

Bacterial etiology and antibiotic susceptibility of conjunctivitis patients isolates from central of Iran

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Abstract

Background: Conjunctivitis is a very common ocular disease, which can be caused by a wide variety of microorganisms. Identification of the bacterial causes of this infection in a specific geographical area can help physicians devise more efficient therapeutic strategies for this disease in the local population. This study bacterial etiology and antibiotic susceptibility of conjunctivitis patients isolates from central of Iran.

Methods: This study was performed of 180 patients referred to department of ophthalmology in Kashan University with symptoms of conjunctivitis from July 2017 to December 2017. Samples were collected from the conjunctival sac and cultured in bacterial culture mediums. Antibiotic susceptibility of the bacteria isolated was done using the Kirby-Bauer method. Methicillin resistance in *staphylococci* isolated from the patients was identified using PCR technique.

Results: In the present study, *Pseudomonas aeruginosa* was most resistant to ampicillin. In the case of *Staphylococcus epidermidis* and *Staphylococcus aureus*, the highest resistance was observed against erythromycin and the least resistance was against rifampicin and linezolid.

Conclusion: In this study, *Staphylococcus aureus* and *Staphylococcus epidermidis* is the most common cause 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.

Background

Conjunctivitis refers to the inflammation 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]. Bacterial agents play a central role in the development of infectious conjunctivitis, especially in children [5]. The major bacterial causes of conjunctivitis are *Staphylococcus*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* [6]. 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 [6].

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, *Staphylococcus 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 [7]. In a study on the prevalence of microbial causes of keratoconjunctivitis in patients examined in Feyz hospital of Isfahan, out of 196 samples tested, 75 had bacterial causes with the most common pathogens being *Staphylococcus aureus* (28.1%), *Staphylococcus coagulase negative* (16.8%), *Bacillus* (6.6%), *Pseudomonas aeruginosa* (5.1%), *Enterobacter* (4.2%), *Klebsiella* (3.6%), and group D *Streptococcus* (3.6%). In this study, the highest antibiotic sensitivity was observed in *Staphylococcus aureus* and *Pseudomonas* under exposure to ciprofloxacin and tobramycin respectively [8].

In a study carried out by Tesfaye et al. (2013), where they examined the bacterial profile and antibiotic susceptibility pattern of external ocular infections, 148 of the cultured 198 samples returned positive, among which the dominant gram-positive and gram-negative organisms were *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively. This study reported that gram-positive bacteria were mostly susceptible to ciprofloxacin and vancomycin and gram-negative bacteria were largely susceptible to amikacin and ciprofloxacin [9]. In a study by Stan et al. (2000) on the bacterial resistance in patients with acute conjunctivitis, it was found that 42% of patients were infected with *Haemophilus influenzae*, 30% with *Streptococcus pneumoniae*, and the rest were negative. This study reported that 69% of *Haemophilus influenzae* cases had Beta-lactamase gene, and 68% of *Streptococcus pneumoniae* cases were penicillin-sensitive and 17% were penicillin-resistant [10]. 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 *Haemophilus influenzae* (82%), *Streptococcus pneumoniae* (16%), and *Staphylococcus aureus* (2.2%) [11]. A study conducted on 548 samples collected from conjunctivitis patients reported that 17% of cases had methicillin-resistant staphylococci [12]. In another study on 3640 patients with extraocular infection, 1088 of the cases (30%) had methicillin-resistant *Staphylococcus aureus* of hospital origin and 2552 (70%) of them had methicillin-resistant staphylococci originating from the general population [13]. 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 [14]. 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 [15].

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 *staphylococcal (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.

Methods

A cross-sectional study was performed on the population of patients with ocular infection referred ophthalmology clinic of Kashan in 2017. The sample consisted of 180 patients with eye infections who were examined at this clinic during July 2017 to December 2017. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments. The protocol of this study was approved by Research Ethics Committee, KAUMS (IR.KAUMS.REC.1396.31), Iran and written informed consent was obtained from all patients. Subjects 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. The prepared plates were incubated at 37°C for 24-48 hours 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, DNAase, chocolate agar were used.

