms can reach the cornea and cause inflammation, usually after a defect in the integrity of the outer epithelial layer of the cornea and as a result of its exposure to external stress, the entry of foreign bodies, or some eye diseases, making it a suitable environment for the growth of microorganisms, thus increasing the possibility of them being infected. Therefore, the types of these infections are numerous. The results showed that the percentage of conjunctiva contamination was (59.49%). The appearance of this high percentage means that the disease is contagious and easily transmitted.

Infection occurs through direct contact of the contaminated hand with the eye, polluted air also causes infection, or from the spread of the pathogen with some respiratory secretions and thus the disease occurs. Also, sometimes some types of bacteria from the nasopharynx may colonize the conjunctiva as a outcome of blockage of the tear duct Hussain et al. (2022). Table (1) shows the percentages of microorganisms isolated from conjunctivitis. It is noted that the highest percentage of infection was for *Staphylococcus aureus* bacteria, the lowest percentage was for *Proteus vulgaris* bacteria, and the highest percentage of microorganisms isolated from keratitis was *S. epidermidis* bacteria. In our study the percentage of their isolation was low, and the reason for this may be due to the fact that streptococcal infections may heal on their own by forming antibodies. Early in the infection, in addition to the fact that streptococci bacteria are sensitive to many antibiotics Furuta et al. (2022).

The increase in the number and diversity of microorganisms, as new genera appear, namely *Klebsiella*, *E. coli*, and *Pseudomonas*, from the group of Gram-negative bacteria. *Pseudomonas* bacteria constitute the most dangerous species present that causes corneal ulcers. This is due to the

frequent use of antibiotics, which is likely to improve bacterial resistance to antibiotics and lead to chronic infections, in addition to the communication of microorganisms that are already resistant to antibiotics from one animal to another. Also, Poulos et al. (2002) *S. aureus* bacteria were considered the most common cause of conjunctivitis, with a percentage of (34%) Cooper et al. (2000). *Staphylococcus aureus* isolates showed resistance to the antibiotic ciprofloxacin. this results agreement with the recent study performed by Abdulridha et al. (2023) Effect of various drugs against the bacteria *S. aureus*. Seven of the *E. coli* isolates studied cause and cause conjunctivitis. It had multiple resistance to antibiotics. *Escherichia coli* has been identified as an ESBL producer encoding a beta-lactamase for the *Bla_{TEM}* gene. this result that similar with study reported by Tsaku et al. (2019) but differ than with study by Pishtiwan and Khadija (2019). The prevalence the produce ESBL-*Proteus spp.* Is increasing worldwide, as well as in the United States, Asia, and Europe. as well as all *proteus vulgaris* isolates in this study was producer gene *Bla_{CTX-M}* this result that not similar with study by Malaki et al. (2022). *Pseudomonas aeruginosa* showed this study found one isolate carries *Bla_{SIM}* gene and result near from reported by Fekri Kohan et al. (2020) in Iran and differ from result (76.1 %) reported by Bunyan et al. (2018). This study showed PVL gene for *staph. Aureus* of present in five isolates and this result agreement with study by Kulangara et al. (2017) and near from study reported by Abdulridha et al. (2023).

CONFLICT OF INTEREST

There is no conflict of interest.

AUTHORS CONTRIBUTION

All authors contributed equally.

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