Isolation of different types of bacteria from polymeric artificial eyes in Iraqi patients

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Abstract : There are several conditions which can cause discomfort and mucoid discharge during wearing polymeric artificial eyes (A.E). This study was performed to investigate the existence of bacteria growth (Gram positive and Gram negative bacteria) in the eyes of A.E wearers. Twenty seven swab samples were taken from patients wearing artificial eyes (A.E). Thirteen samples from (A.E) wearers who were symptomatic after various periods of wearing the (A.E). While other fourteen samples, were taken from artificial eye wearers who don't have symptoms. All these samples were taken through coordination with several hospitals from Iraq / Baghdad that included, Al- Shaheed Ghazi Hospital, Ibn al-Haytham Hospital, and from Hilla Hospital, in addition to Western Hamzah Hospital. It was found that in a total of twenty seven swab specimens, were divided into 13 culture-positive and 14 culture-negative specimens. From positive culture, seven of them were *Staphylococcus aureus* isolates and represented (53.84) %, two of them were *Staphylococcus epidermidis* isolates and represented (15.38) %, while four of them were *Escherichia coli* and coliform bacteria, in (15.38) %, (15.38) % isolation percentage respectively. All growth culture were characterized and identified according to the phenotype standard biochemical and physiological test. Investigation of bacterial isolates to antimicrobial agents was conducted using some, and antibiotics, the results revealed different degree of sensitivity to these antibiotics, Chloramphenicol (CH) and Tetracycline (T) showed the highest sensitivity against all bacterial isolates (92.3, 92.3) % respectively. While Streptomycin (S) and Cefoxitin (T) showed the lowest sensitivity against bacterial isolates (76.9, 76.9) respectively.
Key Words : Artificial eye, Bacteria, Antibiotics, Ocular prosthesis.

Introduction:

Several complications and accidents that can lead to loss of natural eye such as trauma, malignant tumor and end-stage ocular disease which impact on person's self-image. An artificial eye (A.E) is an artificial part replaced for the bulb of eye that could give an esthetic eye socket and maintaining it in normal and natural appearance for the patient[1,2], also play an important role in the preservation ocular muscle function and ton, prevent palpebral collapse, in addition to preventing the accumulation of the fluids in the eye cavity and helping the patient who wearing this prosthesis normal in the community[3]. Wearing of (A.E) for a long period of time can cause several complications such as the eye socket infection, blepharitis, giant papillary conjunctivitis, orbital cellulitis and internal and external hordeolum by several types of microbes that included bacteria, fungi and viruses[4, 5, 6]. One of the most common ophthalmic bacterial species are *Staphylococcus aureus* and *epidermidis*, These types of bacteria are found on the skin and mucosal tissues as commensal bacteria, also can infect the sterile sites on the human and causes disease such as sockets of (E.A)[7,6]. Also, they have reported that

isolation and identification of gram negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa* and gram negative bacilli from patients wear prosthesis. The goals of the present study were to isolation and identification of bacteria from patients who wearing artificial eyes from several Iraqi hospitals, and its susceptibility to antimicrobial agents.

Materials and methods:

Collection of samples:

Twenty seven swab samples were taken from patients wearing artificial eyes (A.E) from several hospitals that include Al- Shaheed Ghazi Hospital in Baghdad, Ibn al-Haytham Hospital in Baghdad, Hilla Hospital and the Western Hamzah Hospital, thirteen samples from artificial eye wearers who were symptomatic after various periods of wearing the (A.E). While other fourteen samples were taken from artificial eye wearers who don't have symptoms.The samples were examined in the laboratories of the biology department in the college of science for women - Baghdad University. The team faced several obstacles during the process of collecting samples. For example, uncooperation of the patient and his unwillingness to deal with a stranger, but with his doctor. In addition to this the problems concerning the examination of samples such as the lack of technical facilities in the hospital laboratories and or the unwillingness of administrators to cooperate with us. All of this led to the damage and the failure of several samples which their collection took a long time and hard effort. Despite the serious difficulties that we faced, the team was able to collect 27 samples which were the subject of this study.