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 to 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 hours of incubation at 37°C, the diameter of the inhibitory area around the discs was measured [15]. The antibiogram was developed for oxacillin, rifampin, ampicillin, amoxicillin, cefepime, ceftazidime, piperacillin ciprofloxacin, chloramphenicol, doxycycline, vancomycin, amoxicillin/ clavulanate, amikacin, tetracycline, cotrimoxazole, erythromycin and gentamicin as instructed in CLSI (2016). After 24 hours of incubation, the inhibitory area was compared with the standard chart [16].

The methicillin resistance (*mecA*) in staphylococci isolated from the patients was identified using the PCR technique. Specific primers with the following sequence were used to investigate the presence of *mecA* gene. The thermal cycle used in the thermocycler device first made an initial denaturation for 5 minutes at 95°C, then 30 cycles at 94°C for 15 seconds, 61°C for 15 seconds and 72°C for 30 seconds. Then final Extension was performed at 72°C for 5 minutes. The volume of the PCR reaction mixture was 25µl for each reaction, including PCR buffer (2.5µl), MgCL₂ (0.8 mM), dNTPs (0.16 mM), Primers (16 pmol of each), Taq polymerase (1u/µl) and 2 microliters 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 bacteria type and demographic variables and then further analyzed using the chi-square test, Fisher's exact tests, and one-way ANOVA at the P-value=0.05 significance level.

Results

This study investigated the bacterial causes of conjunctivitis and their antibiotic resistance pattern 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 that included, *Staphylococcus aureus* (28.2%), *Staphylococcus epidermidis* (53.3%), *Pseudomonas* (3%), *pneumococcus* (2.5%), *streptococcus viridans* (2%), *streptococcus pyogenes* (0.5%), *Micrococcus* (3%), *Klebsiella* (2%), *Enterobacter* (0.5%), *Escherichia coli* (0.5%), *Salmonella* (0.5%), *Proteus* (1%), *Bacillus cereus* (1%) and *Diphtheria* (1%) were isolated.

The frequency distribution of bacterial infections in the sampled conjunctivitis patients in terms of their age and gender is presented in **Tables 1 and 2**, respectively. As can be seen, no significant relationship was found between gender and infectious agents. Also, no significant relationship was found between age groups and infectious agents.

The frequency distribution of bacterial infections in the patients in terms of observed symptoms is provided in **Fig.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 **Fig.2**. In the present study, *Pseudomonas aeruginosa* was most resistant to ampicillin. In the case of *Staphylococcus epidermidis* and *Staphylococcus 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 bacilli (*Pseudomonas aeruginosa*) infections. But for the infections caused by *Staphylococcus aureus* and *Staphylococcus epidermidis*, the preferred antibiotic would be rifampicin and linezolid respectively.

Following PCR to determine *mecA* gene in 11 cefoxitin-resistant staphylococci sample, it was found that despite resistance to disc cefoxitin, none of the resistant strains had *mecA* gene (Fig.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 [5, 17, 18], and such bacterial infections are especially common in children [19, 20]. Among the bacterial strains isolated from the patients of this study, the most common was *Staphylococcus epidermidis*, though it can be considered among the normal flora of the eye tissue [21]. After *Staphylococcus epidermidis*, the most common bacteria species was *Staphylococcus aureus*, which was observed in 55 cases (30.6%). In a study by Ghasemi et al. for example, *Staphylococcus aureus* was the most common (38%) organism isolated from all age groups [22]. On the contrary, Shahriari et al. reported that *pneumococcus* was the most common (52%) organism in an Iranian population [23]. Numerous studies have shown that *Staphylococcus aureus* is the leading cause of ocular infections in many parts of the world [20, 24]. However, in many cases, other bacterial species have been identified as the dominant cause of infection, which is not unreasonable given how this issue is influenced by environmental, geographical, and demographic factors [7, 25]. Other bacterial strains identified in this study, in the order of prevalence among the patients, were *Staphylococcus* coagulase negative, *Bacillus* spp., *Pseudomonas aeruginosa*, *Enterobacter* spp., *Klebsiella pneumoniae*, and D Group *Streptococcus* [26].

