Screening the Effect of Kohl (Al-Ethmed) on the Bacterial Species Isolated from Human Eyes

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Abstract:

Objective: This study aimed to isolate bacteria from the eyelids of that non-cosmetic user group and eye cosmetic user group.

***Materials and Methods*:** The study samples were taken by swabs, and the bacterial isolates were identified by Vitek2 compact system, and the antibacterial activity of Kohl (Al-Ethmed) was performed by well diffusion technique.

Results: The highest percentage of bacterial isolates was gram positive *Staphylococcus. aureus* (21.40%) followed by Coagulase negative *Staphylococcus (CNS)* (19.60%) and *α-hemolytic Streptococcus* (16.10%), while the percentage of gram negative isolates represented by *Echerichia coli, Klebsiella* Spp. and *Proteus* Spp. was (3.60%) and the percentage of *Pseudomonas aeruginosa* was (1.80%). The percentage of most bacterial isolates increased within cosmetic user group. The geographical areas showed that there was some difference in percentage of the species between Erbil, Sulaymania and Kirkuk. According to the ages, *CONS and α-hemolytic Streptococcus* were shown to be the common isolates among all age groups, while according to gender; the results revealed that *S. aureus* (21.20%), *CNS* (15.10%), *Haemophilus* (9%), *E. coli* and *Proteus* (6.60%) and *p. aeruginosa* (3%) were the predominant isolates in females. However, *γ-hemolytics Streptococcus* (18.10%), *α-hemolytic Streptococcus* (22.70%) were of the highest percentage in males followed by *Micrococcus luteus* (13.60%), *S.pyogenes* (9%) and *Klebseilla* (4.50%). The results showed that *S. aureus, CNS, S. pyogenes, α-hemolytic Streptococcus* and *γ-hemolytic Streptococcus* were resistant to (CT) with a percentage of (71.4%), (75%), (66.6%), (60%) and (84%) respectively, while *E. coli, Klebseilla* species, *p. aeruginosa* and *Proteus* showed (100%) resistance to cefuroxime (CX). It is worth mentioning from our study that Kohl (which Prophet Mohammed (Peace be upon him) used it and recommended to use) as cosmetics exhibited considerable antibacterial activities, when the different kohl concentrations were used inhibition of the growth of both *γ-hemolytic Streptococcus* and *p. aeruginosa* was achieved at all concentrations. However, *CNS, Haemophilus* and *Klebseilla* isolates were inhibited at (500), (2500) and (62.5) µg/ml**.**

Keywords: Kohl; Antibacterial; Eye bacteria.

Introduction:

The eye is the vision organ, and vision is one of the five senses which are of great importance for human's life since birth. A great attention has been given to eye's health and cleanliness because it is always exposed to external and internal influences including high temperature, dust, bacteria, fungi, viruses and other infectious agents [(1)].

From ancient times, the traditional eyeliner (Kohl), which is a word of Arabic origins, has been used widely in the Middle East, Far East and Africa for many reasons include religious, cosmetic or medical reasons such as

vision improving and treating of different eye diseases. It is only used in the Arab peninsula among the Arab Bedouins to protect the eyes from the severe sun rays. It is often applied on the eyelid margins for beauty purposes, but may also be put in the conjunctiva to treat different ophthalmic diseases e.g. trachoma, blepharitis and as red eye removers [2, 3]. Misuse of Kohl or eye liner by more than one person or leaving it exposed to air results in the accumulation of dust particles, and therefore contamination with microbes and fungi which are transmitted to the eyes [1].

The conjunctiva and eye lids contain a huge number of bacteria from the external environment known as the normal flora, and these bacteria play a main role in normal body's health and functions by secreting antibiotics and chemical mediators which in turn maintain surface homeostasis and immune regulation. The normal flora also has the ability to compete pathogenic bacteria for nutrition, thereby inhibiting the growth of the pathogenic microbes [4]. These flora do not cause infections in normal conditions, but occasionally become a source of micro organisms that cause ocular diseases [5].

