Research Article

IN-VITRO ANTIBACTERIAL ADHERENCE ACTIVITY STUDY OF ANTIBIOTICS WHICH ARE USED IN THE TREATMENT OF BACTERIAL CONJUNCTIVITIS

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ABSTRACT

It was proven that biofilm formation had an important role in relapse infections and bacterial resistance against antibiotics in recurrent bacterial infections. Bacterial biofilm formation in eye's conjunctiva is considered as a major factor in the chronicity and treatment failure in patients with recurrent bacterial conjunctvitis. According to that, we aimed in our study to evaluate the efficacy of some antibiotics recommended in recurrent bacterial conjunctivitis in preventing the biofilm formation by some bacteria isolated from eye's conjunctiva. We had worked on four aerobic species, isolated from eye's conjunctiva of adults suffering from recurrent conjunctivitis, three were Gram Positive Cocci (*Staphylococcus aureus, Staphylococcus epidermidis*), and two were Gram Negative bacilli (*Klebsiella pneumoniae, Pseudomonas aeruginosa*).We evaluated efficacy of five different antibiotics, Chloramphenicol, Gentamicin, Levofloxacin, Rifamycin, and Tobramycin. Bacterial identification and Disk diffusion test were carried out through standard procedures. Evaluation of the anti adherence efficacy of the antibiotics were carried out by using **"**Enumeration of IOL-Adherent Bacteria". Our study shows that Gentamicin has the best efficacy on *Staphylococcus epidermidis* adherence, followed by Chloramphenicol and Levofloxacin, while Tobramycin increased the adherence's rate, Also Gentamicin has the best efficacy on *Staphylococcus aureus* adherence but followed by Levofloxacin. And it shows that Levofloxacin has the best efficacy on *Klebsiella pneumonia*e, while Rifamycin has the best effect on the adherence of *Pseudomonas aeruginosa.*

Keywords: Biofilm, Eye's conjunctiva, Intraocular lens (IOL), Antibacterial adherence activity.

INTRODUCTION

Most bacterial infections involve biofilms. Biofilms are collections of microorganisms encased in a matrix that is often composed of both bacterial and host materials. They form on natural surfaces such as heart valves or abiotic surfaces such as contact lenses or intraocular lenses[1, 2].

The biofilm matrix promotes adherence of the microbe to smooth surfaces as well as to other cells. Biofilms thereby form large 3 dimensional microbial communities of complex architecture through cell-to-cell communication and coordinated multicellular behavior. The biofilm architecture promotes the exchange of nutrients and waste products.

Biofilms are heterogenous mixtures of bacteria held together by a secreted matrix called extracellular polymeric substances (EPS)[3,4]. They exhibit surprisingly complex multicellular behaviors that are coordinated by cell-to cell signaling networks. Biofilms may consist of cells of several or a single bacterial species interacting cooperatively[1,4]. Cells within a biofilm are physiologically heterogenous because a variety of microniches occur within the biofilm structure[1,4]. Cells on the surface of the structure may have ready access to nutrients and be actively metabolizing and dividing. More internal cells may be largely dormant. This concept of physiological heterogeneity within a biofilm is important because, unlike the relative physiological synchrony of bacteria suspended as individual cells in broth culture, this heterogeneity within biofilms results in cells with vastly different susceptibilities to antibiotic.

As a result, biofilms may be as much as 1000 times more refractory to antibiotic killing than bacterial cells suspended in broth culture. It is likely that this phenomenon underlies the often-observed disparity between in vitro minimum inhibitory concentration values and ineffectiveness of an antibiotic to eradicate a well established infection.

The risk of biofilm related infection of ocular foreign bodies and medical devices depends on location. Biofilms have been observed on contact lenses, where they are believed to contribute to the development of microbial conjunctivitis[5]. It is estimated that more than 30 000 cases of microbial conjunctivitis occur annually in the United States and 100 000 cases annually worldwide[6]. Nonsurgical trauma and contact lens use are the leading predisposing risk factors for microbial conjunctivitis. Contaminated contact lenses, cases and possibly solutions are known to be the source of infection in some instances[7]. In contrast to non–contact lens–associated microbial conjunctivitis, which is more frequently associated with gram-positive organisms, contact lens–related infections in many geographic locations are more commonly associated with gram negative bacteria (particularly *P. aeruginosa*)[5].

