

# Microbial and Molecular Screening of Swimmers Associated with Conjunctivitis from Public Swimming Pools in Erbil Province

Sazan Q. Maulud<sup>1</sup>, Lawin A. Omar<sup>1</sup>, Ahmed N. Hassan<sup>2\*</sup>,  
Rastee H. Saeed<sup>1</sup>

<sup>1</sup>Department of Biology, College of Education, Salahaddin University-Erbil, Erbil, IRAQ

<sup>2</sup>Department of Medical Laboratory Technology, Erbil Health Technical College, Erbil Polytechnic University, Erbil, IRAQ

\*Contact email: [ahmed.nawzad@epu.edu.iq](mailto:ahmed.nawzad@epu.edu.iq)

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## ABSTRACT

Public swimming pools, if not treated well could work as a reservoir of many microorganisms that cause infections among swimmers. Conjunctivitis is one of those common infections that resulted from microbial and non-microbial agents, microbial conjunctivitis caused by viral (mainly Human adenovirus HAdVs) and bacterial infections. This study aims to investigate the prevalence of microbial causative agents of swimming pool conjunctivitis and evaluating the swimming pools in terms of health and the extent of contamination in Erbil province. Eighty-eight specimens were isolated and identified from the swimmers showing signs and symptoms of swimming pool conjunctivitis from different public swimming pools in Erbil city from January to the end of February 2020. Sample identified using bacteriological methods, serology test, and nested PCR for detection of HAdVs. The swimmers samples consisted of 60 males and 28 females, and they were aged between 16-56 years. The obtained results showed that, out of 88 samples, 36 (40.91%) detected as a viral infection and 29 (32.95%) as bacterial infection, while, 23 (26.13%) showed no growth (non-microbial infection). Frequency of swimming pool conjunctivitis among male and female was 60 (68.2%) and 28 (31.8%) respectively. Depending on the obtained results, it can be concluded that conjunctivitis could result from viral, bacterial, and non-microbial agents, a viral infection is the main cause followed by a bacterial infection, also public swimming pools are not a safe place and swimmers are subjected to infection by different pathogens.

**KEYWORDS:** Swimming Pool Conjunctivitis, Viral Conjunctivitis (HAdVs), Bacterial Conjunctivitis, Non-microbial Conjunctivitis, Molecular Diagnosis (Nested PCR).

## الخلاصة

حمامات السباحة العامة، إذا لم تتم معالجتها بشكل جيد، تصبح كخزان للعديد من الكائنات الحية الدقيقة التي تسبب العدوى بين السباحين. التهاب ملتحمه العين هو أحد أنواع العدوى الشائعة التي تنتج عن العوامل الميكروبية وغير الميكروبية، التهاب ملتحمه العين الجرثومي تنتج عن العدوى الفيروسية (بشكل رئيسي الفيروس الغدي البشري HAdVs) والعدوى البكتيرية. الهدف من هذه الدراسة هو التحقق من انتشار العوامل الجرثومية المسببة لالتهاب ملتحمه العين في حمامات السباحة و كذلك تقييم حمامات السباحة العامة من الناحية الصحية ومدى تلوثها في محافظة أربيل. تم عزل وتحديد ثمانية وثمانين عينة من السباحين الذين ظهرت عليهم علامات وأعراض التهاب ملتحمه حمامات السباحة من حمامات السباحة العامة المختلفة في مدينة أربيل من كانون الثاني (يناير) إلى نهاية شباط (فبراير) 2020. تم تشخيص العينات باستخدام الطرق البكتريولوجية واختبار الأمصال، وكذلك فحص PCR المتداخل للكشف عن HAdVs. عينات السباحون تضمنت 60 ذكراً و 28 أنثى، وتراوحت أعمارهم بين 16-56 عاماً. أظهرت النتائج أنه من بين 88 عينة، تم تشخيص 36 (40.91%) عينة كعدوى فيروسية، و 29 (32.95%) عينة كإصابات بكتيرية، و 23 (26.13%) عينة لم تظهر أي نمو (عوامل غير جرثومية). كان معدل الإصابة بالتهاب ملتحمه حمام السباحة بين الذكور والإناث 60 (68.2%) و 28 (31.8%) على التوالي. اعتماداً على النتائج التي تم الحصول عليها، يمكن استنتاج أن التهاب الملتحمه يمكن أن يكون ناتجاً عن عوامل فيروسية وبكتيرية وغير جرثومية، والعدوى الفيروسية هي السبب الرئيسي تليها العدوى البكتيرية، كما أن حمامات السباحة العامه ليست امنه ويتعرض السباحون للإصابة بمسببات الأمراض المختلفة.

