Effect of Sofrodax, Acetic Acid and Ear Wax on Biofilm Formation on Bacterial Isolates from Otitis Media

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Abstract

In this study, one hundred otitis media (OM) swabs were collected from (100) patients who were referred to Hilla Teaching Hospital (ENT unit) and privacy clinic during a period (from November 2013 through March 2014) suffering from OM. The collected samples were investigated for bacterial isolation. Bacterial culture was positive in (96%) patient's verses (4%) patients revealed negative bacterial culture. The most common types of bacterial isolated were *S. epidermidis* (23.6%), followed by *S. aureus* (17%), *Proteus spp.* (14.1%), *Ps. aeroginosa* (9.4%), *Streptococcus spp.*, *K. pneumoniae* and *Enterobacter spp.* (7.6%), *Acinetobacterbummanii* (5.6%), *Serratiamarecence* (5.6%) and *E. coli* (0.9%).Biofilm formation was investigated in all bacterial species, and the results showed that the most isolates that form biofilm in gram negative bacteria and gram positive bacteria in high rate (100%).The effect of ear wax, vinegar and acetic acid on biofilm formation were studied the results showed that these materials have some effect on biofilm formation.

Keywords: otitis media, biofilm formation, ear wax, acetic acid.

الخلاصة:

تم في هذه الدراسة جمع وزراعة (١٠٠)عينة (مسحات اذن وسطى) من (١٠٠) مريض مصابين بالتهاب الاذن الوسطى (حسب التشخيص السريري الاولي من قبل الاستاذ المساعد الدكتور صفاء الطريحي اختصاص الانف والاذن والحنجرة) وممن احيلوا الى مستشفى الحلة التعلمي العام ومن العيادة الخاصة (من تشرين الثاني ٢٠١٣ الى اذار ٢٠١٤) كانت النتائج الزرع الموجبة ل (٩٦) مريض مقابل (٤)نتائج سالبة اتجاه الزرع. تم من خلال الدراسة عزل وتشخيص الانواع البكتيريه التي شملت انواعا بكتيريه موجبة وسالبة لصبغة غرام جاءت في مقدمتها *S. Epidermidis* حيث شكلت نسبة مئوية (٢٣.٦%)،جائت بعدها بكتريا(*S. aureus*17%)،ثم*Proteus* spp. (14%)،ثم *Ps. aeroginosa* (9.4%)، *Streptococcus* spp.، *K. pneumoniae* و(7.6%) *Enterobacter* spp.،ثم*Acinetobacterbummanii* (5.6%)، *Serratia* واخيرا عزله واحدة من بكتريا (*E. coli* 0.9%). تم دراسة تكوين الغشاء الحيوي ايضا لبعض العزلات البكتيرية وقد وجد ان جميع العزلات المنتخبة قادره على تكوين هذا الغشاء الحيوي للبكتريا السالبة والموجبة لصبغه كرام. كما تم دراسة تأثير استخدام الخل على نمو العزلات البكتيرية ووجد ان خلال تمر المحل له تأثير كبير واكثر من خلال تفاح المحلي.

Introduction

Otitis media (OM) is usually an infection and/or inflammation of the middle ear (ME), it constitutes the most common respiratory tract infection of infancy and early childhood [1].The bacterial species associated with otitis media differ according to the type of infection, *S. pneumoniae*, non typable*H. influnezae* and *M. catarrhalis* are most common bacterial of acute otitis media [2]. *Ps. aeroginosa*, *S. aureus* and *Proteus* spp. are predominant pathogen of chronic suppurative otitis media [3].Biofilm is a complex aggregation of microorganisms growing on a solid surface. Biofilm are usually found on solid substances sub merged in or exposed to some aqueous solution [4]. Biofilm on surfaces have a characteristic structure consisting of micro colonies enclosed in a hydrated matrix of microbially produced proteins, nucleic acids, and polysaccharides. In this complex biofilm network, the cells act less as individual entities and more as a

