Prevalence and Molecular Diagnosis of Certain Ocular Bacterial and Viral Infections of Some Patients in Basrah City

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Abstract

The eye, a functionally complex organ, is susceptible to a variety of bacterial, viral, and fungal infections. This study aims to evaluate the results and their accuracy in identifying the most important bacterial and viral infections of the conjunctiva that could be good candidates for this. This research included collecting (60) samples (swabs) from patients' eyes in the form of Swab for bacterial diagnosis and VTM for viral diagnosis for those suffering from conjunctivitis during their visit to Al-Mawani Teaching Hospital and (Al-Basira Clinic specializing) in ophthalmology in Basra Governorate, southern Iraq, during the period from December the second until July (2022). The study confirmed that bacteria and viruses are one of the causes of conjunctivitis, as certain types of viruses were diagnosed using the Rel-Time PCR technique, the highest percentage of which was *Chlamydia trachomates* (73.3%), followed by Varicella zoster virus (68.3%), and HSV-2. (61.6%) and finally HSV-1 (58.3%).

The study subjected samples to direct examination and identification of bacterial colonies using an electron microscope, chemical tests, and the application of the (Vitek2) system to ensure the validity of the results and showed a percentage of *Staphylococcus aureus* bacteria (6.6%) .

This study relied on the gender of the patients in distributing the samples. The percentage of males was (53%) and the percentage of females was (47%). Based on the difference between age groups and the differences in injuries among them, the highest percentage included those between (26-35) (30%) and (36-45) (28%).

Keywords: Conjunctiva, HSV-1, HSV-2, VZV, C. *trachomatis*

Introduction

Conjunctivitis is a generic word for a variety of infections caused by "bacteria, viruses, or fungi" as well as noninfectious instances caused by "allergic, chemical, and mechanical factors." Every year, around 5 million instances of infectious conjunctivitis are reported worldwide (1).

The conjunctiva is a delicate and transparent mucous membrane that covers the inner surface of both the superior and inferior eyelids (referred to as the tarsal conjunctiva) as well and the anterior side surface of the bulbar conjunctiva is the outer layer of the sclera. It maintains its integrity and shape while lining these areas (2).

Conjunctivitis may occur alone or as a complication of ocular inflammation (3).

The presence of "red eye" which refers to significant Conjunctival hyperemia is seen in the Swollen conjunctiva or the palpebral/tarsal conjunctiva, is an anatomical structure symptom in the clinic and caused by an infection in the conjunctiva This symptom is sometimes accompanied by pain and discharge (4).

Acute red eyes account for 1-4% of all general practitioner (GP) appointments in the developed world, with acute bacterial conjunctivitis accounting for 50-75% of all instances (1). Due to the commonality of most clinical presentations, diagnostic testing is seldom required (3).

Typically, viral conjunctivitis affects both eyes and is transmitted via close contact with infected individuals. Allergic conjunctivitis is characterized by bilateral symptoms, including excessive tearing and the presence of grayish, sparse, and stringy mucus. In contrast, bacterial conjunctivitis often affects a discharge, and it affects just one eye including pus, the lid of the eyes sticking, with a clumped look after waking up (2).

When the conjunctiva is infected with bacteria, it becomes inflamed and produces excessive tears. The severity of inflammation and purulent discharge might range from mild and self-limiting to severe (4).

Haemophilus influenzae is a significant cause of acute bacterial conjunctivitis, along with *Staphylococcus aureus* and *Streptococcus pneumoniae*. Bacterial conjunctivitis is distinguished by redness, which may occur in one or both eyes, along with a generally thick and pus-like discharge. Other symptoms include sensitivity to light, excessive tearing, irritation, a sensation of stinging or burning, and discomfort (5).

Chlamydia trachomatis is the primary causative agent of chronic conjunctivitis and the resulting condition known as trachoma (6).

A suggestion has been made to split Chlamydophila from Chlamydia and to classify Chlamydia individuals as Chlamydiales. *C trachomatis* causes trachoma conjunctivitis, which can lead to blindness, pneumonia, and urinary tract infection (7).