MATERIALS AND METHODS

Microorganisms: Study was conducted on 4 types of bacteria [three strains of *S. epidermidis*, strain of *S.aureus*, two *K.pneumoniae* strains and one *P.aeruginosa* strain], isolated from the conjunctiva of adults aged (12-75 years) were suffering from frequently bacterial conjunctivitis. (The isolated strains were identified according to reference methods). Then we conducted susceptibility tests of the disk diffusion test[8, 9] for the selection of susceptible strains and resistant strains to studied antibiotics.

Studied antibiotics: We used commercial drops of Gentamicin (GM), Tobramycin (TM), Levofloxacin (LEV), Rifamycin (RF) and Chloramphenicol (C) of many companies in Syria.

Used culture media: Nutrient Broth, Mueller Hinton Broth, Nutrient Agar, Mueller Hinton Agar, Blood agar, Chocolate agar, Mannitol Salt Agar, EMB Agar and Mackonkey Agar, all of this from (Biolab, Hungary), EMB Agar from (BioMérieux, France). In addition to contact lenses (CooperVision, USA).

Used materials in Enumeration of IOL-Adherent Bacteria method: we put 3 contact lenses in Petri dish contains 10 ml of bacterial suspension (10^5-10^6 CFU/ml) without any antibiotic and is considered as Control in this experience, then we prepare a tube containing 10 ml of the suspension itself with 100 microns of the antibiotic to become an extension 1 / 100, 1 ml taken from it to 9 ml of the suspension to become our extension 1 / 1000, by repetition of this process we get extensions 1 / 10000 and 1 / 100000, we did not note any bacterial growth by using less extensions of these antibiotics.

Evaluate the effectiveness of antibiotics in the prevention of a bacterial biofilm: We studied the effectiveness of anti-adhesion according to the Enumeration of IOL - Adherent Bacteria method[10].

RESULTS

1. *Staphylococcus epidermidis* susceptible to the studied antibiotics: We found by comparing the best concentration for each antibiotic to give maximum effect in the efficiency of anti-adhesion during the first three days of application, that the effectiveness of anti-adhesion for gentamicin is the most effective by (99.3%) with 30µg/ml, followed by Cloramphenicol (98.9%) with 40µg/ml, then levofloxacin (98.3%) with 15µg/ml, while Rifamycin (96.2%) with 10µg/ml, followed by Tobramycin by (91.9%) with 30µg/ml, and Figure (1) illustrates the effectiveness of anti-bacterial adhesion to studied antibiotics with different concentrations.

2. *Staphylococcus epidermidis* intermediate to the studied antibiotics: By comparing the best concentration for each antibiotic to give maximum effect in the efficiency of anti-adhesion during the first three days of application, we found that the effectiveness of anti-adhesion for gentamicin is the most effective by (99.4%) with 30µg/ml, followed by Cloramphenicol (99.1%) with 40µg/ml, and levofloxacin (91%) with 15µg/ml, while (84.9%) of Rifamycin with 10µg/ml followed by Tobramycin (50.4%) with 30µg/ml and Figure (2) illustrates the effectiveness of anti-bacterial adhesion to studied antibiotics with different concentrations.

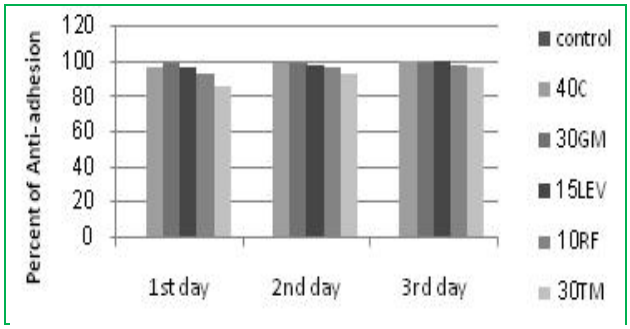

Figure 1: Shows the influence of the effective concentration of the studied antibiotics on adhesion of *S.e* 21

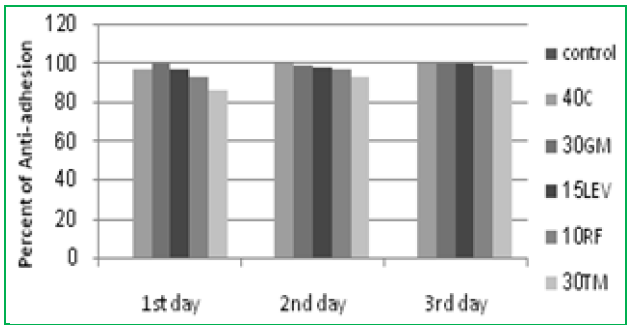

Figure 2: Shows the influence of the effective concentration of the studied antibiotics on adhesion of *S.e* 15

3. *Staphylococcus epidermidis* resistant to the studied antibiotics: The third day had a lower percentage of adhesion of each antibiotic, that the effectiveness of anti-adhesion for the most effective gentamicin was (98.36%) with 30µg/ml, followed by (61.96%) of Cloramphenicol with 40µg/ml, then levofloxacin (61.3%) with 15µg/ml, and then Rifamycin (46.8%) with 10µg/ml followed by Tobramycin (12%) with 30µg/ml and Figure (3) illustrates the effectiveness of anti-bacterial adhesion to studied antibiotics with different concentrations.