Kohl has also been mentioned in many texts of the (Old Testament) as an eye cosmetic [6, 7]. These findings make it clear that past and present secular and religious literature have dealt with Kohl from different physical, chemical, medical, archaeological and cosmetic aspects. In our communities, females began to use beauty products in the age range of (13-17) years, with an increasing number of 'age-defying' products and hence the cosmetic industries appears to focus on 'consumer beautifying' in the later years of their lives. Whilst the majority of cosmetic and eye make-up users are female, more males in the past few years began using cosmetic products, especially those for the face and eyes.

Eye protection against diseases is of a great importance, and many types of eye protection preparations and treatment have been used, and Kohl was one of these antibacterial eyeliner substances that seeps into the conjunctiva of the eye to activate an immune response, an kills pathogenic microbes and prevents infections even before the initiation [8, 9]. Therefore, it is necessary to know the bacterial species of eye and the importance of using cosmetics and eyeliners for the eye's care and health. This study aimed to isolate some bacterial species from the eye with and without cosmetic using and determine the difference in relation with age, gender and geographical distribution. Our study used different Kohl concentrations as antibacterial preparations against bacterial species isolated from the eye, because no previous study on Kohl as antibacterial agent has been conducted in Erbil or in Kurdistan region or even in Iraq.

Materials and Methods:

Isolation of Bacteria:

Bacterial samples were isolated from eyelid of 50 students and teachers at the College of Education, Salahaddin University in Erbil – Iraq [The geographical areas of students and teachers were covered in our study (Erbil, Sulaymania and Kirkuk)]. Samples were taken with sterile cotton tipped swabs moistened in brain heart infusion broth by rubbing the eyelid very carefully without touching the cornea of the eye from both cosmetic users group and non-cosmetic user

group (control group). The swabs were inserted into separate sterile labeled test tubes and transported immediately to the laboratory and cultured by streaking method on nutrient agar, blood agar and MacConkey agar plates, then incubated for (24) and (48) hours at (37) C^o after incubation, different colonies were obtained. The colonies were picked up for Gram staining and morphological screening, then the bacteria were identified depending on the Vitek 2 compact system.

Antibiotic sensitivity test:

Sensitivity to antibiotics was done to determine sensitive and resistant bacteria to the following antibiotics, vancomycin V, gentamicin G cefotaxime CFT erythromycin E tetracycline T and cefuroxime CX ciprofloxacin disc on Mueller-Hinton agar with 24 hours incubation at 37ºC. Then measuring the inhibition zone diameter in millimeters, and then compared with the standard diameters installed in the standard scales [10].

From the pure growth of each tested organism, three colonies were transferred into (5) ml of nutrient broth, and incubated overnight at 37 ºC. In order to obtain approximately 1*106 CFU/ ml, sterile distilled water was used for suspension dilution [11].

Well diffusion technique:

Well diffusion technique was applied to determine antibacterial activity of Kohl (Al-Ethmed). The Nutrient agar plates were seeded with (0.1) ml of the inoculums from each tested bacteria. The inoculums were spread evenly over plate with a sterile loop. A standard cork borer with (8) mm diameter was used to cut uniform wells on the surface of the nutrient agar, and (100) µl of each concentration of Kohl (Al-Ethmed) 1000, 500, 250, 125 and 62.5 Kohl (µg/ml) were placed in the wells, then the plates were incubated for (24) hours at 37 ºC, followed by measuring the inhibition zones to the nearest millimeter (mm).

Results and discussion:

Many studies have been conducted to identify bacterial species in the eyes, but none has studied the effect of Kohl (Al-Ethmed) on these bacterial species in Erbil –Kurdistan Region. Our results revealed a growth of some bacteria in the control group samples (non-cosmetic and non-Kohl users). The highest percentage of the Gram positive normal flora bacterial isolates in this study were *S. aureus* (21.40%), followed by coagulase negative *Staphyloccus* (CONS) (19.60%) and. α-hemolytic *S.* (16.10%), while the percentages of Gram negative isolates represented by *E. coli*, *Klebsiellaspp* and *Proteusspp* were (3.60%) for each one, where *p. Aeruginosa* isolates recorded a percentage of (1.80%) as shown in figure (1).

