IJMCM 14(1) (2024) 2046-2053 Doi: 10.22034/IJMCM.2024.710068

International Journal of
Molecular and Clinical Microbiology

Research Article

Etiology and Antibiotic Resistance Patterns of Endophthalmitis Cases at Khatam-Al-Anbia Hospital, Mashhad (2023)

Arman Hasanzadeh[1], Mahboobeh Nakhaei Moghaddam[2], Mohammad Reza Shayegan[3]

1. Department of Biology, Mashhad Branch, Islamic Azad University, Mashhad, Iran
2. Associate Professor in Microbiology, Department of Biology, Mashhad Branch, Islamic Azad University, Mashhad, Iran
3. Assistant Professor of Ophthalmology, Islamic Azad University, Mashhad Branch, Mashhad, Iran

ARTICLE INFO

Article history:
Received 04 April 2024
Accepted 20 June 2024
Available online 1 July 2024

Keywords:
Endophthalmitis,
Antibiotic Resistance,
Exogenous,
Endogenous,
Bacteria

ABSTRACT

Endophthalmitis is an eye infection that can lead to blindness if untreated. This study aimed to assess bacterial endophthalmitis in patients. It focused on sex differences and antibiotic resistance. It took place in 2023 at Khatam-Al-Anbia Hospital in Mashhad. It involved 60 patients aged 7 to 60. Researchers used biochemical and microbiological methods to identify bacteria. They followed CLSI M100-2023 guidelines to test antibiotic resistance. PCR was also performed to confirm the identification of the isolates. Of 60 samples suspected of ocular endophthalmitis, 15 were infected with bacteria. This included 5 isolates of *Escherichia coli*, 2 isolates of *Pseudomonas aeruginosa*, 4 isolates of *Staphylococcus epidermidis* and 4 isolates of *Staphylococcus aureus*. Gram-positive and Gram-negative bacteria showed the highest resistance to the Clindamycin (70%) and Imipenem (33%), respectively. The results of culture and biochemical tests were the same as those of PCR. Due to unsupervised use, antibiotic resistance is rising. So, we must identify resistance patterns for timely treatment.

1. Introduction

Endophthalmitis is an infection and inflammation of the anterior chamber containing the aqueous humor and the posterior chamber containing the vitreous within the eye, which occurs due to bacterial or fungal invasion. Failure to diagnose and treat this disease in a timely manner can negatively affect a person's vision and cause complete blindness (Valdez-García *et al.*, 2014). Approximately 40 to 80% of all endophthalmitis cases are caused by cataract surgery (Patel *et al.*, 2019). The second most common cause of endophthalmitis is intravitreal injection, and coagulase-negative staphylococci and streptococci are the causative pathogens. Penetrating eye trauma is the third most common cause, accounting for 2%–15% of all Endophthalmitis cases (Garcia *et al*., 2024). Coagulase-negative staphylococci are often the cause of this infection, but other bacteria, such as *Bacillus*, Gram-negative bacilli, and fungi, can also cause the infection. *Bacillus cereus* can also cause post-traumatic endophthalmitis (Sadiq *et al.*, 2015). Endophthalmitis related to the bleb is the fourth most common cause of infection with a bacterial agent, and the pathogens are *Streptococcus pneumoniae*, *Enterococcus pneumoniae*, and *Haemophilus influenzae* in descending order (Sridhar *et al.*,

*Corresponding author: Mahboobeh Nakhaei Moghaddam
E-mail address: Mahboobe_nak@yahoo.com

2015). Keratitis, which leads to the penetration of microorganisms into the cornea, is the fifth most common cause of endophthalmitis, and 50% of keratitis-related cases are caused by fungi, *S. aureus*, *Streptococcus*, and *Pseudomonas*. Endogenous endophthalmitis through blood is the rarest cause of which *Klebsiella pneumoniae* is the most common invader, followed by *Candida*, *Streptococcus*, *S. aureus*, and *E. coli* (Charles and Ta, 2012; Aftab *et al*., 2024). Due to the increase in antibiotic resistance of bacteria, obtaining the antibiotic resistance pattern as soon as possible is extremely important for the treatment of this disease (Rozwandowicz *et al*., 2018; Vaezi *et al*., 2022). This study was conducted with the aim of determining the frequency and etiology of bacterial endophthalmitis infection according to sex and to determine the antibiotic resistance pattern in Khatam-Al-Anbiai Hospital in Mashhad.

