Bacterial Etiology and Antibiotic Susceptibility of Conjunctivitis Patients' Isolates in Kashan, Iran

Abstract

Background: Conjunctivitis is a very common ocular disease, which can be caused by a wide variety of microorganisms. This study was aimed to assess the bacterial etiology and antibiotic susceptibility of conjunctivitis patients' isolates from Central Iran. **Materials and Methods:** This study was performed in 180 patients referred to the Department of Ophthalmology in Kashan University with symptoms of conjunctivitis from July 2017 to December 2017. To detect of different bacteria, Gram staining, morphological characterization, pigment production, biochemical characteristics, coagulase test, optochin and PYR tests, oxidase test, and culture on specific media were used. Antibiotic susceptibility of the bacteria isolated was done using the Kirby–Bauer method. Methicillin resistance in staphylococci isolated from the patients was identified using polymerase chain reaction technique. **Results:** Of the 195 bacteria isolated, about 81.5% were *Staphylococcus epidermidis* and *Staphylococcus aureus* and the remaining 19.5% included other species. In the present study, *Pseudomonas aeruginosa* was most resistant to ampicillin. In the case of *S. epidermidis* and *S. aureus*, the highest resistance was observed against erythromycin and the least resistance was against rifampicin and linezolid. **Conclusion:** In this study, *S. aureus* and *S. epidermidis* are the most common causes of conjunctivitis in all age groups, however, this condition decreases with age and is also influenced by other factors such as season and weather conditions. The results of this study can be helpful in planning more prudent treatment strategies for patients with conjunctivitis in Kashan.

Keywords: *Bacteria, conjunctivitis, drug resistance, Staphylococcus aureus*

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Introduction

Conjunctivitis refers to the inflation or infection of the conjunctiva, which can be caused by a wide range of pathogens. After refractive errors, conjunctivitis is the second most common cause of people going to ophthalmology clinics.[1] Conjunctivitis is also the most common cause of redness of the eye, as the conjunctival tissue (the thin layer that covers the white of the eye) turns red in response to almost any stimuli.[2] Conjunctivitis accounts for 30% of eye complaints, and approximately 15% of people experience some form of this disease in their lifetime.[3] In 78%–80% of infectious conjunctivitis cases, infection is initiated by bacteria.[4] The main features of acute bacterial conjunctivitis are swelling of the eyelids, significant conjunctival hyperemia, a large amount of purulent or purulent secretion, pseudomembrane and typical follicular formation, and subconjunctival hemorrhage.[5] Bacterial

agents play a central role in the development of infectious conjunctivitis, especially in children.[6] The major bacterial causes of conjunctivitis are *Streptococcus pneumoniae* and *Haemophilus influenzae*.[7] Bacterial conjunctivitis epidemics often occur in winter and early spring.[3] If conjunctivitis is caused by viral or bacterial agents, the infection can become contagious. Accurate diagnosis of the type of infection and its etiologic factors and prescription of suitable antibiotics may shorten the duration of the disease as well as transmission time.[7]

Research has shown that the overuse of antibiotics has greatly increased the antibiotic resistance of eye-infecting bacteria and is turning the issue into a serious challenge for the fight against bacterial infections.[8] This highlights the importance of performing regular antibiotic susceptibility tests to monitor the sensitivity of infectious bacteria to different antimicrobial agents. Considering the increased resistance of staphylococci to

methicillin, which leads to increased use of vancomycin, and since there are already several reports of vancomycin resistance, it is necessary to identify methicillin-resistant strains of staphylococci to prevent the overuse of these antibiotics.[9]

Given the multitude of bacterial causes of conjunctivitis and the cost of doing an independent culture test for every conjunctivitis patient, it is necessary to identify the most common causes of these infections in different populations to help physicians devise a general treatment plan for that population accordingly. The increasing antibiotic resistance of many bacterial strains only adds to the importance of monitoring the antibiotic susceptibility of the bacteria involved in conjunctival infections for prescribing effective antibiotic treatments without contributing to the antibiotic resistance problem. This study aimed to identify the bacterial causes of conjunctivitis and their antibiotic resistance patterns and particularly the methicillin-resistant strains of staphylococci (*mecA*) in patients visiting the ophthalmology clinic of Kashan.