## INTRODUCTION

People visit swimming pools for different reasons; the majority of people swim as recreational activities, others swim as sports, while some swim for rehabilitative treatment and physiotherapy. In the last few years, swimming pools became increasingly popular [1]. However, it should be regarded that public swimming pools are subjected to different types of contaminations, and not all swimming pools follows the health standards instructions. Public health problems may occur as a result of possible maintenance failure [2]. The contamination of the pools by the microorganisms resulted in different infections and complications that appear especially in swimmers with immune system deficiencies, diseases, such as diarrhea, skin, ear and upper respiratory infections, particularly if the swimmer's head is submerged [1].

Conjunctivitis is an inflammation or infection of the conjunctiva, which is the thin clear layer that lines the inner surface of the eyelid and covers the white part of the eye [3, 4]. There are many types of swimming pool conjunctivitis, but based on the etiology we can divide them into bacterial conjunctivitis, viral conjunctivitis, fungal conjunctivitis, and parasitic conjunctivitis [5]. Generally, an increased amount of tears, thick yellow discharge, itchy eyes, burning eyes, and blurred vision are among symptoms of conjunctivitis [3, 6]. Viral conjunctivitis (pink or red-eye), the main cause of microbial conjunctivitis, refers to those types of swimming pool conjunctivitis caused by adenovirus, namely human adenovirus (HAdVs), which cause different clinical manifestations [7, 8]. HAdVs can survive for prolonged periods in water and show high stability in the environment; its presence indicates the fecal contamination of water. Ingestion or direct contact with contaminated water is among routes of HAdVs transmission in swimming pools [2, 9].

Bacterial conjunctivitis, the second cause of microbial conjunctivitis, main causative agents of bacterial conjunctivitis includes; *Staphylococcus*, in first place, followed by *Streptococcus*, *Corynebacterium*, and finally *Moraxella* species. The correct diagnosis of causative agents of microbial conjunctivitis is important for correct treatment [6, 10, 11].

Non-bacterial conjunctivitis, is the third common cause of swimming pool conjunctivitis, which includes the chemical substance that added to the water for disinfection purpose [11]. The most important and widely used disinfectant is chlorine compounds. Improper uses of chlorine cause allergic, irritation, and damage of the eye and tear film, leads to conjunctivitis (redness) of the eyes [12].

Recently, public swimming pools became very popular in Erbil city, but unfortunately some of them do not follow standard health instructions and not all of them monitored or supervised by competent authorities and health committees. Continues monitoring and investigations are required to characterize the contaminants of these pools. The present study aimed to determine the main causative agents (viral, bacterial, and non-microbial) of conjunctivitis among swimmers of public swimming pools and to evaluate swimming pools from a healthy point of view.

## MATERIALS AND METHODS

### Specimen collection

Conjunctiva swabs were collected from eighty-eight swimmers who were suspected of having conjunctivitis infection depending on the clinical signs and complications, like; sudden eye pinkness or redness, discomfort, pain, tearing, burning, conjunctiva haemorrhages, eyelid swelling for two months. Samples included 60 male, and 28 female aged from sixteen to fifty-six in swimming pools selected randomly in the center of Erbil province from January to the end of February 2020. Specimens were taken from each participant using two sterile Dacron swabs, one dry collected for bacteriological study and the other collected in 1 ml Phosphate-buffered saline (PBS) and transported immediately to the laboratory on ice and stored at -20 °C [13].

### Bacteriological Examination of Samples

#### Preparation of Bacterial Media

MacConkey, blood, and Chocolate agar (Oxoid, UK), were used to cultivate the isolates. Preparation of the media were prepared according to manufacturer's instructions and sterilized with an autoclave at 121 °C for 15 minute [14].