2. Materials and Methods

2.1. Sample collection

In this cross-sectional study, the samples of 60 male and female patients aged 7–60 years with suspected eye endophthalmitis were collected at Khatam Al-Anbia Hospital in Mashhad in the first half of 2023.

2.2. Sample processing method

The eye fluid samples examined in this study were cultured on blood agar and Eosin methylene blue agar (Merck, Germany) to identify the type of bacteria (Gram positive and Gram negative). To detect fungi, samples were cultured in Sabouraud dextrose agar (Merck, Germany).

Morphological characteristics and biochemical tests were used to detect, confirm, and differentiate bacterial isolates. Catalase, coagulase, DNase, and mannitol salt agar (MSA) tests were used to identify Gram-positive bacteria. For Gram-negative specimens, the oxidase test, mobility and indole test (SIM), triple sugar iron agar (TSI), methyl red test, and Voges–Proskauer (MRVP) test were used (CLSI) (Wayne, 2002; Sharifian *et al.*, 2023).

2.3. Antibiotic assay

In order to check the drug resistance and sensitivity of the isolates, according to the CLSI M100-2023 guidelines, an AntibioGram test was performed using the disc diffusion method of Padten Teb Company, and the samples were cultured on Mueller–Hinton agar medium (Merck, Germany). The antibiotic disks of Cefoxitin (30 µg), Azithromycin (30 µg), Erythromycin (15 µg), and Clindamycin (30 µg) were used to determine the sensitivity of Gram-positive bacteria, and the antibiotic disks of Amikacin (30 µg), Imipenem (10 µg), Ceftazidime (30 µg), and Cefotaxime (30 µg) were used to determine the sensitivity of Gram-negative bacteria. Ciprofloxacin antibiotic disks (30 µg) were also used jointly by both groups.

2.4. DNA Extraction

DNA was extracted from a pure colony by boiling according to the method of Bahmanabadi et al. Then, the desired DNA was stored in a freezer at -20°C until PCR (Bahmanabadi *et al.*, 2018).

2.5. Synthesis and preparation of primers

Synthesis of selected primers sequences was carried out in Sina Clone Company (Iran). The specifications of the primers used in this study are given in the table 1.

2.6. Polymerase chain reaction (PCR)

We used a colored mastermix for PCR. Then, we checked the results with electrophoresis. This confirmed the correctness of the PCR and the amplification of *EC-16S*, *nuc*, *rpoD*, and *EPi*. For visualization, we used 1.5% agarose gels in TBE1x buffer. After electrophoresis, we used a documentation system to photograph the gel's bands.

2.7. Statistical analysis

The statistical analysis of the data and results obtained from the experiments was performed using SPSS version 26 software. The mean values were compared using the analysis of variance test (ANOVA) at a significance level of $P \leq 0.05$.

Table 1. Specifications of the primers used for PCR

Target Gene	Primers Sequences 5′–3′	Amplicon Size (bp)	Organism	Reference
Epi-F Epi-R	GGCAAATTTGTGGGTCAAGA TGGCTAATGGTTTGTCACCA	194	*S. epidermidis*	(Byrne *et al.*, 2007)
Nuc-F Nuc-R	GCGATTGATGGTGATACGGTT AGCCAAGCCTTGACGAACTAAAGC	279	*S. aureus*	(Zhang *et al*, 2004)
EC-16S-F EC-16S-R	AACCTGGGAACTGCATGCATCTGA CAGCACCTGTCTCACGGTTC	433	*E. coli*	(Behnam *et al*, 2022)
rpoD-F	GGGCGAAGAAGGAAATGGTC CAGGTGGCGTAGGTGGAGAA	178	*P. aeruginosa*	(Kaluzny, Abeyrathne and Lam, 2007)

3. Results

Among the 60 collected samples suspected of ocular endophthalmitis, 15 were infected with bacterial infection, which included 5 isolates of *E. coli*, 2 isolates of *P. aeruginosa*, 4 isolates of *S. epidermidis*, and 4 isolates of *S. aureus* (Table 2). Among the positive samples, 60% were female and 40% were male between the ages of 35 and 60. The type of endophthalmitis in 15 cases that were reported to be positive was caused by acute, chronic, and traumatic surgery. 73% of patients had exogenous bacterial endophthalmitis, and 26% of patients had endogenous endophthalmitis and there was no fungal eendophthalmitis.