Since the leading causes of conjunctivitis vary with the location and there is no accurate information about these causes in the population of Kashan, identification of common bacterial causes of conjunctivitis and antibiotic susceptibility patterns of these bacterial agents can contribute to the more prudent use of antibiotics in this area.

Materials and Methods

A cross-sectional study was performed in the population of patients with ocular infection referred to the ophthalmology clinic of Kashan in 2017. The sample consisted of 180 patients with eye infections who were examined at this clinic from July 2017 to December 2017. The design of this study was approved by the Ethics Committee of Kashan University of Medical Sciences (IR.KAUMS. REC.1396.31). Participants were selected by purposive sampling. The inclusion criteria were informed consent and diagnosis of conjunctivitis, and the exclusion criterion was the withdrawal of consent.

After examination by an ophthalmologist and confirmation of conjunctivitis diagnosis, patients were briefly interviewed and then demographic information and conjunctiva samples were collected. For isolation and differential recognition of bacterial causes of conjunctivitis, the collected swab samples were placed in thioglycollate medium and transferred to the microbiology laboratory of Kashan University of Medical Sciences, where inoculation was performed on blood agar, chocolate agar, and MacConkey agar.[9] The prepared plates were incubated at 37°C for 24–48 h with visual mentoring of colonization. Anaerobic culture was also performed on the same mediums in the candle jars. For differential recognition, all bacterial isolates were evaluated in terms of appearance, the morphology

of colonies, pigment production, Gram staining, and biochemical characteristics. To identify different bacteria, tests such as optochin, coagulase, PYR, oxidase, and culture media such as mannitol salt agar, DNase, and chocolate agar were used.[10]

The susceptibility of the isolated bacteria to common antibiotics was determined by the Kirby–Bauer test. Bacterial colonies were dissolved in sterile physiological saline, and turbidity was adjusted to 0.5–1 with the help of McFarland tubes. Then, a sterile swab was used to inoculate this suspension to a plate with Mueller-Hinton medium, and the discs were inserted. After 24 h of incubation at 37°C, the diameter of the inhibitory area around the discs was measured.[9] The antibiogram was developed for oxacillin, rifampin, ampicillin, amoxicillin, cefepime, ceftazidime, piperacillin, ciprofloxacin, chloramphenicol, doxycycline, vancomycin, amoxicillin/clavulanate, amikacin, tetracycline, linezolid, cotrimoxazole, erythromycin, and gentamicin as instructed in CSLI (2016). After 24 h of incubation, the inhibitory area was compared with the standard chart.[10]

The methicillin resistance (*mecA*) in staphylococci isolated from the patients was identified using the polymerase chain reaction (PCR) technique. Specific primers with the following sequence were used to investigate the presence of *mecA* gene [Table 1].[11] The thermal cycle used in the thermocycler device first made an initial denaturation for 5 min at 95°C and then 30 cycles at 94°C for 15 s, 61°C for 15 s, and 72°C for 30 s. Then, the final extension was performed at 72°C for 5 min. The volume of the PCR reaction mixture was 25 μl for each reaction, including PCR buffer (2.5 μl), MgCl2 (0.8 mM), dNTPs (0.16 mM), primers (16 pmol of each), Taq polymerase (1u/μl), and 2 μl of purified bacterial DNA.

Following the protocols of ethics and confidentiality, all participants were informed about the fundamentals and objectives of the study, confidentiality of their information, anonymity of the questionnaire, and their right to refuse to participate or withdraw from the study. After the data collection phase, data were tabulated in terms of bacterial type and demographic variables and then further analyzed using the Chi-square test, Fisher's exact tests, and one-way ANOVA at the $P = 0.05$ significance level.

Results

This study investigated the bacterial causes of conjunctivitis and their antibiotic resistance pattern

Table 1: The sequences of primers and polymerase chain reaction product size mecA gene

Primer	Primer sequence 5'→ 3'	Product Size (bp)
mecA Forward	ACTGCTATCCACCCTCAAAC	286
mecA Reverse	CTGGTGAAGTTGTAATCTGG	

in the patients examined in the ophthalmology clinic of Kashan in 2017. The mean ± SD of the age of participants was $40.45 ± 24.55$. Of the participants, 91 (50.55%) were male and 89 (49.45%) were female. In this study, 195 bacteria were isolated, which included *Staphylococcus aureus* (28.2%), *Staphylococcus epidermidis* (53.3%), *Pseudomonas aeruginosa* (3%), *S. pneumoniae* (2.5%), *Streptococcus viridans* (2%), *Streptococcus pyogenes* (0.5%), *Micrococcus* spp. (3%), *Klebsiella pneumonia* (2%), *Enterobacter* spp. (0.5%), *Escherichia coli* (0.5%), *Salmonella* spp. (0.5%), *Proteus* spp. (1%), *Bacillus* spp. (1%), and *Diphtheroides* (1%).