Isolation and identification

After collection of swabs, all specimens were taken directly to the laboratory of microbiology at the Department of Biology, College of Science (for women) for isolation and identification of all bacteria and fungi that found in these swabs, the obtained isolates transferred and inoculated at sterile media to obtain a pure culture. All isolates identified according to[8]by inoculation at the differential and selective media included MacConky agar, Mannitol salt agar, Blood agar for bacteria and Potato dextrose agar, Sabouraud agar for fungi all plates incubated at 26-28 ℃ for fungal culture for 5-7 days and 37℃ for bacterial culture by placing inside the incubator for 24-48 h. of aerobic conditions. Gram stain was done by staining thin smear to investigate the reaction of all colonies were grown. All isolates were obtained from samples subjected to some biochemical and physiological test like, Catalase,Oxidase, Lactose fermentation, Hydrogen gas production, Methyl red test, and Indol test

Antibiotic susceptibility testing:

All bacterial isolates were isolated in pure culture and tested for antibiotic susceptibility, this was done by using traditional method (Kirby-Bauer) disc diffusion on Mueller-Hinton agar plates. After streaking of bacteria by sterile cotton swab on media and selected antibiotics all plates were incubated inside the Incubator at 37 ℃, after 24 h. The inhibition zone around the antibiotic disc was calculated and determined according to guidelines by[9].

Results and Discussion:

Isolation and identification

A total of 27 patients were wearing (A.E) and who subjected to investigation for isolation and identification of microbial growth. of all, thirteen patients who were symptomatic after various periods of wearing the (A.E), the results of identification and characterization of all bacterial isolates were obtained from culture were reported in the (Table 1 and 2).

Table 1.Biochemical and physiological test of gram positive bacteria

Isolate No.Test	1	2	3	5	7	9	10	12	13
Gram stain	+	+	+	+	+	+	+	+	+
Shape of cells	cocci	cocci	cocci	cocci	cocci	cocci	cocci	cocci	cocci
Catalase	+	+	+	+	+	+	+	+	+
Oxidase	+	+	-	+	+	+	+	-	+
Coagulase	+	+	-	+	+	+	+	-	+
Growth on Mannitol salts agar	+	+	-	+	+	+	+	-	+
Hemolysis on blood agar	β	β	γ	β	β	β	β	γ	β

Table 2.Biochemical and physiological test of gram negative bacteria

Isolate No. Test	4	6	8	11
Gram stain	-	-	-	-
Shape of cells	cocco-bacilli	cocco-bacilli	Rod shape	Rod shape
Catalase	+	+	+	+
Oxidase	-	-	-	-
Lactose fermentation	+	+	-	-
Hydrogen gas production	+	+	+	+
Growth at MacConkey agar	+	+	+	+
Methyl red	+	+	+	+
Indol test	+	+	+	+

Table 3. Types of bacteria isolated from patients wearing artificial eye have symptoms of infection.

Types of bacteria	Types of sample	Total of bacterial	
		Isolates No.	%
Staph. aureus	Artificial eye	7	53.84
Staph. epidermidis	=	2	15.38
E. coli	=	2	15.38
Coliform bacteria	=	2	15.38

The most frequently bacterial isolates were *Staph. aureus* (7) samples isolates (Gram positive cocci with grape shape), all these isolates were positive for coagulase, growth at mannitol salts agar and represented (53.84) %, in addition to *Staph. epidermidis* (2) samples isolates(Gram positive cocci with grape shape), all these isolates were negative for coagulase and positive forcatalase, oxidase while no change in the color of mannitol salt gar (Figure1). *E. coli* (2) samples isolates followed by 2 isolates *Coliform bacteria* (Gram negative) (15.38) % respectively, (Table 3), all Gram negative isolates were hydrogen gas production, catalase, indol and methyl red test positive, whereas negative for oxidase, the isolates of *E. coli* were positive for lactose ferment. The fourteen samples were taken from patients with (A.E.) wearers who don't have symptoms, the results of culture and isolation of fungi showed no growth of any microorganisms.

(A)   **(B)**   **(C)**
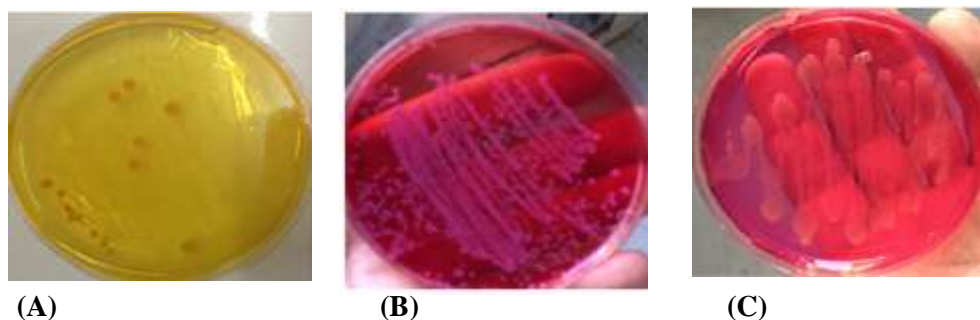
Fig. 1.   Show (A) growth of *Staph. aureus* on Mannitol salt agar, (B) growth of *E. coli* on MacConky agar(Pink colonies) and (C) growth of Coliform Bacteria on MacConky Agar (Colorless colonies).

