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Detection of aac(3)-I gene in *Chlamydia trachomatis* isolated from conjunctivitis by using PCR technique in Al-Najaf province, Iraq

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ABSTRACT

Chlamydia trachomatis causes conjunctivitis to spread throughout the world, it infects people of all ages and can cause blindness if not treated properly. Therefore, the current study aimed to diagnose *C. trachomatis* that causes conjunctivitis and identify the detection of the aac(3)-I gene in *C. trachomatis* isolated from conjunctivitis by using Polymerase chain reaction (PCR) technique. One hundred and seven swabs were collected from patients infected with conjunctivitis from different ages (1 month –70 years) and for both sexes (63 samples from females and 44 samples from males) for the period from 27 November till 19 May 2023 from Al-Najaf province in Iraq. Purulent material from the conjunctiva sac was collected on a sterile swab and placed in phosphate-buffered saline and incubated in a deep freezer after completing the collection. All samples were subjected to a PCR to detect the major outer membrane protein (MOMP) gene to diagnose the *Chlamydia trachomatis*. where the results showed that out of 107 samples, 26 (24.3%) were positive for *C. trachomatis*. The individuals infected with *C. trachomatis* were categorised into multiple age groups. The age group ranging from one month to 14 years exhibited the highest prevalence of conjunctivitis. The age group between 15-28 years was followed by the age group between 57-70 years, while the lowest group for conjunctivitis was the age group between 43-56. The existence of the aac(3)-I gene, which confers resistance to the antibiotic gentamicin routinely employed for the treatment of eye infections, was identified. Among the 26 isolates of *C. trachomatis*, 15 (57.69%) were found to have the aac(3)-I gene. The present investigation demonstrated the significant involvement of *C. trachomatis* in conjunctivitis, with a higher susceptibility observed among women. The prevalence of conjunctivitis was higher in the age range of 1-28 compared to other age groups. The present investigation exhibited the dissemination of the gentamicin resistant aac(3)-I gene among *C. trachomatis* bacteria.

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Introduction

The eye is a unique anatomical part that is constantly exposed to external environmental factors. Eye diseases and complications caused by microorganisms are major health problems all over the world, eye infections can damage the structure of the eye, which may lead to poor vision or blindness if they are diagnosed and treated incorrectly (Diriba et al., 2020). Inflammation (swelling) of the conjunctiva, or conjunctivitis, is a general term that refers to a variety of diseases or disorders that affect the conjunctival membrane and lead to inflammation, despite the presence of many factors that protect eye (Wollenberg et al. 2022).

Conjunctivitis is also called "pink eye" because blood vessels in it will widen and fill with blood, causing the white part of the eye to appear pink or red (Varu et al. 2019). Conjunctivitis varies according to the etiologic agent, some of which are non-infectious, such as allergic conjunctivitis, chemical conjunctivitis, or toxic conjunctivitis, and some are infectious and occur by infection of the eye with bacteria, viruses, or fungi, affecting one or both eyes, and it is rapidly spreading and contagious and infects people at different ages (Azari & Arabi 2020).

C. trachomatis is an obligate intracellular that was once regarded as a virus because, requires the eukaryotic host cells to fuel their metabolism for growth and replication by providing high-energy compounds such as adenosine triphosphate (Tille 2021).

C. trachomatis are Gram-negative bacilli that have lipopolysaccharide (LPS) as a component of the cell wall. The chlamydial LPS, however, has little endotoxic activity. *C. trachomatis* has a major outer membrane protein (MOMP) that is very diverse. The variation in MOMP in *C. trachomatis* is used to separate the species into 15 distinct serovars (Shen et al. 2024; Wan et al. 2023).

Trachoma is the leading infectious cause of blindness worldwide caused by *C. trachomatis*. Trachoma blindness is irreversible. Direct or indirect contact with an afflicted persons eyes or nose might transfer the bacterium that causes conjunctivitis. More children than adults spread the illness. Perhaps as a result of unsanitary living arrangements, a lack of clean water, and inadequate toilets that facilitate the spread of illness. It is a public health issue that affects 42 nations and accounts for 1.9 million cases of blindness or visual impairment (Nash et al. 2023; Tedijanto et al. 2023).