#### Isolation and identification of bacteria

The conjunctiva swabs were inoculated on Blood, MacConkey, and Chocolate agar and incubated at 37 °C for 24hrs. After incubation, slides prepared

for positive culture to identify the morphology using Gram stain. Biochemical tests for identification was used, IMVIC test, urease, oxidase, motility, and catalase test was used to distinguish between *Staphylococcus* (catalase +ve) and *Streptococcus* (catalase -ve). Coagulase test used for identification of different species of *Staphylococcus*, coagulase positive *Staphylococcus aureus* (the pathogenic strains) and the coagulase negative *Staphylococcus species* (the nonpathogenic strains) [15].

### Virological Examination of Samples

**HAdV Immunochromatographic test (IC)** The Immunochromatographic test was performed according to the manufacturer's instruction (Aden test, SA Scientific TM, USA).

Directly after sample collection using sterile swab this test performed in which 4-four drops

(approximately 150µL) of the specimen were put into the kit specimen well. The proper time allowed for the specimen to be filtered through the kit to the specimen position and the control position. The results were read within 15 minutes. Within 30 minute from the application of specimen to the kit, the readings were finalized [8].

### Molecular Test PCR

#### Primer design

Two sets of primers were used ADHEX1, ADHEX2, to approve that the positive results of Immunochromatographic test are HAdVs [16, 17], first primer ADHEX1 used for nested PCR, while the second one ADHEX2 used as a specific primer to detect HAdV DNA. Sequence and base pairs of the primers (see Table 1).

**Table 1.** Primers name, sequences, location, and product size used for detection of HAdV DNA Hexon gene.

Primer	Sequence (5'-3')	Location*	Product size (base pairs)
ADHEX1F	AAC ACC TAY GAC TAC ATG AAC	20380–20400	930 bp.
ADHEX1R	AAT GGG GTA AAG CAT GTT TGC	20836–20854	
ADHEX2F	CCC ATT GAA CCA CCA CCG ATC	20485–20503	473 bp.
ADHEX2R	ACA TCC TTA CCG AAG TTC CAC	20632–20652	

\*Positions as referred to adenovirus 2 Hexon protein-coding region sequence [16].

### HAdV DNA extraction

The genomic matter of HAdV and RNAs were extracted only in thirty-six isolates with positive IC Adenovirus test result using QIAGEN DNA extraction kit (Hilden, Germany). The reagents of the kit were reconstituted before use according to the protocol of the manufacturers' instructions [8].

### Adenovirus Hexon gene amplification

A partial Hexon sequence of HAdV was amplified from all 36 isolates that showed positive results by Immunochromatographic test using nested PCR, described by Avellón, Pérez, Aguilar, Lejarazu and Echevarría [16] with some optimization [8, 18]. The primers used for first-round PCR and nested PCR were specific for the detection of HAdV DNA (see Table 1). A fifty microliter reaction volume for the first-round PCR was prepared. Cycling was carried out by (Gradient thermal cycler Alpha Cycler PCRmax series). PCR conditions (steps, time, and temperature) for

the first primer performed as described (see Table 2). One microliter of the first-round PCR was transferred to a nested PCR mixture containing: 31 µl nuclease-free water, 5 µl 10X PCR buffer, 8 µl of MgCl<sub>2</sub>, 1.5 µl of 10 mmol dNTP mixes, 0.5 µl primer ADHEX2F (100 pmol), 0.5 µl primer ADHEX2R 100 pmol and 2.5 U Taq polymerase (Promega). PCR condition (steps, time, and temperature) for the second primer was carried out as described (see Table 2).

### Detection of PCR products

After amplification, 5µl of PCR product was analyzed using electrophoresis (BioTech-USA) in 1.2% agarose at 75 V for 45 minutes. Safe stain (EvaGreen Fluorescent Gel Stain-Jena Bioscience) was used to visualize the band, DNA marker (1000 bp.) was used as a ruler and bands were visualized under UV trans-illuminator (Benchtop UV-Transilluminator-BioTech-USA).