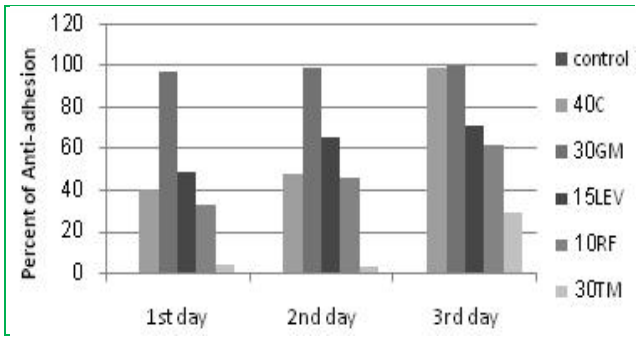

Figure 3: Shows the influence of the effective concentration of the studied antibiotics on adhesion of *S.e* 22

4. *Staphylococcus aureus* resistant to the studied antibiotics: By comparing the best concentration for each antibiotic to give maximum effect in the efficiency of anti-adhesion during the first three days of application, we found that the effectiveness of anti-adhesion for gentamicin by (99.43%) with 30µg/ml, followed by Tobramycin (96.68%) with 30µg/ml, then levofloxacin (92.52%) with 15µg/ml, and less effective was Rifamycin by (74.65%) with 10µg/ml, and finally was Cloramphenicol by (56.2%) with 40µg/ml, and Figure (4) illustrates the effectiveness of anti-bacterial adhesion to studied antibiotics with different concentrations.

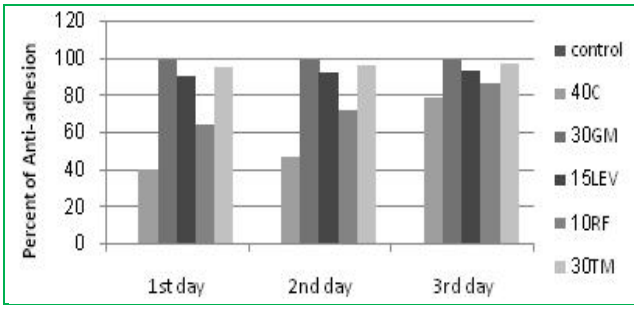

Figure 4: Shows the influence of the effective concentration of the studied antibiotics on adhesion of *S.e* 3

5. *Klebsiella pneumoniae* susceptible to the studied antibiotics: By comparing the best concentration for each antibiotic to give maximum effect in the efficiency of anti-adhesion during the first three days of application, we found that the effectiveness of anti-adhesion for levofloxacin by (100%) with 15µg/ml, followed by Tobramycin (95.6%) with 30µg/ml, then gentamicin (94.6%) with 30µg/ml, and less effective was Cloramphenicol by (94.1%) with 40µg/ml, and finally was Rifamycin by (75%) with 10µg/ml and Figure (5) illustrates the effectiveness of anti-bacterial adhesion to studied antibiotics with different concentrations.

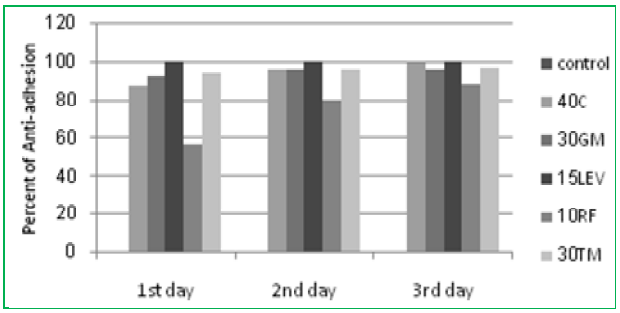

Figure 5: Shows the influence of the effective concentration of the studied antibiotics on adhesion of *K.* 10

6. *Klebsiella pneumoniae* resistant to the studied antibiotics: By comparing the best concentration for each antibiotic to give maximum effect in the efficiency of anti-adhesion during the first three days of application, we found that the effectiveness of anti-adhesion for levofloxacin is the most effective by (98.55%) with 15µg/ml, followed by gentamicin (88.7%) with 30µg/ml, then Tobramycin (85.2%) with 30µg/ml, and less effective was (83.73%) of Cloramphenicol with 40µg/ml, and finally was Rifamycin by (53.4%) with 10µg/ml and Figure (6) illustrates the effectiveness of anti-bacterial adhesion to studied antibiotics with different concentrations.

