Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 8258 - 8277
Received 16 February 2021; Accepted 08 March 2021.

Distribution of *OatA* Alleles Detected by a New Designed Primer in Bacteria Isolated from Eye Infections in Basrah Governorate/Iraq

Mohammed A. Mahdi[1], Munaff J. Abd Al-Abbas[2], Ahmed M. Alsamak[3]

[1]Biology Department, College of Science, University of Basrah, Basrah, Iraq.

[2]Biology Department, College of Science, University of Basrah, Basrah, Iraq.

[3]Ophthalmology Department, Al Zahraa College of Medicine, University of Basrah, Basrah, Iraq.

ABSTRACT

Two hundred and twenty seven samples were collected from infected eyes producing 14(54%) from 26 the contact lens, 67(44%) from 152 conjunctivitis and 19(39%) from 49 keratitis. Bacteria in males (61.62%) was higher than females (38.37%) in conjunctivitis and keratitis, while, they isolated as 100% from conjunctivitis and keratitis together in females only. 16S rDNA sequencing of the 100 isolates showed 14 different bacterial species identified as *Staphylococcus aureus* (33%) and *Staphylococcus epidermidis* (28%) of most species followed by *Pseudomonas aeruginosa* and *Enterococcus faecalis* (7% for both), *Bacillus subtilis* (6%), *Enterobacter hormaechei* (4%). *Streptococcus pyogenes*, *Staphylococcus hominis* and *Proteus mirabilis* (3% for each). *Staphylococcus lugdunensis* (2%). *Bacillus amyloliquefaciens*, *Staphylococcus haemolyticus*, *Enterobacter cloacae* and *Bacillus pumilus* (1% for each). Nevertheless, 6 new global strains were isolated from contact lens cases (42.85%) comparing with 18 of non-contact lens cases (20.93%). *OatA* was detected in 22(91.6%) of *S. aureus* only using a new designed primer producing 2 novel *OatA* alleles from 15 different alleles groups. Generally, Moxifloxacin (96%), Gatifloxacin (95%) and Ofloxacin (95%) were the most effective antibiotics against all 100 isolated species. RAPD test for *S. aureus* strains showed only two strains from two different patients were identical, while all other *S. aureus* isolates were of different strains.

KEYWORDS

OatA, Alleles, Eye Infections.

Introduction

Conjunctivitis (pink eye) is the infection of conjunctiva and the most common causes are viruses, bacteria and allergic (Leibowitz, 2000). Bacterial conjunctivitis may be caused by *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (Azari and Barney, 2013).

Keratitis refers to infection of cornea by entering microorganisms the eye after deep injury causing infection, inflammation and ulceration of the cornea. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Serratia*, *Corynebacterium* and *Haemophilus influenza* are the most common bacteria in keratitis (Schaefer *et al.*, 2001).

Some bacterial pathogens acquired resistance to avoid killing by lysozyme, these bacteria are able to modify their peptidoglycan backbone preventing cell lysis and allow the bacteria to survive in the host mucosal surfaces (Boneca *et al.*, 2007). Peptidoglycan fragments of lysis cells can aid to cause an immune response when recognized by host receptors and that will help in clearing infection of the host (Davis and Weiser, 2011). Peptidoglycan modification can limit its release to play a major role in reducing the immune reaction of the host (Viala *et al.*, 2004). OatA is the enzyme that modifying peptidoglycan by catalyst the O-acetylation of the N-acetylmuramic acid of peptidoglycan (Bera *et al.*, 2005). The alleles are nucleotides variation in specific genes giving at the same time a relatedness between the alleles and the sources of isolates (Chmagh and Abd Al-Abbas, 2019).

Topical antibiotics were used for treating bacterial to accelerate the eradication of bacteria and resolve the symptoms of bacterial conjunctivitis. Bacterial keratitis is an ophthalmic emergency because it can progress rapidly and may cause corneal blindness or visual impairment, therefore, it requires immediate treatment with topical or systemic antibiotics to prevent progression (Whitcher *et al.*, 2001). However, bacterial resistance to antibiotics increasing over the past several decades by changing themselves in some ways to become resistant to antibiotics. Antibiotic resistance evolves naturally via natural selection by random mutation (Fair and Tor, 2014).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 8258 - 8277
Received 16 February 2021; Accepted 08 March 2021.

It is very important to differentiate between various strains of bacterial species in certain cases, this is especially helpful when only some bacterial strains cause illness or resist to specific antibiotic, it is also helpful to identify strain differences and minor the spread of certain species of the bacteria and determine the best antibiotic to eradicate the new strains (Abd Al Wahid and Abd Al-Abbas, 2019)

According to the high infections among people especially with frequent using of contact lens, the present study designed to investigate the role of lysozyme protection, the distribution of bacterial strains and *OatA* alleles among those patients.

Materials and Methods

Samples Collection

Two hundred and twenty seven samples were collected from the eyes of patients with ages ranging between 1 and 80 years (135 males and 92 females) attended to out ophthalmology clinic in Al Basra General Hospital of Basra province between 8/4/2017 to 16/3/2018. The patients were suffering conjunctivitis, keratitis, contact lens wearing conjunctivitis and keratitis. Samples were collected by swabs in the cases of conjunctivitis and scraping by corneal scarper in the cases of keratitis, samples were transferred to tubes containing Brain heart infusion broth as a transport medium and cultured by streaking on Brain heart infusion agar, Chocolate agar and Blood agar plates. Plates were incubated at 37^{o}C for 24-48 hrs.

- **16SrDNA Amplification**

The DNA of 100 isolates were extracted according to the procedure of Presto™ Mini g DNA bacteria kit (Geneaid, Taiwan). *16 S rDNA* gene were amplified using universal primers 27F (5-AGAGTTTGATCCTGGCTCAG-3) and 1492 (5-GGTTACCTTGTTACGACTT-3), the product size is about 1500bp (Wise *et al.*, 1997). 50µl PCR reagent mixture contains 25 µl of Go Taq Green master mix (Bioneer, Korea), 2 µl of DNA template, 2 µl from each primers and 19 µl of Nuclease Free water (Bioneer, Korea). The Verity thermo cycler (Applied Biosystem, USA) was used with conditions for amplifying one cycle at 95°C for 5min. followed by 35 cycles at 95 °C for 30 sec., 55° C for 30 sec and 72°C for 1min, the final extension at 72°C for 5 min. the bands of 1500 bp were detected on by agarose gel electrophoresis and photographed under UV transilluminator (Wisd, Korea).

- **16S rDNA Sequencing**

Twenty µl of *16S rDNA* gene were sent to Macrogen company for sequencing. After proofreading the bacterial species were identified by BLAST "https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch" (Kerbauy *et al.*, 2011).

Phylogenetic Tree

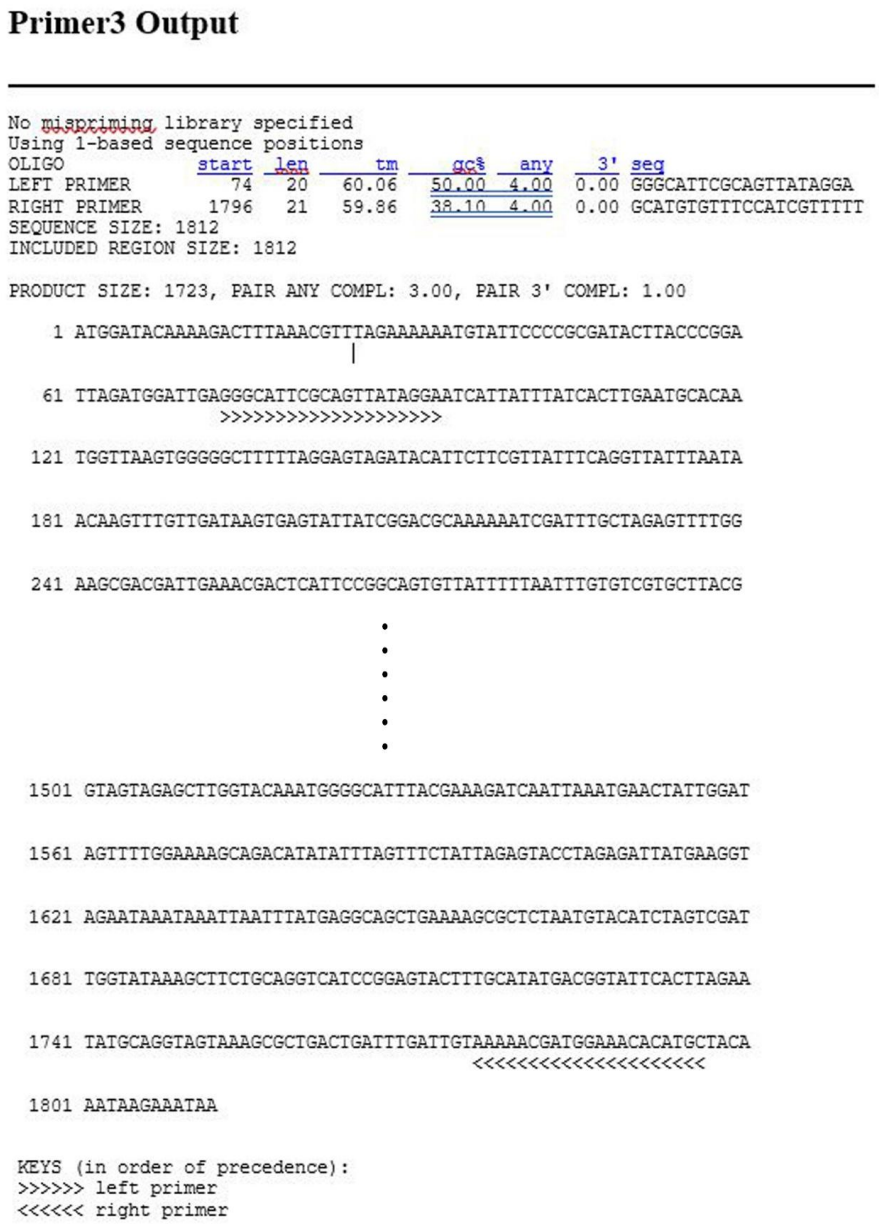
Fourteen *16S rDNA* sequences of different bacterial species detected in the present study were aligned with *16S rDNA* sequences of their reference strains using "CLUSTAL Omega" https://www.ebi.ac.uk/Tools/msa/clustalo/ (Becker *et al.*, 2004). The phylogenetic tree was constructed by using MAFFT (Multiple Alignment using Fast Fourier Transform).

Designing New Primers for *OatA* Gene

The new primers for detecting *OatA* gene were designed in the present study by using primer3: http://bioinfo.ut.ee/primer3-0.4.0 as shown in figure (1). (Rozen and Skaletsky, 2000). The new primer was applied with *OatA* gene sequences from 100 random isolates of *S. aureus* in GenBank using primer3 program to determine the ability of the primer to align with the *OatA* gene sequence. OatA primers were Forward: 5-GGGCATTCGCAGTTATAGGA-3 and Reverse: 5-GCATGTGTTTCCATCGTTTTT-3. 50µl PCR reagent mixture contains 25 µl of Go Taq Green master mix (Bioneer, Korea), 2 µl of DNA template, 2 µl from each primers (Macrogen, Korea) and 19 µl of Nuclease Free water (Bioneer, Korea). The Verity thermo cycler (Applied

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 8258 - 8277
Received 16 February 2021; Accepted 08 March 2021.