C. trachomatis is an acute infection of the conjunctiva that is characterized by edema and erythema of the eyelids and purulent eye discharge. It typically occurs between 5 and 14 days after birth, although it can present earlier. The severity of the symptoms can range from minor to severe (eyelid edema, pseudomembrane development, and severe mucopurulent discharge) according to Beniwal et al. (2023).

Several antibiotics are used to kill or inhibit the bacteria that cause conjunctivitis, but in many infections, the bacteria have developed resistance to the action of the antibiotics (Mubeen et al. 2021).

Therefore, this study aimed to investigate *C. trachomatis* which causes conjunctivitis, and to investigate the gene responsible for the resistance of this bacteria to the antibiotic commonly used in conjunctivitis, gentamicin.

Materials and Methods

Sampling

One hundred and seven cotton swabs from patients with conjunctivitis of different ages and of both sexes (males and females) for the period from 27 Nov. till 19 May 2023, from Al-Najaf province, Iraq. swabs placed in phosphate-buffered saline and incubated in a deep freeze -20°C. The College Faculty of Education for Girls University of Kufa, Najaf, Iraq committee approves the research proposal to be conducted in Partial Fulfilment of the Requirements for the certificate of Doctorate of Philosophy in Biology. None of the investigators and co-investigators participating in this study took part in the decision-making and voting procedure for this study. The College Faculty of Education for Girls Ethics committee expects to be informed about the progress of the study, any serious adverse events occurring in the course of the study, and any revision in the protocol patient information/informed consent.

DNA extraction

DNA was extracted from samples preserved in phosphate-buffered saline to diagnose the MOMP gene specific for *C. trachomatis* by the boiling method with a modification. using Vortex for 5 minutes, then mixed with a micropipette. 200 µL of the sample was taken and placed in an Eppendorf tube; after that, it was placed in a freezer for 5 minutes and then subjected to boiling in a water bath at 99.9 °C for 15 minutes. Immediately, after which it was placed in a freezer for 15 minutes and then placed in the centrifuge for 5 minutes at 1400 rpm. Five microliters of the supernatant were used for the PCR (Ahmed & Dablool 2017).

PCR amplification

The PCR technique was performed by a Thermo cycler device (Applied Biosystems Veriti U.S.A). The primers supplied by Microgen Company where forward primer is 5'-CCTGTGGGGAATCCTGCTGAA-3' and reverse primer is 5'-

GTCGAAAACAAAGTCACCATAGTA-3' targeted major outer membrane protein (MOMP) in *C. trachomatis* the molecular weight 240 bp (Rostami et al. 2017). The primers for detection aac(3)-I gene supplied by Microgen Company the molecular weight 169 bp consists of forward primer 5'-ACCTACTCCCAACATCAGCC-3' and reverse primer 5'- ATATAGATCTCACTACGCGC-3'. Amplification was performed by initial denaturation at 94˚C for 5 minutes, followed by 35 cycles of denaturation at 94˚C for 30 seconds, annealing temperature was (56 ˚C for 30 sec) and extension at 72 ˚C for 30 seconds. The final extension was at 72 ˚C for 7 minutes according to Wang et al. (2016). The agarose gel for electrophoresis was carried out for the detection of amplicon, as mentioned by Sambrook and Russell (2001).

Results

All samples placed in phosphate-buffered saline were subjected to molecular diagnosis using PCR technology based on the diagnostic gene major outer membrane protein (MOMP) of *C. trachomatis*. This protein maintains the structural rigidity of the outer membrane and facilitates porin formation (Collar *et al.* 2022).

Our results showed that out of 107 samples, 26 (24.30%) were positive for *C. trachomatis*, while 81 (75.70%) were negative for *C. trachomatis* as shown in figure 1.

