

# Staphylococcus Epidermidis is the Main Causative Agent of Conjunctivitis

Suhaib Khalid Ibrahim<sup>1</sup>, Najat Mohammed Flyyih<sup>2</sup>

<sup>1,2</sup>College of Health and medical technology /Baghdad; Iraq

Email: [suhaibkhalid2000@yahoo.com](mailto:suhaibkhalid2000@yahoo.com)

## Abstract

**Background:** Staphylococcus epidermidis is a gram-positive coccus, catalase positive, coagulase negative and non mannitol fermenter, it is one of the main commensal microorganisms that colonizes the skin, nose and other sites in the body. It is considered nonpathogenic normal flora but when dislodged to other areas become opportunistic and causes severe infections due to biofilm formation particularly on extraordinary sites like urinary tract infections after catheter acquired infections **Purpose:** To determine the role of Staphylococcus epidermidis as one of the main causative agents of chronic conjunctivitis and its essential factor in increase antibiotic resistance and strong biofilm production in those patients. **Objective:** This study investigates the correlation between the colonization of Staphylococcus epidermidis in the body and chronic eye infections in patients with conjunctivitis. **Methods:** A total of 100 eye swab samples were enrolled in this study, collected from patients with conjunctivitis (n=50) and healthy individuals (n=50). Staphylococcus epidermidis was isolated from the eyes of those groups cultured on special media following standard operating procedures. Antibiotic susceptibility tests against 5 antibiotics by using disk diffusion method were done and biofilm was measured to all groups by using tissue culture plate method. **Results:** In this study, out of 100 samples, there were 36% males and 64% females respectively. A total of 81(81%) were colonized with Staphylococcus epidermidis includes, 31(38.2%) from healthy individuals and 50(61.8%) from patients with conjunctivitis while only 12(12%) were colonized with Staphylococcus aureus includes 8(8%) in healthy individuals and 4(4%) in conjunctivitis patients. Results of antibiotic sensitivity tests to 5 antibiotics revealed all Staphylococcus epidermidis isolates were MDR and high resistant to Methicillin (100%) and sensitive to Vancomycin (100%), also all Staphylococcus epidermidis mainly in conjunctivitis patients were strong biofilm producers much higher than Staphylococcus aureus and Staphylococcus epidermidis isolates in the healthy persons. **Conclusions:** Coagulase negative staphylococci represented by Staphylococcus epidermidis was the most common agent isolated from conjunctiva of Baghdad patients and the most common cause of these chronic infections in those patients accompanied with increase antibiotic resistance rates and strong biofilm production more than coagulase positive Staphylococcus aureus in healthy and infected persons.

**Keywords** Staphylococcus epidermidis, Staphylococcus aureus, Multidrug resistance (MDR).

## 1. Introduction

Staphylococcus epidermidis is a saprophytic bacteria that is the most common inhabitant microorganism of the skin and mucous membrane commensally as natural microbial flora, but it is considered pathogenic in certain conditions that become opportunistic like in hospitals that cause nosocomial infections or when reach to certain area in the body that is not normally found like medical device infections such as catheter , eye infections like conjunctivitis, keratitis [1].

Staphylococcus epidermidis is a gram positive , non-motile , spherical in shape or cocci , it is one of the most important staphylococcus species that differs from the major pathogen staphylococcus aureus by many factors like it is opportunistic pathogen in some circumstances, coagulase negative , major biofilm producers and multidrug resistance(MDR) particularly when transformed to opportunistic form and causes diseases like conjunctivitis, keratitis and catheter related infections [2].

Resistance of Staphylococcus epidermidis in patients with conjunctivitis , catheter infections to several antimicrobial agents like fluoroquinolone , vancomycin, oxacillin and chloramphenicol is associated with formation of very large extracellular biofilm which is composed of glycocalyx, polysaccharide capsule , nucleic acids and proteins [3].