The conjunctivitis that a virus causes may be transmitted from one person to another by close contact with an infected individual or their secretions, or through coming into contact with polluted items or surfaces (8).

Viral conjunctivitis often presents with a watery or mucoserous discharge, accompanied by a burning sensation and a gritty feeling in one eye. Patients may experience the formation of crusts, particularly in the morning, which are then followed by the release of a watery discharge or occasional small amounts of mucus throughout the day (9).

The second eye usually starts to hurt within twenty-four to forty-eight hours. It's conceivable for the tarsal conjunctiva to resemble a follicle. An enlarged and uncomfortable preauricular node is a potential

complication. In many cases, viral conjunctivitis resolves without medical intervention after a few days have passed (8).

Keratitis and conjunctivitis are ocular conditions manifestations keratoconjunctivitis epidemic, which is a specific virus that causes eye inflammation. The most prevalent causes in this instance are adenoviruses of types (8), (19), and (37). (9).

Certain individuals with viral conjunctivitis may also have a sensation of a foreign object in their eyes, which hinders their ability to open their eyes easily. Additionally, they may have many corneal infiltrates that might potentially impair their eyesight. Seeking medical attention is essential if you feel you have keratitis since it has the potential to result in irreversible vision loss (10).

Between 1.3% and 4% of all occurrences of viral conjunctivitis are the result of infection with the herpes simplex virus, the etiology of herpes conjunctivitis (11-13).

Conjunctivitis often affects just one eye and is characterized by a thin, watery discharge and the presence of vesicular ulcers on the lid of the eyes. Refrain from applying glucocorticoids used topically since they may potentially exacerbate the condition (14).

Methods

Sixty samples were collected from people who had a bacterial infection diagnosis. and viral ocular infection and received medical care at Al-Mawani Teaching Hospital and (Al-Basira Clinic specializing) in ophthalmology Within the timeframe of January (2022) to June (2022), in the Basrah Governorate of Iraq. These samples were used as a control group and tested in the current study. The collected samples included eye swabs intended for microbiological investigation. VTM was used for the purpose of transporting viruses into the eye. Physiological and epidemiological samples the results were acquired under the supervision of an ophthalmologist.

Every participant in the research was diagnosed with conjunctivitis by clinical evaluations. The patients displayed a range of clinical symptoms including redness, eye irritation, itching, and discharge. The study excluded individuals who had undergone antimicrobial therapy. Patients' data was gathered using the administration.

This required collecting a wide range of data, including patient demographics, pathological features, inflammatory type, and bacterial species. The patients' swabs were forwarded to the lab for further investigation.

Detection of viruses

The process of extracting DNA

1. The components and tools used in the process of DNA extraction included:

1-1 Sanitation was performed on pipettes, pipette tips, and centrifuge tubes with capacities of (1.5) mL and (2.0) mL.

1-2 For the manufacture of Wash Buffers (1) and (2), (96 to 100) percent ethanol is required.

To process centrifuge work:

- Preparation of VNE-Carrier DNA Buffer

(1 milliliter) of VNE buffer was added to the freeze-dried carrier DNA cylindrical hollow structure. Vertexing was used to thoroughly combine the ingredients, and then the mixture was moved to the VNE Caching on the very first opening. At a temperature of four degrees Celsius, the VNE-carrier DNA buffer was maintained.

- Both Wash Buffer (1) and Wash Buffer (2) are prepared in this instance.

A quantity of ethanol, ranging from (96%) to (100%), was added. Both Wash Buffer (1) and (2), which included ethanol, were kept at temperatures ranging from 15 to 25 degrees Celsius.

2. Using the Centrifuge Method:

- Swabs used as a transport medium

- 1-b(one). The swab was turned and transferred to the tube with great care, and then it was twisted with great care to drop drips that were stuck to the wall of the cylinder.
- 1-b(two). To a microcentrifuge cylinder, a volume of media measuring 140 microliters was transferred.

3. Procedure for lysis of samples:

- A volume of (560) microliters of VNE carrier DNA solution was added.

It was mixed well by stirring gently and then left for ten minutes at room temperature.

Iterative improvement of the binding condition

An amount of 560 microliters of ethanol (96-100% concentration) was added to the sample mixture and well-mixed using a vortex mixer.

