Novel Research in Microbiology Journal (2022), 6(5): 1725-1741
Research Article

(Print) (ISSN 2537-0286)
(Online) (ISSN 2537-0294)
https://nrmj.journals.ekb.eg/
DOI: 10.21608/nrmj.2022.261973

Bacterial conjunctivitis: microbiological profile and molecular characterization of Methicillin-Resistant *Staphylococci* isolated from Minia governorate, Egypt

Fatma Ahmed Abdel Aziz[1]; Gamal Fadl Mahmoud Gad[1]; Ahmed Mohamed Kamal El Shafei[2]; Reham Ali Ibrahem[1*]

[1]Department of Microbiology and Immunology, Faculty of Pharmacy, Minia University, Minia 61111, Egypt; [2]Department of Ophthalmology, Faculty of Medicine, Minia University, Minia 61111, Egypt

[*]Corresponding author E-mail: Rehamee_micro@yahoo.com

Received: 24 August, 2022; Accepted: 27 September, 2022; Published online: 28 September, 2022

Abstract

Ocular infections caused by bacterial sources are a global health issue that can damage the construction of the eye, and lead to disability. The goals of this study were to look at the bacterial species causing conjunctivitis, as well as their antibacterial susceptibility patterns. In addition to emphasizing on detecting the predominance of certain virulence genes of Methicillin Resistant *Staphylococcus aureus* (MRSA), and Methicillin Resistant coagulase negative *Staphylococci* (MR-CoNS), which are known to cause conjunctivitis. In this study, several swabs of bacterial conjunctivitis were sampled from patients who attended to the Ophthalmology department, Minia University and Malawi Ophthalmology hospital, Egypt. A total of 200 eye swab samples were analyzed over the entire period of the study. Results showed that about 133 eye swab samples expressed growth of about 147 pathogenic bacterial spp. The predominant isolated bacteria were *Staphylococcus aureus* (44.89 %), followed by Coagulase negative *Staphylococci* (29.9 %). On the contrary, *Esherichia coli*, *Pseudomonas aeruginosa*, *Proteus* sp., *Streptococcus pneumonia*, *Klebsiella* sp., and *Haemophilus influenza*e, were the least detected bacterial spp. Most of the bacterial isolates tested in this study exhibited high resistance to Amoxacillin-Clavulanic, Sulfamethoxazole-trimethoprim, Cefotaxime, and Cefoperazone. Using Cefoxitin, results of the phenotypic test predicted that 40.9 % of the Staphylococcal spp. were MRSA, and 23.6 % were MR-CoNS. The Polymerase chain reaction (PCR) technique was used to explore the presence of several bacterial pathogenicity genes, including *MecA*, *PVL*, *icaA*, *icaD*, and *Hla* in the MRSA and in MR-CoNS. Results of the PCR revealed that all MRSA and MR-CoNS had *MecA*, *icaA*, and *icaD* genes, whereas 28.9 % of the MRSA had *PVL* and *Hla*. However, no isolate of MR-CoNS recorded the presence of the *PVL* or *HLa* genes. This study showed that prevalence of the bacterial eye conjunctivitis has increased with MRSA dominance. All the MRSA possessed at

least the *icaA*, and *icaD* virulence genes beside the *MecA* gene, which confirm their roles in the pathogenesis of conjunctivitis.

Keywords: Bacterial conjunctivitis, MRSA, MR-CoNS, Resistance, Virulence genes

1. Introduction

Eye is a sense organ that is significant for life. Conjunctivitis involves inflammation of the conjunctiva, which is the transparent mucous membrane that lines the eyelid and covers the sclera. Bacterial conjunctivitis is a common type of pink eye infection, caused by bacteria that infect the eye via various sources of contamination (Bartlett *et* al., 2011). The common pathogenic bacterial spp. that cause the eye conjunctivitis include, *Staphylococci* spp. mainly; *S. aureus* and Coagulase negative *Staphylococci* (CoNS), *Streptococcus pneumoniae* (*S. pneumoniae*), and *Haemophilus influenzae* (*H. influenzae*) (Thomas *et al*., 2019). *S. aureus* and CoNS are common pathogenic bacteria in different types of eye infections, and several previous studies confirmed that they are the major causes of those infections (Kupsik *et al*., 2019; Xu *et al*., 2021).

The pathogenic bacteria cause eye infections due to several factors, including bacterial virulence and/or host's defense mechanisms, which are attributable to different factors such as age, physiology, individual hygiene, nutrition, lifestyle, and socio-economic status (Teweldemedhin *et al*., 2017).

Fluoroquinolones, aminoglycosides, β-lactams, sulfonamides, and tetracyclines are the most frequent antibiotics used in the treatment of eye infections; however recently, an increase in the bacterial resistance rates has been recorded against these antibiotics (Grandi *et al*., 2021). Although, the first-line of treatment against the ocular infections is via the ophthalmic fluoroquinolones, but about 85 % of the MRSA are resistant to these antibiotics.

Identification of the methicillin-resistant *Staphylococci* is required to stop the abuse of antibiotics that causes rising of the staphylococcal resistance to methicillin, which accordingly increases the need for the use of other antibiotics (Bharathi *et al*., 2010). Validation of phenotypic identification of the MRSA *mecA* gene using PCR method has been confirmed previously by Mohammed *et al*., (2020).

The *MecA* gene, which is a DNA fragment known as the staphylococcal cassette chromosomal *mec*, is the gene that causes methicillin resistance (*SCCmec*). This gene produces a protein called penicillin-binding protein (PBP-2a), which prevents β-lactam antibiotics such as methicillin from working. Insertion sites of the other mobile genetic elements including the plasmids and transposons are present in the *mecA* gene complex, which makes it easier to acquire resistance genes for other antibiotics (Rasheed and Hussein, 2020).

Recently, Elkhashab *et al*., (2018) reported that initial involvement, innate virulence, cytotoxicity, avoidance of the host immune system, and antibacterial tolerance/ resistance facilitated by biofilm production, are all serious traits that provide potentials to the pathogenic *Staphylococci*'s to cause conjunctivitis.

Panton-Valentine leukocidin (PVL), which is a pore-forming toxin that causes lysis of the human leukocytes and death of tissues, represents one of these virulence factors that may play a significant role in some of the critical *S. aureus* infections, including conjunctivitis (Shallcross *et al*., 2010; Zaidi *et al*., 2013).

Depending on the ability of Staphylococci to produce and secret slime that is a material produced outside the cell, they possess the potential to form biofilms (An and Friedman, 1998). Slime is an

exopolysaccharide substance synthesized via the *ica* operon that is found in *icaA*, and *icaD* genes. Once activation of the *ica* operon has occurred, the polysaccharide intercedes the intercellular adherence leading to bacterial growth, and massive production of cell groups till forming the bacterial biofilms. Adhesion and biofilm formation are the main reasons of pathogenesis of the staphylococcal bacteria (Montanaro and Arciola, 2000).

One of the most powerful hemolysins produced by *S. aureus* is the alpha-toxin (*HLa*) gene, which acts as a pore-forming cytotoxin (PFT) that is active against a wide range of human cells. The dermonecrotic, hemolytic, and neurotoxic effects of this toxin cause its pathogenicity (Oliveira *et al*., 2018). Moreover, the role of this toxin has been reported in the eye defense system, including evasion and invasion of the eye tissues (Afzal *et al*., 2022). The objectives of the current work were to determine the spread and antibacterial susceptibility responses of the most common bacterial spp. that cause conjunctivitis, and to determine the best antibiotic therapy. In addition to focusing on certain virulence determinants associated with the Methicillin resistant staphylococcal spp. (which were not discussed before in our community), as they represent the most common bacterial pathogens that cause this type of eye infection.