In the present study, *Pseudomonas aeruginosa* was most resistant to ampicillin. In the case of *Staphylococcus epidermidis* and *Staphylococcus 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 bacilli (*Pseudomonas aeruginosa*) infections. But for the infections caused by *Staphylococcus aureus* and *Staphylococcus epidermidis*, the preferred antibiotic would be rifampicin and linezolid respectively. A study by Sohrabi et al. recommended tobramycin and amikacin as main choices for treating gram-negative bacilli (*Pseudomonas aeruginosa*) infections and recommended ciprofloxacin for treating *Staphylococcus aureus* infections [8]. 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 [27, 28]. Therefore, precise monitoring of the antibiotic resistance pattern of common bacteria in each region can help physicians prescribe antibiotics more carefully without risking efficacy [29].

In the present study, 100% of MRSA strains had PCR negative. This result could be related to the presence of a mutation in the methicillin resistance gene in these strains or the presence of physiological differences such as alteration in the bacterial wall [12]. Despite the high resistance to cefoxitine in these

strains, there may be some difficulty regulating *mecA* 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, *Staphylococcus aureus* and *Staphylococcus epidermidis* is the most common cause 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.

Abbreviations

PYR: Pyrrolidonyl arylamidase; PCR: Polymerase chain reaction; MRSA: Methicillin-resistant *Staphylococcus aureus*; CLSI: Clinical & Laboratory Standards Institute.

Declarations

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments.

The protocol of this study was approved by Research Ethics Committee, KAUMS (IR.KAUMS.REC.1396.31), Iran and written informed consent was obtained from all patients.

Consent for Publication

Not applicable.

Availability of data and material

All data generated or analyzed during this study are included in this published article.

Competing interests

No competing financial interests exist.

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Authors' contributions

HF, MM and AA conceptualized, acquired funding, collected the data, analyzed and wrote the first draft of paper. AN and HF validated the data, and analyzed the data. HA and HF assisted in analyzing data and writing the draft manuscript. HF, MS, AN and RM were involved in project administration, supervision of data collection team, revision of draft paper and its finalization. All authors read and approved the final manuscript.

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Tables

Table1. The sequences of primers and PCR product size *mec A* gene.

Primer	Primer sequence 5'	3'	Product Size (bp)
<i>mec A</i> Forward	ACTGCTATCCACCCTCAAAC		286
<i>mec A</i> Reverse	CTGGTGAAGTTGTAATCTGG		

Table2. Frequency of bacterial infections in patients based on age group

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

Table3. Frequency of bacterial infections in patients based on gender

Bacteria		Sex				P-Value
		Male		Female		
		Frequency	percentage	Frequency	percentage	
<i>Staphylococcus aureus</i>	No	62	68.1	63	70.8	0.411
	Yes	29	31.9	26	29.2	
<i>Staphylococcus epidermidis</i>	No	40	44	36	40.4	0.373
	Yes	51	56	53	59.6	
<i>Pseudomonas</i>	No	89	97.8	85	95.5	0.331
	Yes	2	2.2	4	4.5	
<i>pneumococcus</i>	No	88	96.7	87	97.8	0.511
	Yes	3	3.3	2	2.2	
<i>Streptococci viridians</i>	No	89	97.8	87	97.8	0.681
	Yes	2.	2.2	2	2.2	
<i>Streptococcus pyogenes</i>	No	91	100	88	98.9	0.494
	Yes	0	0	1	1.1	
<i>Micrococcus</i>	No	87	95.6	87	97.8	0.352
	Yes	4	4.4	2	2.2	
<i>Klebsiella</i>	No	89	97.8	87	97.8	0.681
	Yes	2	2.2	2	2.2	
<i>Enterobacter</i>	No	91	100	88	98.9	0.494
	Yes	0	0	1	1.1	
<i>Escherichia coli</i>	No	90	98.9	89	100	0.506
	Yes	1	1.1	0	0	
<i>Salmonella</i>	No	90	98.9	89	100	0.506
	Yes	1	1.1	0	0	
<i>Proteus</i>	No	90	98.9	88	98.9	0.746
	Yes	1	1.1	1	1.1	
<i>Bacillus cereus</i>	No	90	98.9	88	98.9	0.746
	Yes	1	1.1	1	1.1	
<i>Diphtheroid</i>	No	91	100	87	97.8	0.243
	Yes	0	0	2	2.2	

