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Assessing *Pseudomonas aeruginosa* in ocular infections: isolation and genetic detection of *ampC* and *oprM* genes

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Abstract

Ocular infection is increasing and presenting a considerable public health concern. *Pseudomonas aeruginosa* is one of the most common bacterial types isolated from eye infections, especially in contact lens users. This study aimed to identify *Pseudomonas aeruginosa* from ocular infections and molecular detection of ampC and oprM genes, and to demonstrate its relation to contact lenses. 100 patients suffering from eye infections were enrolled in this study from January 2024 to May 2024. Their age was between 10 and 60 years, and they were of both genders. Cotton swabs and corneal scrapes were collected from patients aseptically and cultured on blood agar and MacConkey agar. The Vitek2 system was applied for the diagnosis of bacteria, and the PCR protocol was used for the detection of ampC and oprM genes. The results showed that ocular infection related to *Pseudomonas aeruginosa* is significantly higher in females compared to males (P: 0.01). Also, keratitis cases were considerably higher than conjunctivitis. Moreover, patients using contact lenses are at higher risk of developing conjunctivitis than keratitis. The presence of ampC and oprM genes is significantly associated with keratitis. **Conclusion:** Findings of the study indicate that conjunctivitis is more common than keratitis among *P. aeruginosa* infections in the eyes and that females are more likely than males to get these infections. There is a significant association between contact lens use and conjunctivitis, and between the ampC and oprM genes and keratitis. Also, there is a gender-based distribution of ampC and oprM genes detected in *Pseudomonas aeruginosa* isolated from ocular infections.

Keywords: *P. aeruginosa*. Ocular infection, *ampC,* and *oprM* genes, keratitis, conjunctivitis.

Introduction:

The global incidence of ocular infection is increasing and poses a significant public health concern. This type of infection can arise due to exposure to various pathogens, including viruses, bacteria, and fungi. Without proper therapy, ocular infection can significantly damage the eye, leading to vision impairment or blindness (1). The most common ocular infections are keratitis, conjunctivitis, endophthalmitis, and dacryocystitis. Keratitis emerges as corneal inflammation resulting from viral, bacterial, or fungal pathogens and is characterized by eye pain, light sensitivity, blurred vision, and corneal ulcer (2). On the other hand,

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conjunctivitis is the inflammation of the conjunctiva due to pathogen exposure or allergic reaction and manifests as redness, discharge, and eye irritation (3). Early detection through clinical evaluation and laboratory testing by culturing eye swabs and corneal scrapes is crucial to ensure proper antibiotic therapy and to prevent serious complications such as corneal perforation and endophthalmitis (4).

Bacteria are the primary cause of ocular infection globally. Bacterial eye infections can involve single or multiple species that are associated with several predisposing factors, including contact lens wear, surgical procedures, aging, dry eye diseases, persistent nasolacrimal duct blockage, and previous ocular infections (5). *Staphylococci, Streptococci, Pseudomonas aeruginosa, Escherichia coli, and Klebsiella pneumonia* are the most common bacterial species associated with ocular infection (6).

Pseudomonas aeruginosa (*P. aeruginosa*) is a rod-shaped, gram-negative, aerobic pathogen capable of existing in various challenging environments and representing a persistent public health threat. It has been considered the major cause of nosocomial infections, including pneumonia, sepsis, and healthcare-associated urinary tract infections (7). Infectious keratitis and conjunctivitis are frequently caused by this pathogen, especially in contact lens wearers. The pathogen invades the corneal epithelium and damages the corneal barriers, stimulating a strong inflammatory response resulting in corneal ulceration and vision loss (8). *P. aeruginosa* exhibits several virulence factors that contribute to the infection, such as proteases, exotoxin A, and biofilm formation (9). Additionally, the existence of *ampC*, which encodes for a cephalosporin-hydrolyzing enzyme, and *oprM,* which encodes for an outer membrane protein, genes in the bacterial genome enhances the antibiotic resistance of the pathogen and complicates the therapeutic strategy (10). Despite being a dominant vision correction option with more than 140 million users all over the world, contact lenses present a risk factor for keratitis in 2-20 per 100 users yearly (11). Due to its highly virulent nature and rapid clinical deterioration, timely diagnosis and urgent antimicrobial treatment are essential to minimize the vision-threatening damage associated with *P. aeruginosa* (12). Hence, the current study was conducted to isolate and diagnose *P. aeruginosa* from ocular infection and to demonstrate its relationship to contact lenses and ampC and oprM genes.