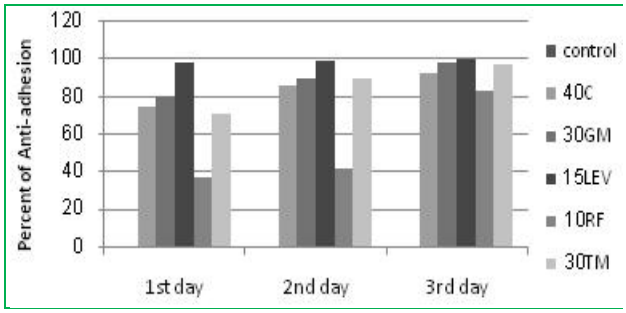

Figure 6: Shows the influence of the effective concentration of the studied antibiotics on adhesion of *K.* 9

7. pathogenic *Pseudomonas aeruginosa*: By comparing the best concentration for each antibiotic to give maximum effect in the efficiency of anti-adhesion during the first three days of application, we found that the effectiveness of anti-adhesion for Rifamycin (100%) with 10µg/ml, followed by Cloramphenicol by (99.13%) with 40µg/ml, then gentamicin by (91,63%) with 30µg/ml, and less effective was (88.3%) of Tobramycin with 30µg/ml, and finally was levofloxacin by (68.65%) with 15µg/ml and Figure (7) illustrates the effectiveness of anti-bacterial adhesion to studied antibiotics with different concentrations.

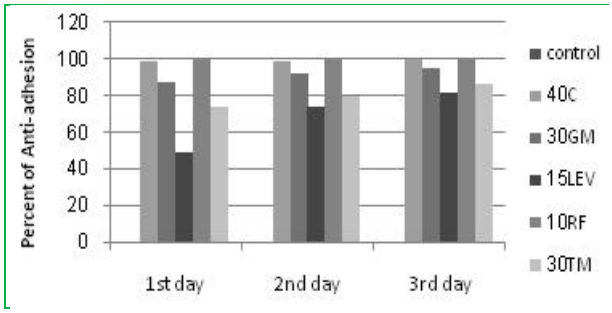

Figure 7: Shows the influence of the effective concentration of the studied antibiotics on adhesion of *P.* 1

DISCUSSION

Our study showed the ability of all studied aerobic bacteria to form bacterial biofilm in vitro and it points to the possibility of formation of a biofilm on the conjunctiva. And this result has coincided with several global studies[1, 4, 11, 12] pointed to the ability of isolated bacteria from the conjunctiva of the eye to form a biofilm in vitro (on contact lenses) and on the conjunctiva of the eye. Note that the degree of bacterial adhesion to the contact lenses depends on the nature of substrate, water content, and electrolyte concentration, polymer composition, and the type of bacterial strain, etc.[13] As for

the effectiveness of antibiotic for anti-bacterial adhesion, our results were as follows:

Staphylococcus epidermidis

When studying the adhesion of strains of *Staphylococcus epidermidis* and the effect of antibiotics used to it, we found that the effectiveness of gentamicin had a good anti-adhesion, followed by Cloramphenicol with lesser grade, then levofloxacin, Rifamycin and Tobramycin. Relatively the good effectiveness of gentamicin due to being one of the antibiotics with enhancer effect for the production of Slime and therefore bacteriocidal results, as well as good effectiveness on planktonic forms[14-16]. As for the Cloramphenicol inhibits expression of the icaADBC operon, which is essential for producing the PIA (polysaccharide intercellular adhesion) which mediated adhesion (cell-to-cell) and thus prevent the production of the biofilm for these bacteria[17]. Significantly, the impact of Tobramycin has shown that increasing bacteria susceptibility with increasing specific growth rate, where it affects newly-divided daughter cells by intervening the cycle of division, although there are some proposed components within the glycocalyx reduce the permeation of antibiotic into biofilm[18]. Previous findings confirm that the use of inadequate doses of antibiotics or higher than the therapeutic concentration is the main reason for occurrence of relapse in recurrent conjunctivitis.

