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Antibiotic susceptibility and efflux pump activity in uncommon bacterial species associated with conjunctivitis

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ABSTRACT

This study investigates the antibiotic susceptibility and phenotypic characteristics of uncommon bacterial species associated with conjunctivitis. A total of 150 conjunctival swab samples were collected from patients presenting with symptoms of conjunctivitis at the Al-Nasiriyah Teaching Hospital and the Al-Haboubi General Hospital (Ophthalmology Consultant Unit; Dhi Qar Governorate, Iraq) between August and December 2024. Bacterial isolates were identified based on cultural and microscopic characteristics, supplemented by the Vitek® 2 Compact System (bioMérieux, France). Gram-positive bacteria predominated, with *Staphylococcus aureus* accounting for 40% of isolates. Several uncommon species were also identified, including *Kocuria kristinae*, *Staphylococcus hominis* subsp. *hominis*, *Escherichia coli*, *Brucella melitensis*, *Gemella bergeri*, *Staphylococcus pseudintermedius*, and *Leuconostoc citreum*. Antibiotic resistance profiles were determined using the Kirby–Bauer disk diffusion method, revealing notable resistance to commonly prescribed agents such as erythromycin and ciprofloxacin. Efflux pump activity (a key mechanism contributing to multidrug resistance) was detected in 37.5% of isolates using the ethidium bromide cartwheel method. These findings highlight the importance of robust antibiotic stewardship and underscore the need for continuous surveillance of bacterial resistance patterns in order to optimize therapeutic strategies for conjunctivitis.

KEY WORDS:
conjunctivitis; antibiotic susceptibility; efflux pumps; uncommon bacteria; common bacteria

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1. Introduction

Conjunctivitis, commonly referred to as "pink eye", is an ocular infection characterized by inflammation of the conjunctiva (a thin mucous

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membrane that may appear pink or crimson), typically caused by bacterial, viral, or allergic agents[1,2]. The most frequently implicated bacterial species include *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Chlamydia* spp., *Moraxella* spp., *Haemophilus influenzae*, and *Neisseria gonorrhoeae*. In addition to these, several uncommon bacterial species have been associated with conjunctivitis, such as *Kocuria kristinae*, *Staphylococcus hominis* subsp. *hominis*, *Escherichia coli*, *Brucella melitensis*, *Gemella bergeri*, *Staphylococcus pseudintermedius*, and *Leuconostoc citreum*[2].

These uncommon bacteria are increasingly recognized for their capacity to develop resistance to multiple antibiotics, largely attributed to the indiscriminate use of antimicrobial agents in hospital settings, which complicates treatment protocols[3]. Efflux pumps (protein transporters embedded in bacterial cell membranes) play a pivotal role in expelling toxic compounds, including antibiotics, thereby contributing to antimicrobial resistance[4]. Accordingly, the present study aimed at isolating and identifying uncommon bacterial pathogens associated with conjunctivitis, assessing their antibiotic susceptibility profiles, and detecting efflux pump activity among the isolates.

2. Methodology

A total of 150 conjunctival swab samples were collected from paediatric patients (aged birth to 7 years) diagnosed with conjunctivitis in the Dhi Qar Governorate (Nasiriyah), Iraq, between August and December 2024. Sampling was conducted at the Ophthalmology Consultant Unit of the Nasiriyah Teaching Hospital and the Al-Haboubi General Hospital. Following clinical examination by a specialist, purulent material from the conjunctiva was collected by using sterile swabs and inoculated onto three types of culture media: blood agar, chocolate agar, and MacConkey agar. The inoculated plates were incubated at 37°C for 24 h.

Phenotypic identification of bacterial isolates was performed by using the Vitek® 2 Compact System (bioMérieux, France), which integrates cultural and microscopic characteristics with biochemical profiling. The system demonstrates high diagnostic accuracy and specificity (95%–99%). Antimicrobial susceptibility testing was conducted in accordance with established protocols[5]. Detection of active efflux pump activity was carried out using the ethidium bromide cartwheel method[6].

Institutional review board approval was obtained from the College of Medicine of the University of Kufa, Iraq (approval number: 5628/2024), in compliance with the Declaration of Helsinki and international guidelines for human research protection. Informed consent was secured from adult participants or the legal guardians of paediatric patients. Data analysis was performed using the SPSS software, version 24.