Biosystem, USA) was used with conditions for amplifying one cycle at 95°C for 5 min. followed by 40 cycles at 95 °C for 30 sec. 56° C for 30 sec and 72°C for 1 min. Final extension at 72°C for 5 min. The bands of 1800 bp were detected on by agarose gel electrophoresis and photographed under UV transilluminator (Wisd, Korea). 20 µl of *OatA* gene were sent to Macrogen company for sequencing, after proofreading the alleles were identified by BLAST "https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch" (Kerbauy et al., 2011).



Primer3 Output

No mispriming library specified
Using 1-based sequence positions

OLIGO	start	len	tm	gc%	any	3'	seq
LEFT PRIMER	74	20	60.06	50.00	4.00	0.00	GGGCATTCGCAGTTATAGGA
RIGHT PRIMER	1796	21	59.86	38.10	4.00	0.00	GCATGTGTTTCCATCGTTTTT

SEQUENCE SIZE: 1812
INCLUDED REGION SIZE: 1812

PRODUCT SIZE: 1723, PAIR ANY COMPL: 3.00, PAIR 3' COMPL: 1.00

```
    1 ATGGATACAAAAGACTTTAAACGTTTAGAAAAAATGTATTCCCCGCGATACTTACCCGGA
                                |

   61 TTAGATGGATTGAGGGCATTCGCAGTTATAGGAATCATTATTTATCACTTGAATGCACAA
                >>>>>>>>>>>>>>>>>>>

  121 TGGTTAAGTGGGGGCTTTTTAGGAGTAGATACATTCTTCGTTATTTCAGGTTATTTAATA

  181 ACAAGTTTGTTGATAAGTGAGTATTATCGGACGCAAAAAATCGATTTGCTAGAGTTTTGG

  241 AAGCGACGATTGAAACGACTCATTCCGGCAGTGTTATTTTTAATTTGTGTCGTGCTTACG
                                .
                                .
                                .
                                .
                                .
                                .

 1501 GTAGTAGAGCTTGGTACAAATGGGGCATTTACGAAAGATCAATTAAATGAACTATTGGAT

 1561 AGTTTTGGAAAAGCAGACATATATTTAGTTTCTATTAGAGTACCTAGAGATTATGAAGGT

 1621 AGAATAAATAAATTAATTTATGAGGCAGCTGAAAAGCGCTCTAATGTACATCTAGTCGAT

 1681 TGGTATAAAGCTTCTGCAGGTCATCCGGAGTACTTTGCATATGACGGTATTCACTTAGAA

 1741 TATGCAGGTAGTAAAGCGCTGACTGATTTGATTGTAAAAACGATGGAAACACATGCTACA
                                        <<<<<<<<<<<<<<<<<<<<<

 1801 AATAAGAAATAA
```

KEYS (in order of precedence):
>>>>>> left primer
<<<<<< right primer

Figure 1. Primer3 output of the new designed primers of OatA, showing the sequence of the primers (forward and reverse) and the size of the amplified gene

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 8258 - 8277
Received 16 February 2021; Accepted 08 March 2021.

Antibiotic Susceptibility

Antibiotic susceptibility test of 11 antibiotics (Azithromycin 15 µg, Chloramphenicol 10 µg, Ciprofloxacin 5 µg, Gatifloxacin 5 µg, Gentamycin 10 µg, Levofloxacin 5 µg, Moxifloxacin 5 µg, Ofloxacin 10 µg, Tetracycline 30 µg, Tobramycin 10 µg and Vancomycin 30 µg) from "Mast group, UK company" were performed for 100 bacterial species using disc diffusion method on Muller Hinton agar according to clinical and laboratory standard institute guideline (CLSI, 2017).

Random Amplified Polymorphic DNA (RAPD)

Eight isolates of *S. aureus* having the same results of the antibiotic susceptibility tests were subjected to RAPD test with three primers, OLP6(5′-GAGGGAAGAG-3′), OLP11(5′-ACGATGAGCC-3′) and OLP13(5′-ACCGCCTGCT-3′) to determine the identical strains according to procedure of Zare *et al.* (2019).

25µl PCR reagent mixture contains 12 µl of Go Taq Green master mix (Bioneer, Korea), 3 µl of DNA template, 1 µl from each primers (Macrogen, Korea) and 7 µl of Nuclease Free water (Bioneer, Korea). The Verity thermo cycler (Applied Biosystem, USA) was used with conditions for amplifying one cycle at 94°C for 5min. followed by 40 cycles at 93 °C for 1min. 37° C for 90 sec. and 72°C for 1 min. Final extension at 72°C for 7 min. The bands were detected on by agarose gel electrophoresis and photographed under UV transilluminator (Wisd, Korea). The dendrogram was constructed by Unweighted pair group method with Arithmetic mean (UPGMA) "www. http://genomes.urv.cat/UPGMA/" by calculating the distance between RAPD bands of 8 isolates according to DNA ladder (Garcia-Vallvé and Puigbo, 2009).

Results

Distribution of Bacterial Isolates among Cases

One hundred (44%) bacterial isolates were obtained from 227 samples of patients with eye infections including 152 Conjunctivitis, 49 Keratitis and 26 Contact lens infections. Contact lens infections had the higher isolation 14(54%) followed by conjunctivitis 67(44%) and keratitis 19(39%) with no significant differences (Table 1).

Table 1. Distribution of eye infection between males and females

	Cases	Number of patients	Isolation	Males	Females
1	Conjunctivitis	152	67(44.07%)	43(64.17%)	24(35.82%)
2	Keratitis	49	19 (38.77%)	10(52.63%)	9(47.36%)
	Total (Conjunctivitis and Keratitis)	201	86 (42.78%)	*53(61.62%)	33(38.37%)
3	Contact lens infections (Conjunctivitis and Keratitis)	26	14(53.84%)	-(0%)	**14(100%)
	Total	227	100(44.05%)	53(53%)	47(47%)

*= $p \leq 0.05$, **= $p \leq 0.01$

In conjunctivitis and keratitis cases, the isolation in males (61.62%) was higher than females (38.37%) with significant difference at $p \leq 0.05$, while in the cases of contact lens conjunctivitis and keratitis was only in females (100%).

Frequency of Bacterial Species in the Eye Infection Cases

16S rDNA gene of 100 isolates were sequenced successfully and the bacterial species were identified as *Staphylococcus aureus* (n=33/ 33%), *Staphylococcus epidermidis* (n=28/ 28%), *Pseudomonas aeruginosa* and *Enterococcus faecalis* (n=7/ 7% for both), *Bacillus subtilis* (n=6 / 6%), *Enterobacter hormaechei* (n=4 / 4%), *Streptococcus pyogenes*, *Staphylococcus hominis* and *Proteus mirabilis* (n=3 / 3% for each), *Staphylococcus lugdunensis* (n=2 / 2%), *Bacillus amyloliquefaciens*, *Staphylococcus haemolyticus*, *Enterobacter cloacae* and *Bacillus pumilus* (n= 1 / 1% for each). Each isolate sequence was aligned with its type strain (ATCC) in NCBI (Figure 2 and Table 2). The similarity of identifications with type strains were ≥ 99%.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 8258 - 8277
Received 16 February 2021; Accepted 08 March 2021.

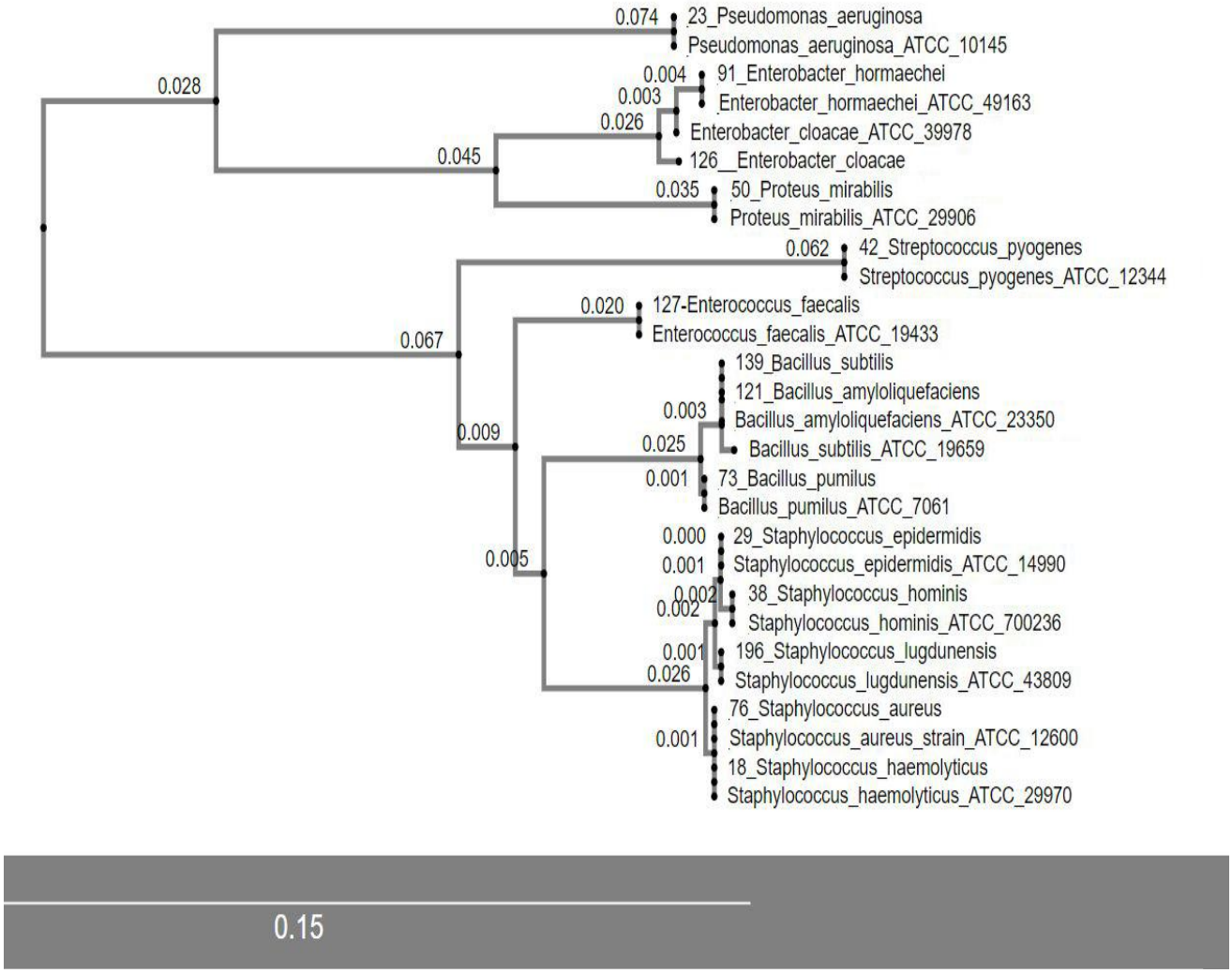


Figure 2. Rooted Neighbor Joining phylogenetic tree constructed from concatenated sequences of 899 bp produced by a MAFFT alignment and visualized using forester version1046. This Neighbor Joining tree showing the distribution and Phylogenetic relationships of 14 different bacterial species isolated from human eye infection with their reference strains (ATCC). All horizontal branch lengths were drawn to scale. Bootstrap values after 1000 repetitions are indicated

S. aureus (33%) and *S. epidermidis* (28%) were the most species isolated from eye infections with high significant difference (P≤0.01) against other species. *B. subtilis* 6(100%), *E. hormaechei* 4(100%), S. *lugdunensis* 2(100%), *B. amyloliquefaciens* 1(100%) and *S. haemolyticus* 1(100) were found only in conjunctivitis with high significant differences (P≤0.01), while *E. cloacae* 1(100%) and *B. pumilus* 1(100%) were found only in keratitis with high significant differences (P≤0.01). But, in the cases of contact lens infections, the highest frequency was in *P. aeruginosa* 6(85.71%) with high significant difference at P≤0.01. Totally, the highest species frequented was in conjunctivitis cases 67(67%) with high significant difference (P≤0.01) than keratitis 19(19%) and contact lens infections 14(14%).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 8258 - 8277
Received 16 February 2021; Accepted 08 March 2021.