Susceptibility to Amikacin, Ciprofloxacin and Ceftazidime was higher among *E. coli* isolates than *P. aeruginosa* ($p<0.05$). Imipenem resistance was higher among *E. coli* and *S. aureus* clinical isolates than other bacteria ($p<0.05$). No significant difference was found between *S. aureus* and *S. epidermidis* for resistance to Ciprofloxacin, but *S. aureus* isolates were more resistant to Imipenem and Ceftazidime.

The highest resistance for *E. coli* and *P. aeruginosa* was to Imipenem (33% resistance and 33% intermediate), while the lowest resistance was to Amikacin (0% resistance and 22% intermediate). The results for *E. coli* and *P. aeruginosa* were that the highest resistance was for the antibiotic Imipenem with 33% resistance and 33% intermediate, and the lowest resistance was for the antibiotic Amikacin with 22% intermediate and there was no resistance for this antibiotic.

Among the 60 endophthalmitis samples collected from patients admitted to the surgery department and outpatients of Khatam-Al-Anbiai Hospital in Mashhad, 15 isolates were confirmed as bacterial infections by PCR, which was equal to the number of isolates identified by culture.

Table 2. Specific biochemical characteristics of bacteria in this study

Isolates	Catalase	Coagulase	DNase	Mannitol Salt Agar	Oxidase	Citrate	Urease	Methyl red	Indole	H2S	Motility	MR	VP
E. coli	+				-	-	-	+	+	-	±	+	-
P. aeruginosa	+				+	+	+	-	-	-	+	+	-
S. aureus	+	+	+	+									
S. epidermidis	+	-	-	-									

Table 3. The frequency distribution of antibiotic resistance for bacterial isolates

	Antibiotics														
Type of bacteria	Cefotaxime			Imipenem			Amikacin			Ciprofloxacin			Ceftazidime		
	R	I	S	R	I	S	R	I	S	R	I	S	R	I	**S**
E. coli (n=5)	2	2	1	2	3	-	-	1	4	-	3	2	2	-	3
P. aeruginosa (n=2)	-	1	1	-	-	2	-	1	1	-	2	-	1	1	-

	Antibiotics														
Type of bacteria	Cefoxitin			Clindamycine			Azithromycin			Ciprofloxacin			**Erythromycin**		
	R	I	S	R	I	S	R	I	S	R	I	S	R	I	**S**
S. aureus (n=4)	-	-	4	4	-	-	3	1	-	2	2	-	1	-	3
S. epidermidis (n=4)	2	1	1	2	2	-	1	1	2	3	1	-	1	3	-

S: sesceptible, R: resistant, I: Intermediate resistant

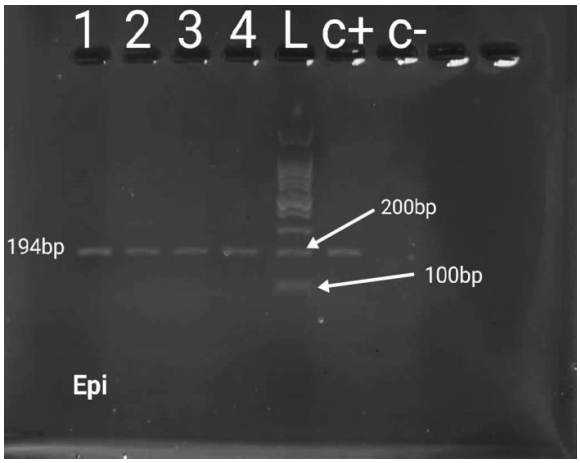

Figure 1. Gel electrophoresis of *Epi* gene PCR products for 4 isolates of *Staphylococcus epidermidis* causing endophthalmitis (L: Leder, C+: positive control, C-: negative control, number 1 to 4 of clinical isolates)