collective living system, often with channels to deliver water and nutrients to the cells at the inner portion of the biofilm. Biofilm organisms are significantly more resistant to environmental stresses or microbially deleterious substances (such as antibiotics and biocides) than planktonic cells. Biofilm cell present on infected tissues or medical devices are less susceptible to host immune responses than planktonic cell [5].The ability of biofilm give an advantages to the growth pattern, bacteria are protected from the inhibitory effects of antimicrobial compounds, biocides, chemical stresses (such as pH and oxygen), and physical stresses (like pressure, heat, and freezing). The polymeric matrix increases the binding of water and leads to decreased chance of dehydration of the bacterial cells-a stress that planktonic cell are subject to. And, close proximity of the microorganism in biofilms allows nutrients, metabolities and genetic material to be readily exchanged [6].Biofilm grown bacteria are refractory to antimicrobial agents and show an increased capacity to evade to the host immune system. Biofilm formation by *Streptococcus pneumonia* is an important human pathogen, using a variety of in vitro model systems. The bacterial cells in these biofilms are held together by an extracellular matrix composed of DNA, proteins and possibly, polysaccharides. Although neither the precise nature of these proteins nor the composition of the putative polysaccharides is clear, it is known that choline-binding proteins are required for successful biofilms formation [7].

Aims of study

These are objectives aims should be mentioned:

1. Isolation and identification of different types of bacteria from acute and chronic otitis media.
2. Study of biofilm formation and the effect of some factors on biofilm formation.

Materials and Methods

Patients:

One hundred samples were collected from patients suffering from otitis media from both sexes with age range (1.5-75 years), these patients have attending to Hilla Teaching Hospital (ENT unit) and privacy clinics during a period from November (2013) to March (2014).

Collection of specimens:

The specimens were generally collected from patients suffering from otitis media. The smears were taken from infected area by sterilized cotton swab, and then samples had been inoculated on the culture media (MacConkey, Blood and Nutrient agar medium) and incubated aerobically at 37oC for 24-48 hrs. the bacterial isolates were identified after staining with gram stain, specific biochemical tests were done to reach the final identification according to [8, 9,10].

Biofilm Production:

Tissue culture plate method (TCP) :

The TCP assay described by [11] is most widely used and was considered as a standard test for detection of biofilm formation. In the present study, all isolates were screened for their ability to form biofilm by TCP method as described [11] with a modification in duration of incubation which was extended to (24) hours according to [12].

Effect of Sofrodax, acetic acid and ear wax on biofilm formation:the same procedure described in (tissue culture plate method for detection biofilm formation) was done with modification. According to[13].

Results and Discussion:

A total of (100) otitis media swabs were subjected for culturing on different types of culture media, the results revealed that 96 samples gave positive bacterial culture, whereas (4) samples showed no bacterial growth even after 48 hours.A total of 106 bacterial isolates were obtained from the 96 samples collected. From the results, it was shown that gram positive bacteria constitutes 51.9% (54/106) from the total isolates and were considered the predominant a etiological agent of OM compared to gram negative bacteria which constitute (48.1%)(51/106). These results were shown in Figure (1).

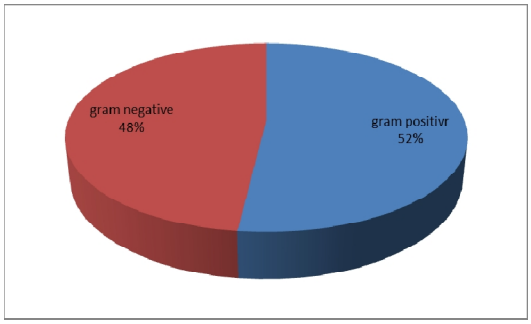

Figure (1): the percentage of gram positive and gram negative among patients with otitis media

The main bacterial types isolated in this study were shown in Table (1). *S. epidermidis* was the most common bacterial species isolated from ear infection (23.6%).*S. aureus* was the second type of bacteria isolated from chronic otitis media (17%), this frequency may be due to firstly, it may enter the middle ear from external canal as a normal flora and by reflux OM when the tympanic membrane was not intact, secondly, *S. aureus* also contain teichoic acid and lipoteichoic acid, capsular material which facilitates the adherence of these bacteria to epithelium this agree with the result mentioned by [14, 15].*Streptococcus* spp. which is considered from the micro flora of nasopharynx in healthy adults, had constituted (7.6%), as shown in Table (1), this microorganisms were reported by many researcher such as [16, 17]*Proteus* spp. was predominantly as invaders from external canal and when the resistance of middle ear is lowered .In this study Proteus spp. constitute (14.1%) as shown in Table (1), and were considered the third bacteria isolated in this study. This finding was incompatible with [16], and compatible with [18]. Who found that *Proteus* spp. represented (13.5%).*Pseudomonas aeroginosa* was the second type of gram negative bacteria isolated from chronic otitis media (9.4%). *Pseudomonas aeroginosa* is the most invaders to middle ear when the resistance is lowered.*Klebsiella pneumonia*,*Enterobacter* spp. and *E. coli* in (7.6%), (7.6%) and (0.9%) respectively as shown in Table (1), the presence of these bacteria may be due to presence of capsular polysaccharide which plays an important role in pathogenicity of *Klebsiella pneumonia* in OM infection. The finding is in agreement with [19], who found that microorganisms were representing in (6.5%, 8% and 1.2%) respectively. Other gram negative bacteria *Acinetobacter* was isolated from OM. *Acinetobacter* represented (6.60%). [20], found that microorganisms was representing in (2.77%), while [21], mentioned that *Acinetobacter* represent with (7.62%)*Serratiamarcecen* was representing in (5.66%) from total pathogens isolated from OM.