Many studies and reports have demonstrated that most of bacterial species present in the eye lid margins or conjunctiva are as normal flora and commensals without infection and disease called *Staphylococcus* species [10]. *Staph. aureus* is one of the most bacterial species that infected patients after cataract surgery, it was characterized as most common pathogens in the skin of the human and causing bacterial infection in the ophthalmic, Conjunctivitis, Keratitis around the world in addition to ocular infection called and[11,6]. Another study, showed present of *Staph. aureus* and coagulase negative *Staphylococci* in percent of (19.9 and 28.6%) respectively. A study by[12]has been demonstrated that *Staph. epidermidis* was the most frequent bacterial species in the lids and conjunctive.

From the present study, several types of bacteria were obtained and observed in the prosthesis eye that including *Staph. aureus, Staph. epidermidis, E. coli* andGram negative bacilli, this result was agreed with literature obtained by[7] who isolated and identified several types of Gram positive and Gram negative bacteria before and after cleaning of the artificial eyes with cleaning solution. Also the results of the present study were consistent with the literature accomplished by[5]who based on the fact, The wearing of artificial eyes for long-term can lead to several complications such as secondary infection, this can lead to change the microorganisms from the state of normal flora to pathogenic microbes of the patients who wearing the prosthesis.

Discharging sockets were associated with *Staph. aureus, Staph.epidermidis*, coliform spp. Bacteria and mixed flora, This result was in agreement with the results findings by[13].

Susceptibility test for antibiotics:

The results of antibiotic susceptibility tests showed variation in the resistant and sensitivity to these antibiotics (Table 4), thus dependent on the types of bacterial isolates and the generation of antibiotics. Most of the examined isolates showed high resistance to Streptomycin (76.9%), Cefoxitin (76.9%), Penicillin (69.2%) and Carbapenems (69.2%), whereas, all bacterial isolates exhibited high sensitivity against Chloramphenicol and Tetracycline (92.3 and 92.3%) respectively. Chloramphenicol showed the most effective antibiotics against external eye infection and is considered as a broad-spectrum antibiotic and is widely used for treatment of infections by Gram negative bacteria such as spectrum β-lactamase (ESBL) which involved *Pseudomonas* spp., Enterobacteriaceae, *E. coli* Etc.[14,15]. While[13] reported that the most effective drug of choice for treatment of external eye infected with *Stahp. aureus*, *Haemophilus influenza* was Chloramphenicol. In a separate study with accomplished by[16]reported that the Methicillin- Resistant *Staph.aureus* (MRSA) isolated from ocular infection was susceptible to Chloramphenicol and Gentamycin, while resistance to the third generation of Fluoroquinolones such as Ciprofloxacin and Ofloxacin in addition to Cefazolin.

Table4. Susceptibility testing of antimicrobial agents against all bacterial isolates.

Bacterial isolates	No of isolates	S/R	Antibiotics								
			P No. %	S No. %	T No. %	CH No. %	E No. %	G No.%	CL No. %	CA No. %	CEF No. %
Staph. aureus	7	S	2 (28.71)	2 (28.5)	6 (85.7)	6 (85.7)	2 (28.5)	3 (42.8)	5 (71.4)	1(14.2)	1 (14.2)
		R	5 (71.48)	5 (71.4)	1 (14.2)	1 (14.2)	5 (71.4)	4 (57.1)	2 (28.5)	6(85.7)	6 (85.7)
Staph. epidermis	2	S	-	1 (50)	2 (100)	2 (100)	1 (50)	1 (50)	2 (100)	1 (50)	-
		R	2 (100)	1 (50)	-	-	1 (50)	1 (50)	-	1 (50)	2 (100)
E. coli	2	S	1 (50)	1 (50)	2 (100)	2 (100)	1 (50)	1 (50)	2 (100)	-	2 (100)
		R	1 (50)	1 (50)	-	-	1 (50)	1 (50)	-	2 (100)	-
Coliform bacteria	2	S	1 (50)	-	2 (100)	2 (100)	1 (50)	2 (100)	1 (50)	1 (50)	-
		R	1 (50)	2 (100)	-	-	1 (50)	-	1 (50)	1 (50)	2 (100)
Total of isolates	13	S	4 (30.7)	4 (30.7)	12 (92.3)	12 (92.3)	5 (38.4)	7 (53.8)	10 (76.9)	3 (23.1)	3 (23.1)
		R	9 (69.2)	9 (69.2)	1 (7.6)	1 (7.6)	8 (61.5)	6 (46.1)	3 (23.1)	10 (76.9)	10 (76.9)

S: Sensitive, R: Resistance
P: Penicillin, S: Streptomycin, T: Tetracycline, CH: Chloramphenicol, E: Erythromycin, G: Gentamycin, CL: Cloxacillin, CA: Carbapenems, CEF: Cefoxitin

Conclusion:

Summary of our study, we isolated and identified Gram positive and Gram negative bacteria from infected patients with symptomatic after various periods of wearing the (A.E), and identification was done according to Berge's Manual of Determinative Bacteriology and antimicrobial susceptibility was done and provide guidance for treatment of infection. The present study revealed that the finding of various bacterial strains, including pathogenic species that can cause acute infection of the eye socket.