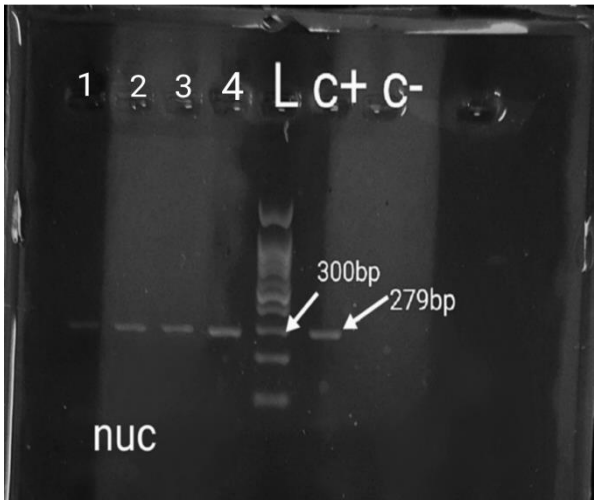

Figure 2. Gel electrophoresis of *nuc* gene PCR products for 4 isolates of *S. aureus* causing endophthalmitis (L: Leder, C+: positive control, C-: negative control, clinical isolates number 1 to 4).

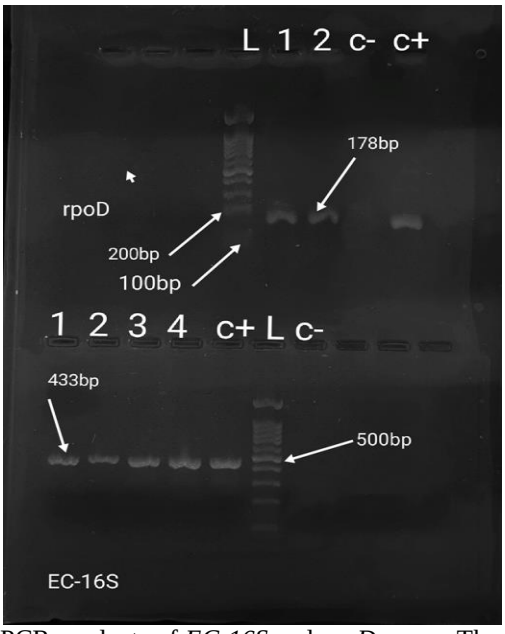

Figure 3. Gel electrophoresis of the PCR products of *EC-16S* and *rpoD* genes. The top of the picture includes two isolates of *Pseudomonas aeruginosa* causing endophthalmitis, positive control and negative control; the bottom of the picture includes 4 isolates of *Escherichia coli* causing endophthalmitis (L: Leder, C+: positive control, C-: negative control, numbers 1 to 4 of clinical isolates)

4. Discussion

One of the common infections in different countries is eye infections, which can have various causes and can cause permanent vision loss. Because eye surgeries are common, the risk of eye infection is high. The results of the present research showed that among the 60 suspected endophthalmitis samples collected, 25% had bacterial infection, of which 13.3% were infected with the Gram-positive bacteria *Staphylococcus aureus* and *S. epidermidis*, and 11.6% were infected with the Gram-negative bacteria *E. coli* and *P. aeruginosa*.

In a study conducted by Jabarvand *et al*. in 2016 on endophthalmitis after surgery, the prevalence of endophthalmitis among patients was 23% (15). In the research conducted by Maliki *et al*. in 2013 at Al-Zahra Ophthalmology Hospital, it was shown that of 24 eye samples, 25% had bacterial endophthalmitis. The results

of these studies are consistent with the present study.

According to the findings of the present study, the most common pathogens causing endophthalmitis were *E. coli* (8.3%), *S. aureus* (6.6%), and *S. epidermidis* (6.6%). Similar to this study, in a study by Thapa *et al*. in 2017 at the Nepal Eye Research Center, out of 8 cases of acute endophthalmitis caused by cataract surgery, 87.5% (7) had isolation in the aqueous humor and vitreous, and all patients had infection caused by *E. coli* (Thapa *et al.*, 2017). In 2020, Liu *et al*. conducted a study on 1003 endophthalmitis samples and showed that the most common isolates were staphylococcal species, with *S. epidermidis* having a frequency of 30.2% and *S. aureus* having a frequency of 11.3% (Liu *et al.*, 2020). In the research conducted by parmar et al. in 2023 at a tertiary center in Central India, on endophthalmitis samples and showed that the most common bacterial cause of infectious endophthalmitis was *Pseudomonas* followed by *Staphylococcus* and *Bacillus* (Parmer *et al*., 2023). In addition, in the retrospective study by Jindal *et al*. (2013) and the study by Ramesh *et al*. in 2010, Gram-positive cocci were the most common isolates (Ramesh *et al.*, 2010; Jindal *et al.*, 2013). In this study, after *E. coli*, *S. aureus* and *S. epidermis* isolates were the most common isolates among the samples, which followed these studies.