The reason for more biofilm production in patients with conjunctivitis , keratitis and other device related infections is related to the presence of pathogenic strains

of Staphylococcus epidermidis that carried several adhesion genes in the eyes of infected patients attacked by host innate immunity represented by lysozyme and antimicrobial peptides that is differentiated from strains of Staphylococcus epidermidis normal habitant flora in the eyes of healthy individuals which is recognized by host innate immunity as non-pathogens [4].

## 2. Materials and Methods

### Collection of Samples

100 eye swab samples were included in this study. 50 samples were collected from patients suffered from chronic conjunctivitis and 50 samples collected from apparently healthy individuals with age ranged (15-50) years during a period from February 2020 to April 2020 from Baghdad teaching hospital/ consultant clinic in the medical city [4].

### Bacterial isolation

All eye swab specimens were cultured on blood agar, brain heart agar and mannitol salt agar plates, incubated overnight at 37°C. Colonies of Staphylococcus epidermidis were identified according to colonial morphology on blood agar , fermentation ability on mannitol agar and biochemical tests like catalase and coagulase [5].

### Antibiotic sensitivity tests

Five antibiotic discs were performed for all Staphylococcus epidermidis isolates according to the clinical and laboratory standards institute (CLSI) guidelines by disc diffusion tests. Antibiotics were methicillin (10 µg), vancomycin (30 µg), fluoroquinolones (10 µg) and oxacillin (25 µg) [6]. All antibiotic disks were provided from Bioanalyse Group UK, Ltd.

### Biofilm assay tests

Assay of bacterial biofilm for all Staphylococcus epidermidis isolates was determined by microtiter tissue culture plate method depending on crystal violet tests [7]. Staphylococcus epidermidis was inoculated in 10 ml of special medium called of tryptic soy broth (TSB) supplemented with 0.2% glucose for enhancement, incubation overnight at 37 C° then washing with sterile phosphate buffered saline (PBS) three times to remove non adherent cells, the cells were fixed with methanol (200 µl) for 10 min. the plate was stained with 200 µl of 1% crystal violet stain and left for 10 min., then washed by distilled water to remove the excess stain and finally 250 µl of absolute ethanol was added and optical density was measured at 570-600 nm by ELISA reader. one well left with TSB medium only that considered as negative control while another well with broth and culture only considered as positive control, the cutoff point was 0.200nm so any results less than cutoff was negative while other results equal or greater than 0.200nm considered positive [8].

## 3. Statistical Analysis

The data were statistically analyzed by using (SPSS) ver. (22.0), for significant correlation between the frequency of Staphylococcus epidermidis in eyes and patients with chronic conjunctivitis. P values of less than 0.05 were regarded as statistically significant.

## 4. Results

### Distribution of study groups according to sex

All 100 eye swab samples were 50 (50%) collected from patients with conjunctivitis and 50 (50%) were from healthy controls. The total number of males in all study groups were 60 (60%) more than females there were 40 (40%), in conjunctivitis group the number of males were 33 (66%) while females were 17 (34%), whereas in healthy control group the number of males were 27 (54%) while females were 23 (46%) with a statistically non-significant differences between study groups regarding sex distribution at (p=0.08), as shown in (Table 1).

sex	Conjunctivitis group	Healthy group	Total	P-value
Male (No / %)	33 ( 66%)	27 ( 54%)	60 ( 60%)	**
Female (No / %)	17 ( 34%)	23 ( 46%)	40 ( 40%)	0.08
Total (No / %)	50 ( 50%)	50 ( 50%)	100	NS

\* Highly significant; \*\* non-significant

### Staphylococcus species in conjunctivitis and healthy persons

A total of 100 eye swab samples were 50 collected from patients with chronic conjunctivitis and 50 collected from healthy

persons as controls, 30(60%) patients with conjunctivitis were more infected with Staphylococcus epidermidis as an opportunistic pathogen, while only 10 (20%) healthy colonized as a normal microbiota in eyes of control group, in contrast with 18 (36%) patients with chronic conjunctivitis were infected with S.aureus pathogens and no anyone of controls. Statistically there is a highly significant differences between S. aureus and S.epidermidis distribution among healthy controls and patients with conjunctivitis at (p = 0.0009), as shown in (Table 2), white big circular colonies of S.epidermidis on mannitol agar (figure 1).