A study by Ansari *et al* [12] found that (52.4%) cases had bacterial positive cultures in the eyes, with 79 (88.8%) of them having coagulase-negative *Staphylococcus* (CONS) and 82(95.3%) cases having *Staphylococcus aureus*. However, another study performed by Fernandez-Rubio *et al* [13] found that CONS had the highest prevalence (88.3%) followed by *Streptococci* (23.1%), and *Staphylococcus aureus* (10.2%), while the prevalence rate of Gram-negative rods was (4.5%). Another study reported that the commonest flora isolated was Coagulase Negative *Staphyloccus (CONS),* with (50%) being in the right eye & (52%) in the left eye, followed by Coagulase positive *Staphylococcus (COPS)* (53%), *Micrococcus* (913%), Gram positive bacilli (21%) and *Streptococcus* species (20%). In a study by Akrayi which

included isolation of bacteria from eyelid of 30 persons (includes 10 eye cosmetic users (group A), 10 natural eyeliner (natural alkohl) users (group B), and 10 control (group C: don't use anything of male (3) and female (7)) found that the percentage of each of *S. aureus* and *S. epidermidis* was (40%), (40%) and (20%) for group (A), (B) and (C) respectively, and the percentage of *S. capitis* was (30%), (10%) and (60%) for group (A), (B) and (C) respectively, while the percentage of Gram negative isolates represented by *E. coli* was (70%) and (30%) for group (A) and (B) respectively [(14)].

All the above-mentioned studies showed that the predominant flora of eye were CONS and COPS. The predominance of these bacteria can be attributed to the fact that they are the common skin's and mucous membranes resident flora and are transmitted to the conjunctiva from the adjacent eyelid figure (2) represented the normal flora of the eye isolated from cosmetic using group. The percentage of *S. aureus* increased to (27.30%), *S. pyogenes* to (10.70%) and *α-hemolyticS* to (21.30%). Also the percentage of gram negative isolates *E. coli*, *Klebsiella* and *Proteus* increased to (6%), while the percentage of *p. aeruginosa* recorded (3%).

It is estimated that about (70%) of women used cosmetic products around the eyes. Cosmetics are often used in the lid areas, in the lashes and also in areas close to Meibomian gland ducts on the lid margin. The manufacturers of eye make-up have recently improved their products depending on their ability to resist fading, smudging or wearing off over time in order to make their removal more difficult. Cosmetics may play an important role as the most common cause of eyelid dermatitis. Several factors could contribute to eyelid blepharitis such as cosmetics used as eyeliners, mascara, eyelash curlers, eye shadow and eye make-up remover. Other studies found that (67% to 100%) of the samples were contaminated with *Staph*, *Strep* and even *E. coli* bacteria. Moreover, Misusing of Kohl or eyeliner by more than one person or leaving it exposed to air results in the accumulation of dust particles, and therefore contamination with microbes and fungi which are transmitted to the eyes [(1)].

The naturally-occurring facial cosmetics (Calabash stone & Black antimony) have been assessed for their antibacterial activity and heavy metal contents in a study performed by [(15)], who also used the agar broth diffusion assay to screen cosmetics against selected gram positive and gram negative bacteria. Calabash chalk is relatively safer to use than the black antimony due to its higher metal concentrations. Calabash chalk was found to have moderate antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus pyogenes*, but it showed no bioactivity against *Pseudomonas aeruginosa*, while a remarkable antibacterial activity was shown by black antimony even against *P. aeruginosa.* Users of these cosmetics will be exposed to low levels of metal poisoning when they use them continuously, entirely or as additives. Bioaccumulation of the metals may be harmful to human health and due to the high lead and cadmium contents in the black antimony, its use as a cosmetic should be discouraged.