Table 2. Frequency of bacterial species in the eye infection cases

No.	Bacterial species	n	conjunctivitis	keratitis	contact lens infection
1	*Staphylococcus aureus*	*33	20(60.6%)	9(27.27%)	4(12.12%)
2	*Staphylococcus epidermidis*	*28	21(75%)	4(14.28%)	3(10.71%)
3	*Pseudomonas aeruginosa*	7	0%	1(14.28%)	*6(85.71%)
4	*Enterococcus faecalis*	7	*6(85.71%)	1(14.28%)	0%
5	*Bacillus subtilis*	6	*6(100%)	0%	0%
6	*Enterobacter hormaechei*	4	*4(100%)	0%	0%
7	*Streptococcus pyogenes*	3	2(66.66%)	1(33.33%)	0%
8	*Staphylococcus hominis*	3	2(66.66%)	0%	1(33.33%)
9	*Proteus mirabilis*	3	2(66.66%)	1(33.33%)	0%
10	*Staphylococcus lugdunensis*	2	*2(100%)	0%	0%
11	*Bacillus amyloliquefaciens*	1	*1(100%)	0%	0%
12	*Staphylococcus haemolyticus*	1	*1(100%)	0%	0%
13	*Enterobacter cloacae*	1	0%	*1(100%)	0%
14	*Bacillus pumilus*	1	0%	*1(100%)	0%
	Total	100	*67(67%)	19(19%)	14(14%)

$P \leq 0.01$

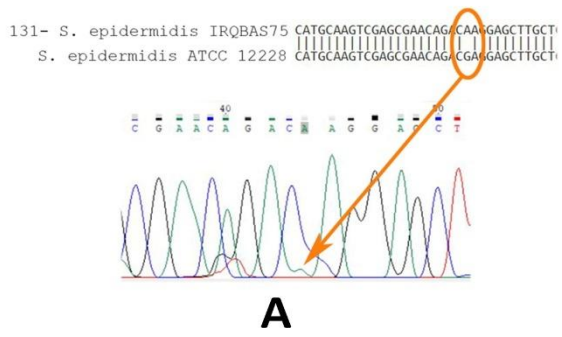
Detection of New Strains

Twenty four new global bacterial strains were identified by comparing the nucleotides with their type strains. The new strains were recorded in DNA Data Bank of Japan (DDBJ) and published on The National Center for Biotechnology Information (NCBI) and the GenBank (Table 3 and Figures 3).

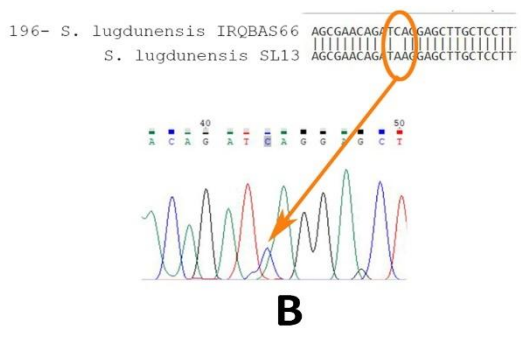
Table 3. List of new detected strains

	Bacterial species	Strain	Mutation	Closely related to	Similarity	Figure
1	131-*Staphylococcus epidermidis*	IRQBAS75 "LC499781.1"	Transition (A instead G) at 44 bp	ATCC 12228	99.93%	A
2	196-*Staphylococcus lugdunensis`*	IRQBAS66 "LC499613.1"	Transversion (C instead A) at 43 bp	SL13	99.93%	B
3	16-*Staphylococcus epidermidis*	IRQBAS65 "LC499612.1"	Insertion T at 283 bp	IBK-11	99.93%	C
4	142-*Staphylococcus epidermidis*	IRQBAS72 " LC499747.1"	Transversion (C instead G) at 591 bp	LH-Y4	99.93%	D
5	29-*Staphylococcus epidermidis*	IRQBAS70 " LC498579.1"	Transversion (C instead G) at 41 bp.	IAE142	99.93%	E
6	128-*Staphylococcus aureus*	IRQBAS62 " LC499609.1"	Two transition (T instead C) at 86 bp and 105 bp subsequently	UP_338	99.93%	F
7	38-*Staphylococcus hominis*	IRQBAS84 " LC499790.1"	Insertion T at 314 bp	FCu1	99.93%	G
8	50-*Proteus mirabilis*	IRQBAS63 " LC499610.1"	Insertion A at 802 bp	UFV 131	99.93%	H
9	4-*Staphylococcus epidermidis*	IRQBAS86 " LC499610.1"	Transition (T instead C) at 268 bp	KCOM 1912	99.93%	I
10	138-*Staphylococcus epidermidis*	IRQBAS81 " LC499787.1"	Transition (A instead G) at 997 bp and transversion (A instead C) at 1069 bp	H42	99.93%	J
11	117-*Staphylococcus epidermidis*	IRQBAS77 "LC499783.1"	Deletion A at 571 bp	UFVCC1197	99.93%	K
12	39-*Staphylococcus aureus*	IRQBAS74 " LC499749.1"	Transversion (C instead G) at 904 bp and T instead G at 971 bp	CFSAN082783	99.77%	L
13	189-*Staphylococcus aureus*	IRQBAS80 "LC499786.1"	Transition (T instead C) at 636 bp	MRSA-5227	99.87%	M
14	146-*Bacillus subtilis*	IRQBAS79 " LC499785.1	Transversion (C instead G) at 374 bp	yxw4	99.87%	N
15	41-*Staphylococcus aureus*	IRQBAS78 " LC499784.1"	Transition (A instead G) at 636 bp	LHICA120	99.87%	O
16	144-*Staphylococcus aureus*	IRQBAS76 " LC499782.1"	Transition (A instead G) at 637 bp	SGC801	99.87%	P
17	97-*Staphylococcus aureus*	IRQBAS69 "LC498828.1"	Transition (A instead G) at 641 bp and insertion A at 671 bp	Be62	99.40%	Q
18	136-*Staphylococcus aureus*	IRQBAS67 "LC499745.1"	Transversion (C instead G) at 681 bp and insertion T at 689 bp	LHICA120	99.70%	R
19	77-*Staphylococcus aureus*	IRQBAS64 "LC499611.1"	Transversion (C instead G) at 583 bp	MR10	99.8%	S
20	162-*Enterobacter hormaechei*	IRQBAS92 "LC576389.1"	Insertion A and G at 238 bp and 266 bp respectively	SSBB1	99.75%	T
21	212-*Enterobacter hormaechei*	IRQBAS94 "LC576391.1"	Transition (A instead G) at 674 bp	E70	99.87%	U
22	7-*Staphylococcus epidermidis*	IRQBAS95 "LC576392.1"	Insertion T at 489 bp	1910ICU248	99.86%	V
23	137-*Proteus mirabilis*	IRQBAS96 "LC576393.1"	Transversion (C instead G) at 674 bp	S_1	99.86%	W
24	120-*Staphylococcus aureus*	IRQBAS101 "LC576398.1"	Transversion (G instead C) at 1116 bp	Gv69	99.77%	X

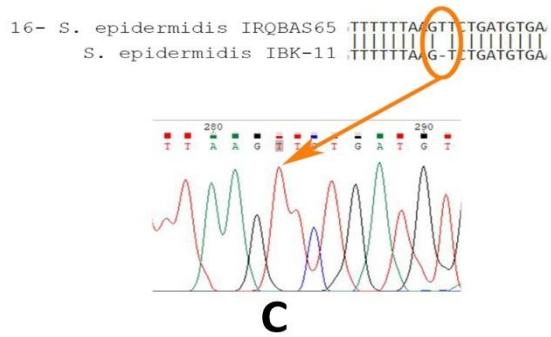
Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 8258 - 8277
Received 16 February 2021; Accepted 08 March 2021.