2. Materials and methods

2.1. Isolation and identification of the pathogenic bacteria causing conjunctivitis

A total of 200 conjunctival swabs were collected by ophthalmologists from patients attending to the ophthalmology department at Minia University, and Malawi hospital of ophthalmology. About two swabs were taken from each patient. One of the 2 swabs was used for direct smear on a glass slide, stained by Gram stain, and then examined under a microscope. The other swab was directly inoculated onto the surface of different culture media, including Mannitol salt agar, blood agar, MacConkey agar, and chocolate agar, and then all plates were incubated aerobically at 37°C for 24-48 h. However, the inoculated chocolate agar plates were kept in candle jar extinction for providing an anaerobic condition. After incubation, all the plates were examined for the development of bacterial growth. The isolated bacterial colonies were purified, and then identified according to their morphological, microscopical, and biochemical characteristics (Benson, 2002; Yang *et al*., 2018). The bacterial isolates were preserved in 20 % glycerol vials at -70 °C, till further use.

2.2. Antibiotic sensitivity assay

The Kirby-Bauer Disk Diffusion assay was used to test the sensitivity of the bacterial isolates to 11 different antibiotics, in reference to Hudzicki, (2009). The tested bacterial isolates were prepared in dilutions equivalent to 0.5 McFarland standards (1.5×10^8 cells/ ml). Approximately 0.1 ml of each bacterial suspension was inoculated and then spread individually on the surface of two Muller Hinton (MH) agar plates for each tested bacterial sp. using a sterile cotton swab. Using a sterile forceps, about 4 antibiotic discs were placed individually on the surface of each plate, and allowed to air dry before being incubated at 37°C for 24 h. The used antibiotic discs, include Ciprofloxacin (5 µg), Tobramycin (10 µg), Cefoperazone (75 µg), Chloramphenicol (30 µg), Erythromycin (15 µg), Gentamicin (10 µg), Moxifloxacin (5 µg), Amoxacillin-clavulanic (20/ 10 µg), Fusidic acid (10 µg), Cefotaxim (30 µg), and Sulfamethoxazole-Trimethoprim (1.25/ 23.75 µg). Cefoxitin antibiotic disc (30 µg) was used to detect the Methicillin resistant Staphylococcal spp. All the used antibiotics discs were provided from Oxoid, Basingstoke, UK. After incubation, the inhibition zone diameter around each disc was measured using a calibrated ruler. The tested bacterial isolates were classified as sensitive or resistant according to the inhibition zones diameters of the Clinical Laboratory Standards Institute (Wayne, 2018).

2.3. DNA extraction and amplification of *MecA* gene and other virulence genes

The *MecA* gene and several other virulence genes, including *PVL*, *icaA*, *icaD*, and *HLa* of all the 71 Methicillin resistant Staphylococcal isolates (45 MRSA and 26 MR-CoNS) were detected using PCR assay. DNA extraction kit (Qiagen, Germany) was used to extract the DNA template from the tested bacterial isolates according to the instructions of the manufacturer's. The sequences of primers used in this study are listed in Table (1), whereas the conditions of processing the PCR products are recorded in Table (2). The annealing temperatures differed according to the type of the tested primers. The PCR assay was performed using 5 µl of template DNA in 25 µl reaction mixture. The PCR products of the amplified DNA were analyzed at 100-V for 30 min. through gel electrophoresis in 2 % agarose gel, stained with ethidium bromide, and finally visualized under a trans-illuminator. The band sizes were defined through comparison with a standard ladder of a 100 bp (Lee *et al*., 2012).

Table (1): List of the primers sequences used in this study

Virulence genes	Primer Sequence	References
MecA	F 5'AAA ATC GAT GGT AAA GGT TGGC-3' R 5'AGT TCT GGC ACT ACC GGA TTT TGC-3'	Kalorey *et al*., (2007)
PVL	F5'ATCATTAGGTAAAATGTCTGGACATGATCCA3' R 5'GCATCAAGTGTATTGGATAGCAAAAGC3'	McClure *et al*., (2006)
icaA	F 5'TCTCTTGCAGGAGCAATCAA-3' R 5'TCAGGCACTAACATCCAGCA-3'	Mckenney *et al*., (1999)
icaD	F 5'ATGGTCAAGCCCAGACAGAG-3' R 5'CGTGTTTTCAACATTTAATGCAA-3'	Mckenney *et al*., (1999)
Hla	F 5'CTGATTACTATCCAAGAAATTCGATTG3' R 5'CTTTCCAGCCTACTTTTTTATCAGT-3'	Jarraud *et al*., (2002)

Table (2): PCR conditions of amplifying the tested bacterial DNA, adopted according to the manufacturer's

Gene	Initial denaturation temp. (˚C)	Denaturation temp. (˚C)	Annealing temp. (˚C)	Extension temp. (˚C)	Final extension temp. (˚C)	Cycles	Product size (bp)
MecA	95 °C for 5 min.	95 °C for 45 sec	54 for 45 sec	72°C for 1 min.	72 °C for10 min.	40	533 bp
PVL	95 °C for 5 min.	95 °C for 45 sec	50 for 45 sec	72 °C for1 min.	72 °C for 10 min.	40	433 bp
icaA	95 °C for 5 min.	95 °C for 45 sec	52 for 45 sec	72 °C for 1min.	72 °C for10 min.	45	188 bp
icaD	95 °C for 5 min.	95 °C for 30 sec	54 for 30 sec	72 °C for1 min.	72 °C for10 min.	40	198 bp
Hla	95 °C for 5 min.	95 °C for 45 sec	58 for 45 sec	72 °C for1 min.	72 °C for10 min.	40	209 bp

2.4. Statistical analysis

Statistical analysis of the data was carried out using the IBM SPSS 26.0 statistical package software (IBM; Armonk, New York, USA). To compare the different categorical variables, the Chi square test or Fisher's exact test were used. A *p*-value less than 0.05 was considered as significant.

3. Results

3.1. Isolation and identification of the pathogenic bacteria that cause conjunctivitis

Out of the 200 collected swabs, about 133 (66.5 %) only yielded bacterial growth. A total of 147 bacterial isolates were recovered, and the majority of patients 119/ 133 (89.5 %) gave a single bacterial isolate, while only 14/ 133 (10.5 %) yielded mixed bacterial cultures. After morphological, microscopical, and biochemical identification of the purified cultures, the most commonly recovered bacteria were *S. aureus* (44.89 %), and CoNS (29.9 %). The remaining bacterial isolates, include *E. coli* (8.16 %), *P. aeruginosa*, *Proteus* sp. (4 %), *S. pneumonia* (3.4 %), whereas both *Klebsiella* sp. and *H. influenza* represented 2.72 %.

Patients enrolled in this study were divided into four age groups, which were: less than 1 year, 1-3 years old, 3-15 years old, and more than 15 years old. The highest frequency of bacterial isolates (29.2 %) was recorded in the age group from 3 - 15 years old, whereas the lowest frequency of isolates (18.4 %) was observed in the age group less than 1 year, as shown in Table (3). There was no statistical significance between the isolated bacteria and the different age groups ($p > 0.05$).