Given that *E. coli*, the most frequent cause of endophthalmitis, accounts for 8.3% of cases and antibiotic resistance heightens with excessive usage, an investigation into antibiotic resistance patterns is essential. Biotic properties need to be identified in various geographical locations. The bacterium exhibited the greatest resistance to Imipenem and the least resistance to Amikacin among the tested antibiotics.

In 2021, Chen *et al*. examined the antibiotic susceptibility of endophthalmitis bacteria isolated at a Taiwanese hospital towards vancomycin, Ceftazidime, and Amikacin. In the study, Ceftazidime and Amikacin proved effective for treating Gram-negative bacteria (Chen *et al.*, 2021). In the present study, *E. coli* and *P. aeruginosa* did not exhibit resistance to Amikacin, aligning with Chen *et al*.'s findings.

In 2024, Gaur *et al*. examined the antibiotic susceptibility of endophthalmitis bacteria isolated at a centre Northern India towards vancomycin, Ceftazidime, Cefazolin, Ciprofloxacin and Tobramycin. In the study, Vancomycin, Ciprofloxacin and Tobramycin proved effective for treating Gram-negative bacteria (Gaur *et al.*, 2024). In the present study *E. coli* and *P. aeruginosa* did not exhibit resistance to Ciprofloxacin, it was not aligned Gaur *et al 's* findings.

In our study, the majority of the patients belong to the low socioeconomic strata, were between 35–60 years of age, and travel from remote areas for treatment, with 60% of the population being female. This was similar to a study conducted by Parmar *et al*. On the contrary in Gaur, India, in which they also documented male predominance in the total number of cases of endophthalmitis bacteria presenting in their cornea.

Conclusion

There are very few studies that have evaluated the etiological cause and antibiotic susceptibility in the Mashhad of Iran. This study will therefore be of help in deciding the empirical treatment in endophthalmitis patients. *E. coli* and *P. aeruginosa* among Gram-negatives, and *S. aureus, S. epidermidis* among Gram-positives, are major bacterial causes of eye infections due to antibiotic misuse and resistance; swift antibiotic treatment, especially for *P. aeruginosa*-linked endophthalmitis, is crucial for preventing vision loss. In the initial treatment of ocular endophthalmitis infections, Ceftazidime's usage should be reduced due to a higher resistance rate compared to other antibiotics. The sample size of our study was also small compared to the prevalence of the disease. Thus, the resistance pattern cannot necessarily be extrapolated outside of Mashhad. A larger study population from various geographical locations is needed to confirm the findings of this study in general.

Acknowledgments

I would like to express my gratitude to Khatam-Al-Anbia Hospital for cooperation in examining Endophthalmitis patients.

Conflict of interest

There is no conflict of interest between the authors.

Refereces

Aftab, O. M., Dupaguntla, A., Khan, H., Uppuluri, A., Zarbin, M. A., & Bhagat, N. (2024). Regional Variation of Infectious Agents Causing Endogenous Endophthalmitis in the United States: A National Database Analysis. Ophthalmology Retina.

Bahmanabadi, R. et al. (2018) 'Evaluation of the prevalence of typical and atypical enteropathogenic Escherichia coli isolated from stool specimens of patients with diarrhea admitted to Tehran Children's Hospital by the PCR Method', Feyz Medical Sciences Journal. Feyz Medical Sciences Journal, 22(2), pp. 222–229.

Byrne, F. J. et al. (2007) 'Development of a molecular methodology to quantify Staphylococcus epidermidis in surgical washout samples from prosthetic joint replacement surgery', European Journal of Orthopaedic Surgery & Traumatology. Springer Nature BV, 17(5), pp. 449–456.

Charles, Q. Y. and Ta, C. N. (2012) 'Prevention of postcataract endophthalmitis: evidence-based medicine', Current opinion in ophthalmology. LWW, 23(1), pp. 19–24.

Chen, K.-J. et al. (2021) 'Susceptibility of bacterial endophthalmitis isolates to vancomycin, ceftazidime, and amikacin', Scientific Reports. Nature Publishing Group UK London, 11(1), p. 15878.