References:

1. Lucci LMD, Holfing-Lima AL,Erwenne CM, Cassano EMd-T. Artificial eye amplitudes and characteristics in enucleated socket with porous polyethylene spherical and quad-motility implant. Arquivos BrasileirosOphthalmology.2007 Sept,70(5):831-838.
2. Pine K,Franzco BS, Stewar J, Jacobs RJ.Concerns of ophthalmic patients wearing artificial eyes.Clinical and experimental ophthalmology.2011 Jan, 39 (1): 47-52.
3. Kohlhaas M, Schulz D.The complex facial prosthesis: the value of bone-anchored maxillofacial prostheses in the treatment of extensive loss of facial tissue. Revue de stomatologie et de chirurgie maxillo-faciale. 2001 Aug, 102 (5): 261-256.
4. Sarac O.Erdener U.Irkec M.Gungen Y.Tear eotaxin levels in giant papillary conjunctivitis associated with OP. Ocular immunology and inflammation. 2003 Sep11, (3): 223-230.
5. YangJW.Choi JW. Leea SG. Kimb DS.Antibacterial properties of artificial eyes containing Nano-sized particle silver.The international Journal on Orbital Disorders, Ocuoplastic and Lacrimal surgery.2011 Feb15;30 (2):77-81.
6. Nadig SS. Velusamy N. Kar S.Sharma S.Arakere G.*Staphylococcus aureus* eye infection in two Indian hospitals: emergence of ST772 as a major clone. Clinical Ophthalmology.2012 Jan 23, 6: 165-173.
7. Paranhos RMZ.Batalhao CH.M. Semprini S. Regalo CH.Ito IY.Mattos MdGCd. Evaluation of ocular prosthesis biofilm and anophthalmic cavity contamination after use of three cleansing solutions.Journal of Applied Oral Science.2007 Feb22,15 (1): 33-38.
8. Holt JG.Krieg NR.Sneath PHA.Staley JT.S. Williams T.Berge's Manual of Determinative Bacteriology. Williams and Wilkins, USA Baltimore, M.S. eds. 1994.
9. Kahlmeter G.Brown DF.Golstein FW.Macgowan AP.Mouton JW. Odentholt I.Rodloff A. Soussy CJ. SteinbakkM. Soriano F. Stetstiou O. European committee on antimicrobial susceptibility testing (EUCAST) technical notes on antimicrobial susceptibility. Clinical Microbiologyand Infection.2006 Jun,12(6): 501-503.
10. Iskeleli G. Bahar H. Eroglu E. Torun MM. Ozkan S. (2005). Microbial changes inconjunctival flora with 30-day continuous-wear silicone hydrogel contact lenses. Eye Contact Lens.2005 May, 31 (3): 124-126.
11. Behlau I.Gilmore MS.Microbial biofilms in ophthalmology and infectious disease. Archives ophthalmology. 2008Nov, 126(11): 1572-158.
12. McCulleyPJ.Dougherty JM.Deneau DG.Classification of chronic blepharitis. Ophthalmology.1982 Oct, 89(10): 1173-1180.
13. Seal, DV.Barrett SP.McGill JI.Aetiology and treatment of acute infection of the external eye.The British Journal of Opthalmology.1982 Jun, 66(6): 357-360.
14. Torres JA.Villegas MV.QuinnJP.Current concept in antibiotic-resistant gram-negative bacteria.Expert Review of Anti-infective Therapy.2007 Oct, 5 (5): 833-843.., 2007.
15. Wang N.HuangQ.Tan YW.Lin LP.Wu K L.Bacterial spectrum and resistance patterns in corneal infections at a Tertiary Eye Care Center in South China.International Journal of Ophthalmology.2016 Mar, 9 (3): 384-389.
16. Shanmuganathan VA.Armstrong M.Buller A. TulloAB.External ocular infections due to Methicillin-Resistant *Staphylococcus aureus* (MRSA) Eye (lond).2005 Mar, 19 (3):284-291.

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