Materials and methods:

Study population:

One hundred patients with ocular infections who visited IBN Al-Haytham Teaching Eye Hospital, Ghazi-Al-Hariri Hospital, Baghdad Teaching Hospital, and Children Welfare Hospital between January 2024 to May 2024, were the subjects of the current study. They are both male and female and range in age from 10 to 60 years old.

Sample collection:

Cotton swabs were used to collect samples from the external ocular surface, and a surgical blade was used to obtain corneal scrabs to diagnose keratitis. The ophthalmologist scraped using 4% lignocaine, which was then directly inoculated onto the plates. Afterward, eye swabs were taken by transport medium, stored until transported to the laboratory, and incubated at 37°C for 24 hours (13). Each patient had read and signed the consent form and answered the questionnaire provided by the author, which included age, gender, and occupation.

Ethical statement:

The study was conducted under the ethical standards outlined by the Ethics Committee of Mosul Medical Institute. Informed consent was obtained from all participants before their involvement in the study.

Diagnosis of *P. aeruginosa*:

Swabs from each patient were inoculated on blood agar and MacConkey agar. Then, the single, separated colonies suspected of *P. aeruginosa* were

subcultured on cetrimide agar and incubated overnight at 37 °C. *P. aeruginosa* was diagnosed by subjecting single-separated colonies to the catalase oxidase tests, and then the diagnosis was confirmed by Vitek2 (Biomerieux France) system tools using GN diagnostic kits performed by Biomerieux France, 2023 (14).

Molecular detection of *ampC* and *oprM* genes of *P. aeruginosa*:

DNA extraction:

According to the manufacturer's kit, the genomic DNA of a 24-hour-old liquid pure culture of P. aeruginosa was extracted using a kit made by Geneaid Tech Ltd. (New Taipei City, Taiwan) (14).

Primer preparation:

PCR was performed to detect *ampC* and *oprM* genes of *P. aeruginosa* using specific primers manufactured by Macrogen Co., Korea, as presented in Table 1. Nuclease-free water was used to reconstruct lyophilized primers to achieve a stock concentration of 100 pmol/µl as a stock solution. To prepare a working solution of 10 pmol/µl, 10 µl of primer stock solution (which was stored in a freezer at -20 ℃ was mixed with 90 µl of nuclease-free water.

The master mix components (Macrogen Co., Korea) and primers were prepared freshly at a final volume of 20 µl per tube (19.5 µl of master mix and 0.5 µl of primers) and then put in the thermal cycler (Eppendorf, Germany). The PCR program is optimized and then run according to the program presented in Table 2.

Table 1: Primers used in the current study

Primer	Sequence	Productsize	Annealing Temp.	Reference
ampC-F	5'-CGATACCAGATTCCCCTGCC-3'	**282 bp**	**60 ˚C**	**This study**
ampC-R	5'-GTGAAGGTCTTGCTCACCGA-3'			
oprM -F	5'-TCAACCTGCCGATCTTCACC-3'	**209 bp**		
oprM -R	5'-GAGCTGGTAGTACTCGTCGC-3'			

Table 2: PCR program

Steps	Temp. °C	m: s	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	60	00:30	
Extension	72	01:00	
Final extension	72	07:00	1
Hold	10	10:00	

Electrophoresis and DNA Loading:

Electrophoresis was conducted to confirm PCR amplification using agarose gel electrophoresis with a 1X TAE buffer system. The amplified DNA samples were mixed with ethidium bromide (10 mg/ml) and loaded into the gel alongside a DNA ladder (manufactured by Macrogen Co., Korea) (15). A gel containing (10 µl) of PCR products (10) was loaded directly into the wells. An electric field of 100 v/m Ampis was applied for 75 min. causing DNA fragments to migrate to the positive electrode based on their molecular weight. To visualize the DNA bands under UV light, the gel was stained with ethidium bromide (10 mg/ml), and the gel tray was transported to a UV transilluminator at 302 nm (manufactured by Macrogen Co., Korea) (16).

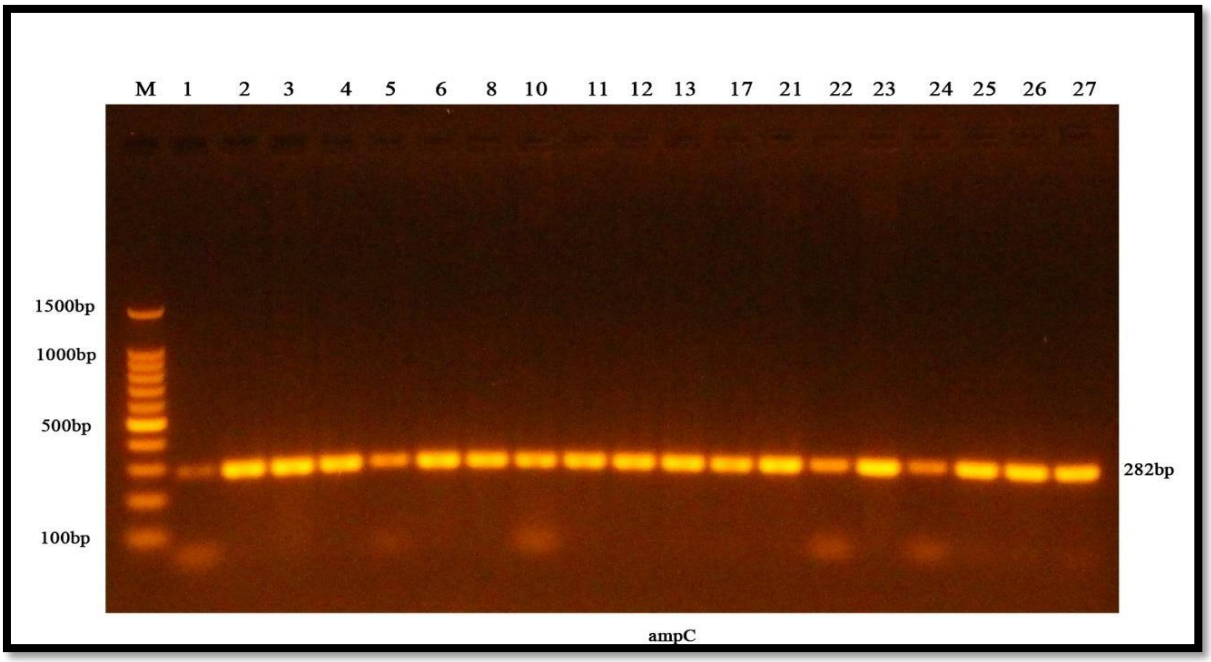

Figure 1: PCR results of DNA bands (282bp) of *ampC* gene after electrophoresis at 100v/mAmp for 75 min.