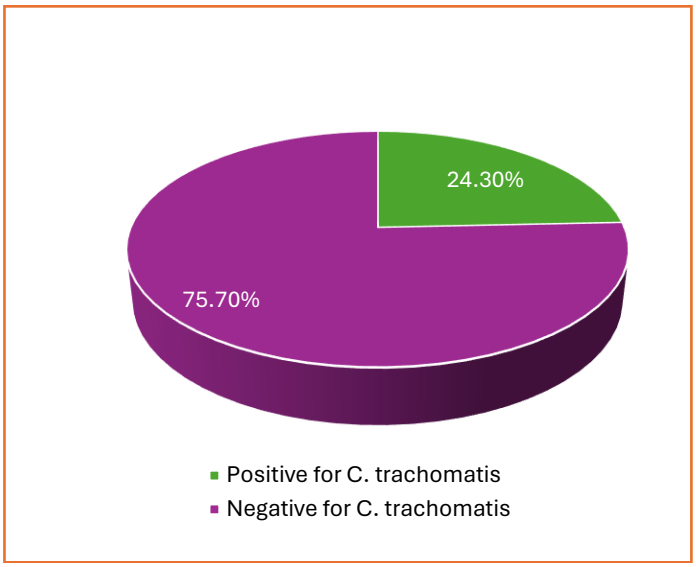

Fig 1. The percentage of conjunctivitis samples that showed positive and negative presence of *Chlamydia trachomatis*.

The gene amplification results show by comparison with band amplification and DNA ladder, it was found that the resulting band had a molecular weight of 240 bp, as shown in figure (2).

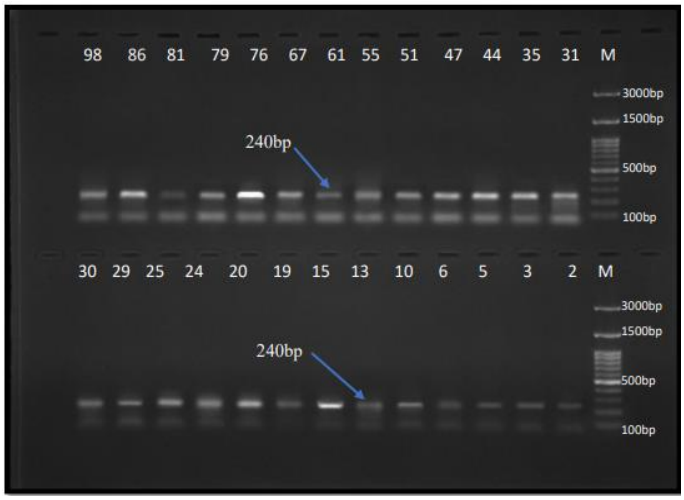

Fig 2: Electrophoresis on agarose gel of *C. trachomatis* using primer MOMP with product (240 bp) amplification products for bacterial isolates where: M is the DNA ladder (100bp), 2/3/5/6/10/13/15/19/20/24/25/29/30/31/35/44/47/ 51/55/61/67/ 76/ 79/81/ 86/98 samples contain *C. trachomatis*.

Out of the 107 samples, 63 (58.88%) samples were collected from females suffering from conjunctivitis, of which 17 (65.38%) samples were positive for *C. trachomatis* while 46 (42.99%) samples were negative.

44 samples were collected from males suffering from conjunctivitis where 9 samples representing 8.41%) were positive for *C. trachomatis*, while 35 (32.71%) samples were negative for the pathogen as shown in Table (1).

Table 1: The distribution of samples by gender.

Gender	Total number	Positive	Negative
Female	63 (58.88%)	17 (15.89%)	46(42.99%)
Male	44 (41.12%)	9 (8.41%)	35(32.71%)
Total	107 (100%)	26 (24.30%)	81 (75.7%)

The ages of the infected samples ranged from a month to 70 years divided into several groups. The group from one month and 14 years, showed the highest incidence of conjunctivitis, followed by the age group between 15-28 years, followed by the age group between 57-70 years, and the age group between 43-56 was the lowest group for conjunctivitis, followed by the age group between 29-42 years (table 2).

Table 2: The prevalence of conjunctivitis with *C. trachomatis* by age.

Age	Total
1 month to 14 years	11
15-28 years	6
29-42 years	3
43-56 years	1
57-70 years	5
Total	26

The results of agarose gel electrophoresis of the amplification products of the aac(3)-I gene in 26 *C. trachomatis* showed that of 15 (57.69%) possess aac(3)-I possess the gene by comparison between the amplification bands and the DNA ladder bands, with the appearance of a band with a molecular weight of 169 bp as shown in figure (3).