Figure (3) showed the percentage of bacterial isolates from eye lids of cosmetic users according to district

wise. The geographical areas covered in our study showed difference in the percentage of bacteria species in Erbil, Sulaymania and Kirkuk. So, there no correlation was found in isolation of flora regarding the geographical distribution. Figure (4) demonstrated the percentage of the isolated bacteria from eye in cosmetic users according to age groups. The students and teachers in our study were divided into three age groups (21-30) (31-40) and (41-50) years. It was shown that CONS and *α-hemolytices S.* were the most common organisms isolated among all age groups. The highest percentage of these species (20.9%) was observed in younger populations (21-30) years and (50%) in the age group (31-40), while with the increasing age (41-50)years, the eyes were found to be increasingly colonized with *S. aureus* (28.5%), CONS, γ- *hemolytic S. ,α- hemolytic S.* and *Proteus* (14.3%) and *P.aeruginosa* (41.3%), however, cultures of the age groups (21-30) showed growth of all species except *P.aeruginosa*. Our result did not agree with the findings of Singer *et al* [16] who reported in their study that conjunctival cultures from (229) eyes showed that the species isolated from adults per eye was greater than isolates from younger subjects. Our results also showed a consistent increase in the percentage of *α-hemolytic S.* (50%) with the increasing age, and showed an increase in the percentage of *S. aureus* (23.2%) in younger age groups. The shift in floral composition may be associated with lack of aqueous tear with age, goblet cell changes, and lipid deregulatory states which accumulates over time.

Regarding the percentage of normal flora isolated from eyes of cosmetic users according to gender, table (5) revealed that the percentages of normal flora in females were as follows: *S. aureus* (21.20%), *CNS* (15.10%) *Haemophilus* (9%) *E. coli* and *Proteus* (6.60%) and *p. aeruginosa* (3%). However, in males*, γ-hemolytic s* and *α-hemolytic s* recorded the highest percentage (18.10%) and (22.70%) respectively, followed by *M.luteus* (13.60%), *pyogenes* (9%) and *Klebseilla* (4.50%). The result also showed that (100%) of the bacterial species were found in females in different percentages, while the male swabs contained only (72.7%) of these species. A study conducted by [17] indicated that males had the highest percentage of normal flora growth (68.1%) in comparison with the females (31.9%), while (18) found no statistically significant difference between males and females in the prevalence of normal flora.

In evaluation of the effect of different antibiotics on eye flora, results in figure (6) showed that *S. aureus, CNS, S. pyogenes, α-hemolytic S.* and *γ-hemolyticS* were resistant to (CX) with a percentage of (71.4%), (75%), (66.6%), (60%) and (84%) respectively, while *E. coli, Klebseilla* species, *p. aeroginosa* and *Proteus* showed (100%) resistance to (CT).

The impact of these antibiotics to decrease flora of the eye were also studied and showed reduced bacterial growth of most species with antibiotic (V) when compared with the effect of other antibiotics used in the study. Ancient civilizations have used several kinds of eye preparations to protect eyes from diseases; Kohl was one of these preparations which is closely related to almost all human civilizations including Greeks, Egyptians, Romans, Indians,

Chinese, Japanese, Phoenicians and Muslims [19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29].

For the rapeutical purposes, Kohl is the most popular eye product reported in almost every human civilizations used to prevent and treat eye diseases like blepharitis, chalazion, pterygium, conjunctivitis, trachoma, cataract and ectropion, as well as to prevent recurrent trichiasis [30]. Kohl is also used to improve vision, strengthen and keep the eyes healthier [31]. It was found that the black and shiny particles of galena (lead sulphide), which is a major constituent of Kohl, protect the eyes from the reflection and glare of the sun, and thus protecting the eyes from the harmful effects of UV rays present in sunlight [32]. Zinc oxide in Kohl may also be useful due to its powerful natural sun blocking properties [33], which may possibly promote the protective ability of galena against the sun glare. Neem or Azadirachta *indica* is widely used all over the world because of its strict and antibacterial characteristics [34]. Similar to silver leaves, neem is also characterized by the possession of anti-viral activity [35]. Chaksuor (*Cassia absus*) which is often used for strengthening the eye sight is also useful in treating conjunctivitis purulent opthalmia. While, the extract of fennel or (*Foeniculumvulgare*) plant is actually used to process Kohl stone or (Galena). Mamiran or (*Coptististeeta*) is very important in the treatment of rheumatic and catarrhal conjunctivitis and also used as eye salve or as a paste on sores [31].