Table (1): distribution of bacterial isolated from patient with otitis media according to the isolates

Bacteria	Single isolates	Mixed isolates	Total isolate	%
S. epidermidis	**21**	**4**	**25**	**23.6**
S. aureus	**15**	**3**	**18**	**17**
***Streptococcus* spp.**	**5**	**3**	**8**	**7.6**

***Proteus* spp.**	**14**	**1**	**15**	**14.1**
Pseudomonas aeroginosa	**9**	**1**	**10**	**9.4**
Klebsiella pneumonia	**6**	**2**	**8**	**7.6**
***Enterobacter* spp.**	**7**	**1**	**8**	**7.6**
Acinetobacterbaumanii	**7**	**0**	**7**	**6.60**
Serratiamarcecen	**6**	**0**	**6**	**5.6**
E. coli	**1**	**0**	**1**	**0.9**
Total	**91**	**15**	**106**	**100%**

A total of (37) isolates from different types of bacteria were tested for their ability to produce biofilm. From these isolates, (34), (21) were from strong and moderate biofilm respectively (100%) and no isolates showed weak/non biofilm formation (0%) as shown in Table (2), (3).Six isolates of *S. aureus* (100%) and (100%) of *S. epidermidis* were formed biofilm. This approximately agree with [12] who found that *S. aureus* were formed biofilm after incubation (24) hours.Biofilms are surface-associated, sessile bacterial communities. A mature biofilm is formed when planktonic cells initially colonize a surface, aggregate and/or grow into multicellular colonies, and embed themselves in an exopolysaccharide matrix [22].

Table (2) Production of biofilm in gram negative bacteria

Bacterial isolates (No.)	**Biofilm**			
	Strong	**Moderate**	**% of biofilm formation**	**Weak**
***Proteus* spp.(6)**	**5**	**1**	**100%**	**0(0%)**
***Klebsiella pneumonia*(6)**	**5**	**1**	**100%**	**0(0%)**
***Pseudomonas aeroginosa*(6)**	**4**	**2**	**100%**	**0(0%)**
***Enterobacter*spp.(6)**	**1**	**5**	**100%**	**0(0%)**
***Serratiamarsc*(6)**	**3**	**3**	**100%**	**0(0%)**
***Acinetobacterbaumanii*(6)**	**3**	**3**	**100%**	**0(0%)**
***E. coli* (1)**	**1**	**0**	**100%**	**0(0%)**

Table (3) Production of biofilm in gram positive bacteria

Bacterial isolates (No.)	**Biofilm**			
	Strong	**Moderate**	**% of biofilm formation**	**Weak**
***S. aureus* (6)**	**4**	**2**	**100%**	**0(0%)**
***S. epidermidis* (6)**	**4**	**2**	**100%**	**0(0%)**
***Streptococcus* spp. (6)**	**4**	**2**	**100%**	**0(0%)**

Biofilm formation after adding Ciprofloxacin drop:

In gram positive bacterial, *S. aureus* bacteria, the percentage of biofilm formation change from (100%) to (50%), this result approximately agree with [23] who found the between Fluroquinolonesuse and incidence of healthcare-associated infection by resistant *S. aureus*.Both of *S. epidermidis* and *Streptococcus* spp. was investigated to biofilm formation, and it was found that the biofilm change from (100%) to (0%), (66.7%) respectively. These results showed that both of these bacteria are sensitive to this drug or antibiotic (Ciprofloxacin) as reported by [24]. The resistance of *Streptococcus* spp. to Fluroquinolones is used for ambulatory treatment of community-acquired pneumonia only after antibiotic classes have been tried and failed, or in those demonstrated drud-resistant Streptococcus spp. [25, 26].