Results documented using a 16-megapixel camera for photography.

Chi-square test was performed for variables and P<0.05 values were regarded as significant differences.

**Statistical analysis**

**Table 2.** PCR protocol and conditions, including time and temperature of all steps for ADHEX1 and ADHEX2 primers.

Gene	Initial Denaturation		Denaturation		Annealing		Extension		Final extension		No. of cycles
	Temp	Time	Temp	Time	Temp	Time	Temp	Time	Temp	Time	
ADHEX1	94°C	2min	93°C	1min	50 °C	1min	72°C	1min	72°C	6min	30
ADHEX2	94°C	2min	93°C	1min	55 °C	1min	72°C	1min	72°C	6min	30

**RESULTS**

Eighty-eight conjunctiva samples were isolated and identified from the swimmers suffering from pink eyes conjunctivitis at the different public swimming pools in Erbil city. These patients consisted of 60 (68.2%) males and 28 (31.8%) females, and they were aged between 16-56 years (see Table 3).

Results of bacterial cultivation on Blood (BA), MacConkey, and Chocolate agar, morphological identification, and biochemical tests detected 29

positive isolates, in which 22 (25%) of the samples confirmed as *Staphylococcus aureus* (β-hemolysis on BA, gram-positive cocci in clusters, positive urease, catalase and coagulase, negative for oxidase and motility), and 7 (7.95%) of the samples were *Streptococcus pneumonia* (α-hemolysis on BA, gram-positive diplococci, negative for urease, oxidase, motility, catalase, and coagulase), results of detecting HAdV using Immunochromatographic test (IC) detected 36 (40.91%) as positive samples, (see Table 3).

**Table 3.** The age, gender, and infectious agents of swimmers according to the present study.

Age/Years	Gender		Bacterial		Viral		Non-microbial						
	No.	%	No.	%	No.	%	No.	%	No.	%			
16-56	Male	60***	68.2	<i>Staphylococcus aureus</i>		22***	25	HAdVs		36	40.91	23	26.14
	Female	28***	31.8	<i>Streptococcus pneumonia</i>		7***	7.95						
Total		88	100			29*	32.95			36*	40.91	23*	26.14

\*\*\* p < .0001; \*\*\*p < .0002; \* p < .0376

The results of PCR amplification for HAdVs detection, in which 36 isolates amplified using specific primers, all 36 samples showed positive results when the amplified product were analyzed by agarose gel electrophoresis, as shown in Figure 1.

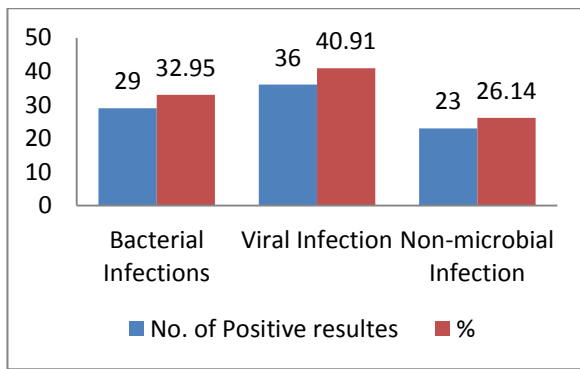
The overall result showed both viral and bacterial infections, out of 88 samples, 36 (40.91%) viral infected, 29 (32.95%) bacterial infection, and 23 (26.13%) no growth (non-microbial infections), as shown in Figure 2.

Results of statistical analysis using Chi-square test showed high significance differences (p < .0001) between males and females. Also, there were significant differences (p < .0376) among conjunctivitis types (viral, bacterial, and non-microbial infections), and (p < .0002) between bacterial conjunctivitis (*Staphylococcus aureus* and *Streptococcus pneumoniae*).



**Figure 1.** Agarose gel electrophoresis analysis 1.2% (stained with safe stain) of PCR products presenting gene amplified with ADHEX2 primer that showed the expected size 473 bp. Lane 1: is 1000 bp. DNA Ladder; Lane 2 negative control: Lanes 3-18: gene product amplified from HAdV genome run on 75V for 45min including samples from 1 to 16. All samples showed positive results for HAdV Hexon gene.