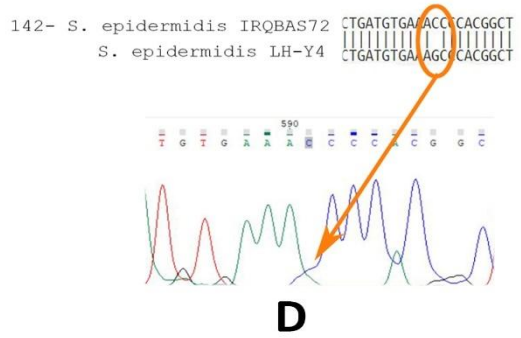
A



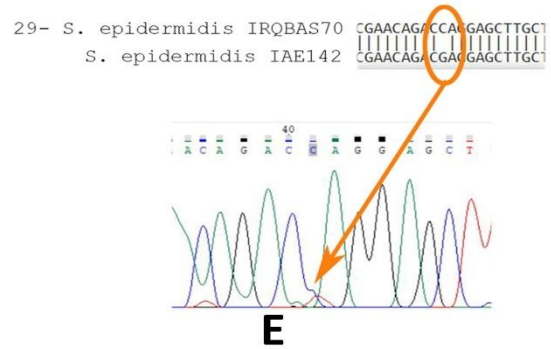
B



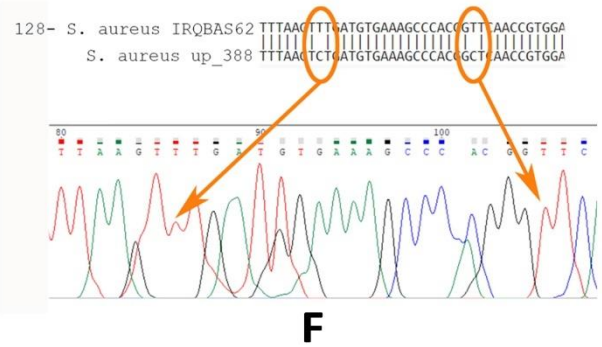
C



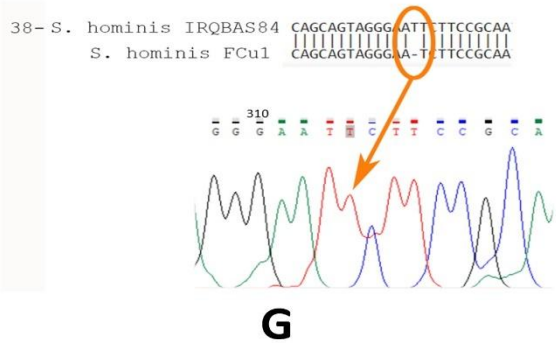
D



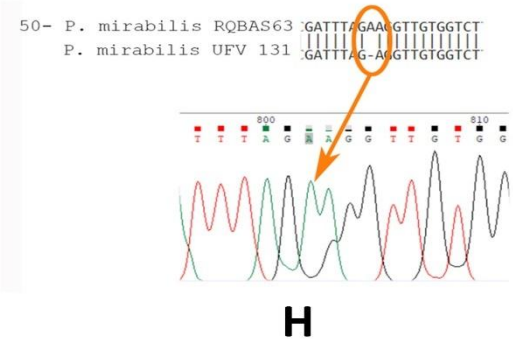
E



F

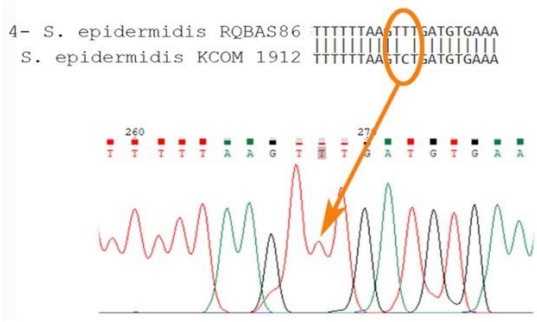


G

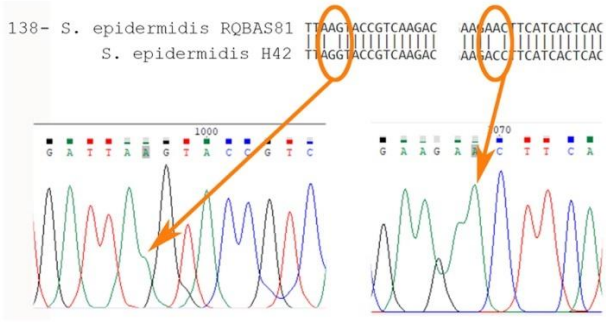


H

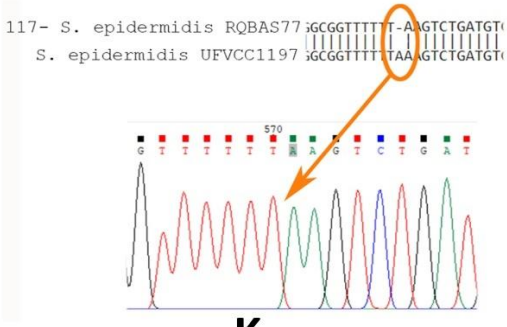
Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 8258 - 8277
Received 16 February 2021; Accepted 08 March 2021.



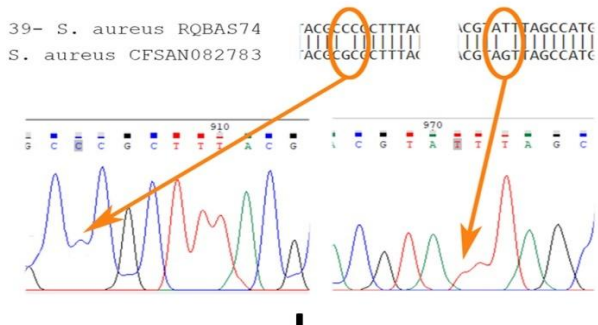
I



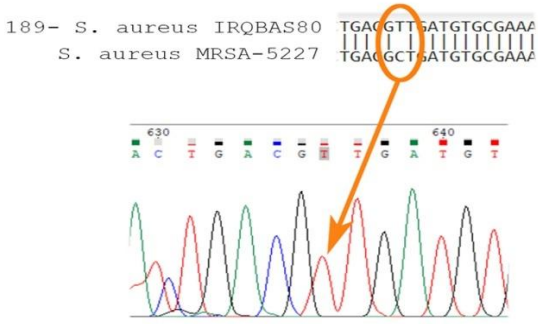
J



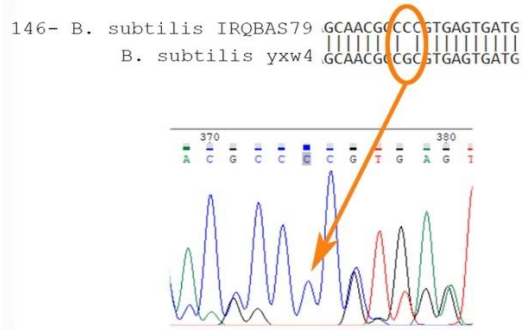
K



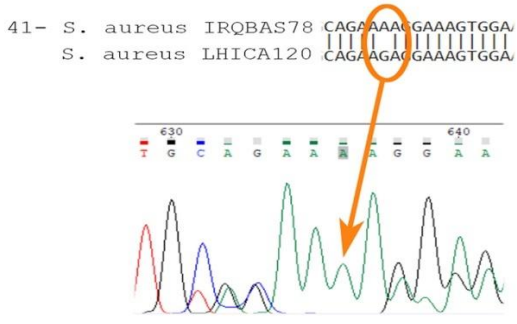
L



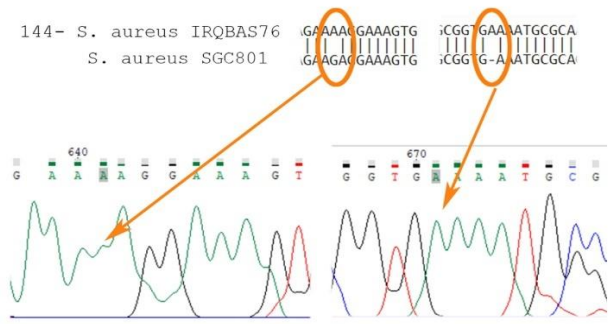
M



N



O



P

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 8258 - 8277
Received 16 February 2021; Accepted 08 March 2021.

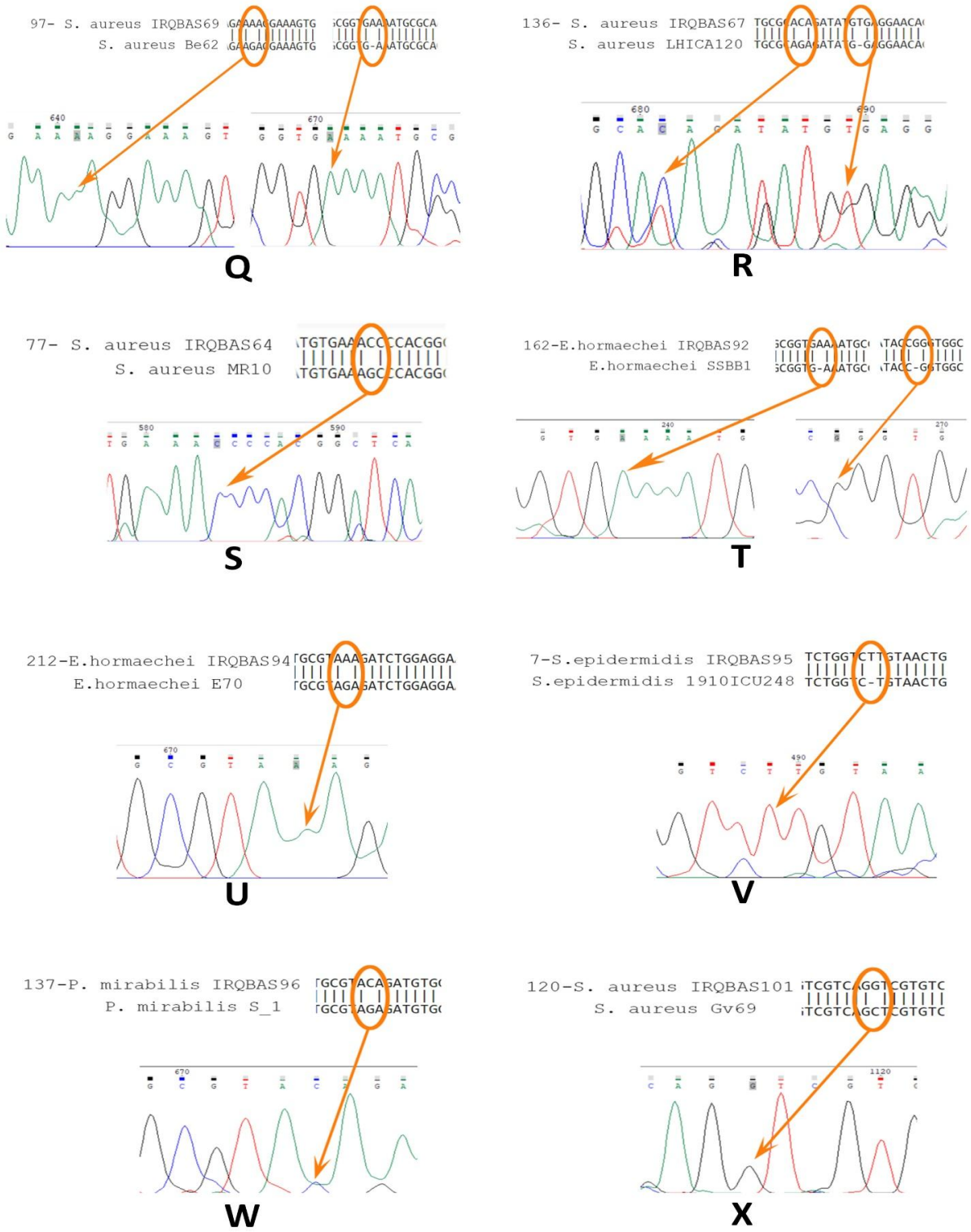


Figure 3. Comparison of 16S rDNA nucleotide sequence for isolates from present study with its type strains

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 8258 - 8277
Received 16 February 2021; Accepted 08 March 2021.

The frequency of new strains isolated from contact lens cases were 6 (42.85%) while that from non-contact lens cases were 18 (20.93%) with high significant difference at P≤0.01 (Table 4).

Table 4. Frequency of the bacterial new strains in contact lens and non-contact lens cases

	Source	Total isolates	New strains n(%)
1	Contact lens	14	*6 (42.85%)
2	Non-contact lens	86	18 (20.93%)
	Total	100	24

*= P≤0.01

Amplification and Sequencing of *OatA* Gene

The *OatA* gene was detected in only 22 (22%) of all the 100 tested isolates. Particularly, all the 22 *OatA* positive isolates were only *S. aureus* of the total *S. aureus* 33(67%) as Figure (4).

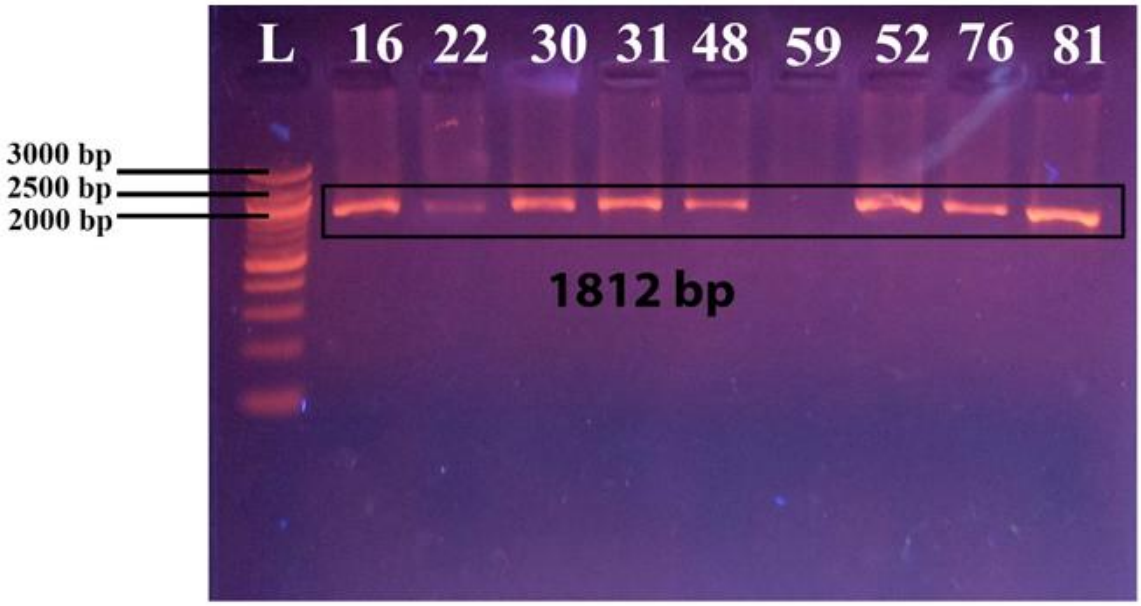


Figure 4. Agarose gel electrophoresis showing amplified *OatA* gene (1812 bp). Lane L: 100 bp Marker, Lane (16, 22, 30, 31, 48, 52, 76 and 81): *OatA* gene bands of *S. aureus*

The *OatA* gene PCR products of 22 *S. aureus* isolates were sequenced successfully and aligned with type sequencing in NCBI. The similarity of identifications were ≥ 99%.