Staphylococcus aureus

The results were convergent with results of *Staphylococcus epidermidis*, except Tobramycin which rank second one of effectiveness of anti-adhesion and thus aminoglycosides (Gentamicin and Tobramycin) have the best effect of anti-adhesion of these resistant bacteria. This is consistent with the study[19] where it was found that the aminoglycosides generate a proton-motive force which facilitates their uptake by these bacteria with their effect on persisters, although dormant, but they are programmed to uptake drug metabolites in addition to the central metabolism and respiration and thus reduce the rate of adhesion. Then followed by levofloxacin, and then Rifamycin, and finally came Cloramphenicol. We have noted that the used antibiotics with high concentration were effective in reducing adhesion and this seems to return to the used strain which were resistant to all antibiotics. The fact that resistance to antibiotics is a growing problem worldwide, especially ophthalmic bacterial infections by Staphylococcus aureus[20]. In general, both Staphylococcus aureus and *Staphylococcus epidermidis* are the main causes of ophthalmic infections by producing multi-saccharide biofilm to protect its colonies on the surface as one of pathogenic mechanisms. Studies have shown that the biofilm of Staphylococcus *epidermidis* strains was more virulent than negative strains biofilm[21,22]. This ability depends on production of polysaccharide intercellular adhesion (PIA) molecules, encoded by the intercellular adhesion (*ica*) locus including the *ica*A gene, *ica*B gene, *ica*C gene, *and ica*D gene[23], and high prevalence of antibiotic resistance may be due to the biofilm production by bacterial strains[24].

Klebsiella pneumonia

Our study showed the highest effectiveness of anti-adhesion of Klebsiella pneumonia only with high concentrations of levofloxacin. While each of Rifamycin and Cloramphenicol were the last, however gentamicin and Tobramycin caused lesser impact than levofloxacin even with high concentrations against adhesion. It has been shown through the studies[25-27] that the fluoroquinolones have had a better effect in inhibition and/or decrease a biofilm formed by Klebsiella pneumonia. These bacteria are hydrophobic and hydrophobic effect of aminoglycosides on the binding surface is a slight effect, although aminoglycosides decrease the forming of biofilm by Klebsiella pneumonia, in most cases, moderately increase the sensitivity to oxidative pressure and decrease the effectiveness of lipase[28].

Pseudomonas aeruginosa

It is one of the most common prevalent pathogenic bacteria on contact lenses and associated with keratitis[29]. This organism can form a biofilm on contact lenses, storage cases, and other surfaces; the biofilm helps it adheres to surfaces and shields it from chemical disinfectants[30]. Its cell surface contains small protein structures (pili) that interact with surface receptors on corneal epithelial cells. Another protein on the surface of P. aeruginosa (exoenzyme S) is believed to assist with adherence. Also, the carbohydrate constituent of the lipopolysaccharide on the surface of a P. aeruginosa cell is thought to play a role in adhesion[30]. Once P. aeruginosa attaches to the cornea, it synthesizes alginate, a polysaccharide that protects it from phagocyte attack, thus reducing the effects of the host immune response[31].

Differently from the rest of other organisms, Rifamycin showed that the effectiveness of anti-adhesion is very high whereas no bacterial adhesion using the highest concentration of it and thus became the first in terms of effectiveness of anti-adhesion, followed by gentamicin then Cloramphenicol, Tobramycin. Differently, levofloxacin has become the last, but fairly all studied antibiotics have good effect of anti-adhesion. There is convergence in terms of effectiveness of anti-adhesion between all studied antibiotics except Tobramycin and levofloxacin, we have found that Tobramycin could penetrate these bacteria biofilm, but it failed to inactivate the bacteria[32], generally, aminoglycosides are molecules for which poor penetration has been reported in biofilms of an alginate (the mucoid EPS)-producing strain of these bacteria[33]. For levofloxacin, there was increasing resistance over the years[34].

In general we noticed that all the studied antibiotics do not lead to decrease the adhesion completely for all bacteria. It gets varying decreased in the rate of adhesion, and is due to the fact that complete removal of the

bacteria within biofilm requires adoption of pharmaceutical combinations of more than one antibiotic, but it is worth noting that Rifamycin is completely reducing the adhesion of Pseudomonas aeruginosa.

CONCLUSION

Adoption of combination (Gentamicin with Levofloxacin) as an initial option in predictive treatment for recurrent bacterial conjunctivitis. And obligation to antibiotics doses is recommended by international institutions. It is also proposed to add Rifamycin to the sterile solutions for contact lenses.

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