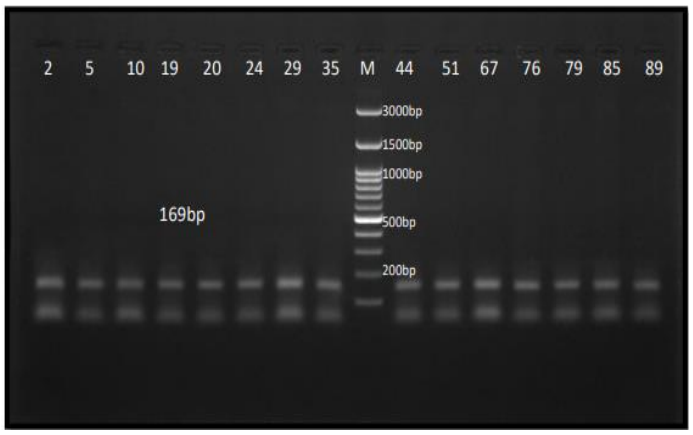

Fig 3. Electrophoresis on agarose gel of *aac(3)-I* (169bp) gene amplification products for *C. trachomatis* isolates. Where M is the DNA ladder (100bp), 2 / 5 /10 /19 / 20 / 24 / 29 / 35 / 44 / 51 / 67 / 76 /79 / 85 /98 isolates that possess the *aac(3)-I* gene.

Discussion

This study showed a high incidence of *C. trachomatis* bacteria in the occurrence of conjunctivitis. In a study conducted in Buenos Aires, Argentina, adults with venereal symptoms were included as well as infants (less than 30 days old) with neonatal ophthalmia. Neonatal conjunctivitis has been found to have a very high incidence of *C. trachomatis* bacteria (Vaulet et al. 2010). Eye C. trachomatis infection causes trachoma, the leading infectious cause of blindness worldwide (Pickering et al., 2019).

While Lynch et al. (2022) conjunctival swabs for PCR testing were collected from 100% of samples enrolled in the study no *C. trachomatis* was found when diagnosed by PCR in any of the samples and there were no positive PCR results for other pathogens.

Several tests can be performed to detect *C. trachomatis*, such as the Giemsa stain ELISA and PCR. In a study conducted by Lee and Chen (2022) comparing the three methods, it was found that the sensitivity of Giemsa cytology was only 36%. While the sensitivity of the ELISA for detecting *C. trachomatis* ranged from 40% to 71%, moreover, the detection of C. trachomatis DNA from eye swabs using commercial PCR assays was correct and reached 95.71% in terms of sensitivity. Thus, the tests may be PCR is a rapid and ideal tool for detecting eye *C. trachomatis* infection, then Giemsa stain and ELISA.

The major outer membrane protein (MOMP) of *C. trachomatis* is a protein that helps maintain the structural rigidity of the outer membrane, facilitating porin formation, and allowing the diffusion of solutes through the intracellular reticulate body membrane. It also plays a role in pathogenesis and possibly adhesion. The MOMP along with the lipopolysaccharide, makes up the surface of the elementary body cell (Collar et al. 2022; Rostami et al. 2016).

This study showed that the incidence of conjunctivitis is higher in females than in males, and several researchers have found a higher incidence of infection in females, as in the study of Alajbegovic-Halimic et al. (2023) found out of 53 patients, 23 males 43.39% and 30 females 56.63%. The study by Ahmed and Hamdan, (2016) reported that females 59.6% were more infected than males 40.4%. While Ibrahim et al., (2022) reported a high incidence of conjunctivitis in males 61.3%, while the incidence in females was 38.7%.

The high incidence of conjunctivitis in females may be due to the use of some eye cosmetics, which can constitute sources of transmission of infection in the eye, as exchanging cosmetics or using them incorrectly may cause the transmission of bacterial pathogens to other people.

As Hassan and Majed (2018) showed the incidence of conjunctivitis in children is 53%, with the predominance being between 1 and 10 years old. Ahmed and Hamdan (2016) The highest frequency. of isolates was 41.0%. which was in the age group (<0-15 years). followed by the age group (16-30). years which were 26.3%. while the lowest frequency of isolates was14.1% in the age group of (>50 years). In contrast, Abdullah et al. (2013) found that conjunctivitis was more common among the elderly with 34.4% belonging to the age group between 61 to 70 years.

The high incidence of conjunctivitis in the age group from one month to 28 years because this age group is at the peak of its activity, as children between the ages of months and 6 years go through several stages, such as crawling, walking, and exploring things around them, which leads to contamination of the hands with bacteria and thus contact with the eyes. The reason may be attributed to their

incomplete immune system, so they are more susceptible to bacterial infection than others (Hassan & Majed 2018).