3. Results and Discussion

Of the 150 specimens analysed, 85 (56.67%) exhibited positive bacterial growth on culture media after 24 h of incubation at 37°C, while 65 (43.33%) showed no growth even after 48 h. Among the 85 culture-positive specimens, 74 (87.06%) were identified as Gram-positive and 11 (12.94%) as Gram-negative.

The most frequently isolated species was *Staphylococcus aureus* (34 isolates; 40%), followed by *Staphylococcus hominis* (7; 8.24%), *Staphylococcus pseudintermedius* (5; 5.88%), *Staphylococcus epidermidis* (4; 4.71%), *Kocuria kristinae* (2; 2.35%), *Gemella bergeri* (1; 1.18%), *Leuconostoc citreum* (1; 1.18%), *Escherichia coli* (9; 10.59%), and *Brucella melitensis* (2; 2.35%). Additionally, 20 isolates (23.52%) were classified as "unidentified" organisms.

Antibiotic resistance profiles varied across species. *S. aureus* exhibited high resistance to erythromycin and doxycycline, with moderate resistance to vancomycin and the highest recorded resistance to ciprofloxacin (maximum: 36). *S. hominis* showed notable resistance to erythromycin and tetracycline, though overall resistance was lower than that of *S. aureus*. *S. pseudintermedius* demonstrated extensive resistance, particularly to vancomycin and ciprofloxacin (maximum: 46). *S. epidermidis* displayed relatively lower resistance, with erythromycin and doxycycline being the most affected. *K. kristinae* showed generally low resistance, although some isolates were significantly resistant to ciprofloxacin. *G. bergeri* and *L. citreum* had limited data points, but appeared to exhibit low resistance across tested antibiotics. *E. coli* presented variable resistance, especially to ciprofloxacin and amoxicillin–clavulanate, with

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Table 1. Results of phenotypic detection of efflux pump activity in uncommon bacterial isolates associated with conjunctivitis, using varying concentrations of ethidium bromide (EtBr) dye in tryptic soy agar.

EtBr (mg/L) and degree of fluorescence produced				
Isolate (and its number)	**1 mg/L**	**2 mg/L**	**3 mg/L**	**Efflux activity**
Staphylococcus aureus (1)	-	-	-	Negative
Staphylococcus aureus (4)	-	-	-	Negative
Kocuria kristinae	+	+	+	Positive
Staphylococcus hominis	-	-	-	Negative
Escherichia coli	+	+	+	Positive
Brucella melitensis	-	-	-	Negative
Gemella bergeri	-	-	-	Negative
Staphylococcus epidermidis (10)	-	-	-	Negative
Staphylococcus aureus (13)	-	-	-	Negative
Staphylococcus aureus (14)	-	-	-	Negative
Staphylococcus epidermidis (15)	-	-	-	Negative
Staphylococcus pseudintermedius (16)	-	+	+	Intermediate
Staphylococcus hominis (17)	+	+	+	Positive
Leuconostoc citreum (19)	+	+	+	Positive
Staphylococcus pseudintermedius (20)	+	+	+	Positive
Staphylococcus pseudintermedius (22)	+	+	+	Positive

some isolates fully resistant to ampicillin. Finally, *B. melitensis* showed inconsistent resistance, with some isolates highly resistant to imipenem and cefotaxime. Overall, erythromycin, ciprofloxacin, and doxycycline were associated with higher resistance rates across multiple species, whereas vancomycin and imipenem retained relatively lower resistance levels.

The efflux pump activity was assessed in 16 isolates of uncommon bacterial species using the ethidium bromide–agar cartwheel method. Six isolates (37.5%) tested positive for efflux pump activity, as determined by the lowest concentration at which fluorescence was absent under ultraviolet light exposure (Table 1).

The findings suggest that bacterial pathogens are the predominant cause of conjunctivitis, potentially exacerbated by prior exposure to antibiotics and corticosteroids. A comparative study conducted in the Babil Governorate, Iraq, has reported that 52.5% of the conjunctival specimens were culture-positive, while 47.5% were culture-negative[7].