Throughout the year, the most predominant bacterial isolates that were recovered in the summer and the autumn were; *S. aureus* (43.9 % and 34.8 %), and CoNS (16.7 % and 66.7 %), respectively. Most the bacterial isolates were recorded at autumn (49.6 %), and the least bacterial isolates were observed at the spring (9.5 %).

Overall, there was significant correlation observed between the seasonal distribution and appearance of Staphylococci spp. only ($p < 0.05$), while the rest of bacterial isolates showed no such significant correlation. Results presented in Table (4) summarize the frequency of recovery of the bacterial isolates and their seasonal distribution.

3.2. Antibiotic resistance assay

Results of antibiotics resistance patterns of the different bacterial isolates recovered from the conjunctival swabs are demonstrated in Table (5). *S. aureus* expressed high resistance to both of Amoxacillin-Clavulanic and Sulfamethoxazole-trimethoprim (71.2 %), Cefotaxim (69.7 %), Cefoxitin (68.2 %), Cefoperazone (57.6 %). On the other hand, CoNS demonstrated resistance against Cefotaxim (75 %), Cefoperazone and Cefoxitin (59.1 %), and Sulfamethoxazole –trimethoprim (54.5 %).

The phenotypic assay carried out using Cefoxitin antibiotic had shown that 45 isolates (40.9 %) of *Staphylococci* spp. were MRSA, while 26 isolates (23.6 %) were MR-CoNS. *E. coli* showed low resistance to both of Chloramphenicol and Moxifloxacin (25 %); however, high resistance was observed against Cefotaxim (100 %). *P. aeruginosa* had low resistance to Cefotaxim (16.7 %). *Proteus* sp. exhibited low resistance to Erythromycin (16.7 %), and high resistance was observed against Cefotaxim (100 %). *S. pneumonia* had low resistance against Sulfamethoxazole-trimethoprim (20 %), and high resistance was recorded against Amoxacillin-Clavulanic (60 %). *Klebsiella* sp. expressed moderate resistance against Cefoperazone, Sulfamethoxazole-trimethoprim (50 %), and high resistance against Cefotaxim (75 %). Finally, *H. influenzae* had low resistance to Tobramycin (25 %).

Table (3): Distribution of the recovered bacterial isolates causing conjunctivitis in the different age groups

Bacteria **N (%)**	**Age groups**				***p** value**
	< 1 year N (%)	**1-3 years N (%)**	**3-15 years N (%)**	**>15 years N (%)**	
S. aureus 66 (44.89 %)	10(37.04)	16(39.02)	19(44.18)	21(58.33)	0.242
CoNS 44 (29.9 %)	11(40.74)	14(34.15)	12(27.91)	7(19.44)	0.242
E. coli 12 (8.16 %)	0(0)	6(14.63)	5(11.63)	1(2.78)	0.779
P.aeruginosa 6 (4 %)	2(7.41)	0(0)	1(2.33)	3(8.33)	0.607
Proteus sp. 6 (4)	3(11.11)	0(0)	2(4.65)	1(2.78)	0.607
S. pneumonia 5 (3.4 %)	0(0)	1(2.44)	1(2.33)	3(8.33)	0.449
Klebsiella sp. 4 (2.72 %)	0(0)	2(4.88)	2(4.65)	0(0)	-
H. influenzae 4 (2.72 %)	1(3.7)	2(4.88)	1(2.33)	0(0)	0.779
Total (147)	27(18.4)	41(27.9)	43(29.2)	36(24.5)	0.091

Where; N refers to the number of bacterial isolates. Results were analyzed using Chi square test or Fisher's exact test. **p* value < 0.05 is considered significant

Table (4): Frequency of recovery of the bacterial isolates with respect to seasonal distribution

Bacterial spp. **N (%)**	**Season**				***p** value**
	Spring N (%)	**Summer N (%)**	**Autumn N (%)**	**Winter N (%)**	
S. aureus 66 (44.89 %)	6 (42.86)	29(70.73)	23(31.51)	8(42.11)	0.028*
CoNS 44 (29.9 %)	6(42.85)	8(19.51)	28(38.36)	2 (10.53)	< 0.0001*
E. coli 12 (8.16 %)	0(0)	4(9.76)	5(6.85)	3(15.79)	-
P. aeruginosa 6 (4 %)	0(0)	0(0)	6(8.22)	0(0)	-
Proteus sp. 6 (4 %)	0(0)	0(0)	5(6.85)	1(5.26)	0.1

S. pneumonia 5 (3.4 %)	1(7.14)	0(0)	1(1.37)	3(15.79)	0.449
Klebsiella sp. 4 (2.72 %)	1(7.14)	0(0)	3(4.11)	0(0)	0.317
H. influenzae 4 (2.72 %)	0(0)	0(0)	2(2.74)	2(10.53)	-
Total (147)	14(9.5)	41(28)	73(49.6)	19(12.9)	-

Where; N refers to the number of bacterial isolates. Results were analyzed using Chi square test or Fisher's exact test. *p value < 0.05 is considered significant

Table (5): Resistance patterns of the bacterial isolates recovered from conjunctival swabs against 11 different antibiotics

Antibiotics	***S. aureus* (66)**	***CoNS* (44)**	***E. coli* (12)**	***P. aeruginosa* (6)**	***Proteus* sp. (6)**	***S. pneumonia* (5)**	***Klebsiella* sp. (4)**	***H. influenzae* (4)**
	N (%)*	**N (%)***	**N (%)***	**N (%)***	**N(%)***	**N (%)***	**N (%)***	**N (%)***
Ciprofloxacin (5 µg)	15(22.7)	5(11.4)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Tobramycin (10 µg)	7(10.6)	1(2.3)	0(0)	0(0)	0(0)	0(0)	0(0)	1(25)
Cefoperazone (7 5µg)	38(57.6)	26(59.1)	0(0)	3(50)	2(33.3)	0(0)	2(50)	0(0)
Chloramphenicol (30 µg)	4(6.1)	0(0)	3(25)	0(0)	0(0)	Not tested	0(0)	0(0)
Erythromycin (15 µg)	16(24.2)	5(11.4)	0(0)	0(0)	1(16.7)	0(0)	0(0)	Not tested
Gentamicin (10 µg)	2(3)	1(2.3)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Moxifloxacin (5 µg)	21(31.8)	11(25)	3(25)	0(0)	0(0)	0(0)	0(0)	0(0)
Amoxacillin-clavulanic (20/10 µg)	47(71.2)	21(47.7)	0(0)	3(50)	2(33.3)	3(60)	3(75)	0(0)
Fusidic acid (10 µg)	18(27.3)	12(27.3)	0(0)	0(0)	0(0)	Not tested	0(0)	Not tested
Cefotaxim (3 0µg)	46(69.7)	33(75)	12(100)	1(16.7)	6(100)	0(0)	3(75)	0(0)
Sulfamethoxazole-Trimethoprim (1.25/23.75 µg)	47(71.2)	24(54.5)	6(50)	2(33.3)	3(50)	1(20)	2(50)	0(0)

Where; N refers to the number of bacterial isolates, *Percentage was correlated to total number of each bacterial isolate

3.3. Antibiotic resistance among MR-Staphylococcal isolates

Table (6) shows the MRSA and MR-CoNS resistance patterns against the different antibiotics. The MRSA isolates were highly resistant to Amoxacillin-clavulanic and Cefotaxim (57.8 %); however they had low resistance to Gentamicin (4.4 %). Meanwhile, the MR-CoNS isolates exhibited high resistant to Cefoperazone (96.1 %).