Table4. Frequency of bacterial infections in patients based on place

Bacteria		Location				P-Value
		City		Village		
		Frequency	percentage	Frequency	percentage	
<i>Staphylococcus aureus</i>	No	113	70.6	12	60	0.234
	Yes	47	29.4	8	40	
<i>Staphylococcus epidermidis</i>	No	67	41.9	9	45	0.485
	Yes	93	58.1	11	55	
<i>Pseudomonas</i>	No	154	96.3	20	100	0.488
	Yes	6	3.8	0	0	
<i>pneumococcus</i>	No	155	96.9	20	100	0.551
	Yes	5	3.1	0	0	
<i>Streptococci viridans</i>	No	156	97.5	20	100	0.622
	Yes	4	2.5	0	0	
<i>Streptococcus pyogenes</i>	No	159	99.4	20	100	0.889
	Yes	1	0.6	0	0	
<i>Micrococcus</i>	No	154	96.3	20	100	0.488
	Yes	6	3.8	0	0	
<i>Klebsiella</i>	No	156	97.5	20	100	0.622
	Yes	4	2.5	0	0	
<i>Enterobacter</i>	No	159	99.4	20	100	0.889
	Yes	1	0.6	0	0	
<i>Escherichia coli</i>	No	159	99.4	20	100	0.889
	Yes	1	0.6	0	0	
<i>Salmonella</i>	No	159	99.4	20	100	0.889
	Yes	1	0.6	0	0	
<i>Proteus</i>	No	158	98.8	20	100	0.79
	Yes	2	1.3	0	0	
<i>Bacillus cereus</i>	No	158	98.8	20	100	0.79
	Yes	2	1.3	0	0	
<i>Diphtheroid</i>	No	158	98.8	20	100	0.79
	Yes	2	1.3	0	0	