Table 2: distribution of Staphylococcus species in study groups.

Study groups	Samples NO.	S.epidermidis positive		S.aureus positive		P-value
		NO.	%	NO.	%	
Healthy controls	50	10	20	0	0	0.0009 (HS)*
Conjunctivitis	50	30	60	18	36	
Total groups	100	40	40	18	18	

\* Highly significant; \*\* non-significant

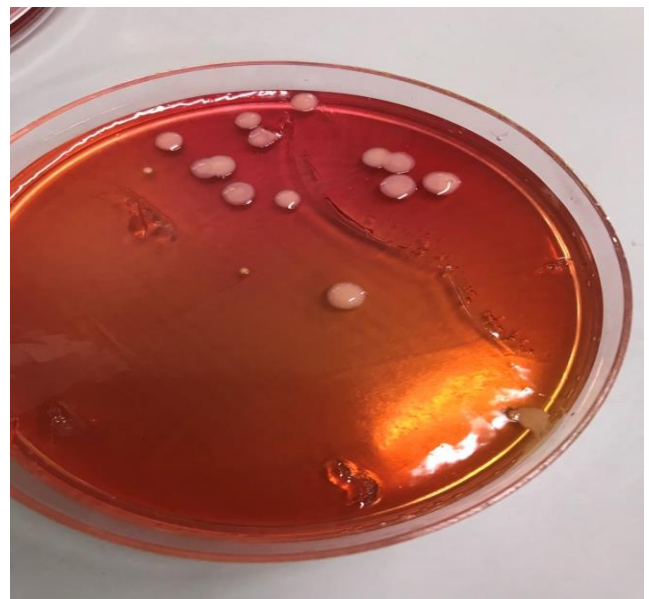


Figure 1: colonies of S.epidermidis on mannitol salt agar invitro.

### Antimicrobial sensitivity pattern

Four types of antibiotics was tested against S.aureus and S.epidermidis isolates in all study groups according to CLSI. S.epidermidis in conjunctivitis patients group were more resistant to antibiotics (62.5%) than healthy control group (27.5%). Furthermore, S.aureus and S.epidermidis isolates in conjunctivitis group showed complete resistance to Methicillin (100%) whereas (50%) Methicillin resistance of S.epidermidis in healthy control, also all S.aureus isolates showed high resistance to Oxacillin and Fluoroquinolones but S.epidermidis isolates showed more resistance to these antibiotics (73%) and (70%) respectively, than S.aureus isolates in the same study group. All staphylococcus isolates revealed very high sensitivity rate to vancomycin, statistically there is a highly significant correlation in the rate of antibiotic resistance between S.aureus and S.epidermidis isolates in patients with conjunctivitis accompanied with healthy group at (p=0.00), as shown in (Table 3).



**Table 3: Antibiotic resistance pattern of staphylococcus species in eye isolates.**

Study groups	Conjunctivitis		Healthy control		P-value
	S.aureus	S.epidermidis	S.aureus	S.epidermidis	
Antibiotics	No.(%)	No.(%)	No.(%)	No.(%)	
Methicillin ME	18(100 % )	30(100% )	-	5(50%)	*0.00
Vancomycin [VA]	1(5.5% )	2(6.6 %)	-	0	**1.00
Oxacillin [OX]	10(55% )	22(73 % )	-	4 (40%)	*0.00
Fluoroquinolones [fl]	12(67 %)	21(70%)	-	2 (20% )	*0.00
Total resistance	56.8%	62.5%	-	27.5%	*0.000