Table (6): *In vitro* resistance pattern of the MRSA and MR-CoNS isolates recovered from the conjunctival swabs against 11 different antibiotics

Antibiotics	**MRSA (45)**	**MR-CoNS (26)**
	N (%)*	**N (%)***
Ciprofloxacin (5 µg)	15(33.3)	4(15.4)
Tobramycin (10 µg)	7(15.5)	1(3.8)
Cefoperazone (75 µg)	18(40)	25(96.1)
Chloramphenicol (30 µg)	4(8.9)	0(0)
Erythromycin (15 µg)	14(31.1)	4(15.4)
Gentamicin (10 µg)	2(4.4)	1(3.8)
Moxifloxacin (5 µg)	17(37.8)	11(42.3)
Amoxacillin-clavulanic (20/10 µg)	26(57.8)	17(65.4)
Fusidic acid (10 µg)	14(31.1)	10(38.5)
Cefotaxim (30 µg)	26(57.8)	20(76.9)
Sulfamethoxazole-Trimethoprim (1.25/23.75 µg)	23(51.1)	22(84.6)

Where; N refers to the number of bacterial isolates, *represent the resistance percentage correlated to total number of each bacterial isolate

3.4. Detection of virulence genes using PCR

All the MR Staphylococcal 71 isolates were tested for existence of the *MecA*, *PVL*, *ica*A, *icaD*, and *HLa* virulence genes. PCR results showed that *MecA*, *IcaA*, and *IcaD* were recorded by 100 % in both of MRSA and MR-CoNS isolates. The *PVL* and *HLa* genes were observed in 13(28.9 %) of the MRSA isolates only; however, no MR-CoNS isolate possessed neither *PVL* nor *Hla* genes, as presented in Table (7).

The *MecA*, *icaA*, and *icaD* genes were detected together in 32 MRSA isolates (71.1%), while the *MecA* gene and all the other studied virulence genes (i.e., *PVL*, *icaA*, *icaD*, and *Hla*) coexisted together in 13 (28.9 %) of the MRSA isolates. On the other hand, all the MR-CoNS isolates recorded the existence of *MecA* gene together with the *icaA* and *icaD* genes only with absence of the *PVL* and *Hla* genes.

Using PCR assay, the bands recovered from the MRSA and MR-CoNS isolates on the agarose gel for detecting the existence of *MecA*, *PVL*, *icaA*, *icaD*, and *Hla* genes are illustrated in Fig. 1 (a-e).

Table (7): Frequencies of detection of the *MecA* and several virulence genes among the MRSA and MR-CoNS isolates

Virulence genes	MRSA (45) N (%)*		MR-CoNS (26) N (%)*	
	Present	**Absent**	**Present**	**Absent**
MecA	45(100)	0(0)	26(100)	0(0)
PVL	13(28.9)	32(71.1)	0(0)	26(100)
icaA	45(100)	0(0)	26(100)	0(0)
icaD	45(100)	0(0)	26(100)	0(0)
Hla	13(28.9)	32(71.1)	0(0)	26(100)
Hla	13(28.9)	32(71.1)	0(0)	26(100)

Where, N refers to the number of bacterial isolates containing virulence gene. * Percentage of gene detection correlated to the total number of each bacterial isolate

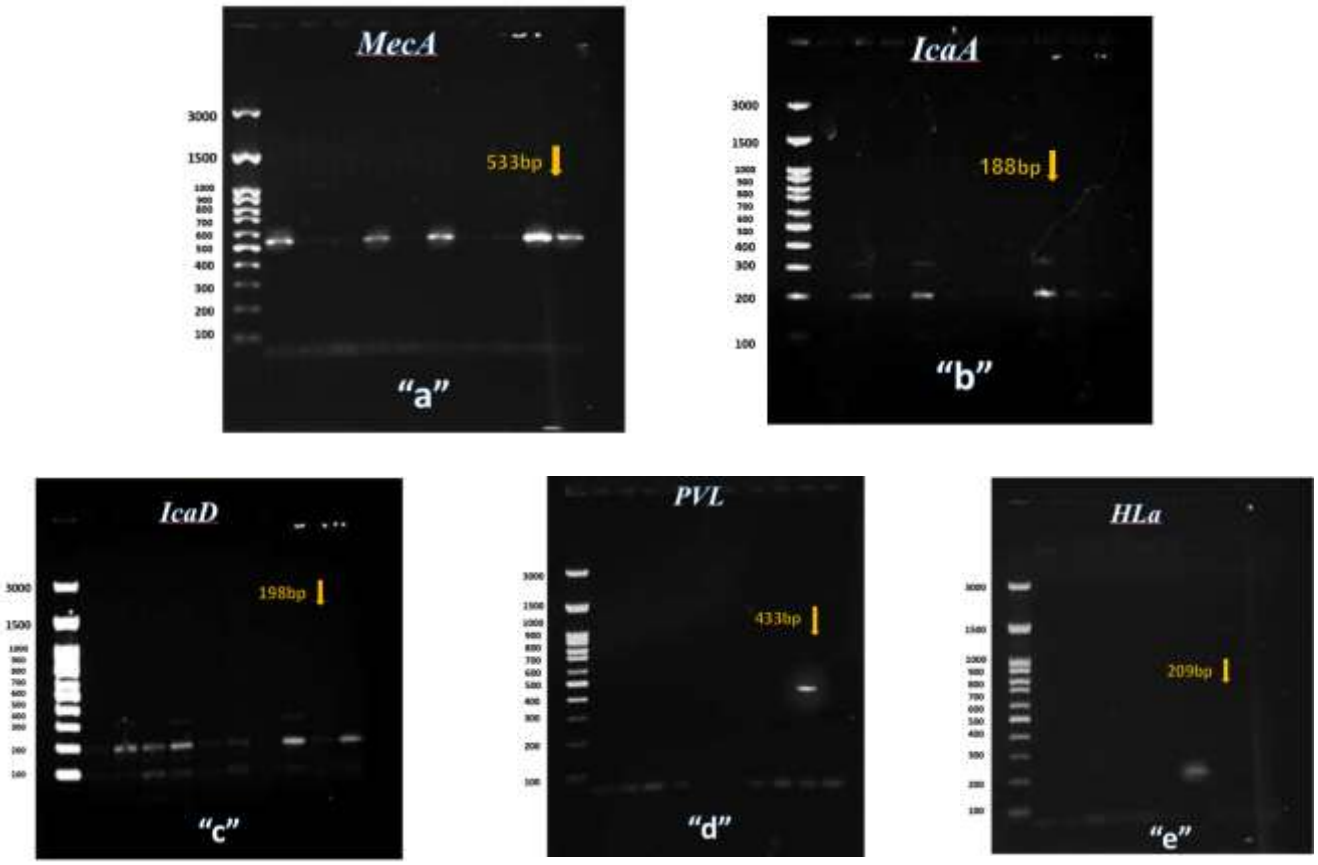

Fig. 1: Amplification product of the different virulence genes in MRSA and MR-CoNS isolates; where (a) *MecA* gene with detected bands at 533 bp in both MRSA and MR-CoNS, (b) *IcaA* gene detected bands at 188 bp in both MRSA and MR-CoNS, (c) *IcaD* gene with recovered bands at 198 bp in both MRSA and MR-CoNS, (d) *PVL* gene band at 433 bp observed in MRSA only, and (e) *HLa* gene band at 209 bp detected in MRSA only

4. Discussion

One of the popular ophthalmic infections often observed by the clinical practitioners is the bacterial conjunctivitis (Ni *et al*., 2015; Grzybowski *et al*., 2017). This disease affects all ages causing serious health and economic problems worldwide (Schneider *et al*., 2014; Chen and Yang, 2019).