Two novel *OatA* alleles were frequented in five *S. aureus* strains of present study. The first novel allele IRQBAS_19 "LC556094.1" was found in 4 strains (No.19, 22,128 and 223) which are closely related (99.94%) to *S. aureus* Up_620 with a transition mutation (G instead A) at 494 bp (Figure 5). The second novel *OatA* allele IRQBAS_moh99 "LC575194.1" was found in 97- *S. aureus* which is closely related (99.79%) to *S. aureus* 78 with a transition mutation (G instead A) and transversion mutation (C instead A) at 943 bp and 967 bp respectively (Figure 6). The two novel *OatA* alleles were recorded The National Center for Biotechnology Information (NCBI) and the GenBank DNA sequences.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 8258 - 8277
Received 16 February 2021; Accepted 08 March 2021.

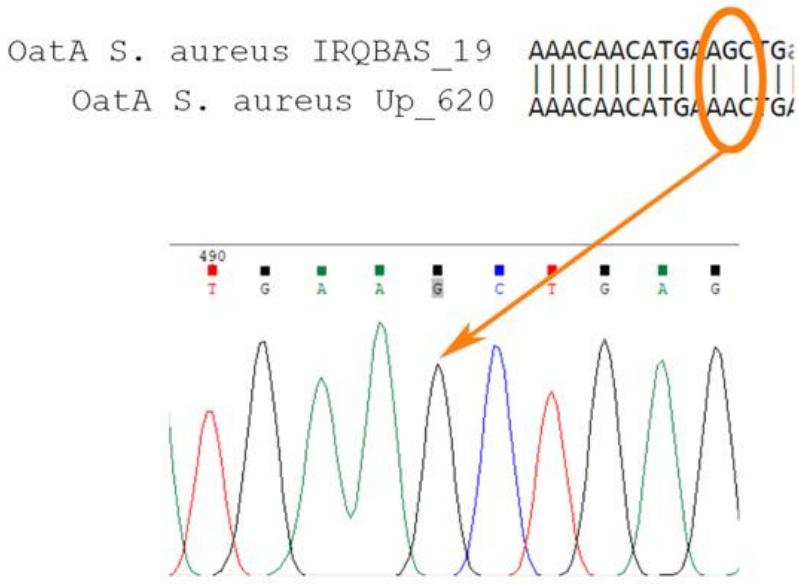


Figure 5. Comparison of OatA nucleotide sequence for isolate IRQBAS_19 from present study with OatA nucleotide sequence of type strain UP_620, a transition mutation (G instead A) at 494 bp.

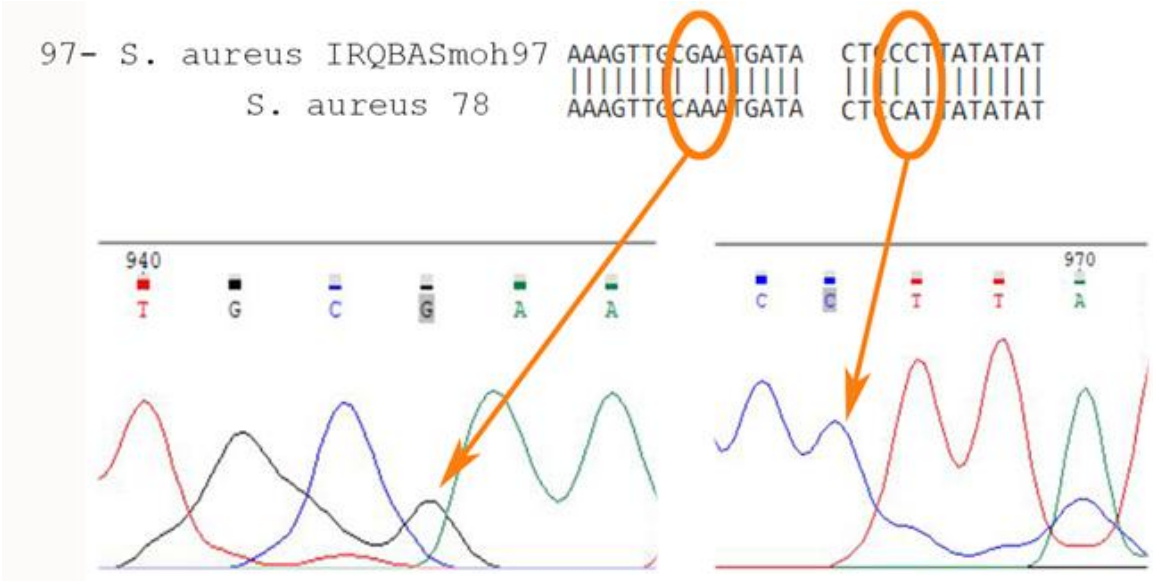


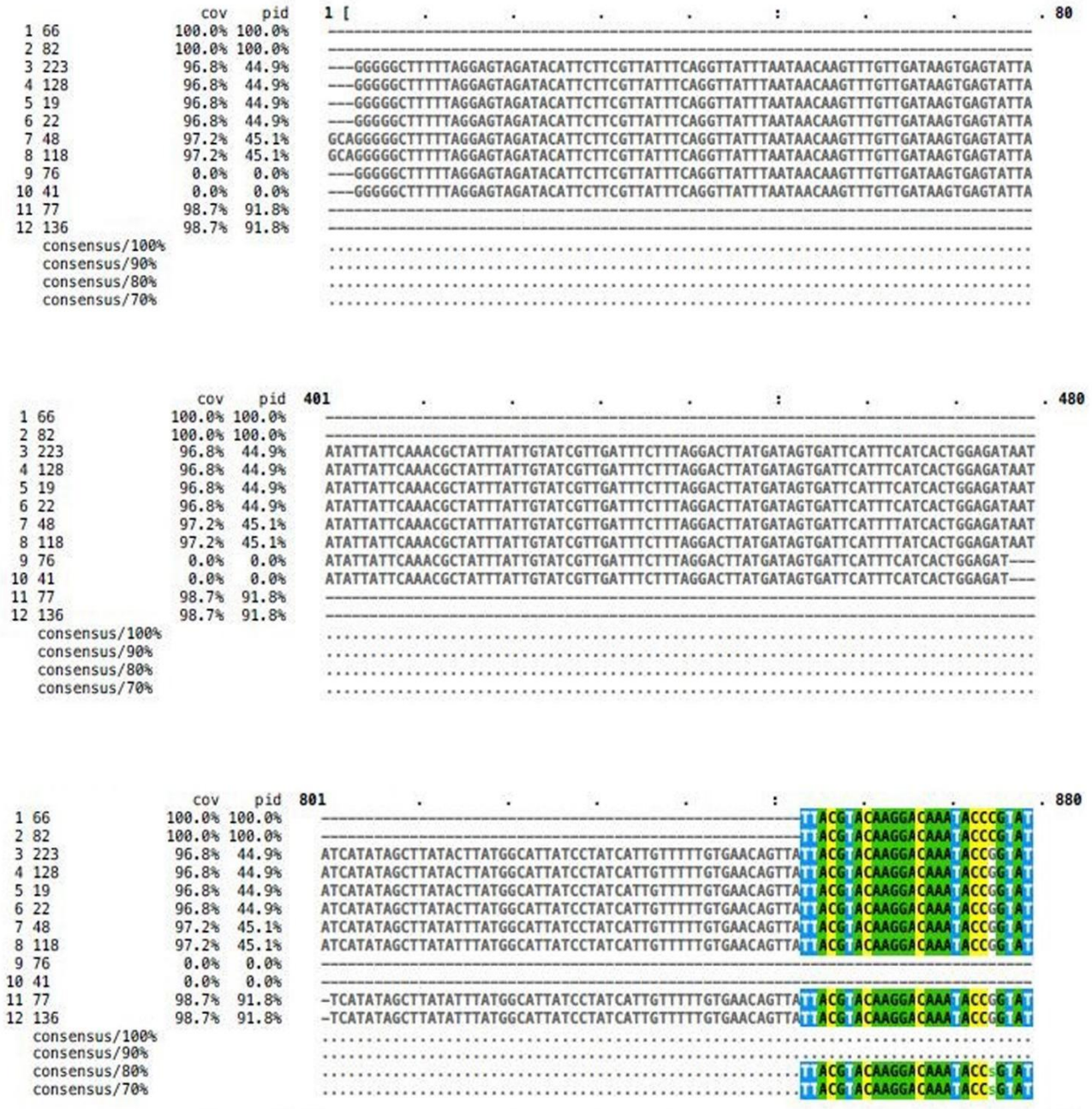
Figure 6. Comparison of OatA nucleotide sequence for isolate IRQBASmoh99 from present study with OatA nucleotide sequence of type strain 78, a transition mutation (G instead A) and transversion mutation (C instead A) at 943 bp and 967 bp