4. Attach viral DNA to the surface of the column membrane.

5. Wash column membrane.

6. Dry membrane.

Detection of molecules with the use of real-time PCR

Procedures taken

- **The first extraction should be carried out.**

Extraction of a sample and implementation of a negative control (NC):

- The negative control (NC) and the internal control (IC) were solubilized.
- Positive controls (PC) were not removed since they were in the form of plasmids, which makes them susceptible to degradation. This was the reason why they were not extracted.

Main PCR setup procedure

- **The experiment was set as for Rotor-Gene Q:**

1. This procedure makes use of the dissolving reagents from the fast-track master mix, which can be recognized by its light blue cap, as well as Vesic PP, CT/AV PP, positive control (PC), and 2x RT-PCR buffer. Each of the two halves included both the (PC) and the (NC) that had been removed. To get the chemicals ready for usage, they were first thoroughly dissolved, then they were combined by a brief process of vortexing, and last, they were shaken for a short period. After that, the (PC) were allowed to thaw at room temperature for a period of (twenty) to (thirty) minutes, and they were suitably prepared before being used. To preserve its integrity, the 25-fold enzyme RT-PCR (Fast-track master mix, orange cap) that is derived from the Fast-track master mix is always kept in either the freezer or the cooling block.
2. The remaining amounts of (PP) and (PC) needed to be refrozen as soon as possible (it was important).

- Following **FTD-15.1-32**, each ppmix is enough for thirty-two reactions, plus the inaccuracy of pipetting. It is possible to have a minimum of one patient and a maximum of thirty patients or more, in addition to PC and NC.
- Following **ftd-15.1-64**, each ppmix is enough for sixty-four reactions, plus the inaccuracy of pipetting. With the addition of PC and NC, it is feasible to have a minimum of one patient and a maximum of sixty-two patients.

Rotor-Gene Q was used to make a 36-well plate):

1. The Rotor-Gene Q-compliant 64-well plate was selected for use.
2. Pipette up and down to create a short mixture.
3. Rotor-Gene Q optical adhesive film was used to make the board secure.
4. Shortly after that, the plate was spun and centrifuged to separate the particles.
5. Incorporating the board into the Rotor-Gene Q instrument

Table (1): the amounts of chemicals that are necessary for a variety of well counts are shown here, namely (1), (9), (32), and (64).

Total count of reactions		**1**	**9**	**32**	**64**
ftd-15.1-32/64	Buffer	12.5 µl	112.5 µl	400 µl	800 µl
	Ppmix	1.5 µl	13.5 µl	48 µl	96 µl
	Enzyme	1 µl	9 µl	32 µl	64 µl
	Total	15 µl	135 µl	480 µl	960 µl

Configuring the thermocycler for programming

Table (2): Provides a comprehensive summary of the programming used for the sample, control, and detector configurations.

Programming for the Fast Finder device, including both sample and control programming.			The programming of detectors			
"Prefix" (nametag of the sample type)	Nametag suffix for mix description	last-minute programming	Master mix	Infectious Agent	Pigment	Wavelength of detection (in nanometers)
pc nc sample name	-ey1	pc-ey1 nc-ey1 …-ey1	Vesic	HSV-1	GREEN	520
				HSV-2	YELLOW	550
				IC(MCMV)	ORANGE	610
				VZV	RED	670
pc nc sample name	-ey2	pc-ey2 nc-ey2 …-ey2	CT/AV	IC(MCMV)	YELLOW	550
				C. trachomatis	ORANGE	610
				HAdV	RED	670