In this study, the frequency of conjunctival samples that yielded positive bacterial growth was 66.5%. This result is similar to the previous study conducted in El- Khartoum state, Sudan, by Ahmed and Hamdan, (2016), as its positive results were 78 %, but is incompatible with the study carried out in Baghdad by Rahama *et al*., (2017), which showed high positive results (97 %). On the other hand, Nuhu and Usman, (2021) reported that 30.9 % only of the collected conjunctival specimens yielded bacterial growth. These variations in results may be attributed to the geographical, culture, and socioeconomic variations among the different countries.

Currently, about 89.5 % of the conjunctival swabs had a single bacterial isolate, while only 10.5 % showed the existence of mixed bacterial cultures. This result agree with the previous study of Mahmoud *et al*., (2003) that was performed on the outpatient clinic of ophthalmology department at El-Minia University hospital, Egypt, which recorded mixed bacterial cultures in 8.5 % of the specimens. However, this result was slightly lower than the results revealed by Bhattacharyya *et al*., (2020) in Assam medical college hospital (India), as they recorded a pure single culture by 80 %, and mixed bacterial cultures by 20 %.

In this study, *S. aureus* (44.89 %) was the commonly recovered bacterial sp., followed by CoNS (29.9 %). Meanwhile, *E. coli* (8.16 %), *P. aeruginosa* and *Proteus* sp. (each 4 %), *S. pneumonia* (3.4 %), and both of *Klebsiella* sp. and *H. influenza*e (2.72 % each), were isolated less frequently. These findings are consistent with the previous studies conducted by Mahmoud *et al*., (2003); Shaeri *et al*., (2020); Tang *et al*., (2022), who found that *S. aureus* had the highest incidence among the bacterial spp. recovered from several cases of acute bacterial conjunctivitis by 44.1 %, 28.2 %, and 29 %, respectively. Bhattacharyya *et al*., (2020); Tang *et al*., (2022) obtained similar results in accordance to the current study recording the CoNS isolates (29.1 % and 27 %, respectively), but in contrast to the results of Oikonomakou *et al*., (2019); Woreta and Kebdo, (2022), who recorded CoNS as the predominant isolates in their studies. These variations may be attributed to the difference among the countries, and to the difference in climates.

Despite that conjunctivitis is very common in young persons and disappears stepwise at puberty, and the etiology of bacterial conjunctivitis is more common in older children (Teoh and Reynolds, 2003); however, in the current study, the high frequency of bacterial isolates was recorded the age group from 3-15 years old. No significance was observed between the bacterial spp. and the different age groups, and also no significance was observed between the same age group and the different bacterial spp. These results are in agreement with the previous works of Oikonomakou *et al*., (2019); Shaeri *et al*., (2020). On the contrary, Ahmed and Hamdan, (2016) in Khartoum, Allwaldin eye hospitals, Sudan, reported statistically significant age frequency.

In this study, the highest conjunctival infections were observed at Autumn (49.6 %) followed by the Summer (28 %), whereas the lowest were at spring (9.5 %). The most predominant bacterial spp. that were isolated in the summer and the autumn were *Staphylococci* spp. Significant correlation was detected between the seasonal distribution and appearance of *Staphylococci* spp. only ($p < 0.05$), however the rest of the bacterial spp. did not show

such significant correlation. These results are inconsistent with the previous results of Bhattacharyya *et al*., (2020), who reported maximum number of conjunctival infections in the summer, in addition to Agarwal *et al*., (1967) who previously documented the high frequency of bacterial conjunctivitis during the summer, which decreased suddenly in autumn and remained low during the winter. These variations might be attributed to the differences in weather, socioeconomic status, and hygienic conditions worldwide.

In agreement with the previous results of Muluye *et al*., (2014), most of the current bacterial spp. showed high resistance to Amoxacillin-Clavulanic, Sulfamethoxazole-trimethoprim, Cefotaxim, and Cefoperazone. Using Cefoxitin disk (30 µg), the phenotypic assay was used to identify the MR Staphylococcal spp. Results revealed that 71 of the isolates were MR Staphylococcal spp., including 40.9 % of MRSA and 23.6 % of MR-CoNS. Similarly, Thomas *et al*., (2019) in the United States highlighted that 36.6 % of the *Staphylococci* were MRSA that is close to the current results, whereas the MR-CoNS isolates were 48.6 %, which were extremely higher than this study.

The MRSA isolates showed high resistance to Amoxacillin-clavulanic and Cefotaxim (57.8 %), and low resistance to Gentamicin (4.4 %). On the other hand, the isolates of MR-CoNS were highly resistant to Cefoperazone (96.1 %), and all the isolates were sensitive to Chloramphenicol. Thomas *et al*., (2019) agreed with these findings in decreased resistance of the MRSA to the Aminoglycosides, and in decreased resistance of MR-CoNS to Chloramphenicol (1.2 %).

All the MRSA and MRCoNS isolates were selected to examine the existence of the *MecA* gene and several other virulence genes in them. These genes are supposed to be responsible for the pathogenesis of these Staphyloccocal isolates (Gordon and Lowy, 2008).

Results of this study revealed that all the MRSA and MR-CoNS isolates possessed the *MecA* gene. Similarly, previous results of Zahan *et al*., (2009); Rahama et al., (2017) were close to the current study as 85.37 %, 81 % of the MRSA were positive for *MecA*; respectively. In addition, Farina and Samudio, (2017) study detected the *MecA* gene in 70 % of the *S. epidermidis* isolates. On the contrary, García-Álvarez *et al*., (2011) reported the absence of *MecA* gene in the MRSA strains, while Lee *et al*., (2006) documented in their research that few isolates of *Staphyloccci* were positive for *MecA* (36.4 %).

Recently, Afzal *et al*., (2022) revealed that 46 % of the MRSA had the *MecA* gene. In all these previous studies, although most of the MRSA isolates were phenotypically resistant to Oxacillin; however, they did not possess the *MecA* gene. This may be attributed to a mutation in the *MecA* gene that is not reported in the current study, as all MRSA and MR-CoNS possessed this gene. On the other hand, the *ica* operon carries four genes encoding for proteins necessary for production of the polysaccharide intercellular adhesion (PIA). These *icaA* and *icaD* are the major genes in the structure of the exopolysaccharide and biofilm formation (Arciola *et al*., 2015). The ability of *Staphylococci* to be adhering to the artificial surfaces in addition to the living cells, and their abilities to produce biofilm structures are important features that influence the development of conjunctivitis. Adhesion to surfaces is a critical first step in pathogenesis of the *Staphylococci*. The conjunctiva and the area around the eyeball contain components that promote the bacterial growth, particularly those bacteria that produce massive amounts of external protein substances. Adhesion and formation of biofilm structures are key factors, which influence the chronic and even the weeks-long nature of conjunctivitis (Josse *et al*., 2017). Accordingly, great interests were given to detect the existence of certain

virulence genes that contribute to this step of pathogenesis.