Figures

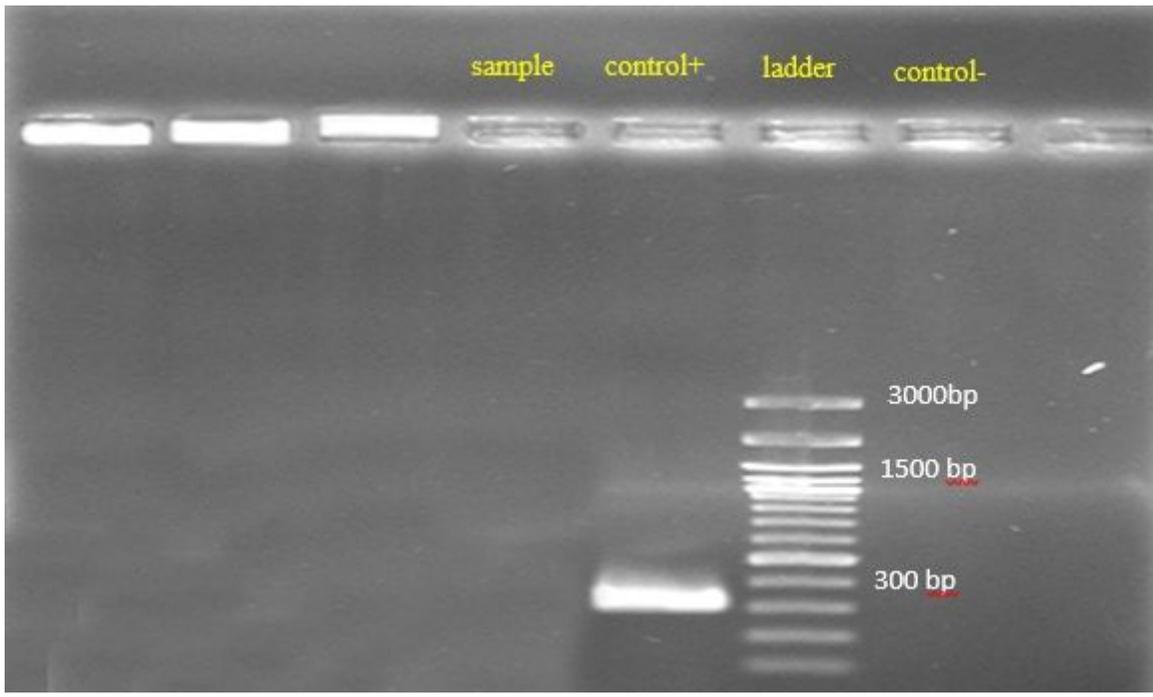


Figure 1

MecA gene product

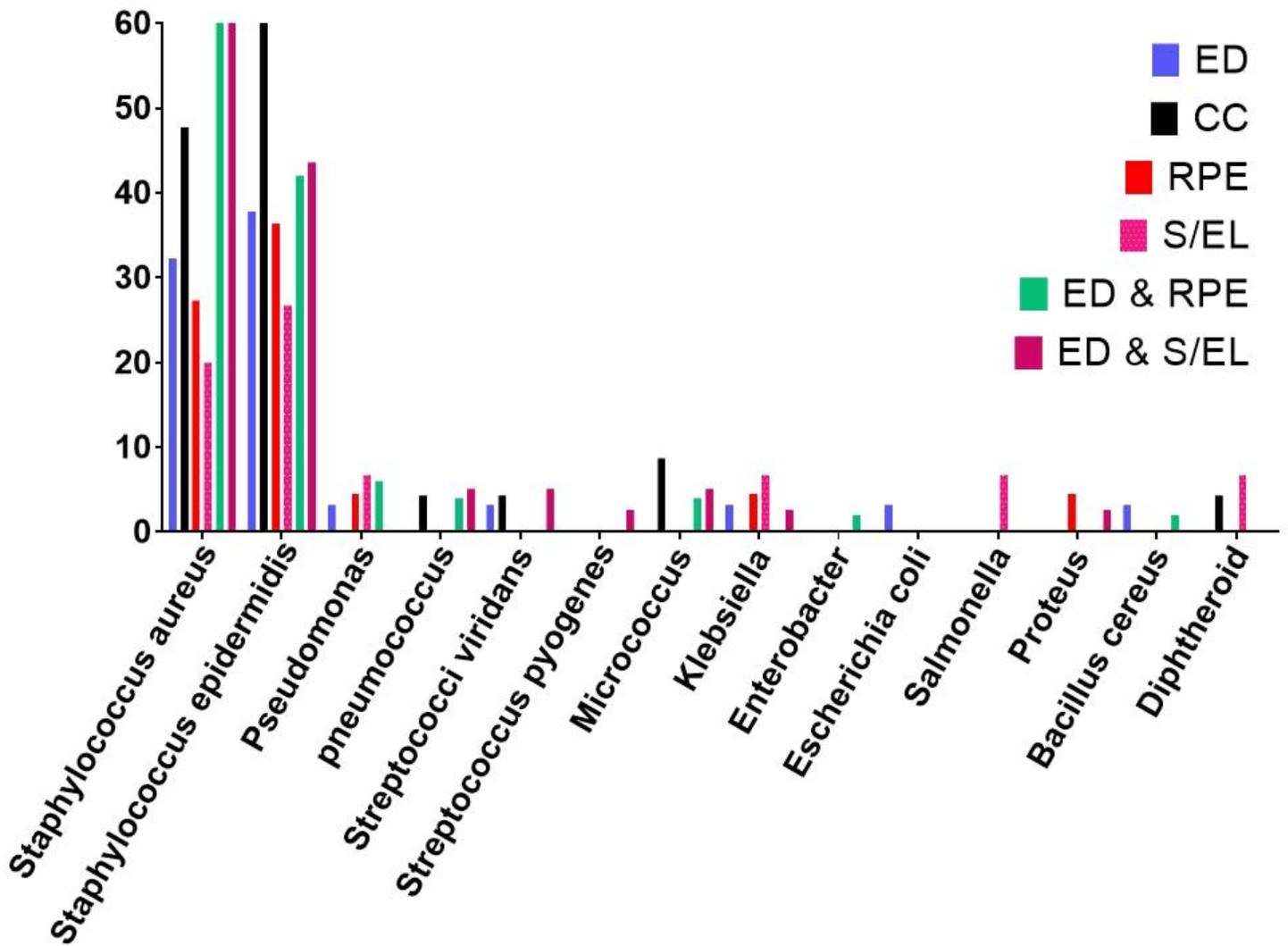


Figure 2

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)