\* Highly significant; \*\* Non significant

## Biofilm formation

In the present study the optical density of S.aureus and S.epidermidis biofilm was measured by ELISA reader including the biofilm cutoff point of S.epidermidis was (0.200) nanometer whereas the cutoff point of S.aureus biofilm was only (0.150) nanometer in all study groups. About 60% Of S.epidermidis isolates have the ability to strong biofilm production in patients with conjunctivitis while 55% of S.aureus in conjunctivitis patients forming

strong biofilm in the same study group. Statistically there is a highly significant differences in the biofilm formation ability according to cutoff point (weak, moderate and strong) of S.aureus and S.epidermidis correlated with chronic conjunctivitis patients at (  $p = 0.00$  ), as shown in (Table 4). The final step of biofilm formation by microtiter plate method determined by crystal violet was showed in figure 2 (A and B ) before and after addition of crystal violet stain respectively.

Bacterial biofilm	Conjunctivitis patients No. (%)		Healthy eyes No. (%)		P-value
	S.aureus	S.epidermidis	S.aureus	S.epidermidis	
Cutoff (0.150 , 0.200)					
Weak $\leq 0.200$	1(5.5)	2 (6.6)	0	8 (80 )	*0.00
Moderate (0.200-0.400)	7 (38.8 )	10 (33)	0	1 (10)	*0.001
High $\geq 0.400$	10 (55 )	18 (60 )	0	1 (10)	*0.003
Total	18 (36)	30 (60 )	0	10 (20)	*0.000
	48 (48)		10 (10)		

\* Highly significant; \*\* non significant



Figure 2: The final step of biofilm formation invitro.

## 5. Discussion

In this study two staphylococcal species were isolated from patients with chronic conjunctivitis during a period from February 2020 to April 2020 , Staphylococcus epidermidis and staphylococcus aureus were the most frequent and common cause of ocular infections like conjunctivitis [9], due to S.epidermidis was considered as an opportunistic pathogen in immunocompromized patients like those with devise related infections and ocular infections in elderly patients , in addition these bacteria considered the major biofilm producers that contribute to increase virulence of bacteria and severity of eye infections. This study agreed with other study was conducted in Mexico by Juárez-Verdayes et al. [6].

In the present study regarding distribution of study groups concerning sex , the total males were 60% and females were 40% from all study groups , in conjunctivitis patients were

66% males and 34% females , while in healthy control males were 54% and females were 46% , the reason for slightly increasing males than females in current study due to the eye samples were collected randomly from patients and controls , heard life style of males and frequently working outdoor, this study coincide with study of African Journal of Microbiology Research by O. A. Adeyeba et al. [10], who reported that the frequency of bacterial conjunctivitis in Nigeria patients were 63.9% males and 36.1 were females but the current study concerning sex distribution not identical with other study was published in Ethiopia by Cevallos et al. [11], who reported that results including randomly conjunctival swabbing of 148 adults in rural Ethiopia were 112 females and 36 males.

The present study also included 60% of patients suffering from conjunctivitis caused by opportunistic S.epidermidis with 20% healthy colonized eyes with these bacteria while only 36% of chronic conjunctivitis patients caused by S.aureus pathogens with no anyone of healthy control eyes was colonized with coagulase positive staphylococci , the explanation of these results about the presence of S.epidermidis in eyes of infected patients that constitute the more predominant and frequent cause of chronic ocular infections than the pathogenic S.aureus give more indication about the nature opportunistic and adaptation resistant mechanisms of these bacteria to lacrimal enzymes represented by more antibiotic resistance and huge , tough , rigid biofilm formation with other resistant genes that differs from S.aureus human pathogen in action , nature of resistant and mechanism of invasion , the present study identical with other study conducted in Italy by Papa et al. [12], who reported that results were