Resistance trends were particularly concerning for *S. pseudintermedius*, which demonstrated the highest resistance levels, especially to ciprofloxacin and vancomycin, posing a significant clinical challenge. In contrast, *S. epidermidis* and *K. kristinae* exhibited lower resistance rates, particularly to vancomycin and ciprofloxacin, suggesting a more favourable susceptibility profile[8]. *S. aureus* remains a global concern due to its high resistance to erythromycin and doxycycline. Conversely, *E. coli* and *B. melitensis* exhibited relatively low resistance to critical antibiotics such as imipenem, thereby indicating that standard treatment protocols may remain effective.

The observed resistance to ciprofloxacin and amoxicillin underscores a pressing public health issue, necessitating revised treatment guidelines and alternative therapeutic options for resistant pathogens such as *S. pseudintermedius* and *S. aureus*[9]. These data reinforce the importance of surveillance programs and antimicrobial stewardship to track resistance patterns and inform clinical decision-making. Further research is warranted to elucidate the genetic and environmental factors driving resistance[10].

4. Conclusion

This study confirms that bacterial pathogens

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– particularly Gram-positive species – are the primary aetiological agents of conjunctivitis. Notably, several Gram-negative isolates exhibited efflux pump activity, which contributes to antibiotic resistance and complicates treatment outcomes.

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Conflicts of interest

None exist.

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References

1. Mahoney M.J., Bekibele R., Notermann S.L., Reuter T.G., Borman-Shoap E.C. Pediatric conjunctivitis: a review of clinical manifestations, diagnosis, and management. *Children (Basel)* 10(5), 808, 2023. DOI: 10.3390/children10050808
2. Al-Buhamrah N., Hussain J., Saadon Q. Detection of aac(3)-I gene in *Chlamydia trachomatis* isolated from conjunctivitis by using PCR technique in Al-Najaf province, Iraq. *Microb. Biosyst.* 9(1), 27–32, 2024. DOI: 10.21608/mb.2024.283952.1097
3. Saba C.K.S., Amenyona J.K., Kpordze S.W. Prevalence and pattern of antibiotic resistance of *Staphylococcus aureus* isolated from door handles and other points of contact in public hospitals in Ghana. *Antimicrob. Resist. Infect. Control* 6, 44, 2017. DOI: 10.1186/s13756-017-0203-2
4. Blair J.M., Webber M.A., Baylay A.J., Ogbolu D.O., Piddock L.J. Molecular mechanisms of antibiotic resistance. *Nat. Rev. Microbiol.* 13(1), 42–51, 2015. DOI: 10.1038/nrmicro3380
5. Shaeri M., Nazari-Alam A., Fathizadeh H., Moniri R., Akbari H., Mansoori M., *et al.* Bacterial etiology and antibiotic susceptibility of conjunctivitis patients' isolates in Kashan, Iran. *Adv. Biomed. Res.* 9, 49, 2020. DOI: 10.4103/abr.abr_118_20
6. Baiomy A.A., Shaker G.H., Abbas H.A. Sensitizing multi drug resistant *Staphylococcus aureus* isolated from surgical site infections to antimicrobials by efflux pump inhibitors. *Afr. Health Sci.* 20(4), 1632–1645, 2020. DOI: 10.4314/ahs.v20i4.16
7. Kareem Rhumaid A., Alak Mahdi Al-Buhilal J., Al-Rubaey N.K.F., Yassen Al-Zamily K. Prevalence and antibiotic susceptibility of pathogenic bacteria associated with ocular infections in adult patients. *Arch. Razi Inst.* 77(5), 1917–1924, 2022. DOI: 10.22092/ARI.2022.359510.2437
8. Szemraj M., Glajzner P., Sienkiewicz M. Decreased susceptibility to vancomycin and other mechanisms of resistance to antibiotics in *Staphylococcus epidermidis* as a therapeutic problem in hospital treatment. *Sci. Rep.* 13(1), 13629, 2023. DOI: 10.1038/s41598-023-40866-3
9. An N.V., Hai L.H.L., Luong V.H., Vinh N.T.H., Hoa P.Q., Hung L.V., *et al.* Antimicrobial resistance patterns of *Staphylococcus aureus* isolated at a general hospital in Vietnam between 2014 and 2021. *Infect. Drug Resist.* 17, 259–273, 2024. DOI: 10.2147/IDR.S437920
10. Munita J.M., Arias C.A. Mechanisms of antibiotic resistance. *Microbiol. Spectr.* 4(2), 10.1128/microbiolspec.VMBF-0016-2015, 2016. DOI: 10.1128/microbiolspec.VMBF-0016-2015

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