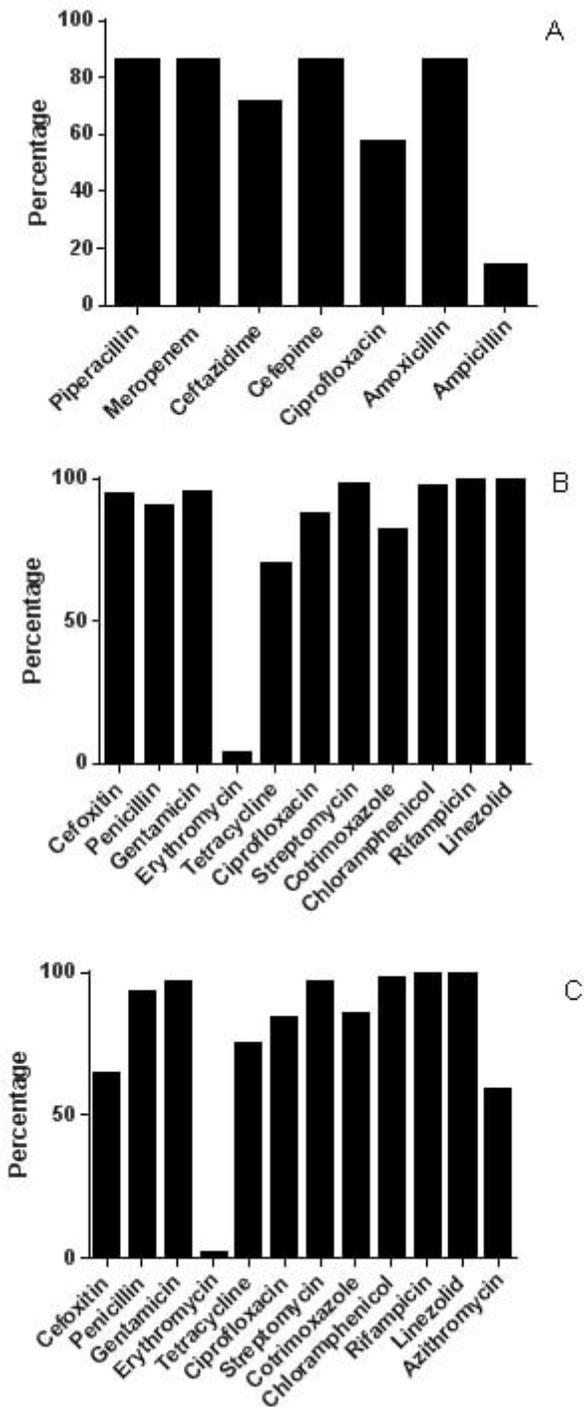


Figure 3

The antibiotic sensitivity of organisms isolated from conjunctivitis patients (A: Pseudomonas, B: Staphylococcus epidermidis, C: Staphylococcus aureus)