It is observed from table (1) that kohl as a cosmetic showed a considerable antibacterial activity, when the concentrations (1000), (500), (250), (125), and (62.5) µg/ ml of kohl were used against the normal flora isolates of eyes, and inhibition of the growth of both *γ-hemolytics* and *p. aeroginosa* was achieved at all concentration. *CNS, Haemophilus* and *Klebseilla* isolates were inhibited at (500), (250) and (62.5) µg/ ml. however, the results showed that the complete inhibition of all species occurred at (125) and (62.5) µg/ ml concentrations.

When a group of French researchers in 2010 analyzed (52) kohl container samples present at the Louvre museum in Paris, they concluded that trace quantities of four uncommon lead species were contained in kohl: Galena (PbS), Cerussite (PbCO3), Phosgenite (Pb2Cl2CO3) and Laurionite (Pb (OH) Cl) [36]. When the lead sulfate present in kohl was exposed to the skin cells, it was found that lead ions have induced high immunological response. The cultured cells released one of the most important messaging gaseous molecule in the immune system (nitric oxide gas (NO) was released by cultured cells and this molecule serves as an activating messenger to bacteria-engulfing macrophages and stimulates blood flow by increasing blood capillary diameter, thereby encouraging rapid immune cell movement in the circulation (8). A 240-fold increase in NO production was noticed in the presence of lead ions, a bona fide tsunami of molecules flooding surrounding cells to respond to the invading bacteria. This strong biochemical interaction indicates that kohl was not only a beautifying cosmetic and the precursor of sunglasses, but also an important antibacterial eye ointment.

Using of Kohl by Prophet Mohammed (Peace be upon him) suggests that it is beneficial and not harmful to humans as he himself used to use Kohl Al-Ethmed three times every night in each eye as mentioned in the Hadeeth narrated by Ibn Abbas [37].

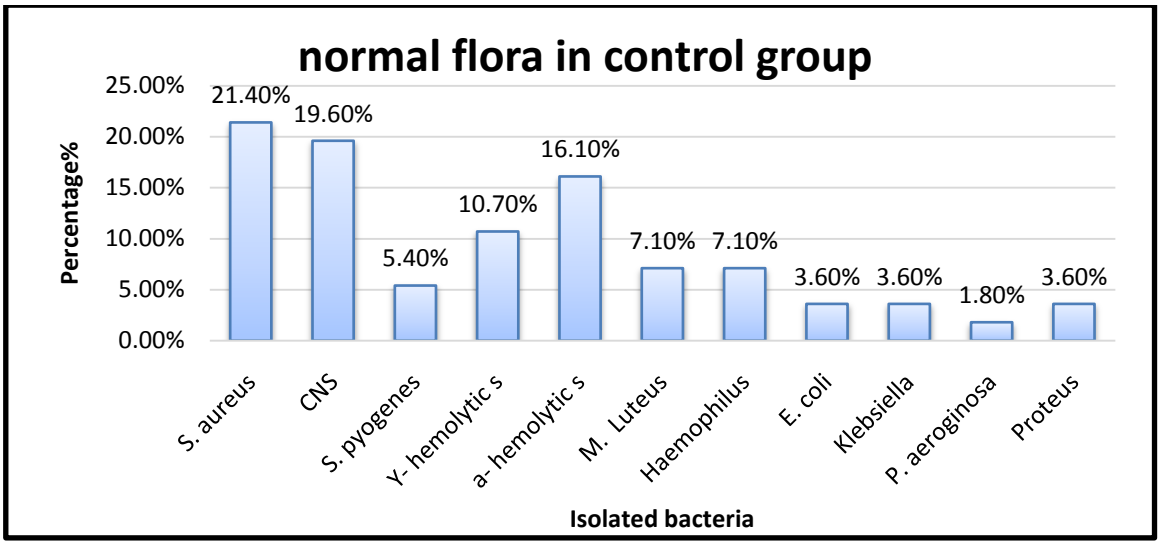

Figure (1): Percentage of eye bacterial normal flora in the control group.