Results

Study population

The salient characteristics of the research population are outlined in Table 3. The present investigation included the collection of sixty ocular infection samples from patients diagnosed with conjunctivitis at Al-Mawani Teaching Hospital and Al-Basira Clinic, which specializes in ophthalmology, in Basrah City. During the period of research spanning from March (2022) to July (2022). There were 32 men, accounting for 53% of the patients, and 28 females, accounting for 47% of the patients. The patients were categorized into seven age cohorts, and the outcomes of cases within each cohort are as follows: 3 individuals, accounting for 5% of the total, are between the ages of 5 and 15 years old. 10 individuals, representing 7% of the total, fall within the age range of (16) to (25) years old. (18) individuals, making up (30%) of the total, are between the ages of (26) and (35) years old. (17) individuals, comprising (28%) of the total, fall within the age range of (36) to (45) years old. (7) individuals, accounting for (12%) of the total, are between the ages of (46) and (55) years old. (3) individuals, representing (5%) of the total, fall within the age range of (56) to (65) years old. Lastly, (2) individuals, making up (3%) of the total, are between the ages of (66) and (75) years old. Conjunctivitis was most prevalent in the age range of (26-35) years, followed by the age group of (36-45) years, and finally by the age group of (16-25) years.

Table (3) provides a comprehensive description of the common clinical signs and symptoms of conjunctivitis seen in individuals of different age groups. The observed indicators were as follows: redness in (37) instances (61.6%), discharge in (31) cases (51.6%), conjunctival congestion in (30) cases (50%), discomfort in (20) cases (33.3%), and impaired vision in (20) cases (33.3%). Along with these symptoms, (5) instances (8%) of DM and subconjunctival hemorrhage were seen, and (9) cases (15%) of corneal abscesses were identified. Out of all the instances, only (4) (6.6%) had an anterior chamber response.

Although samples were taken at various times of the year, the highest concentration was (22) in June (36.7%), then (18.3%) in May, (16.7%) in July, (15%) in April, and (13.3%) in March. Table 3 presents the information.

Detection of viruses and C. trachomatous

All samples were analyzed using real-time PCR with specific primers for HSV-1, HSV-2, VZV, and *C. trachomatis*. The findings may be seen in Table 4. Out of the (60) samples analyzed, HSV-1 was found in (35) samples (58.3%), HSV-2 in (37) samples (61.6%), VZV in (41) samples (68.3%), and *C. trachomatis* in (44) samples (73.3%). Just as Table (4) shows. The predominant Infectious Agent is *C. trachomatis*, followed by VZV, HSV-2, and finally HSV-1. However, this investigation did not find any bacteria often linked to conjunctivitis, save for a small number of *S. aureus* cases.

Table (3): depicts the features of the study population in their respective distributions.

Different factors		Number of instances	The proportion
Sex	MALE	32	53%
	FEMALE	28	47%
	Total	60	100%
Age groups	26-36	18	30%
	36-46	17	28%
	16-26	10	17%
	46-56	7	12%
	5-16	3	5%
	56-65	3	5%
	66-75	2	3%
	Total	60	100%
Clinical signs and symptoms	Redness	37	61.66%
	Discharge	31	51.66%
	Conjunctiva congestion	30	50%
	Pain	20	33.33%
	Blurred vision	20	33.33%
	Contact lens wear	13	21.66%
	Sub-epithelial infiltrate	9	15%
	Corneal abscess	7	11.66%
	Sub-conjunctival hemorrhage	5	8.33%
	DM	5	8.33%
	Anterior Chamber reaction	4	6.66%
Time of collection	June	22	36.7%
	May	11	18.3%
	July	10	16.7%
	April	9	15%
	March	8	13.3%
	January	0	0%
	February	0	0%
	Total	60	100%

Table (4): This table shows the number of viruses and bacteria that were discovered in clinical samples taken from patients who had been diagnosed with conjunctivitis.

Infectious Agent	Number of instances	The proportion
C. trachomates	44	73.3%
VZV(Hadv)	41	68.3%
HSV-2	37	61.6%
HSV-1	35	58.3%

PCR amplification of a given gene in real-time

The magnifying the effect of a particular We took advantage of genes to identify every virus under research, as well as *C. trachomatous*.