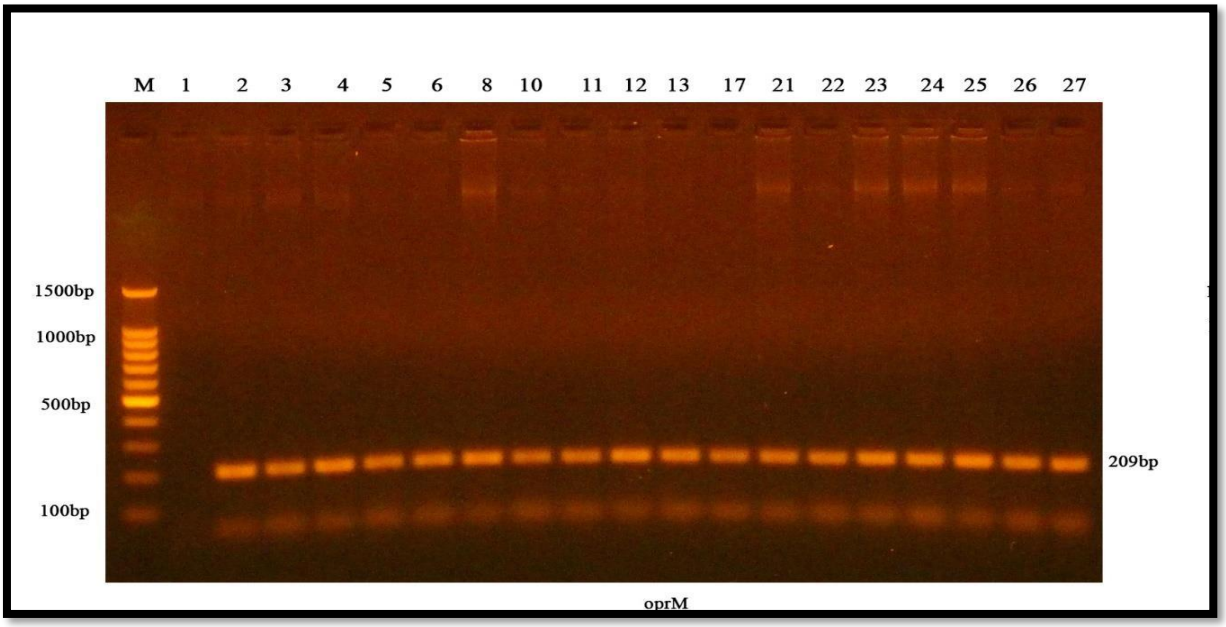

Figure 2: PCR results of DNA bands (282bp) of the *oprM* gene after electrophoresis at 100v/mAmp for 75 min.

Statistical analysis:

The study data were expressed as mean, standard deviation, and percentage. The results of the current study were analyzed using the SPSS software package (Version 24, supplied by IBM Corp., USA). An independent t-test was used to test the significance of the means. Also, a Cross-tabulation test was used to clarify the association between parameters. In cases where the P-value is ≤ 0.05, the result was considered statistically significant.

Results:

The results of Table 3 showed the demographic and clinical data of the patients with ocular infections. Females (60.8%) were more significantly affected than males (39.2%) (P value = 0.01). Also, patients aged 51-60 years (35.3%) were the most affected, but age was not statistically significant. Contact lens use (51%) showed no significant association. Conjunctivitis (68.6%) was more prevalent than keratitis (31.4%), with a significant difference (P value = 0.013).

The data presented in Table 4 indicate a significant relationship between contact lens use and the type of ocular infection. Among individuals who used contact lenses, 84.61% developed conjunctivitis, while only 15.38 % had keratitis. In contrast, among non-contact lens users, 48% developed keratitis, and 52% developed conjunctivitis. The odds ratio (0.2063, 95% CI: 0.1063-0.04005, P<0.001) suggests that contact lens users had a significantly lower likelihood of developing keratitis compared to non-users. These findings indicate that non-contact lens wearers may be at high risk for keratitis due to other predisposing factors, such as environmental or immunological influences.

Table 3: Distribution of patients by sex, age, contact lens use, and type of ocular condition.