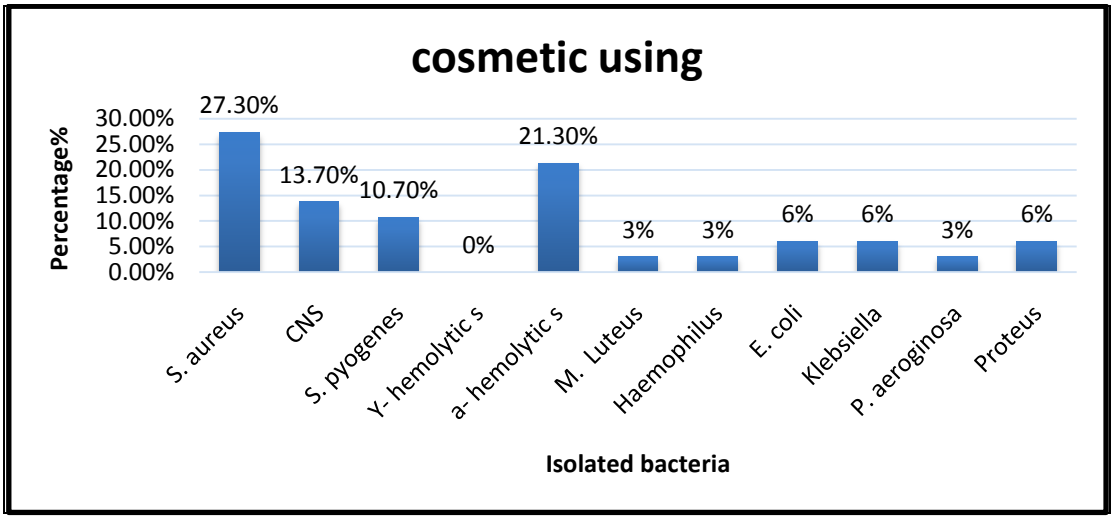

Figure (2): Percentage of eye normal flora among cosmetic using group.

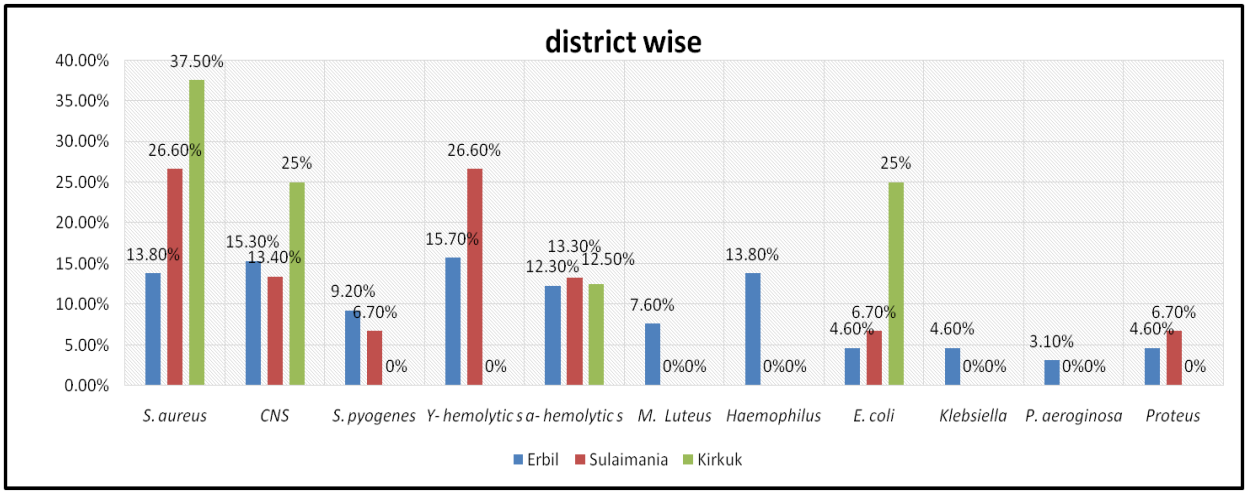

Figure (3): Percentage of normal flora from eye of cosmetic users according to district wise.