The current PCR results showed that *MecA*, *icaA*, and *icaD* genes were detected by 100 % in both of the MRSA and MR-CoNS isolates, and these findings suggest that the strains of *Staphylococci* that were currently involved in the etiology of conjunctivitis had nearly the same ability to form biofilm. This was typically recorded previously by Arciola *et al*., (2001), who reported that all the staphylococcal strains were positive for both of *icaA*, and *icaD* genes. However, Satorres and Alcaráz, (2007) found 35.2 % of *S. aureus* and 48.4 % of *S. epidermidis* were both positive for *icaA* and *icaD* genes. Recently, Jasińska *et al*., (2021) disagreed with this result, as they reported that the *ica* genes were detected in 26.9 % of the coagulase negative Staphylococci and in 42.3 % of the *S. aureus* isolates.

Another virulence factor of the *Staphylococci* is the two-component toxin named PVL (Panton-Valentine leukocidin), which stimulates the leukocytolysis (Rasigade *et al*., 2010), and participates in the occurrence of conjunctivitis (Zaidi *et al*., 2013). A previous study conducted by Melles *et al*., (2006) documented that possession of *PVL* gene is extremely linked with the community-acquired MRSA. In the current study, a lower proportion of the strains possessed the *PVL* gene (28.9 % of MRSA), which was totally absent in the MR-CoNS isolates, suggesting that this toxin more likely has not been acquired in the community. These findings were compatible with the previous results of Rahama *et al*., (2017), who reported that 31.71 % of the MRSA isolates carried the *PV*L gene. Vandenesch *et al*., (2003) revealed that all the *S. aureus* isolates contained the *PVL* locus, while AL-Bakri *et al*., (2013) highlighted that only two MRSA isolates hardboard this *PVL* gene; however, 54.2 % of the MR-CoNS possessed this gene. On contrary to this study, Ali *et al*., (2012) reported that the *PVL* gene in MRSA isolates was detected in lower percentage in several countries, including UK (4.9 %), France (less than 5 %), and in Saudi Arabia (8.1 %).

The staphylococcal alpha-toxin (Hla) has been observed to be connected with the corneal epithelial wound healing, and to support the bacterial pathogens invasion within the inner surface of the cornea, thus globally causing ocular tissue destruction and inflammation (Astley *et al*., 2019). In this study, the *Hla* gene was detected only in MRSA by 28.9 %, and was absent in MR-CoNS. Such current results differ from those obtained by Astley *et al*., (2019), who reported the occurrence of *Hla* gene in all *S. aureus* isolated from the ocular infections in USA, and differ also from the results recorded by Nasaj *et al*., (2020), who detected the *Hla* gene in 87.9 % of the MRSA.

In this study, the MR-CoNS isolates were less aggressive than the MRSA, as the MR-CoNS isolates possessed neither the *PVL* nor the *Hla* gene. The *MecA* gene and all the other studied virulence genes currently coexisted together in 28.9 % of the bacterial isolates.

Conclusion

Data from this study revealed that different bacterial spp. were the causative agents of bacterial conjunctivitis, but the most common bacterial sp. was the *Staphylococci*, where most of them were MRSA and MR-CoNS. Moreover, antibiotic resistance persisted in high pattern among the conjunctival bacterial isolates, and thus there is an urgent need for implementation of the antibiotic surveillance programs in the hospitals. All the MR *Staphylococci* possessed at least the *icaA* and *icaD* virulence genes beside the *MecA* gene, which confirms their roles in pathogenesis of the bacterial conjunctivitis. Moreover, low proportion (%) of the isolates harbored the *PVL* and *Hla* genes, which might increase their aggressiveness.

Acknowledgement

None.

Conflict of interest

The authors declare no conflict of interests.

Funding source

The current study did not receive fund from any organization.

Ethical approval

This research was carried out in accordance with the ethical principles. The approval code of the Faculty of Pharmacy, Minia University Ethical Committee was provided (HV30/2019). The patient's consents and statement of protection of the patient's privacy are provided.

5. References

Afzal, M.; Vijay, A.K.; Stapleton, F. and Willcox, M. (2022). Virulence Genes of *Staphylococcus aureus* Associated With Keratitis, Conjunctivitis, and Contact Lens-Associated Inflammation. Transationl Vision Science and Technology. 11(7): 5. https://doi.org/10.1167/tvst.11.7.5

Agarwal, H.; Raichaudhury, M. and Munsi, N. (1967). Bacterial flora in acute conjunctivitis. Indian Journal of Ophthalmology 15(2): 58-66. https://doi.org/10.1128%2Fjcm.30.5.1156-1161.1992

Ahmed, O.B. and Hamdan, E.M. (2016). Profile of bacterial conjunctivitis in Sudan. Scholars Journal of Applied Medical Sciences. 4(4B): 1217-1221.

Al-Bakri, A.G.; Al-Hadithi, H.; Kasabri, V.; Othman, G.; Kriegeskorte, A. and Becker, K. (2013). The epidemiology and molecular characterization of methicillin-resistant *Staphylococci* sampled from a healthy Jordanian population. Epidemiology and Infection. 141(11): 2384-2391. https://doi.org/10.1017/s0950268813000010

Ali, M.A.M.; Alaa, H.A.C.; Fadhil, H.N. and Miaad, K.A.K. (2012). Community-acquired methicillin-resistant *Staphylococcus aureus* carrying *mecA* and Panton-Valentine leukocidin (*PVL*) genes isolated from the holy shrine in Najaf, Iraq. African Journal of Bacteriology Research 4(2): 15-23. https://doi.org/10.5897/JBR12.005

An, Y.H. and Friedman, R.J. (1998). Concise review of mechanisms of bacterial adhesion to biomaterial surfaces. Journal of Biomedical Materials Research. 43(3): 338-348.

Arciola, C.R.; Campoccia, D.; Ravaioli, S. and Montanaro, L. (2015). Polysaccharide intercellular adhesin in biofilm: structural and regulatory aspects. Frontiers in Cellular and Infection Microbiology. 5: 7. https://doi.org/10.3389/fcimb.2015.00007

Arciola, C.R.; Baldassarri, L. and Montanaro, L. (2001). Presence of *icaA* and *icaD* genes and slime production in a collection of staphylococcal strains from catheter-associated infections. Journal of Clinical Microbiology. 39(6): 2151-2156. https://doi.org/10.1128%2FJCM.39.6.2151-2156.2001

Astley, R.; Miller, F.C.; Mursalin, M.H. and Coburn, P.S. (2019). An Eye on *Staphylococcus aureus* Toxins: Roles in Ocular Damage and Inflammation. Toxins. 11(6):356. https://doi.org/10.3390/toxins11060356

Bartlett, J.D.; Karpecki, P.M.; Melton, R. and Thomas, R.K. (2011). Diagnosis and treatment algorithms for ocular surface disease states: keratitis part four of an ongoing series new paradigms in the understanding and management of keratitis. Review of Optometry. 148(11)**:** /S1+.