**Figure 2.** The percentage of infections in swimmer infected conjunctivitis.

## DISCUSSIONS

The present study detected microbial (viral and bacterial), and non-microbial eye conjunctivitis among swimmers (males and females) in public swimming pools in different frequencies, in which viral conjunctivitis detected as main causative agent followed by bacterial and non-microbial agents, respectively.

Regarding the results of sex group of the present study, there were significant differences between males and females ( $p < .0001$ ); males showed higher frequency than the females with (68.2%) and (31.8), respectively. The explanation of male prevalence over female could be understood regarding the cultural differences and rules, as Erbil population are highly restricted to cultural rules and female not allowed to visit public swimming pools like the male. Detecting a higher rate in male rather than the female is approved by other studies as well [19, 20].

Results from the current study regarding the causative agents of swimming pools conjunctivitis revealed that the most prevalence swimming pool conjunctivitis was caused by viral infection with (40.91%) frequency as shown in Figure 2. The obtained results showed that viral conjunctivitis is prevalence over bacterial and other conjunctivitis, such a result approved by a research carried out by Azari and Barney in 2013, that collected results of 86 researches that investigated causative agents of conjunctivitis over 10 years, they showed that viral conjunctivitis is the most common overall cause of infectious conjunctivitis [21]. Another explanation for the prevalence of adenovirus infection in the current study may be resulted from time of sample collection (January and

February), as adenovirus tend to peak in the winter and early spring [22]. Conjunctiva waterborne outbreaks resulted from the prevalence of HAdVs in the swimmers such as pharyngoconjunctivitis and possibly gastroenteritis at these swimming pools [23]. Several other researches worldwide approved the result of the current study for detecting HAdV as the main causative agent for swimming pool conjunctivitis [2, 20, 24, 25]. Being highly stable in the environment, surviving for prolonged periods in water, and having different routes of transmission made HAdVs to be the main causative agent of swimming pool conjunctivitis [2].

The second conjunctivitis infection was a bacterial infection in which *Staphylococcus aureus* showed the highest prevalence (25%) compared to *Streptococcus pneumonia* (7.95%), with ( $p < .0002$ ) significant differences. Bacterial conjunctivitis became a major issue as it increases year after year; Cavuoto, Zutshi, Karp, Miller and Feuer [26] reported an increase from 4.4% in 1994 to 42.9% in 2003 with bacterial conjunctivitis. According to a study carried out in 2017 that covers researches of 14 countries worldwide, revealed that *Staphylococcus aureus* is the major cause of bacterial conjunctivitis in significantly higher frequencies than *Streptococcus pneumonia*, which agree with the results of the current study. In an approach in Iraq to detect the most common pathogens for conjunctivitis a study carried out in 2017 and their results showed that *Staphylococcus aureus* was the main pathogen rather than *Streptococcus spp* [27]. Furthermore, Taiwo and his colleagues in 2004, reported a prevalence rate of 34.7% of *S. aureus* in the North Central part of Nigeria [28]. Another study reported an approximately the same result of the current study carried by Saberianpour and Momtaz in 2016 [29].

The third causative agent for swimming pool conjunctivitis was a non-microbial infection, with (26.14%). Detecting of such a high level of chemical conjunctivitis is related to using chlorine, which is considered as the main cause of eye irritation after swimming and consequently causes conjunctivitis [9]. Chlorine dehydrates eyes and consequently removes the tear film, the redness occurs when the blood vessels near the

surface of the eye become larger and then dilate [30]. Most public swimming pools in Erbil city use chlorine as a disinfectant and they do not restrict to the standards for the amount of chlorine that should be added regarding the volume of water in the pool, A study carried out in 2009 approved that the amount of chlorine differed among the public swimming pools in Erbil city [31].

Results of the current study revealed that public swimming pools are not a safe place, especially if they are not sufficiently treated and disinfected, they could play a significant role in spreading several diseases like conjunctiva, such results are approved by other researches [2, 32]. The microbiological quality of all the water sources was poor and contaminated with different microbial organisms; they are pathogenic. Swimmers using public swimming pools request from pool responsible and health authorities for more strict surveillance measures for the protection of swimmer's health [33].