Parameter		**No**	**%**	***P* value**
Gender	Male	20	39.20%	0.01*
	Female	31	60.80%	
Age (year)	10-20	6	11.80%	NS
	21-30	14	27.50%	
	31-40	8	15.70%	
	41-50	5	9.80%	
	51-60	18	35.30%	
Contact lenses	Yes	26	51%	NS
	No	25	49%	
Infection	Keratitis	16	31.40%	0.013**
	Conjunctivitis	35	68.60%	

***Results are significant at the 0.05 level. **Results are significant at the 0.01 level. NS: Results are non-significant**

Table 4: Cross-tabulation between ocular infection with contact lenses and the *ampC* and *oprM* genes.

Infection * Contact lenses and genes		Infection		Odd ratio	95 % CI:	*P* value
		Keratitis No. (%)	Conjunctivitis No. (%)			
Contact lenses use	Yes	4 (15.38%)	22 (84.61)	0.2063	0.1063 to 0.4005	<0.001**
	NO	12 (48%)	13 (52%)			
ampC	+	10 (38.46%)	16 (61.54%)	2.0246	1.1001 to 3.7261	0.0234*
	-	6 (24%)	19 (76%)			
oprM	+	12 (40%)	18 (60%)	2.1111	1.1484 to 3.8808	0.0162*
	-	4 (16%)	17 (68%)			
**Results are significant at the 0.01 level. *Results are significant at the 0.05 level.						

The presence of the *ampC* gene was also significantly associated with keratitis. Among *ampC*-positive cases, 38.46% had keratitis, while 61.54% had conjunctivitis. On the other hand, 24% of *ampC*-negative cases had keratitis, and 76% had conjunctivitis. The odds ratio (2.0246, 95% CI: 1.1004 - 3.7261, P= 0.0234) suggests that the individuals infected with the *ampC* gene-containing *P. aeruginosa* are over twice as likely to develop keratitis compared to those infected with the same pathogen but without this gene. This result implies that the *ampC* gene may contribute to the pathogenicity of *P. aeruginosa,* leading to keratitis.

Table 5 presents an analysis of the association between gender and the presence of *ampC* and *oprM* genes in *P. aeruginosa* isolated from ocular infections. The results showed that there is a significant association between the presence of the *ampC* gene and gender (P<0.0001). Among males, 61.53% tested positive for the *ampC* gene, whereas only 38.47% of females carried a *P. aeruginosa* isolate with this gene. Conversely, *ampC*-negative cases were predominantly females (79.18%) compared to males (20.83%). The odds ratio (5.8840, 95% CI: 3.1434-11.0140, P<0.0001) suggests that males were nearly six times more likely to harbor bacteria with the *ampC* gene. This may indicate a gender-specific susceptibility to *ampC-mediated* antibiotic resistance or differences in bacterial colonization patterns.

The presence of the *oprM* gene in *P. aeruginosa* isolated from ocular infection was also significantly linked to differences in gender (P= 0.0003). However, the distribution patterns differed from those of *ampC*. In this case, bacterial isolates isolated from females had a higher prevalence of the *oprM* gene (68.97%) compared to males (31.03%). In contrast, *OprM* negative cases were more frequent among males (57.15%) than females (42.82%). The odds ratio (0.3389, 95% CI: 0.1898-0.6053, P=0.0003) indicates that males were less likely to carry P. aeruginosa isolates with the *oprM* gene compared to females.

Table 5: Cross-tabulation between gender and *ampC* and *oprM* genes of *P. aeruginosa*

Gene * Gender		Male		Female		Odd ratio	95 % CI:	P value
		No	%	No	%			
ampC	**Positive**	16	61.53%	10	38.47%	5.8840	3.1434 to 11.0140	<0.0001**
	Negative	5	20.83%	19	79.175			
oprM	**Positive**	9	31.03%	20	68.97%	0.3389	0.1898 to 0.6053	0.0003**
	Negative	12	57.15%	9	42.85			
****Results are significant at the 0.01 level.**								

Discussion:

The results of this study found that ocular infections (conjunctivitis and keratitis) caused by *P. aeruginosa* were significantly higher among females of any age; however, a significant association was found between the use of contact lenses and only conjunctivitis caused by P. *aeruginosa*, as presented in Table 1.