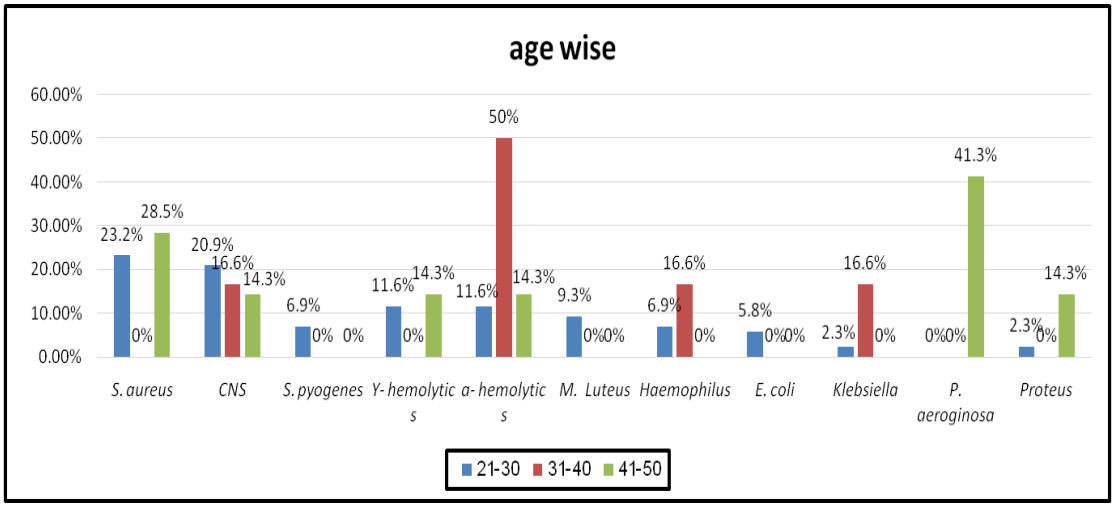

Figure (4) Percentage of the normal flora from eye of cosmetic users according toage.

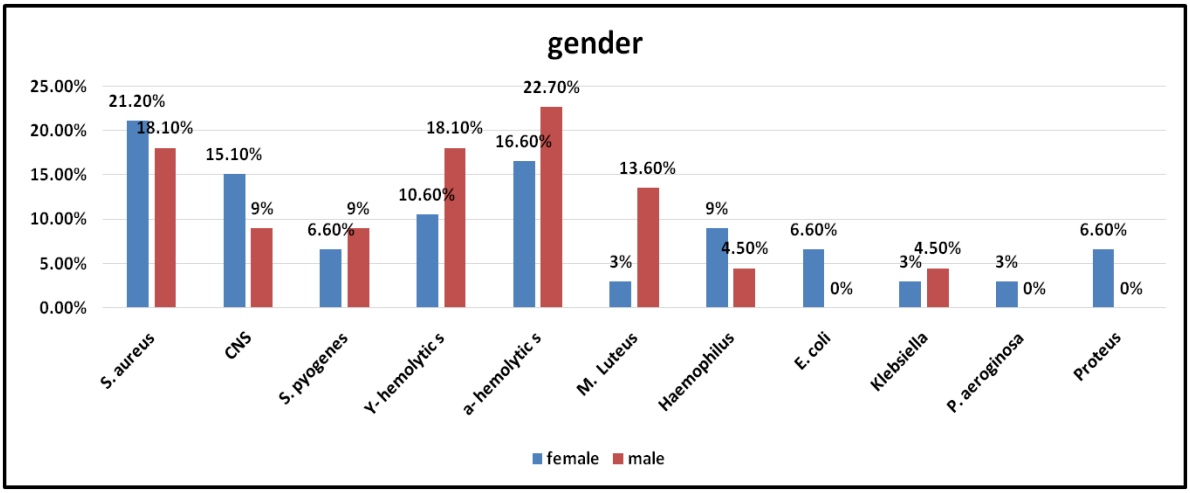

Figure (5): Percentage of the normal flora from eye of cosmetic users according to gender.