According to the findings, no significant relationship was found between gender and infectious agents [Table 2]. Furthermore, no significant relationship was found between age and living place groups with infectious agents [Tables 3 and 4]. The frequency distribution of bacterial infections in the patients in terms of observed symptoms is provided in Figure 1. According to the observations, no significant relationship was observed between symptoms and infectious agents.

The study also investigated the antibiotic resistance of organisms isolated from conjunctivitis patients and their antibiotic resistance patterns. The results of this investigation are presented in Figure 2. In the present study, *P. aeruginosa* was most resistant to ampicillin. In the case of *S. epidermis* and *S. aureus*, the highest resistance was observed against erythromycin and the least resistance was against rifampicin and linezolid. According to the results, piperacillin, meropenem, cefepime, and amoxicillin are the top choices for treating Gram-negative *Bacillus* (*P. aeruginosa*) infections. However, for the infections caused by *S. aureus* and *S. epidermis*, the preferred antibiotic would be rifampicin and linezolid, respectively.

Following PCR to determine *mecA* gene in 11 cefoxitin-resistant staphylococcal samples, it was found that despite resistance to disc cefoxitin, none of the resistant strains had *mecA* gene [Figure 3].

Discussion

Considering the wide variety of microorganisms that can be involved in the development of conjunctivitis,[1] studying the bacterial causes of this disease may help us better understand its epidemiology and devise appropriate therapeutic strategies accordingly. Many bacterial and viral agents can contribute to the development of ocular infections,[6,12,13] and such bacterial infections are especially common in children.[14,15] *S. pneumoniae*, *S. aureus*, and *P. aeruginosa* are important worldwide pathogens, causing a variety of systemic diseases such as pneumonia and bacteremia. These bacteria are also common causes of ocular infections including keratitis and conjunctivitis.[16] Among the bacterial strains isolated from the patients of this

Table 2: Frequency of bacterial infections in patients based on gender

Bacteria	Sex, frequency (%)		*P*
	Male	**Female**	
Staphylococcus aureus			
No	62 (68.1)	63 (70.8)	0.411
Yes	29 (31.9)	26 (29.2)	
Staphylococcus epidermidis			
No	40 (44)	36 (40.4)	0.373
Yes	51 (56)	53 (59.6)	
Pseudomonas aeruginosa			
No	89 (97.8)	85 (95.5)	0.331
Yes	2 (2.2)	4 (4.5)	
Streptococcus pneumoniae			
No	88 (96.7)	87 (97.8)	0.511
Yes	3 (3.3)	2 (2.2)	
Streptococcus viridans			
No	89 (97.8)	87 (97.8)	0.681
Yes	2 (2.2)	2 (2.2)	
Streptococcus pyogenes			
No	91 (100)	88 (98.9)	0.494
Yes	0 (0)	1 (1.1)	
Micrococcus			
No	87 (95.6)	87 (97.8)	0.352
Yes	4 (4.4)	2 (2.2)	
Klebsiella pneumoniae			
No	89 (97.8)	87 (97.8)	0.681
Yes	2 (2.2)	2 (2.2)	
Enterobacter			
No	91 (100)	88 (98.9)	0.494
Yes	0 (0)	1 (1.1)	
Escherichia coli			
No	90 (98.9)	89 (100)	0.506
Yes	1 (1.1)	0 (0)	
Salmonella			
No	90 (98.9)	89 (100)	0.506
Yes	1 (1.1)	0 (0)	
Proteus			
No	90 (98.9)	88 (98.9)	0.746
Yes	1 (1.1)	1 (1.1)	
Bacillus cereus			
No	90 (98.9)	88 (98.9)	0.746
Yes	1 (1.1)	1 (1.1)	
Diphtheroid			
No	91 (100)	87 (97.8)	0.243
Yes	0 (0)	2 (2.2)	