Studies have shown that bacterial eye infection and its sequelae are considered a significant health issue and are associated with elevated degrees of blindness and visual morbidity globally (16-17). Numerous factors influence the distribution of ocular infections in the population, including wearing contact lenses, surgery, trauma, prior eye infections, nasolacrimal duct obstruction, age, and dry eye (18).

P. aeruginosa was considered the second most prevalent commensal bacterium in healthy conjunctival sacs. These bacteria are free-living and found in many different parts of the environment, such as contact lens solutions and water (19-21). It is pointed out that Pseudomonas keratitis is one of the most common causes of infectious keratitis, especially in developed countries where there is an increased prevalence of contact lens use (22).

P. aeruginosa can easily contaminate the cornea and penetrate or destroy corneal cells, causing corneal damage. It also produces a variety of virulence factors, including pili, flagella, elastase, exotoxin A, a type III secretion system (T3SS), and others (23). When the corneal epithelial barrier is damaged by injury or contact lens wear, *P. aeruginosa* can cause infectious keratitis, leading to blindness (24).

According to age, it has been demonstrated that bacterial eye infections affect people of all ages, but primarily those between the ages of 30 and 55 years (25). The working age group's ocular trauma and contact lenses were identified as the main underlying risk factors for such infection (26).

Moreover, most studies revealed no indication of a gender difference in the prevalence of bacterial eye infections (27). However, whenever gender difference was found, it is usually associated with the underlying risk factors. for instance, it has been shown that trauma-related keratitis is more common in men, but contact lens-associated keratitis is more common in women. (28-32).

Our study's findings indicate that wearing contact lenses is linked to conjunctivitis more than keratitis (Table 1). Contrary to our findings, using contact lenses has been identified as one of the most prevalent risk factors for keratitis, especially in industrialized nations (33-34). On the other hand, because fewer people in developing nations wear

contact lenses, contact lens-related keratitis is far less common there (35).

However, our study demonstrated a significant association between contact lens use and conjunctivitis, which is caused by *P. aeruginosa*, and also revealed a positive association between gene distribution and correlation with genders, with the *ampC* gene deemed a risk factor in the male infection, whereas the *oprM* gene was also associated with females (Table 2, 3). Our agreement with the study indicated that contact lenses with the eye are the major risk factors for conjunctivitis (36). Furthermore, given the fact that a considerable proportion of travelers have decreased visual acuity and many are contact lens wearers (37), Various microorganisms that colonize contact lenses can cause infection and inflammation, but microbial keratitis is the leading cause of eye damage and corneal perforation if not treated properly (38).

P. aeruginosa has employed numerous cells associated with extracellular virulence, such as proteases, which help to invade and kill corneal cells (39). Additionally, the microbial infection was associated with ocular infection, which was linked positively with the antimicrobial ability of tears and also increased the adhesion ability for bacteria to adhere to the lens and cause the disease (40). Furthermore, in ocular microbial infection, similar numbers of men and women had active and inactive cases. Men were more likely to have primary active disease, while women were more prone to recurrent active disease (41). Another study demonstrated differences between men and women in ocular anomalies and disease, emphasizing the importance of understanding sex- and gender-based disparities in eye health. Researchers suggest that including both genders in clinical studies can aid in a comprehensive understanding and treatment (42).

Conclusion

The findings of the study indicate that conjunctivitis is more common than keratitis among *P. aeruginosa* infections in the eyes and that females are more likely than males to get these infections. A significant correlation between conjunctivitis and the AmpC and OprM genes and a relationship between the use of contact lenses and the incidence of conjunctivitis, was observed. It is important to monitor and manage microbial contamination in contact lens wearers to prevent ocular infections, as these data show that bacterial strains expressing these resistance genes may be associated with conjunctivitis in lens wearers. In *P. aeruginosa* strains isolated from infected eyes, the distribution of ampC and oprM genes appears to be influenced by gender.

Conflict of interest: NIL

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