Benson, H.J. (2002). Microbiological Applications: Laboratory Manual in General Microbiology, 8th Edition, McGraw Hill, New York. 4: 1-478.

Bharathi, M.J.; Ramakrishnan, R.; Shivakumar, C.; Meenakshi, R. and Lionalraj, D. (2010). Etiology and antibacterial susceptibility pattern of community-acquired bacterial ocular infections in a tertiary eye care hospital in south India. Indian Journal of Ophthalmology. 58(6): 497-507. https://doi.org/10.4103%2F0301-4738.71678

Bhattacharyya, A.; Sarma, P.; Sarma, B.; Kumar, S.; Gogoi, T. and Kaur, H. (2020). Bacteriological pattern and their correlation with complications in culture positive cases of acute bacterial conjunctivitis in a tertiary care hospital of upper Assam: A cross sectional study. Medicine. 99(7): e18570. https://doi.org/10.1097%2FMD.0000000000018570

Chen, R. and Yang, J. (2019). Global Associations of Air Pollution and Conjunctivitis Diseases: A Systematic Review and Meta-Analysis. International Journal of Environmental Research and Public Health . 16(19): 3652. https://doi.org/10.3390/ijerph16193652

Elkhashab, T.H.T.; Adel, L.A.; Nour, M.S.; Mahran, M. and Elkaffas, M. (2018). Association of intercellular adhesion gene A with biofilm formation in *Staphylococci* isolates from patients with conjunctivitis. Journal of Laboratory Physicians. 10(3): 309-315. https://doi.org/10.4103/jlp.jlp_122_17

Fariña, N. and Samudio, M. (2017). Methicillin resistance and biofilm production of *Staphylococcus epidermidis* isolates from infectious and normal flora conjunctiva. International Ophthalmology. 37(4): 819-825. https://doi.org/10.1007/s10792-016-0339-8

García-Álvarez, L.; Holden, M.T.; Lindsay, H.; Webb, C.R.; Brown, D.F.; Curran, M.D. et al. (2011). Methicillin-resistant *Staphylococcus aureus* with a novel *mecA* homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infectious Diseases .11(8): 595-603. https://doi.org/10.1016/S1473-3099(11)70126-8

Gordon, R.J. and Lowy, F.D. (2008). Pathogenesis of methicillin-resistant *Staphylococcus aureus* infection. Clinical infectious diseases. 46(Supplement_5): S350-S359. https://doi.org/10.1086/533591

Grandi, G.; Bianco, G.; Boattini, M.; Scalabrin, S.; Iannaccone, M.; Fea, A. et al. (2021). Bacterial etiology and antimicrobial resistance trends inocular infections: A 30-year study, Turin area, Italy. European Journal of Ophthalmology .31(2): 405-414. https://doi.org/10.1177/1120672119896419

Grzybowski, A.; Brona, P. and Kim, S.J. (2017). Microbial flora and resistance in ophthalmology: a review. Graefe's Archive for Clinical and Experimental Ophthalmology. 255(5): 851-862. https://doi.org/10.1007/s00417-017-3608-y

Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. American Society for Microbiology. 15: 55-63. https://doi.org/10.3389/fmicb.2017.02433

Jarraud, S.; Mougel, C.; Thioulouse, J.; Lina, G.; Meugnier, H.; Forey, F. et al. (2002). Relationships between *Staphylococcus aureus* genetic background, virulence factors, age groups (alleles), and human disease. Infection and Immunity. 70(2): 631-641. https://doi.org/10.1128/IAI.70.2.631-641.2002

Jasińska, E.; Bogut, A.; Magryś, A. and Olender, A. (2021). Evaluation of the role of *Staphylococci* in the pathomechanism of conjunctivitis. International Ophthalmology. 41(7): 2585-2600. https://doi.org/10.1007/s10792-021-01818-w

Josse, J.; Laurent, F. and Diot, A. (2017). Staphylococcal Adhesion and Host Cell Invasion: Fibronectin-Binding and Other Mechanisms. Frontiers in Microbiology. 8: 2433. https://doi.org/10.3389/fmicb.2017.02433

Kalorey, D.R.; Shanmugam, Y.; Kurkure, N.V.; Chousalkar, K.K. and Barbuddhe, S.B. (2007). PCR-based detection of genes encoding virulence determinants in *Staphylococcus aureus* from bovine subclinical mastitis cases. Journal of Veterinary Science. 8(2): 151-154. https://doi.org/10.4142%2Fjvs.2007.8.2.151

Kupsik, M.; Sulo, S.; Katz, A. and Memmel, H. (2019). What do women really think? Patient understanding of breast cancer risk. Breast Journal. 25(6): 1320-1322. https://doi.org/10.1111/tbj.13472

Lee, P.Y.; Costumbrado, J.; Hsu, C.Y. and Kim, Y.H. (2012). Agarose gel electrophoresis for the separation of DNA fragments. Journal of Visualized Experiments. (62):3923. https://doi.org/10.3791%2F3923

Lee, D.G.; Urbach, J.M.; Wu, G.; Liberati, N.T.; Feinbaum, R.L.; Miyata, S. et al. (2006). Genomic analysis reveals that *Pseudomonas aeruginosa* virulence is combinatorial. Genome Biology. 7(10): R90. https://doi.org/10.1186/gb-2006-7-10-r90

Mahmoud, S.M.; Amgad, I.A.O.; Saher, A.A.E. and Noha, A.H. (2003). A study of acute bacterial conjuntivitis in children in Minia governorate. El-Minia Medical Bulletin. 14 (2): 249-260.

McClure, J.A.; Conly, J.M.; Lau, V.; Elsayed, S.; Louie, T.; Hutchins, T. et al. (2006). Novel multiplex PCR assay for detection of the staphylococcal virulence marker Panton-Valentine leukocidin genes and simultaneous discrimination of methicillin-susceptible from-resistant *Staphylococci*. Journal of Clinical Microbiology. 44(3): 1141-1144. https://doi.org/10.1128/JCM.44.3.1141-1144.2006

Mckenney, D.; Pouliot, K.L.; Wang, Y.; Murthy, V.; Ulrich, M.; Doring, G.; Lee, J.C.; Goldmann, D.A. and Pier, G.B. (1999). Broadly protective vaccine for *Staphylococcus aureus* based on an *in vivo*-expressed antigen. Science. 284(5419): 1523-1527. https://doi.org/10.1126/science.284.5419.1523

Melles, D.C.; van Leeuwen, W.B.; Boelens, H.A.; Peeters, J.K.; Verbrugh, H.A. and van Belkum, A. (2006). Panton-Valentine leukocidin genes in *Staphylococcus aureus*. Emerging Infectious Diseases. 12(7): 1174-1175. https://doi.org/10.3201%2Feid1207.050865

Mohammed, N.A.; Omer, A.F.A. and Saeed, M.I. (2020). Molecular Characterization of Methicillin Resistant *Staphylococcus aureus* Isolates from Sudanese Conjunctivitis Patients. Biomedical Journal of Scientific Technical Research. 31(2): 24048-24053. http://dx.doi.org/10.26717/BJSTR.2020.31.005072

Montanaro, L. and Arciola, C.R. (2000). Studying bacterial adhesion to irregular or porous surfaces. Handbook of Bacterial Adhesion, Springer**.** pp. 331-343.