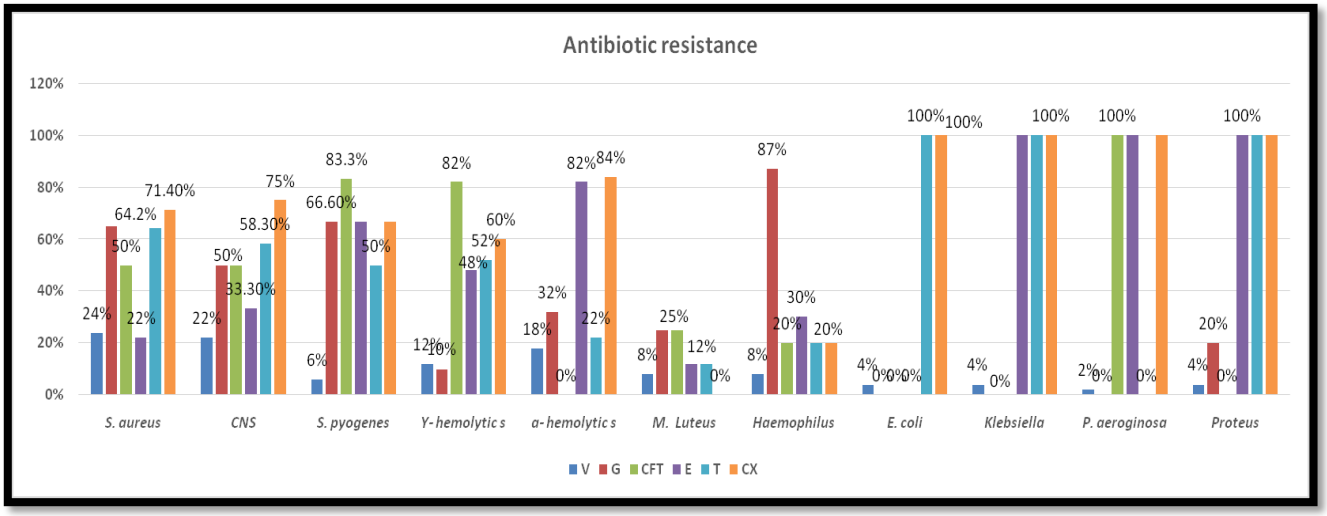

Figure (6): The sensitivity of normal flora from eye to some types of antibiotics.

Table (1): The antibacterial activityof Kohl (Al-Ethmed) against bacterial isolates.

Bacterial species	Inhibition zone (mm)					
	1000	500	250	152	62.5	Control (V)
S. aureus	12	10	0	0	0	s
CNS	8	5	0	0	0	s
S. pyogenes	6	6	0	0	0	s
Y- hemolytic s	0	0	0	0	0	s
a- hemolytic s	6	6	0	0	0	s
M. Luteus	4	3	1	0	0	s
Haemophilus	6	0	0	0	0	s
E. coli	12	10	3	0	0	s
Klebsiella	10	0	0	0	0	s
P. aeroginosa	0	0	0	0	0	s
Proteus	12	11	5	0	0	s

Conclusion:

Our recommendation in this study was that eye cosmetics are usually clean and safe, but sometimes dangerous bacteria grow in these substances. Also the risk of contamination may be even greater with "testers" at retail stores, where many people are using the same sample cosmetics. Adding water to moisten the cosmetic may introduce bacteria and dilute the preservative that is intended to protect against microbial growth.

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