Likewise, the group older than 6 years may be school and university students, which allows the infection to spread among this group through contact with eye secretions or the upper respiratory tract of infected people or through contaminated fingers. In addition, this group practices various activities such as swimming and football, which makes the conjunctiva of the eye an easy target for infection and contamination with bacteria (Ahmed & Hamdan 2016).

The increase in infection in the age group 56-70 may be due to the lack of immunity of these patients, or perhaps the patients have recurring or chronic conjunctivitis infections that have led to bacteria becoming resistant to antibiotics, which leads to failure of treatment for this age group and thus an increase in the transmission of infection among them (Abdullah et al. 2013).

aac(3)-I gene recorded in a large number of Enterobacteriaceae and other Gram-negative clinical isolates (Ramirez & Tolmasky 2010). However, the current study proved that the aac(3)-I gene is widespread in *Chlamydia trachomatis* bacteria.

Aminoglycosides are among the most commonly used antibiotics in the treatment of conjunctivitis by both Gram-negative and Gram-positive bacteria. They bind to the ribosomes and thus interfere with protein synthesis (Ahmed et al. 2020). The ability of bacterial isolates to resist gentamicin through the gene is attributed, perhaps to the repeated use of this antibiotic with bacterial conjunctivitis, which led to a genetic mutation in the aac(3)-I gene, which is one of the genes responsible for resistance to the antibiotic gentamicin, or perhaps to the presence of this gene on plasmids that leads to ease of transmission from one bacteria to another. With multiple bacterial infections, including *C. trachomatis*, there were secondary bacteria with it, which may have led to the transfer of this gene from one bacterium to another.

Conclusion

The current study showed that *Chlamydia trachomatis* has an important role in conjunctivitis and that women are more susceptible to conjunctivitis. Conjunctivitis was more common in the age group of 1-28 than in other age groups. The current study demonstrated the spread of the gentamicin resistance aac(3)-I gene among *C. trachomatis* recovered bacteria.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Abdullah FE , Khan MI, Waheed S. (2013). Current pattern of antibiotic resistance of clinical isolates among conjunctival swabs. Pakistan journal of medical sciences, 29(1), 81.

Ahmed OB, Dablool AS. (2017). Quality improvement of the DNA extracted by boiling method in gram-negative bacteria. International Journal of Bioassays, 6(4), 5347-5349.

Ahmed OB, Hamdan EM. (2016). Profile of bacterial conjunctivitis in Sudan. Sch J App Med Sci, 4(4B), 1217-21.

Ahmed S, Sony SA, Chowdhury MB, Ullah M, Paul S, Hossain T. (2020). Retention of antibiotic activity against resistant bacteria harboring aminoglycoside-N-acetyltransferase enzyme by adjuvants: a combination of in-silico and in-vitro study. Scientific Reports, 10(1), 19381.

Alajbegovic-Halimic J, Jovanovic N, Halimic T. (2023). Microbiological evaluation of bacterial conjunctivitis in children. Acta Medica Saliniana, 53(1).

Azari A, Arabi A. (2020). Conjunctivitis: a systematic review. Journal of ophthalmic & vision research, 15(3), 372.

Beniwal N, Lekha VC, Vins A, Parvez R, Sharmila F, Dhodapkar R, Muruganandam N. (2023). *Chlamydia trachomatis*: Conjunctivitis in the Andaman and Nicobar Islands population. Cureus. 51241

Collar AL, Linville AC, Core SB, Frietze KM. (2022). Epitope-based vaccines against the *Chlamydia trachomatis* major outer membrane protein variable domain 4 elicit protection in mice. Vaccines, 10(6), 875.

Diriba K, Kassa T, Alemu Y, Bekele S. (2020). In vitro biofilm formation and antibiotic susceptibility patterns of bacteria from suspected external eye infected patients attending ophthalmology clinic, Southwest Ethiopia. International journal of microbiology, 2020, 1-12.

Hassan TM, Majid BT. (2018). Identification of Common Aerobic Bacterial Isolates among Conjunctivitis in Sulaymaniyah Province/Iraq. Iraqi Journal of Medical Sciences, 16(2).