Muluye, D.; Wondimeneh, Y.; Moges, F.; Nega, T. and Ferede, G. (2014). Types and drug susceptibility patterns of bacterial isolates from eye discharge samples at Gondar University Hospital, Northwest Ethiopia. BMC Research Notes. 7(1): 1-5. https://doi.org/10.1186/1756-0500-7-292

Nasaj, M.; Saeidi, Z.; Asghari, B.; Roshanaei, G. and Arabestani, M.R. (2020). Identification of hemolysin encoding genes and their association with antimicrobial resistance pattern among clinical isolates of coagulase-negative *Staphylococci*. BMC Research Notes. 13(1): 68. https://doi.org/10.1186/s13104-020-4938-0

Ni, N.; Nam, E.M.; Hammersmith, K.M.; Nagra, P.K.; Azari, A.A.; Leiby, B.E. et al. (2015).

Seasonal, geographic, and antimicrobial resistance patterns in microbial keratitis: 4-year experience in eastern Pennsylvania. Cornea. 34(3): 296-302. https://doi.org/10.1097/ico.0000000000000352

Nuhu, A. and Usman, A. (2021). Bacterial Conjunctivitis among Patients of Different Age Groups Attending Some Eye Clinics in Sokoto, Nigeria. Himalayan Journal of Applied Medical Sciences and Research. 2(5):161-165.

Oikonomakou, M.Z.; Makri, O.E.; Panoutsou, E.; Kagkelaris, K.; Plotas, P.; Garatziotou, D. et al. (2019). Bacteriology and Antimicrobial Susceptibility Patterns of Childhood Acute Bacterial Conjunctivitis in Western Greece. Medical Hypothesis, Discovery, Innovation Ophthalmology Journal. 8(4): 266-271.

Oliveira, D.; Borges, A. and Simões, M. (2018). *Staphylococcus aureus* toxins and their molecular activity in infectious diseases. Toxins. 10(6): 252. https://doi.org/10.3390/toxins10060252

Rahama, H.A.; Ali, Q.A.N. and Mustafa, A.A. (2017). Molecular Study of Most Common Pathogenic Bacteria Isolated From Conjunctivitis Patients In Baghdad. Al-Kufa University Journal for Biology 9(3).

Rasheed, N.A. and Hussein N.R. (2020). Methicillin-resistant *Staphylococcus aureus* carriage rate and molecular characterization of the staphylococcal cassette chromosome mec among Syrian refugees in Iraq. International Journal of Infectious Diseases. 91: 218-222. https://doi.org/10.1016/j.ijid.2019.12.006

Rasigade, J.P.; Laurent, F.; Lina, G.; Meugnier, H.; Bes, M.; Vandenesch, F. et al. (2010). Global distribution and evolution of Panton-Valentine leukocidin positive methicillin-susceptible *Staphylococcus aureus*, 1981-2007. Journal of Infectious Diseases. 201(10):1589-1597. https://doi.org/10.1086/652008

Satorres, S.E. and Alcaráz, L.E. (2007). Prevalence of *icaA* and *icaD* genes in *Staphylococcus aureus* and *Staphylococcus epidermidis* strains isolated from patients and hospital staff. Central European Journal of Public Health. 15(2): 87-90. https://doi.org/10.21101/cejph.a3396

Schneider, J.E.; Scheibling, C.M.; Segall, D.; Sambursky, R.; Ohsfeldt, R.L. and Lovejoy, L. (2014). Epidemiology and economic burden of conjunctivitis: a managed care perspective. Journal of Managed Care Medicine. 17(1): 78-83.

Shaeri, M.; Nazari-Alam, A.; Fathizadeh, H.; Moniri, R.; Akbari, H.; Mansoori, M. et al. (2020). Bacterial Etiology and Antibiotic Susceptibility of Conjunctivitis Patients' Isolates in Kashan, Iran. Advanced Biomedical Research. 9: 49. https://doi.org/10.4103%2Fabr.abr_118_20

Shallcross, L.J.; Williams, K.; Hopkins, S.; Aldridge, R.W.; Johnson, A.M. and Hayward, A.C. (2010). Panton-Valentine leukocidin associated staphylococcal disease: a cross-sectional study at a London hospital, England. Clinical Microbiology and Infection .16(11): 1644-1648. https://doi.org/10.1111/j.1469-0691.2010.03153.x

Tang, X.J.; He, J.T.; Liu, Q.; Chen, X.K. and Chen, L. (2022). Severe ophthalmia neonatorum in Southwest China: a 5-year review of demographics, microbiological results, and risk factors. International Ophthalmology .42(7): 2185-2193. https://doi.org/10.1007/s10792-022-02218-4

Teoh, D.L. and Reynolds, S. (2003). Diagnosis and management of pediatric conjunctivitis. Pediatric Emergency Care. 19(1): 48-55. https://doi.org/10.1097/00006565-200302000-00014

Teweldemedhin, M.; Gebreyesus, H.; Atsbaha, A.H.; Asgedom, S.W. and Saravanan, M. (2017). Bacterial profile of ocular infections: a systematic review. BMC Ophthalmology. 17(1): 212. https://doi.org/10.1186/s12886-017-0612-2

Thomas, R.K.; Melton, R. and Asbell, P.A. (2019). Antibiotic resistance among ocular pathogens: current trends from the ARMOR surveillance study (2009-2016). Clinical Optometry (Auckl). 11: 15-26. https://doi.org/10.2147%2FOPTO.S189115

Vandenesch, F.; Naimi, T.; Enright, M.C.; Lina, G.; Nimmo, G.R. et al. (2003). Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerging Infectious Diseases. 9(8): 978-984. https://doi.org/10.3201/eid0908.030089

Wayne, P. (2018). CLSI. Performance Standards for Antimicrobial Susceptibility Testing. CLSI Supplement M100. Clinical and Laboratory Standards Institute . 28th Edition: 30-102.

Woreta, A.N. and Kebede, H.B. (2022). Antibiotic Susceptibility Pattern and Bacterial Spectrum Among Patients with External Eye Infections at Menelik II Referral Hospital in Addis Ababa, Ethiopia. Infection and Drug Resistance. 15: 765-779. https://doi.org/10.2147/idr.s352098

Xu, S.; Guo, D.; Liu , X.; , Jin, X.; Shi, Y.; Wang, Y. et al. (2021). Ocular pathogens and antibiotic resistance in microbial keratitis over three years in Harbin, Northeast China. Acta Ophthalmology. 99(8): 909-915. https://doi.org/10.1111/aos.14789

Yang, Y.; Lin, Y. and Qiao, L. (2018). Direct MALDI-TOF MS Identification of Bacterial Mixtures. Analytical Chemistry. 90(17): 10400-10408. https://doi.org/10.1021/acs.analchem.8b02258

Zahan, N.A..; Hossain, M.; Musa, A.; Shamsuzzaman, A.; Mahamud, M.; Mamun, A. et al. (2009). PCR for *mecA* gene of methicillin resistant *Staphylococcus aureus*. Mymensingh Medical Journal: MMJ 18(1): 21-26.

Zaidi, T.; Zaidi, T.; Yoong, P. and Pier, G.B. (2013). *Staphylococcus aureus* corneal infections: effect of the PantonValentine Leukocidin (*PVL)* and antibody to *PVL* on virulence and pathology. Investigative Opthalmology Visual Science. 54(7): 4430-4438. https://doi.org/10.1167/iovs.13-11701