study, the most common was *S. epidermidis*, though it can be considered among the normal flora of the eye tissue.[17] After *S. epidermidis*, the most common bacteria species was *S. aureus*, which was observed in 55 cases (30.6%). Parul *et al*. carried out a study on the samples collected from children under 3 years of age with bacterial conjunctivitis. In this study, 78% of cultures returned positive, with the most common causes being *H. influenzae* (82%), *S. pneumoniae* (16%), and *S. aureus* (2.2%).[18] A study

conducted on 548 samples collected from conjunctivitis patients reported that 17% of cases had methicillin-resistant staphylococci.[19] In another study on 3640 patients with extraocular infection, 1088 of the cases (30%) had methicillin-resistant *S. aureus* (MRSA) of hospital origin and 2552 (70%) of them had methicillin-resistant staphylococci originating from the general population.[20] In a study of 92 conjunctivitis patients at Labbafinejad Hospital in Tehran, 58.5% of cultures returned positive, of which 57.1% were for aerobic and 7.4% for anaerobic

organisms. Among aerobic organisms, *S. epidermidis* was the most common (30%). This study recommended that given the multitude of bacterial causes of conjunctivitis, it is best to postpone the antibiotic treatment until after identifying the pathogenic microorganism.[21]

In a study by Ghasemi *et al*., for example, *S. aureus* was the most common (38%) organism isolated from all age groups.[22] On the contrary, Shahriari *et al*. reported that *S. pneumoniae* was the most common (52%) organism in

Table 3: Frequency of bacterial infections in patients based on age group

Bacteria	Age group, frequency (%)			*P*
	<20	20-59	>60	
Staphylococcus aureus				
No	36 (75)	60 (73.2)	29 (58)	0.115
Yes	12 (25)	22 (26.8)	21 (42)	
Staphylococcus epidermidis				
No	18 (37.5)	33 (40.2)	25 (50)	0.405
Yes	30 (62.5)	49 (59.8)	25 (50)	
Pseudomonas aeruginosa				
No	44 (91.7)	80 (97.6)	50 (100)	0.059
Yes	4 (8.3)	2 (2.4)	0 (0)	
Streptococcus pneumoniae				
No	46 (95.8)	80 (97.6)	49 (98)	0.783
Yes	2 (4.2)	2 (2.4)	1 (2)	
Streptococcus viridans				
No	48 (100)	80 (97.6)	48 (96)	0.399
Yes	0 (0)	2 (2.4)	2 (4)	
Streptococcus pyogenes				
No	48 (100)	81 (98.8)	50 (100)	0.548
Yes	0 (0)	1 (1.2)	0 (0)	
Micrococcus				
No	47 (97.9)	78 (95.1)	49 (98)	0.572
Yes	1 (2.1)	4 (4.9)	1 (2)	
Klebsiella pneumoniae				
No	48 (100)	79 (96.3)	49 (98)	0.390
Yes	0 (0)	3 (3.7)	1 (2)	
Enterobacter				
No	47 (97.9)	82 (100)	50 (100)	.251
Yes	1 (2.1)	0 (0)	0 (0)	
Escherichia coli				
No	47 (97.9)	82 (100)	50 (100)	0.251
Yes	1 (2.1)	0 (0)	0 (0)	
Salmonella				
No	47 (97.9)	82 (100)	50 (100)	0.251
Yes	1 (2.1)	0 (0)	0 (0)	
Proteus				
No	48 (100)	81 (98.8)	49 (98)	0.635
Yes	0 (0)	1 (1.2)	1 (2)	
Bacillus cereus				
No	48 (100)	81 (98.8)	49 (98)	0.635
Yes	0 (0)	1 (1.2)	1 (2)	
Diphtheroid				
No	48 (100)	81 (98.8)	49 (98)	0.635
Yes	0 (0)	1 (1.2)	1 (2)	