***OtaA* Alleles Groups**

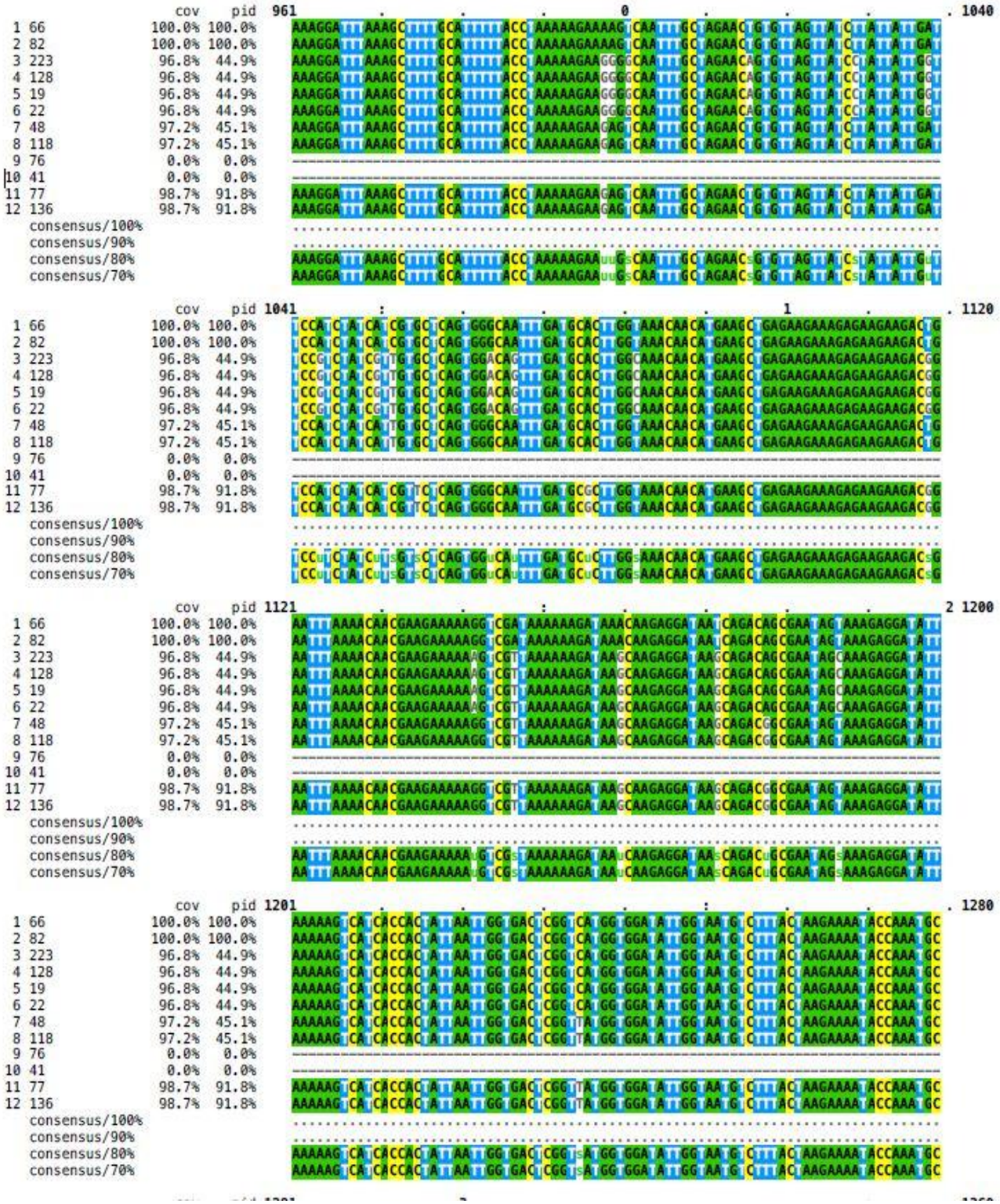
The *OtaA* gene sequences were compared among 22 *S. aureus* strains in the present study using multiple sequence alignment (MSA) "https://www.ebi.ac.uk/submission/". The sequences were divided into two groups, Group 1 contains *OatA* allele sequences of 12 strains and Group 2 contains *OatA* gene sequences of other 10 strains. The comparison results showed that there are 15 different alleles of *OtaA* gene. In group 1, the first allele was found in 2 strains "48 and 118" with size 1590 bp, the second allele was found in 4 strains "223, 128, 19 and 22" with size 1584 bp, the third allele was found in 2 strains "82 and 66" with size 757 bp, the fourth allele was found in 2 strains "77 and 136" with size 800 bp and the fifth allele was found in 2 strains "76 and 41" with size 475 bp (Figure 7). In

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 8258 - 8277
Received 16 February 2021; Accepted 08 March 2021.

group 2, the 10 different alleles were distributed in the 10 different strains (209, 12, 206, 58, 111, 97, 144, 68, 102 and 120) with different sizes (558,795, 696, 1031, 1083, 943, 765, 1017,972 and 538) respectively, as shown in Figure (8) and Table (5).



Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 8258 - 8277
Received 16 February 2021; Accepted 08 March 2021.



Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 8258 - 8277
Received 16 February 2021; Accepted 08 March 2021.

Table 5. Distribution of *OatA* alleles among *S. aureus* strains

Groups	No. of alleles	No. of *S. aureus* strains	Size from 1812 bp
1	1	48 118	1590
	2	223 128 19 22	1584
	3	82 66	757
	4	77 136	800
	5	76 41	475
2	6	209	558
	7	12	795
	8	206	696
	9	58	1031
	10	111	1083
	11	97	943
	12	144	765
	13	68	1017
	14	102	972
	15	120	538

Antibiotic Susceptibility

The results of antimicrobial susceptibility test for 100 isolates were shown in Table (6). Most isolates were susceptible to MXF, GAT and OFX (96%, 95% and 95%) respectively, while less to T, VA and C (53%, 55% and 56%) respectively, with significant differences at p≤0.01.

Table 6. Antibiotic susceptibility of bacterial species isolates against antibiotics

No.	Bacterial species	n	C	LEV	AZM	T	CIP	TOB	OFX	GM	VA	MXF	GAT
1	*Staphylococcus aureus*	33	10 (30.3%)	25 (75.75%)	10 (30.3%)	8 (24.24%)	29 (87.87%)	8 (24.24%)	29 (87.87%)	10 (30.3%)	11 (33.33%)	31 (93.93%)	29 (87.87%)
2	*Staphylococcus epidermidis*	28	20 (71.42%)	28 (100%)	21 (75%)	17 (60.71%)	24 (85.71)	22 (78.57%)	28 (100%)	24 (85.71%)	22 (78.57%)	26 (92.85%)	27 (96.42%)
3	*Pseudomonas aeruginosa*	7	4 (57.14%)	7 (100%)	4(57.14 %)	5(71.42 %)	5(71.42 %)	6(85.71 %)	7(100%)	5(71.42 %)	1(14.28 %)	7(100%)	7(100%)
4	*Enterococcus faecalis*	7	4 (57.14%)	7 (100%)	4 (57.14%)	4 (57.14%)	4 (57.14%)	4 (57.14%)	6 (85.71%)	7 (100%)	5 (71.42%)	7 (100%)	7 (100%)
5	*Bacillus subtilis*	6	4 (66.66%)	6 (100%)	6 (100%)	4 (66.66%)	6 (100%)	4 (66.66%)	6 (100%)	6 (100%)	3 (50%)	6 (100%)	6 (100%)
6	*Enterobacter hormaechei*	4	4 (100%)	2 (50%)	0 0%	3 (75%)	3 (75%)	4 (100%)	4 (100%)	4 (100%)	4 (100%)	4 (100%)	4 (100%)
7	*Streptococcus pyogenes*	3	2 (66.66%)	3 (100%)	3 (100%)	1 (33.33%)	3 (100%)	1 (33.33%)	3 (100%)	1 (33.33%)	1 (33.33%)	3 (100%)	3 (100%)
8	*Staphylococcus hominis*	3	3 (100%)	3 (100%)	0 0%	2 (66.66%)	3 (100%)	3 (100%)	3 (100%)	3 (100%)	2 (66.66%)	3 (100%)	3 (100%)
9	*Proteus mirabilis*	3	1 (33.33%)	3 (100%)	3 (100%)	3 (100%)	3 (100%)	2 (66.66%)	3 (100%)	2 (66.66%)	2 (66.66%)	3 (100%)	3 (100%)
10	*Staphylococcus lugdunensis*	2	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	1 (50%)	2 (100%)	2 (100%)
11	*Bacillus amyloliquefaciens*	1	0 0%	0 0%	100 100%	100 100%	100 100%	100 100%	100%	100%	100%	100%	100%
12	*Staphylococcus haemolyticus*	1	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
13	*Enterobacter cloacae*	1	0%	100%	100%	100%	100%	100%	100%	100%	0%	100%	100%
14	*Bacillus pumilus*	1	100%	100%	100%	100%	100%	100%	100%	0%	100%	100%	100%
	Total	100	56%	89%	57%	53%	86%	60%	95%*	67%	55%	96%*	95%*

*= P≤0.01

Chloramphenicol (C), Levofloxacin (LEV), Azithromycin (AZM), Tetracycline (T), Ciprofloxacin (CIP),

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 8258 - 8277
Received 16 February 2021; Accepted 08 March 2021.

Tobramycin (TOB), Ofloxacin (OFX), Gentamicin (GM), Vancomycin (VA), Moxifloxacin (MXF), Gatifloxacin (GAT)

RAPD-PCR for *S. Aureus* Strains

The bands of RAPD-PCR for 8 isolates (identical in antibiotic susceptibility) of *S. aureus* were shown on agarose gel (Figure 9).

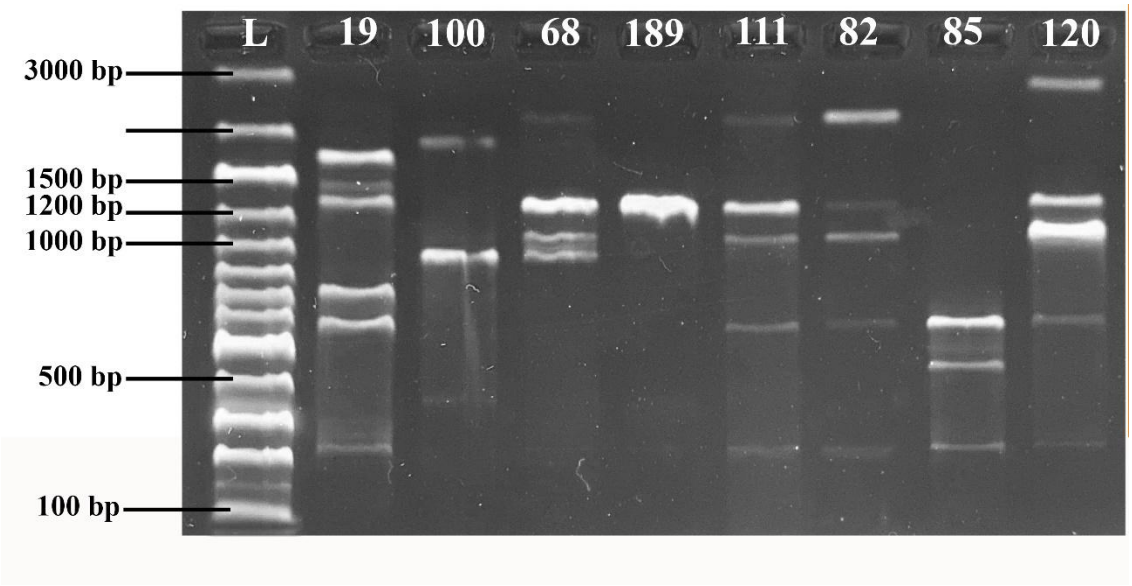


Figure 9. Agarose gel electrophoresis showing RAPD pattern of *S. aureus* bands

The phylogenetic tree showed that strains 111 and 82 are identical and closely related to strain 120 (Figure 10) and (Table 7)

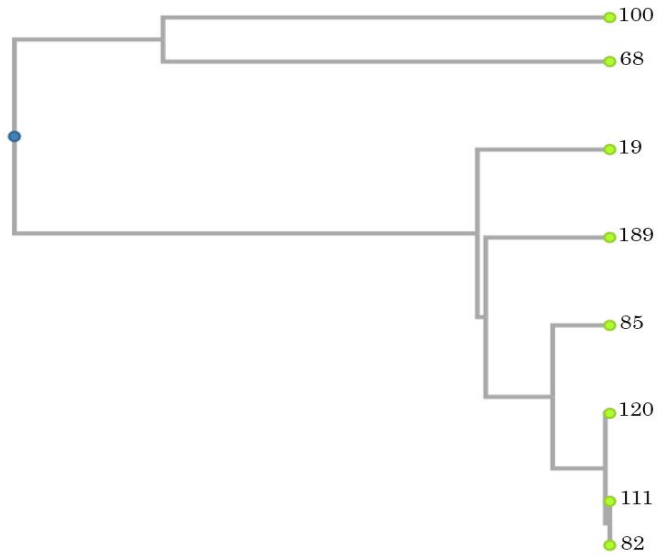


Figure 10. Dendrogram of 8 *S. aureus* strains 19 and 100, 68 and 189, 111 and 82, 85 and 120 constructed by a set of variables RAPD bands using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm. Bootstrap values after 100 repetitions are indicated.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 8258 - 8277
Received 16 February 2021; Accepted 08 March 2021.