He J, Chen A, Zou M, Young CA, Jin L, Zheng D, Congdon N. (2023). Time trends and heterogeneity in the disease burden of trachoma, 1990–2019: a global analysis. British Journal of Ophthalmology, 107(3), 337-341.

Ibrahim SK, Flyyih NM. (2022). *Staphylococcus epidermidis* is the Main Causative Agent of Conjunctivitis. HIV Nursing, 22(2), 1644-1648.

Lee WJ, Chen C. (2022). Adult inclusion conjunctivitis diagnosed by polymerase chain reaction and Giemsa stain. IDCases, 27, e01367.

Lynch KD, Brian G, Ahwang T, Newie T, Newie V, Perrett, C, Lambert S B. (2022). Discord between the presence of follicular conjunctivitis and *Chlamydia trachomatis*

infection in a single Torres Strait Island community: a cross-sectional survey. Australian and New Zealand Journal of Public Health, 46(2), 155-160.

Mubeen B, Ansar A N, Rasool R, Ullah I, Imam S, Alshehri S, Kazmi I. (2021). Nanotechnology as a novel approach in combating microbes providing an alternative to antibiotics. Antibiotics, 10(12), 1473.

Nash SD, Chernet A, Weiss P, Nute AW, Zerihun M, Sata E, Callahan EK. (2023). Prevalence of ocular *Chlamydia trachomatis* infection in Amhara region, Ethiopia, after 8 years of trachoma control interventions. The American Journal of Tropical Medicine and Hygiene, 108(2), 261.

Pickering H, Palmer C, Houghton J, Makalo P, Joof H, Derrick T, Holland, M. J. (2019). Conjunctival microbiome-host responses are associated with impaired epithelial cell health in both early and late stages of trachoma. Frontiers in Cellular and Infection Microbiology, 9, 297.

Ramirez M, Tolmasky ME. (2010). Aminoglycoside modifying enzymes. Drug resistance updates, 13(6), 151-171.

Rostami M, Rashidi BH, Aghsaghloo F, Nazari R. (2016). Comparison of clinical performance of antigen based-enzyme immunoassay (EIA) and major outer membrane protein (MOMP)-PCR for detection of genital *Chlamydia trachomatis* infection. International Journal of Reproductive BioMedicine, 14(6), 411.

Rostami M, Rashidi BH, Nazari R, Aghsaghloo F, Habibi A. (2017). A multiplex assay of Trichomonas vaginalis, *Chlamydia trachomatis* and Neisseria gonorrhoeae infections in genital samples. The Journal of Infection in Developing Countries, 11(11), 833-839.

Sambrook J, Russell D. (2001). Molecular Claning Laboratory Manual . 3rded . Cold Spring Harbor , New York . USA . 1044.

Shen L, Gao L, Swoboda AR, Ouellette SP. (2024). Targeted repression of topA by CRISPRi reveals a critical function for balanced DNA topoisomerase I activity in the *Chlamydia trachomatis* developmental cycle. mBio, 15(2), 02584-23.

Tedijanto C, Solomon AW, Martin DL, Nash SD, Keenan JD, Lietman TM, Arnold BF. (2023). Monitoring transmission intensity of trachoma with serology. Nature Communications, 14(1), 3269.

TilleP. (2021). Bailey & Scott's Diagnostic Microbiology 15th Edition -E-Book. Elsevier Health Sciences.

Varu DM, Rhee MK, Akpek EK, Amescua G, Farid M, Garcia-Ferrer F, Dunn SP. (2019). Conjunctivitis preferred practice pattern®. Ophthalmology, 126(1), P94-P169.

Vaulet LG, Entrocassi C, Corominas AI, Fermepin MR. (2010). Distribution study of Chlamydia trachomatis genotypes in symptomatic patients in Buenos Aires, Argentina: association between genotype E and neonatal conjunctivitis. BMC Research Notes, 3(1), 1-6.

Wan W, Li D, Jiao J. (2023). Advances in genetic manipulation of *Chlamydia trachomatis*. Frontiers in Immunology, 14, 1209879

Wang N, Huang Q, Tan YW., Lin LP, Wu, K. L. (2016). Bacterial spectrum and resistance patterns in corneal infections at a Tertiary Eye Care Center in South China. International journal of ophthalmology, 9(3), 384.