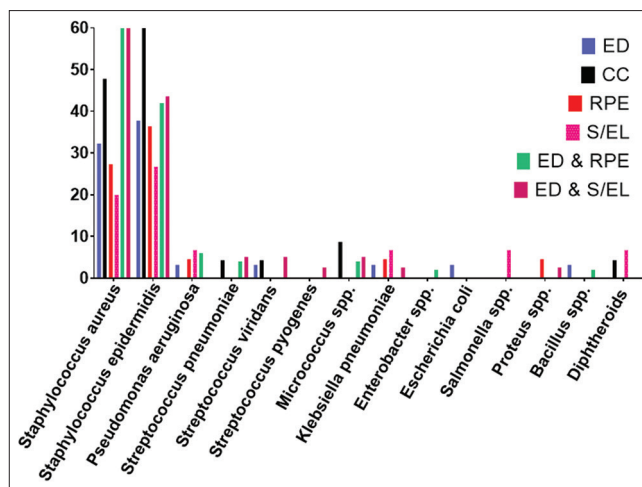| Table 4: Frequency of bacterial infections in patients based on place			
Bacteria	**Location, frequency (%)**		***P***
	City	**Village**	
Staphylococcus aureus			
No	113 (70.6)	12 (60)	0.234
Yes	47 (29.4)	8 (40)	
Staphylococcus epidermidis			
No	67 (41.9)	9 (45)	0.485
Yes	93 (58.1)	11 (55)	
Pseudomonas aeruginosa			
No	154 (96.3)	20 (100)	0.488
Yes	6 (3.8)	0 (0)	
Streptococcus pneumoniae			
No	155 (96.9)	20 (100)	0.551
Yes	5 (3.1)	0 (0)	
Streptococcus viridans			
No	156 (97.5)	20 (100)	0.622
Yes	4 (2.5)	0 (0)	
Streptococcus pyogenes			
No	159 (99.4)	20 (100)	0.889
Yes	1 (0.6)	0 (0)	
Micrococcus			
No	154 (96.3)	20 (100)	0.488
Yes	6 (3.8)	0 (0)	
Klebsiella pneumoniae			
No	156 (97.5)	20 (100)	0.622
Yes	4 (2.5)	0 (0)	
Enterobacter			
No	159 (99.4)	20 (100)	0.889
Yes	1 (0.6)	0 (0)	
Escherichia coli			
No	159 (99.4)	20 (100)	0.889
Yes	1 (0.6)	0 (0)	
Salmonella			
No	159 (99.4)	20 (100)	0.889
Yes	1 (0.6)	0 (0)	
Proteus			
No	158 (98.8)	20 (100)	0.79
Yes	2 (1.3)	0 (0)	
Bacillus cereus			
No	158 (98.8)	20 (100)	0.79
Yes	2 (1.3)	0 (0)	
Diphtheroid			
No	158 (98.8)	20 (100)	0.79
Yes	2 (1.3)	0 (0)	

Figure 1: Frequency of bacterial infections in patients based on symptoms (ED: Eye discharge, CC: Chronic conjunctivitis, RPE: Red or pink eye, S/EL: Sticky or eyelashes)

an Iranian population.[23] Numerous studies have shown that *S. aureus* is the leading cause of ocular infections in many parts of the world.[15,24] However, in many cases, other bacterial species have been identified as the dominant cause of infection, which is reasonable given how this issue is influenced by environmental, geographical, and demographic factors.[21,25] Other bacterial strains identified in this study, in the order of prevalence among the patients, were *Staphylococcus* coagulase negative*, Bacillus* spp., *P. aeruginosa*, *Enterobacter* spp., *K. pneumoniae*, and D group *Streptococcus*.[26]

In the present study, *P. aeruginosa* was most resistant to ampicillin. In the case of *S. epidermidis* and *S. aureus*, the highest resistance was observed against erythromycin and the least resistance was against rifampicin and linezolid. According to the results, piperacillin, meropenem, cefepime, and amoxicillin are the top choices for treating Gram-negative *Bacillus* (*P. aeruginosa*) infections. However, for the infections caused by *S. aureus* and *S. epidermidis*, the preferred antibiotic would be rifampicin and linezolid, respectively. A study by Sohrabi *et al*. recommended tobramycin and amikacin as the main choices for treating Gram-negative *Bacillus* (*P. aeruginosa*) infections and recommended ciprofloxacin for treating *S. aureus* infections.[27] A study conducted by Bhattacharyya *et al*. was reported that all the major organisms were highly sensitive to aminoglycosides, cephalosporins, chloramphenicol, vancomycin, and linezolid, whereas a high level of resistance was seen toward fluoroquinolones (ciprofloxacin and moxifloxacin).[28] Unfortunately, because of the careless use of antibiotics for the treatment of bacterial infections of the conjunctiva, there has been a worrying increase in the emergence of drug-resistant strains of some of the bacteria that infect this part more regularly.[29,30] Therefore, precise monitoring of the antibiotic resistance pattern of common bacteria in each region can help physicians prescribe antibiotics more carefully without risking efficacy.[31]