Table 7. Distance Matrix between RAPD-PCR bands of *S. aureus* strains

	19	**100**	**68**	**189**	**111**	**82**	**85**	**120**
19	0	136.649	129.558	30.292	27.434	27.434	18.314	26.308
100		0	87.542	118.024	130.570	130.570	119.780	129.379
68			0	102.992	91.905	91.905	125.351	93.324
189				0	23.937	23.937	25.701	23.653
111					0	0.000	11.263	0.865
82						0	11.263	0.865
85							0	10.986
120								0

Discussion

Out of 227 samples, 100 (44%) were axenic cultures grown, axenic culture means only a single species or pathogen grown from sample after the first culture. The low rate of positive cultures from the total samples may be due to the randomly use of antibiotics in treatment of eye infections or because of non bacterial infections such as viruses and fungi, this result is inconsistent with Azari and Barney (2013); Mohammed *et al.* (2020). Althogh the isolated bacteria from males 53(53%) were higher than females 47(47%) but with no significant difference in the frequency of bacteria in agreement with Sthapit and Tuladhar (2014). Since, the high percentage of bacteria in females, despite the low percetntage of samples compared with males due to the samples of contact lens infection which were collected from females only.

According to the cases, the higher isolation of bacteria was in the samples from contact lens infections (53.48%) followed by conjunctivitis (44.07%) and keratitis (38.77%) but with no significant differences. However, the bacteria from conjunctivitis and keratitis accompanied with contact lens (53.84%) was higher than the total from conjunctivitis and keratitis without contact lens cases (42.78%). These differences may indicate that the wearing of contact lens with less disinfection care will increase the chance of bacterial infection. Since, there was a strong link between microbial keratitis, storage case hygiene and the replacement with microbial contamination of the storage case (Stapleton, 2020). Present study showed bacterial conjunctivitis was higher than keratitis in agreement with some researches finding the conjunctivitis is more common than keratitis (Roberts, 2010; Lee *et al.*, 2018). The increased risk of developing microbial keratitis associated with contact lens has been accompanied with the corneal epithelium modification providing a niche for carrying microorganisms in the ocular surface and limiting the eye natural mechanisms for clearing microbes (Carnt *et al.*, 2010; Willcox *et al.*, 2010).

The bacterial isolation was higher in males than females in cases of conjunctivitis and keratitis without contact lens with significant differences, this agreed with Panda *et al.* (2007); Fumilayo *et al.* (2020). While in the cases of contact lens (conjunctivitis and keratitis) the bacteria were only of females (100%) because females using contact lens much more than males in our society, and during the period of present study, no male patient attend to the hospital suffering from infection due to contact lens wearing. This result agreed with Ibrahim *et al.* (2018) for the same reason reportinng the isolation from conjunctivitis and keratitis was only in male cases without contact lens. Interestingly, the bacteria from conjunctivitis and keratitis in females were higher in contact lens cases (100%) than without contact lenses (38.37%), this is may be due to the low hygiene uses of contact lenses increasing the hypoxia of the cornea with low humidity and physical irritation to increase bacterial pathogens (POLSE, 1990).

Distribution and phylogenetic relationships among the studied bacterial species and their identical type strains were shown in Figure (2). Sequences of 14 bacterial species were chosen as one species from each similar species to avoid the overlapping in the tree concatenated with 899 bp according to the shorter sequence in the present study. *S. aureus* and *S. epidermidis* were the most common species (33%) in agreement with many studies (Kerr and Stern, 1992; Azari and Barney, 2013; O'Callaghan, 2018). Since, these species are commensal on the skin and can opportunistically colonize the ocular surface (Petrillo *et al.*, 2020) or because the high number of lysozyme resistance strains (Bera *et al.*, 2005). *B. subtilis, E. hormaechei*, S. *lugdunensis*, *B. amyloliquefaciens* and *S. haemolyticus* were found only in the cases of conjunctivitis, this is agreed with Boiko *et al.* (2014); Di Ianni *et al.* (2015); Haq *et al.* (2013); Land *et al.* (2018). Interestigly, *B. pumilus* and *E. cloacae* were only isolated from keratitis cases this is agreed with Peng *et al.* (2018); Teweldemedhin *et al.* (2017). While, in the contact lens cases the species with the

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 8258 - 8277
Received 16 February 2021; Accepted 08 March 2021.

highest frequency was *P. aeruginosa* in agreement with Fleiszig *et al.* (2020); Stapleton (2020). This is because of motility,pili, flagella, biofilm forming and binding sites of *P. aeruginosa* can facilitate adhesion process (Duran *et al.*, 1987; Tran, 2011; Zimmerman, 2016).

According of the results from many studies that suggested a range between 0.5% and 1% of differences (99.5 to 99% similarity) is usually very useful in classification (Abd Al-Abbas *et al.*, 2012; Mossong *et al.*, 2013). In the present study, 24 isolates from conjunctivitis and keratitis were recorded as new strains. The similarities of strains against their referece strains were more than 99% (1% difference in *16S rRNA* sequence). These new strains may be resulted from mutations caused by unrepaired damage of DNA or RNA strand usually caused by radiation or chemical mutagens such as antibiotics, the mutation may caused by error in the process of DNA replication including insertion or deletion (Burrus and Waldor, 2004; Aminetzach *et al.*, 2005). On other hand, the frequency of new strains isolated from contact lens cases (42.85%) was higher than those from non-contact lens (20.93%) with high significant difference. Since, the contact lens may provide an environment that protect bacteria from host defenses and prolong the retention time of bacteria on ocular surface allowing the replication and the preferential selection of certain virulence factors. The changing of bacterial genotypic in the eye is associated with the long using of the contact lens. These changes may be due to bacterial biofilm formation deposition of lysozyme, albumin, immunoglobulin, lipids, in addition to materials of lens, storing case and solution on the lens (Stapleton and Carnt, 2012; Boost *et al.*, 2017). Contact lens wearing may lead to molecular changes causing resistance to antibiotics and antiseptic, a study compared spectacle wearers with long-term contact lens users revealed that the latter group had significantly more isolates with antiseptic resistance genes (Shi *et al.*, 2015).

S. aureus was chosen for designing primer because the first *OatA* primer in the history was designed for *S. aureus* (Bera *et al.*, 2005). The new designed primer were tested toward 100 *OatA* sequences from NCBI for lysozyme resistant *S. aureus* to be sure that the primer will align to all *OatA* sequences. The new designed primer were used to detect the *OatA* gene in all 100 studied isolates and showed a specificity to amplify the *OatA* gene in *S. aureus* only, because the gene depending to designed *OatA* primer was for *S. aureus* referring that the different bacterial species may be needs different specific *OatA* primer. However, the genes amplified by PCR were sequenced and matched with the standard gene sequence from NCBI. From 24 *S. aureus* positive to lysozyme resistance assay, 22(91.6%) isolates were *OatA* positive. Because the new primer was amplified the *OatA* from *S. aureus* then it considered as a specific primer.

Two novel *OatA* alleles were published in NCBI referring to the new mutation in the *OatA* gene responsible for lysozyme resistance. By using multiple sequence alignment (MSA), the *OatA* gene sequences were compared among 22 *S. aureus* strains in the present study. Because the degree of heterogenicity between *OatA* alleles and for better alignment, the sequences were divided into two groups (Group 1 and Group 2). The comparison results showed there were 15 different alleles of *OatA* gene. This is may indicate to the changing in the gene among the strains as a result to the randomly mutations. On other hand, the strains with similar alleles are likely to be identical and descended from common ancestor strain infected patient before weeks or months and transmitted it to other patients (Enright *et al.*, 2000).

Most isolates of the present study were susceptible to MXF, GAT and OFX, this is agreed with Kowalski *et al.* (2003); Wong *et al.* (2012). While the lowest susceptibility were to T, VA and C in agreement with Shalchi *et al.* (2011).

Bacterial strain typing is a way for phenotyping and genotyping systems to identify bacteria at the strain level for ascertaining whether the strains derived from the single parental bacteria. Antibiotic susceptibility typing determines the resistance of bacteria growing on media in the presence of antibiotics (disk diffusion, agar dilution and gradient test) which provide qualitative and quantitative values can be used to detect phenotypic variations between strains (Maugeri *et al.*, 2019). In the present study, 8 strains of *S. aureus* were identical in the results of antibiotic susceptibility test, meaning they cannot be discriminated and they need to be differentiated by genotyping system.

Bacterial species and strains need to be accurately described for its importance in epidemiology and ecology. Closely related isolates are very similar and difficult to recognize and differentiate by biochemical methods. Because phenotypes not variable enough for discriminating between closely related strains, while due to its high resolution, genotyping which discriminates bacterial strains based on their genetic content has become widely used for bacterial

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 8258 - 8277
Received 16 February 2021; Accepted 08 March 2021.

strain typing (Wenjun Li, 2009). Therefore, genetic differences of similar *S. aureus* strains were done in the present study by random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR). The benefit of using more than single primers is improve the differentiation power of RAPD technique (Abd Al-Abbas et al., 2012). Two patients of 8 (25%) have the same strains of *S. aureus* strains (Figure 10 and Table 6) referring to these two strains are descended from the same ancestor and may infected other patients. This is a sign to the ability of the same strain to transmit among the humans causing the same infection.

Conclusions

S. aureus and *S. epidermidis* were the most species in eye infections. Some *S. aureus* have the ability to resist the eye lysozyme with different *OatA* alleles and the same bacterial strain could be transmitted between patients. Strains with contact lens infection had more DNA mutation.

Acknowledgement

Grateful to the ophthalmologists Dr. Zainab K. Aldoraky, Dr. Mohammed A. Al Ashoor and Dr. Maha A. Mohammed for their help in collecting samples.

References

[1] Abd Al-Abbas, M.J., Al-Samak, A.M., Al-Luaibi, Y.Y., & Mohammed, H.F. (2012). RAPD-PCR Typing to Confirm the Role of Nasopharyngeal Staphylococcus Epidermidis as a Source of Conjunctivitis in the Same Patient. *American Journal of Scientific Research*, 130-141.

[2] Abd Al Wahid, Z., & Abd Al-Abbas, M.J. (2019). Detection of E. Coli Strains Isolated from Water Sources and Diarrhea Cases by Random Amplified Polymorphic DNA in Basrah Governorate. *J International Journal of Sciences, 8*, 68-83.

[3] Aminetzach, Y.T., Macpherson, J.M. and Petrov, D.A. (2005). Pesticide resistance via transposition-mediated adaptive gene truncation in Drosophila. *Science, 309*, 764-767.

[4] Azari, A.A., & Barney, N.P. (2013). Conjunctivitis: a systematic review of diagnosis and treatment. *Jama, 310*, 1721-1730.

[5] Becker, K., Harmsen, D., Mellmann, A., Meier, C., Schumann, P., Peters, G., & Von Eiff, C. (2004). Development and evaluation of a quality-controlled ribosomal sequence database for 16S ribosomal DNA-based identification of Staphylococcus species. *Journal of clinical microbiology, 42*, 4988-4995.