Garcia O'Farrill, N., Abi Karam, M., Villegas, V. M., Flynn Jr, H. W., Grzybowski, A., & Schwartz, S. G. (2024). New Approaches to Overcoming Antimicrobial Resistance in Endophthalmitis. Pharmaceuticals, 17(3) , 321.

Gaur, N., Takkar, B., Chandra, P., Puri, S., Satpathy, G., & Sharma, Y. R. (2024). Clinical Profile, Antibiotic Resistance and Outcomes in Bacterial Endophthalmitis: Coagulase-Negative Staphylococcus Endophthalmitis as Compared to Other Organisms. Cureus, 16(2).

Jindal, A. et al. (2013) 'Polymicrobial endophthalmitis: prevalence, causative organisms, and visual outcomes', Journal of ophthalmic inflammation and infection. Springer, 3, pp. 1–6.

Kaluzny, K., Abeyrathne, P. D. and Lam, J. S. (2007) 'Coexistence of two distinct versions of O-antigen polymerase, Wzy-alpha and Wzy-beta, in Pseudomonas aeruginosa serogroup O2 and their contributions to cell surface diversity', Journal of bacteriology. Am Soc Microbiol, 189(11), pp. 4141–4152.

Li, H. et al. (2020) 'SARS-CoV-2 and viral sepsis: observations and hypotheses', The Lancet. Elsevier, 395(10235), pp. 1517–1520.

Liu, C. et al. (2020) 'Microbiological Isolates and Antibiotic Susceptibilities in Cases of Posttraumatic Endophthalmitis: A 15-Year Review', Journal of Ophthalmology. Wiley Online Library, 2020(1), p. 5053923.

Parmar, G. S., Meena, A. K., Borde, P., & Prasad, S. (2023). Microbial keratitis and antibiotic sensitivity patterns: A retrospective analysis at a tertiary center in Central India. Indian Journal of Ophthalmology, 71(6), 2455-2459.

Patel, S. N. et al. (2019) 'Changes in management based on vitreous culture in endophthalmitis after intravitreal anti-vascular endothelial growth factor injection', American Journal of Ophthalmology. Elsevier, 207, pp. 224–231.

Ramesh, S. et al. (2010) 'Prevalence of bacterial pathogens causing ocular infections in South India', Indian Journal of Pathology and Microbiology. Medknow, 53(2), pp. 281–286.

Relhan, N., Forster, R. K. and Flynn Jr, H. W. (2018) 'Endophthalmitis: then and now', American journal of ophthalmology. Elsevier, 187, pp. xx–xxvii.

Rozwandowicz, M. et al. (2018) 'Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae', Journal of Antimicrobial Chemotherapy. Oxford University Press, 73(5), pp. 1121–1137.

Sadiq, M. A. et al. (2015) 'Endogenous endophthalmitis: diagnosis, management, and prognosis', Journal of

ophthalmic inflammation and infection. Springer, 5, pp. 1–11.

Sharifian, H. K. et al. (2023) 'Evaluation of Shiga toxin 2 genes in Enterobacteriaceae bacteria isolated from stool and urine specimens of the patients referred to the Falavarjan Therapeutic Clinic in Isfahan', International Journal of Molecular and Clinical Microbiology. Islamic Azad University-Tonekabon Branch, 13(1), pp. 1810–1819.

Sridhar, J. et al. (2015) 'Endophthalmitis caused by Pseudomonas aeruginosa: clinical features, antibiotic susceptibilities, and treatment outcomes', Retina. LWW, 35(6), pp. 1101–1106.

Thapa, M. et al. (2017) 'Antibiotic sensitivity pattern on cluster endophthalmitis caused by Gram negative organism', Journal of Institute of Medicine Nepal, 39(2), pp. 38–42.

Vaezi, A. et al. (2022) 'Prevalence and Determination of Pattern of Antibiotic Resistance of Enterobacteriaceae Causing Urinary Tract Infection in Clienteles Referred to the Laboratory of Valiasr Clinic in Mashhad in 2022', International Journal of Molecular and Clinical Microbiology. Islamic Azad University-Tonekabon Branch, 12(1), pp. 1650–1656.

Valdez-García, J. E. et al. (2014) 'Anterior chamber bacterial contamination in cataract surgery', BMC ophthalmology. Springer, 14, pp. 1–4.

Wayne, P. A. (2002) 'National committee for clinical laboratory standards', Performance standards for antimicrobial disc susceptibility testing, 12, pp. 1–53.