In the present study, 100% of MRSA strains had PCR negative. This result could be related to the presence of a mutation in the *mecA* gene in these strains or the presence of physiological differences such as alteration in the bacterial wall.[19] Despite the high resistance to cefoxitin in these strains, there may be some difficulty regulating *mecA*
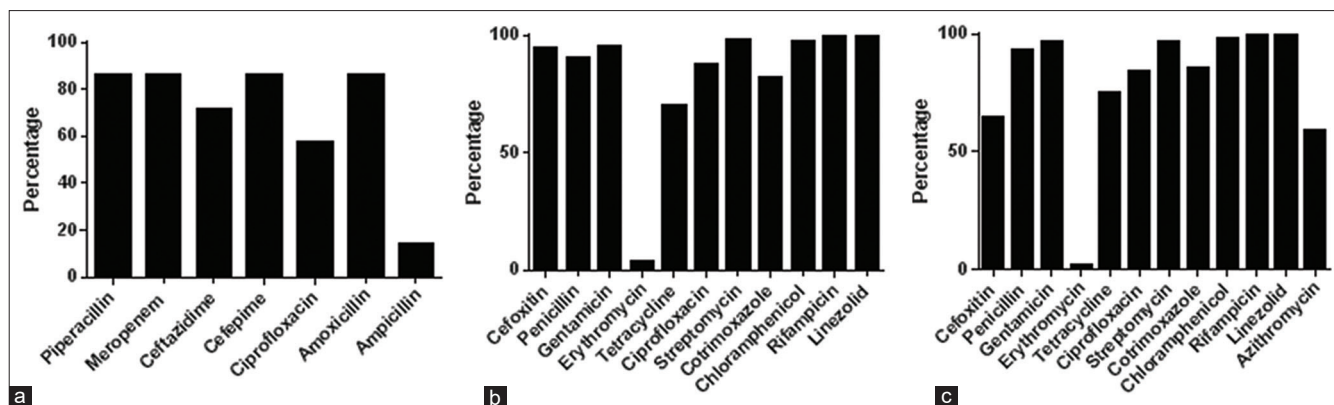
Figure 2: The antibiotic sensitivity of organisms isolated from conjunctivitis patients (a: *Pseudomonas aeruginosa*, b: *Staphylococcus epidermidis*, c: *Staphylococcus aureus*)
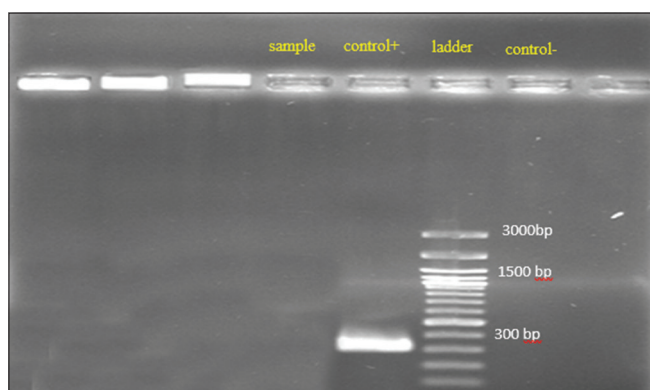

Figure 3: *MecA* gene product

gene expression. In future studies, it is recommended to conduct research in this area.

This report is one of the most comprehensive studies ever conducted on the relative frequency of bacterial and adenoviral causes of conjunctivitis in the population of Kashan and their antibiotic resistance patterns. These results can contribute to the planning and adjustment of therapeutic strategies and, especially antibiotic medication, for patients with conjunctivitis in this region.

Conclusion

In this study, *S. aureus* and *S. epidermidis* are the most common causes of conjunctivitis in all age groups, however, this condition decreases with age and is also influenced by other factors such as season and weather conditions. The results of this study can be helpful in planning more prudent treatment strategies for patients with conjunctivitis in Kashan.

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Conflicts of interest

There are no conflicts of interest.

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