



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

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Abstract



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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, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus* sp., *Streptococcus pneumoniae*, *Klebsiella* sp., and *Haemophilus influenzae*, were the least detected bacterial spp. Most of the bacterial isolates tested in this study exhibited high resistance to Amoxicillin-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 secrete 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), Amoxicillin-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
<i>MecA</i>	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)
<i>PVL</i>	F5'ATCATTAGGTAAAATGTCTGGACATGATCCA3' R 5'GCATCAAGTGTATTGGATAGCAAAAGC3'	McClure et al., (2006)
<i>icaA</i>	F 5'TCTCTTGCAGGAGCAATCAA-3' R 5'TCAGGCACTAACATCCAGCA-3'	Mckenney et al., (1999)
<i>icaD</i>	F 5'ATGGTCAAGCCCAGACAGAG-3' R 5'CGTGTTTTCAACATTTAATGCAA-3'	Mckenney et al., (1999)
<i>Hla</i>	F 5'CTGATTACTATCCAAGAAATTCGATTG3' R 5'CTTCCAGCCTACTTTTTTATCAGT-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)
<i>MecA</i>	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
<i>PVL</i>	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
<i>icaA</i>	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
<i>icaD</i>	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
<i>Hla</i>	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 Amoxicillin-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 Amoxicillin-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				<i>p</i> * value
	< 1 year N (%)	1-3 years N (%)	3-15 years N (%)	>15 years N (%)	
<i>S. aureus</i> 66 (44.89 %)	10(37.04)	16(39.02)	19(44.18)	21(58.33)	0.242
<i>CoNS</i> 44 (29.9 %)	11(40.74)	14(34.15)	12(27.91)	7(19.44)	0.242
<i>E. coli</i> 12 (8.16 %)	0(0)	6(14.63)	5(11.63)	1(2.78)	0.779
<i>P.aeruginosa</i> 6 (4 %)	2(7.41)	0(0)	1(2.33)	3(8.33)	0.607
<i>Proteus</i> sp. 6 (4)	3(11.11)	0(0)	2(4.65)	1(2.78)	0.607
<i>S. pneumonia</i> 5 (3.4 %)	0(0)	1(2.44)	1(2.33)	3(8.33)	0.449
<i>Klebsiella</i> sp. 4 (2.72 %)	0(0)	2(4.88)	2(4.65)	0(0)	-
<i>H. influenzae</i> 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				<i>p</i> * value
	Spring N (%)	Summer N (%)	Autumn N (%)	Winter N (%)	
<i>S. aureus</i> 66 (44.89 %)	6 (42.86)	29(70.73)	23(31.51)	8(42.11)	0.028*
<i>CoNS</i> 44 (29.9 %)	6(42.85)	8(19.51)	28(38.36)	2 (10.53)	< 0.0001*
<i>E. coli</i> 12 (8.16 %)	0(0)	4(9.76)	5(6.85)	3(15.79)	-
<i>P. aeruginosa</i> 6 (4 %)	0(0)	0(0)	6(8.22)	0(0)	-
<i>Proteus</i> sp. 6 (4 %)	0(0)	0(0)	5(6.85)	1(5.26)	0.1

<i>S. pneumonia</i> 5 (3.4 %)	1(7.14)	0(0)	1(1.37)	3(15.79)	0.449
<i>Klebsiella</i> sp. 4 (2.72 %)	1(7.14)	0(0)	3(4.11)	0(0)	0.317
<i>H. influenzae</i> 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	<i>S. aureus</i> (66)	<i>CoNS</i> (44)	<i>E. coli</i> (12)	<i>P. aeruginosa</i> (6)	<i>Proteus</i> sp. (6)	<i>S. pneumonia</i> (5)	<i>Klebsiella</i> sp. (4)	<i>H. influenzae</i> (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 (7.5 µg)	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)
Amoxicillin-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 (30 µg)	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 Amoxicillin-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)
Amoxicillin-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*, *icaA*, *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
<i>MecA</i>	45(100)	0(0)	26(100)	0(0)
<i>PVL</i>	13(28.9)	32(71.1)	0(0)	26(100)
<i>icaA</i>	45(100)	0(0)	26(100)	0(0)
<i>icaD</i>	45(100)	0(0)	26(100)	0(0)
<i>Hla</i>	13(28.9)	32(71.1)	0(0)	26(100)
<i>Hla</i>	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