included 72.5% of positive isolates were 58% *S.epidermidis* and 15% were *S.aureus*, while other study disagreed with the current study was published in India by Solanke et al. [13], who reported that the major isolates of bacterial conjunctivitis were 55.93 % due to *S.aureus* whereas only 28% of conjunctivitis due to *S.epidermidis*. In the present study regarding estimation of antibiotic resistance rate to all staphylococcus isolates by disc diffusion method against Methicillin, Vancomycin, Oxacillin and Fluoroquinolones, in chronic conjunctivitis group the patients showed high resistance rate of *S.epidermidis* isolates against methicillin, Oxacillin and Fluoroquinolones in a percentage about 100%, 73% and 70% respectively, while in *S.aureus* isolates the percentage of resistance to these antibiotics was slightly lower than rate of resistance to *S.epidermidis* isolates against Oxacillin(55%) and Fluoroquinolones(67%) with exception to all staphylococcus species isolates in this study were showed complete resistance to methicillin (100%), the indication of these results depend upon the nature of these bacteria and different strains other than saprophytic bacteria that cause several infections due to different mutations in antibiotic resistance genes and emergence of new novel resistant genes like quinolones mutation gene [14], oxacillin-resistance and other infectious virulence factors make *S.epidermidis* and *S.aureus* the most frequently isolated in patients with chronic conjunctivitis and other eye infections [15], the results of this current study regarding antibiotic resistance pattern to methicillin, Oxacillin and Fluoroquinolones in agreement with study of Blanco et al. [16] was published in Colombia, who reported that all coagulase positive and negative staphylococci was complete resistance to oxacillin and methicillin, furthermore these bacterial isolates in the present study showed high rate of susceptibility to vancomycin in conjunctivitis patients, this result agreed with study of Papa et al. [12], who reported that all staphylococci isolates were complete sensitivity to vancomycin, while the study by Juárez-Verdayes et al. [6] conducted in Mexico, reported that 28% of conjunctivitis patients were intermediate vancomycin resistance. Finally current study dealing with estimation and measurement of bacterial biofilm optical density (O.D.) by ELISA reader in all study groups to all staphylococcus isolates involving coagulase positive and negative staphylococci, which a tough, rigid layer of extracellular matrix glue substances containing polypeptide, proteins and lipids, capsular polysaccharide with other substances [8], these biomaterials responsible for attachment of bacteria to other surfaces, increase virulence of bacteria to invade host cells, prevent phagocytosis and increase resistance of bacteria many antimicrobial drugs [17]. The results of biofilm in this study revealed very thick and high density production of biofilm represented by *S.epidermidis* about 60% in conjunctivitis patients depending of cutoff point O.D. was (0.200), while the cutoff point of *S.aureus* isolates was (0.150) with only 10% were high biofilm producer isolates in the same patients group correlated with 55% and 10% strong biofilm formation by *S.aureus* isolates in conjunctivitis and

healthy eyes respectively, the reason for high production of biofilm particularly by *S.epidermidis* depends upon the highly pleomorphic phase variation of these bacteria and heterogeneous adhesion genes expression more than any bacteria even *S.aureus* like intercellular adhesion (*ica*) locus genes that responsible for formation of many biofilm adhesion genes to cause many problems in immunocompromized infected persons [18, 19], the biofilm results of this study agreed with study conducted in Poland by Rypuła et al. [20], who reported that more *S.epidermidis* isolates were high biofilm formers with > 1.296 optical density at 0.324 cutoff level, whereas the current study not identical with other study was conducted in China by Hou et al. [8], who reported that the major biofilm producer isolates were (51.90%) of *S.aureus* and only (28.13%) biofilm were *S.epidermidis* isolates.

## 6. Conclusion

In this study results we conclude that the healthy persons eyes inhabit a limited population of *Staphylococcus epidermidis* (20%) with no any colonization of *S.aureus* in conjunctiva of those healthy individuals, these low isolates not recognized by host innate immune system which considered as a part of normal microbial flora fight pathogens, in contrast to high population staphylococci mainly other opportunistic strains of *Staphylococcus epidermidis* (60%) that opportunists low immunity particularly in children and elderly to invade conjunctiva by strong attachment mechanism and adhesion genes with huge biofilm construction that finally become more antibiotics resistant and severe eye infections like conjunctivitis and keratitis.

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