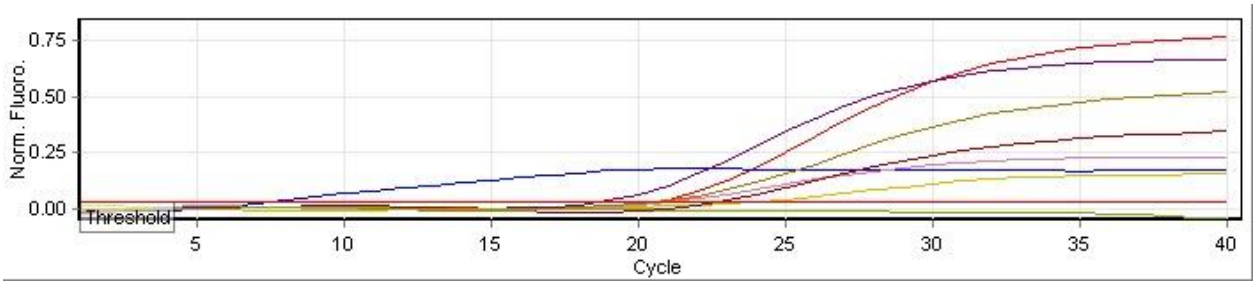

Figure (1): HSV-1 The threshold for the amplification curve was established automatically by the machine

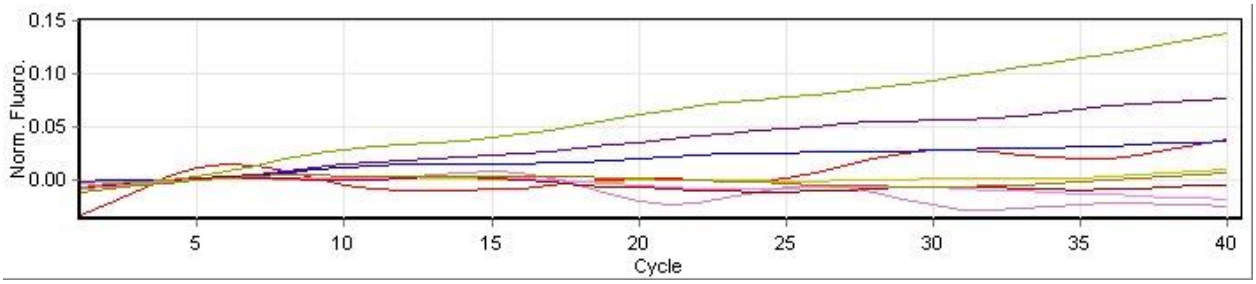

Figure (2): HSV-2 the threshold for the amplification curve was established automatically by the machine

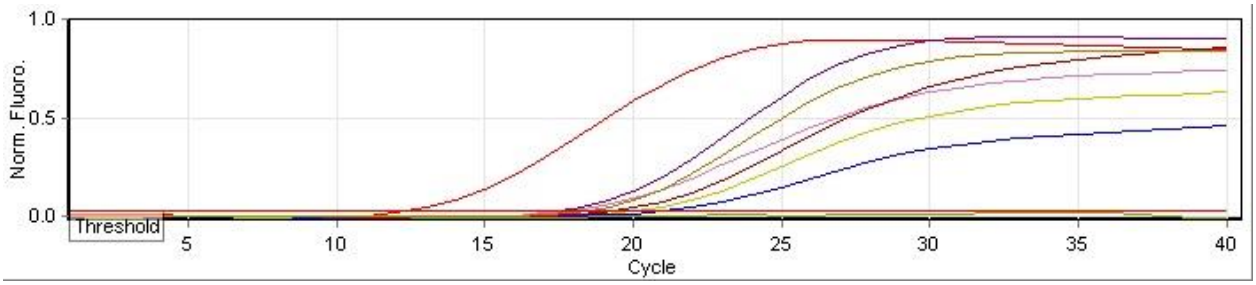

Figure (3): VZV the threshold for the amplification curve was established automatically by the machine