[6] Bera, A., Herbert, S., Jakob, A., Vollmer, W., & Götz, F. (2005). Why are pathogenic staphylococci so lysozyme resistant? The peptidoglycan O-acetyltransferase OatA is the major determinant for lysozyme resistance of Staphylococcus aureus. *Molecular microbiology, 55*, 778-787.

[7] Boiko, E.V., Pozniak, A.L., Maltsev, D.S., Suetov, A.A. and Nuralova, I.V. (2014). High frequency of latent conjunctival C. trachomatis, M. hominis, and U. urealyticum infections in young adults with dry eye disease. *Journal of Ophthalmology*, 2014.

[8] Boneca, I.G., Dussurget, O., Cabanes, D., Nahori, M.A., Sousa, S., Lecuit, M., Psylinakis, E., Bouriotis, V., Hugot, J.P., & Giovannini, M. (2007). A critical role for peptidoglycan N-deacetylation in Listeria evasion from the host innate immune system. *Proceedings of the National Academy of Sciences, 104*, 997-1002.

[9] Boost, M., Cho, P., & Wang, Z. (2017). Disturbing the balance: effect of contact lens use on the ocular proteome and microbiome. *Clinical experimental optometry, 100*, 459.

[10] Burrus, V., & Waldor, M.K. (2004). Shaping bacterial genomes with integrative and conjugative elements. *Research in microbiology, 155*, 376-386.

[11] Carnt, N., Willcox, M.D., Keay, L., Flanagan, J., & Stapleton, F. (2010). Pathogenesis of contact lens-associated microbial keratitis. *Optometry Vision Science, 87*, 612-613.

[12] Chmagh, A.A., & Abd Al-Abbas, M.J.J.G.R. (2019). Comparison between the coagulase (coa and vwb) genes in

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 8258 - 8277
Received 16 February 2021; Accepted 08 March 2021.

Staphylococcus aureus and other staphylococci., *16*, 100410.

[13] CLSI (2017). Performance standards for antimicrobial susceptibility testing M100. *Journal*.

[14] Davis, K.M., & Weiser, J.N. (2011). Modifications to the peptidoglycan backbone help bacteria to establish infection. *Infection immunity*, *79*, 562-570.

[15] Di Ianni, F., Dodi, P.L., Cabassi, C.S., Pelizzone, I., Sala, A., Cavirani, S., Parmigiani, E., Quintavalla, F., & Taddei, S. (2015). Conjunctival flora of clinically normal and diseased turtles and tortoises. *BMC veterinary research*, *11*, 91.

[16] Duran, J.A., Refojo, M.F., Gipson, I.K., & Kenyon, K.R. (1987). Pseudomonas attachment to new hydrogel contact lenses. *Archives of Ophthalmology*, *105*, 106-109.

[17] Enright, M.C., Day, N.P., Davies, C.E., Peacock, S.J., & Spratt, B.G. (2000). Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones ofStaphylococcus aureus. *Journal of clinical microbiology*, *38*, 1008-1015.

[18] Fair, R.J., & Tor, Y. (2014). Antibiotics and bacterial resistance in the 21st century. *Perspectives in medicinal chemistry*, *6*, PMC. S14459.

[19] Fleiszig, S.M., Kroken, A.R., Nieto, V., Grosser, M.R., Wan, S.J., Metruccio, M.M., & Evans, D.J. (2020). Contact lens-related corneal infection: Intrinsic resistance and its compromise. *Progress in retinal eye research*, *76*, 100804.

[20] Fumilayo, A.J.,, O.O.,, A.K.,, A.R.A.,, A.S., & Buru (2020). Incidence of Bacterial Conjunctivitis among Patients attending Obafemi

[21] Awolowo University Teaching Hospital Complex, Ile-Ife, Nigeria. *Appli Microbiol open Access*, 6, 5.

[22] Garcia-Vallvé, S., & Puigbo, P. (2009). DendroUPGMA: a dendrogram construction utility. *Universitat Rovira i Virgili*.

[23] Haq, A., Wardak, H., & Kraskian, N. (2013). Infective conjunctivitis–its pathogenesis, management and complications. *Journal of forensic sciences*, *21*.

[24] Ibrahim, N.K., Seraj, H., Khan, R., Baabdullah, M., & Reda, L. (2018). Prevalence, habits and outcomes of using contact lenses among medical students. *Pakistan Journal of Medical Sciences*, *34*, 1429.

[25] Kerbauy, G., Perugini, M., Yamauchi, L.M., & Yamada-Ogatta, S.F. (2011). Vancomycin-dependent Enterococcus faecium vanA: characterization of the first case isolated in a university hospital in Brazil. *Brazilian Journal of Medical Biological Research*, *44*, 253-257.

[26] Kerr, N., & Stern, G.A. (1992). Bacterial keratitis associated with vernal keratoconjunctivitis. *Cornea*, *11*, 355-359.

[27] Kowalski, R., Dhaliwal, D., Karenchak, L., Romanowski, E., Mah, F., Ritterband, D., & Gordon, Y. (2003). Gatifloxacin and moxifloxacin: an in vitro susceptibility comparison to levofloxacin, ciprofloxacin, and ofloxacin using bacterial keratitis isolates. *American journal of ophthalmology*, *136*, 500.

[28] Land, A., Penno, K., & Brzezinski, J. (2018). Identification of Microorganisms Isolated From Counterfeit and Unapproved Decorative Contact Lenses. *Journal of forensic sciences*, *63*, 635-639.

[29] Lee, J.Y., Kim, J.W., Kim, E.J., Lee, M.Y., Nam, C.W., & Chung, I.S. (2018). Spatial analysis between particulate matter and emergency room visits for conjunctivitis and keratitis. *Annals of occupational environmental medicine*, *30*, 41.

[30] Leibowitz, H.M. (2000). The red eye. *New England Journal of Medicine*, *343*, 345-351.

[31] Maugeri, G., Lychko, I., Sobral, R., & Roque, A.C. (2019). Identification and antibiotic-susceptibility profiling of infectious bacterial agents: a review of current and future trends. *Biotechnology journal*, *14*, 1700750.

[32] Mohammed, A.A., Ali, M.M., & Zenebe, M.H. (2020). Bacterial etiology of ocular and periocular infections, antimicrobial susceptibility profile and associated factors among patients attending eye unit of Shashemene comprehensive specialized hospital, Shashemene, Ethiopia. *BMC ophthalmology*, *20*, 1-8.

[33] Mossong, J., Gelhausen, E., Decruyenaere, F., Devaux, A., Perrin, M., Even, J., & Heisbourg, E. (2013).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 8258 - 8277
Received 16 February 2021; Accepted 08 March 2021.

Prevalence, risk factors and molecular epidemiology of methicillin-resistant Staphylococcus aureus (MRSA) colonization in residents of long-term care facilities in Luxembourg, 2010. *Epidemiol Infect, 141*, 1199-206.

[34] O'Callaghan, R.J. (2018). The pathogenesis of Staphylococcus aureus eye infections. *Pathogens, 7*, 9.

[35] Panda, A., Satpathy, G., Nayak, N., Kumar, S., & Kumar, A. (2007). Demographic pattern, predisposing factors and management of ulcerative keratitis: evaluation of one thousand unilateral cases at a tertiary care centre. *Clinical experimental ophthalmology, 35*, 44-50.

[36] Peng, M.Y., Cevallos, V., McLeod, S.D., Lietman, T.M., & Rose-Nussbaumer, J.J.C. (2018). Bacterial keratitis: isolated organisms and antibiotic resistance patterns in San Francisco, *37*, 84.

[37] Petrillo, F., Pignataro, D., Lavano, M.A., Santella, B., Folliero, V., Zannella, C., Astarita, C., Gagliano, C., Franci, G., & Avitabile, T. (2020). Current Evidence on the Ocular Surface Microbiota and Related Diseases. *Microorganisms, 8*, 1033.

[38] Polse, K. (1990). Contact lens use under adverse conditions: Applications in military aviation.

[39] Roberts, A. (2010). Eye Infections.

[40] Rozen, S., & Skaletsky, H. (2000). Primer3 on the WWW for general users and for biologist programmers. *Bioinformatics methods and protocols.* Springer.

[41] Schaefer, F., Bruttin, O., Zografos, L., & Guex-Crosier, Y. (2001). Bacterial keratitis: a prospective clinical and microbiological study. *The British Journal of Ophthalmology, 85*, 842-847.

[42] Shalchi, Z., Gurbaxani, A., Baker, M., & Nash, J. (2011). Antibiotic resistance in microbial keratitis: ten-year experience of corneal scrapes in the United Kingdom. *Ophthalmology, 118*, 2161-2165.

[43] Shi, G., Boost, M., & Cho, P. (2015). Prevalence of antiseptic-resistance genes in staphylococci isolated from orthokeratology lens and spectacle wearers in Hong Kong. *Investigative ophthalmology visual science, 56*, 3069.

[44] Stapleton, F. (2020). Contact lens-related corneal infection in Australia. *Clinical Experimental Optometry*.

[45] Stapleton, F., & Carnt, N. (2012). Contact lens-related microbial keratitis: how have epidemiology and genetics helped us with pathogenesis and prophylaxis. *Eye, 26*, 185-193.

[46] Sthapit, P.R., & Tuladhar, N.R. (2014). Conjunctival flora of normal human eye. *JSM Ophthalmol, 2*, 1021.

[47] Teweldemedhin, M., Gebreyesus, H., Atsbaha, A.H., Asgedom, S.W., & Saravanan, M. (2017). Bacterial profile of ocular infections: a systematic review. *BMC ophthalmology, 17*, 212.

[48] Tran, V.B. (2011). *Pseudomonas Aeruginosa Adhesion to Soft Contact Lenses: Effects of Cell Surface and Substrate Chemistry*. UC Berkeley.

[49] Viala, J., Chaput, C., Boneca, I.G., Cardona, A., Girardin, S.E., Moran, A.P., Athman, R., Mémet, S., Huerre, M.R., & Coyle, A.J. (2004). Nod1 responds to peptidoglycan delivered by the Helicobacter pylori cag pathogenicity island. *Nature immunology, 5*, 1166-1174.

[50] Wenjun Li, D.R., Pierre-Edouard Fournier (2009). Bacterial strain typing in the genomic era *FEMS Microbiology Reviews, 33*, 892–916.

[51] Whitcher, J.P., Srinivasan, M., & Upadhyay, M.P. (2001). Corneal blindness: a global perspective. *Bulletin of the world health organization, 79*, 214-221.

[52] Willcox, M.D., Carnt, N., Diec, J., Naduvilath, T., Evans, V., Stapleton, F., Iskandar, S., Harmis, N., De la Jara, P.L., & Holden, B.A. (2010). Contact lens case contamination during daily wear of silicone hydrogels. *Optometry Vision Science, 87*, 456-464.

[53] Wong, R.L., Gangwani, R., Yu, L.W., & Lai, J.S. (2012). New treatments for bacterial keratitis. *Journal of ophthalmology*, 2012.

[54] Zare, S., Derakhshandeh, A., Haghkhah, M., Naziri, Z., & Broujeni, A.M. (2019). Molecular typing of Staphylococcus aureus from different sources by RAPD-PCR analysis. *Heliyon, 5*, e02231.

[55] Zimmerman, A.B.J.T. (2016). Contact lens associated microbial keratitis: practical considerations for the optometrist, *155*.