## CONCLUSIONS

Microorganisms, rather than other contaminations, cause most conjunctivitis among swimmers. Microbial conjunctivitis resulted from viral and bacterial infections. It can be concluded that public swimming pools are not a safe place, and swimmers in public swimming pools are subjected to viral and bacterial infections. Many swimming pools are not obeying health instruction, and most swimmers are not aware of the risks of contaminated water of these pools. Further studies are needed to determine the prevalence rate of different microorganisms related to conjunctivitis.

## RECOMMENDATIONS

- 1- Swimming pools must strictly apply healthy instructions.
- 2- Periodic microbial tests must be done for the water of swimming pools.
- 3- Swimmers must not neglect any signs and symptoms of conjunctivitis; they must contact their health providers as soon as possible to treat any infections.
- 4- More research and investigations are recommended in this field.

## REFERENCES

[1] Leoni, E., *Recreational Water Illnesses*. 2019: MDPI AG.

- [2] Bonadonna, L. and Rosa, G. La, *A Review and Update on Waterborne Viral Diseases Associated with Swimming Pools*. *International journal of environmental research and public health*, 2019. 16(2): p. 166.
- [3] Chaudhry, I., *Common Eye Infections*. 2013: IntechOpen.
- [4] Ryder, E.C. and Benson, S., *Conjunctivitis*, in *StatPearls*. 2020, StatPearls Publishing Copyright © 2020, StatPearls Publishing LLC.: Treasure Island (FL).
- [5] Sthapit, P., *Conjunctival Flora of Normal Human Eye*. *JSM Ophthalmology*, 2014. 2(2): p. 1021.
- [6] Rumelt, S., *Advances in Common Eye Infections*. 2016: IntechOpen.
- [7] Azari, A.A. and Arabi, A., *Conjunctivitis: A Systematic Review*. *Journal of Ophthalmic & Vision Research*, 2020. 15(3): p. 372-395.
- [8] Maulud, S., *Molecular epidemiology of human Adenovirus among patients with Keratoconjunctivitis*. 2020. 31.
- [9] Ahmad, S.S., *Water related ocular diseases*. *Saudi journal of ophthalmology : official journal of the Saudi Ophthalmological Society*, 2018. 32(3): p. 227-233.
- [10] Kellerman, R.D. and Rakel, D., *Conn's Current Therapy 2019*. 2018: Elsevier Health Sciences.
- [11] Watson, S., Cabrera-Aguas, M. and Khoo, P., *Common eye infections*. *Australian prescriber*, 2018. 41(3): p. 67-72.
- [12] van Veldhoven, K., van Veldhoven, K., Keski-Rahkonen, P., Barupal, D.K., et al., *Effects of exposure to water disinfection by-products in a swimming pool: A metabolome-wide association study*. *Environment international*, 2018. 111: p. 60-70.
- [13] Rosado-Filho, E.N., Cardoso, S.A., Marçal, L.N. et al., *Prevalência de conjuntivite adenoviral em clínica oftalmológica no município de Viçosa (MG)*. *Revista Brasileira de Oftalmologia*, 2015. 74: p. 203-208.
- [14] Vandepitte, J., Engbaek, K., Rohner, P, Piot, P., Heuck, C.C. et al. *Basic laboratory procedures in clinical bacteriology*. 2003, 2<sup>nd</sup> ed., World Health Organization.
- [15] Bascomb, S. and Manafi, M., *Use of enzyme tests in characterization and identification of aerobic and facultatively anaerobic gram-positive cocci*. *Clinical microbiology reviews*, 1998. 11(2): p. 318-340.
- [16] Avellón, A., Pérez, P., Aguilar, J. C., Lejarazu, R., and Echevarría, J. E., *Rapid and sensitive diagnosis of human adenovirus infections by a generic polymerase chain reaction*. *J Virol*