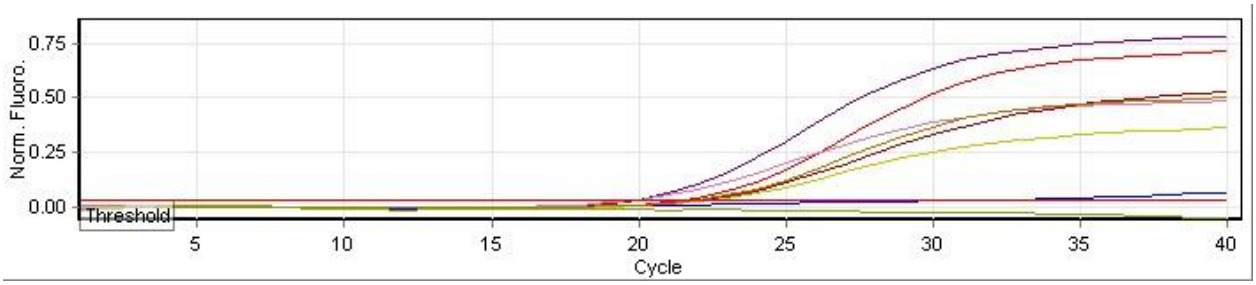

Figure (4): *C. trachomatis* the threshold for the amplification curve was established automatically by the machine

Discussion

We selected and analyzed (60) patients attending ophthalmology clinics in Basrah city who were infected with conjunctivitis with the use of real-time PCR. Contrary to the findings of earlier studies. We were chosen to look into the relationship between the patient's age to the clinical symptoms, months in the year, and how they affected study results.

As compared to alternative Furthermore, other variables influence the occurrence of eye infections, such as gender, age, and immune system health. The majority of eye diseases are caused by microbes, such as viruses and bacteria. The purpose of this research was to identify the most common bacterial and viral causes of eye infections seen by patients at Al-Mawani Teaching Hospital in Basrah Governorate.

The research found that the prevalence of eye infections was almost the same in men and females; (32) patients, or (53%) of the total, were male and 28 patients, or (47%) of the total, were female. The results of our study indicated that the percentage of males is higher than that of females, in contrast to some previous studies, such as the study conducted in the United States of America Which showed that infection more common in women than in men (15). This percentage is due to the large number of men who visit medical clinics or because of customs and traditions in our society, and also because of the nature of men's work compared to women, which exposes them to infection.

As for age groups, it was noted that age groups ranging between (26-36) years (30%) and (36-46) years (28%) are the most susceptible to infection. This study was similar to some previous studies in enhancing the outcome of ocular conjunctivitis, as in a study conducted in a hospital in Tokyo, Japan, The results of this study demonstrated that the incidence of conjunctivitis is very low throughout the first two decades of a person's life and that its frequency gradually rises during the third decade of life (16). Based on these findings, the aging factor is the most critical in conjunctivitis development.

The most common clinical signs and symptoms of conjunctivitis among patients of different ages are also listed. These signs included (redness, secretions, conjunctival hyperemia, blurred vision, and others). The study also showed that the percentage of redness was the highest at different ages for both sexes (61.6%).

A previous study in a Nigerian hospital for eye patients confirmed that redness of the eye is one of the most common causes of

presentation to the eye clinic and it affected both sexes almost equally (17).

The study included collecting samples throughout several months, with the (22) number of samples (36.7%) being taken in June. This was followed by (11) samples (18.3%) in May, (10) samples (16.7%) in July, (9) samples (15%) in April, and (8) samples (13.3%) in March. The prevalence of inflammation throughout the summer and spring seasons was almost high, and there is a potential for an outbreak of diseases. It was also found in a study of a group of patients visiting ophthalmology clinics in western Iran that the highest prevalence of the disease is in the summer (18).

Also, in a study conducted in Turkey, a high percentage of samples were diagnosed in the summer (19).

In summer and spring, there is probably a higher chance of getting conjunctivitis because of the increased frequency of activities like walking and swimming outside during the warm seasons; in winter, there is probably a lower chance of these activities due to the cold weather.

Acknowledgments

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Ethical Statement:

This study was approved by the University of Basra/College of Science Ethical Committee.

-The decision of the Research Committee in the Ministry of Health/Basra Health Department to approve the implementation of the research project after meeting the ethical and scientific standards approved by the Ministry of Health.

- Approval of the Training and Human Development Center in the Basra Health Department to facilitate the research mission to work on completing the requirements in health institutions.
- Approval of the Ministry of Higher Education/University of Basra/College of Science to facilitate the task of completing the work requirements in the central laboratory in the College of Medicine/University of Basra and approval of the central laboratory to complete the work.

Conflicts of interest:

The authors declare there is no conflict of interest.

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