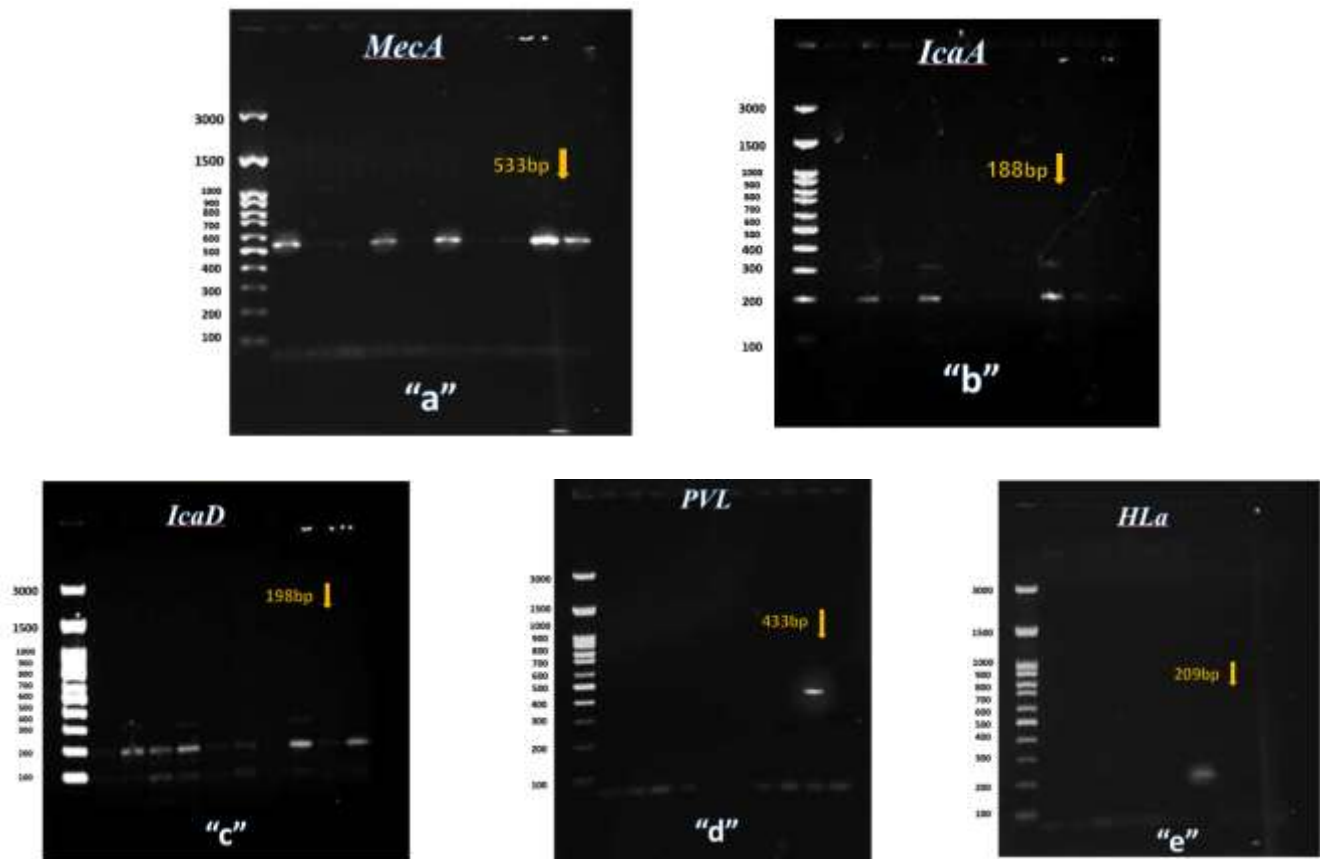


Fig. 1: Amplification product of the different virulence genes in MRSA and MR-CoNS isolates; where (a) *MecA* gene with detected bands at 533 bp in both MRSA and MR-CoNS, (b) *IcaA* gene detected bands at 188 bp in both MRSA and MR-CoNS, (c) *IcaD* gene with recovered bands at 198 bp in both MRSA and 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. influenzae* (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 *Staphylococcal* 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 *Staphylococci* 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 PVL 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 harbored 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.

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Conflict of interest

The authors declare no conflict of interests.

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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 are included with the journal.

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