- Methods, 2001. 92(2): p. 113-20.
- [17] van Heerden, J., Ehlers, M. M., Heim, A. and Grabow, W. O., Prevalence, Quantification and Typing of Adenoviruses Detected in River and Treated Drinking Water in South Africa. *Journal of applied microbiology*, 2005. 99: p. 234-42.
- [18] van Heerden, J., Ehlers, M. and Grabow, W., Detection and risk assessment of adenoviruses in swimming pool water. *Journal of applied microbiology*, 2005. 99: p. 1256-64.
- [19] Ajaiyeoba, A. I., Isawumi, M. A., Adeoye, A. O. and Oluleye, T. S., Prevalence and Causes of Eye Diseases amongst Students in South-Western Nigeria. *Annals of African Medicine*, 2006. 5 (4): p. 197-203
- [20] Akçay, E., Çarhan, A., Hondur, G., Tufan, Z. K., Duru, N., Kılıç, S., Ensari, E. N., Uğurlu, N. and Çağıl, N., Molecular identification of viral agents associated with acute conjunctivitis: a prospective controlled study. *Brazilian Journal of Infectious Diseases*, 2017. 21: p. 391-395.
- [21] Azari, A.A. and N.P. Barney, Conjunctivitis: a systematic review of diagnosis and treatment. *JAMA*, 2013. 310(16): p. 1721-1729.
- [22] Dela Cruz, C.S., Pasnick, S., Gross, J. E., Keller, J., Carlos, W.G., Cao, B. and Jamil, S., Adenovirus Infection and Outbreaks: What You Need to Know. *Am J Respir Crit Care Med*, 2019. 199(7): p. 13-14.
- [23] Shih, Y.J., Tao, C.W., Tsai, H.C., Huang, W.C., Huang, T.Y., Chen, J.S., Chiu, Y.C., Hsu, T.K. and Hsu, B.M., First detection of enteric adenoviruses genotype 41 in recreation spring areas of Taiwan. *Environmental science and pollution research international*, 2017. 24: p. 18392–18399.
- [24] Balasopoulou, A., Kokkinos, P., Pagoulatos, D., Plotas, P., Makri, O.E., Georgakopoulos, C.D., & Vantarakis, A., A molecular epidemiological analysis of adenoviruses from excess conjunctivitis cases. *BMC Ophthalmology*, 2017. 17(1): p. 51.
- [25] Santiago, L.A., Silva, J., Azevedo, O., and Vasconcelos, P., Comparative study on the efficacy of non-steroidal, steroid and non-use of anti-inflammatory in the treatment of acute epidemic conjunctivitis. *Acta Cirurgica Brasileira*, 2019. 34.
- [26] Cavuoto, K., Zutshi, D., Karp, C. L., Miller, D., & Feuer, W., Update on bacterial conjunctivitis in South Florida. *Ophthalmology*, 2008. 115(1): p. 51-6.
- [27] Rahama, H.A., Ali, Q.A., Mustafa, A.A. Molecular Study of Most Common Pathogenic Bacteria Isolated From Conjunctivitis Patients In Baghdad. *Medical Journal of Babylon*, 2017. 14 (4): p. 706-713.
- [28] Taiwo, S.S., Onile, B.A., and Akanbi II, A. A., Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Ilorin, Nigeria. *African Journal of Clinical and Experimental Microbiology*, 2004. 5: p. 189-192.
- [29] Saberianpour, S., Momtaz, H., Ghanbari, F., Mahmodi, F., Assessment of Bacterial and Fungal Contamination in Public Swimming Pools in Shahrekord - IRAN. *Journal of Tropical Diseases*, 2016. 4(2): p. 190.
- [30] Chakrabarti, A., Research into Design for a Connected World: Proceedings of ICoRD 2019 Volume 2. 2019: Springer Singapore.
- [31] Toma, J., Determinants of the microbiological characteristics of Erbil city public swimming pools. *The Botulinum J*, 2009. 12: p. 1-13.
- [32] Barna, Z. and M. Kádár, The risk of contracting infectious diseases in public swimming pools. A review. *Ann Ist Super Sanita*, 2012. 48(4): p. 374-86.
- [33] Okesola, A.O. and Salako, A.O., Microbiological profile of bacterial conjunctivitis in Ibadan, Nigeria. *Ann Ib Postgrad Med*, 2010. 8(1): p. 20-24.