Table (4) Effect of Ciprofloxacin drops in biofilm formation in gram positive bacteria

Bacterial isolates (No.)	Biofilm after adding Ciprofloxacin drops			
	Strong	Moderate	% of biofilm formation	Weak
***S. aureus* (6)**	**0**	**3**	**50%**	**3(50%)**
***S. epidermidis* (6)**	**0**	**0**	**0%**	**6(100%)**
***Streptococcus* spp.(6)**	**0**	**4**	**66.7%**	**2(33.3%)**

According to gram-negative bacteria, the results showed that the biofilm formation of *Proteus* spp. reaches to (0%) after using of Ciprofloxacin, while normally reach to (100%). Also, *Klebsiella pneumonia* showed the same effect.The same results presents with other gram negative bacteria after treated with Ciprofloxacin. These results agree with [27] who pointed that the Ciprofloxacin is the most frequently prescribed Fluroquinolones for different infections.*Serratia* app. is sensitive to Ciprofloxacin biofilm. These results agree with result obtained by [28].

E. coli bacteria biofilm formation (100%) change to (0%). This results in agreement with [29], who pointed that *E. coli* resistant (88.2%), to the Ciprofloxacin.

Table (5) Effect of Ciprofloxacin drops in biofilm formation in gram negative bacteria

Bacterial isolates (No.)	Biofilm after adding Ciprofloxacin drops			
	Strong	Moderate	% of biofilm formation	Weak
***Proteus* spp.(6)**	**0**	**0**	**0%**	**6(100%)**
***Klebsiella pneumonia*(6)**	**0**	**0**	**0%**	**6(100%)**
***Pseudomonas aeroginosa*(6)**	**0**	**0**	**0%**	**6(100%)**
***Enterobacter*spp.(6)**	**0**	**0**	**0%**	**6(100%)**
***Serratiamarsc*(6)**	**0**	**0**	**0%**	**6(100%)**
***Acinetobacterbaumanii*(6)**	**0**	**0**	**0%**	**6(100%)**
***E. coli* (1)**	**0**	**0**	**0%**	**1(100%)**

Effect of wax in biofilm formation:

In gram positive bacteria, *S. aureus*, *S. epidermidis*, in this study, showed that the percentage of biofilm formation change from (100%) for all bacteria to (50%), (0%) respectively, and in gram negative bacteria, *Proteus, Klebsiella pneumonia, Pseudomonas, Enterobacter, Serratia, Acinetobacter, E. coli*, the percentage of biofilm formation change from(100%) for all bacteria to (33.3%), (50%), and (0%) for another bacteria. These results refer to the effect of ear wax in the lowest biofilm formation and prevent the infection with otitis media.The function off ear wax is carries out the important task of keeping the ear canal moist and also provides some protection from bacteria, fungi, insects and water [30].

Table (6) Effect of ear wax in biofilm formation in gram positive bacteria

Bacterial isolates (No.)	Biofilm after adding ear wax			
	Strong	Moderate	% of biofilm formation	Weak
***S. aureus* (6)**	**0%**	**3**	**50%**	**3(50%)**
***S. epidermidis* (6)**	**0%**	**0**	**0%**	**6(100%)**
***Streptococcus* spp. (6)**	**0%**	**6**	**100%**	**0(0%)**

Table (7) Effect of ear wax in biofilm formation in gram negative bacteria

Bacterial isolates (No.)	Biofilm after adding ear wax			
	Strong	Moderate	% of biofilm formation	Weak
***Proteus* spp.(6)**	**2**	**0**	**33.3%**	**4(66.7%)**
***Klebsiella pneumonia*(6)**	**0**	**3**	**50%**	**3(50%)**
***Pseudomonas aeroginosa*(6)**	**0**	**0**	**0%**	**6(100%)**
***Enterobacter*spp.(6)**	**0**	**0**	**0%**	**6(100%)**
***Serratiamarsc*(6)**	**0**	**0**	**0%**	**6(100%)**
***Acinetobacterbaumanii*(6)**	**0**	**0**	**0%**	**6(100%)**
***E. coli* (1)**	**0**	**0**	**0%**	**1(100%)**

Effect of vinegar (acetic acid) in biofilm formation:

The effect of vinegar (acetic acid) on biofilm formation was studied; the results showed that using of vinegar have a great effect on biofilm formation. The biofilm formation affected by using vinegar (acetic acid).According to *S. aureus, S. epidermidis*and *Streptococcus* spp. the percentage of biofilm formation change from (100%) to (66.7%), (0%) and (83.3%) respectively.According to gram negative bacteria the results showed that the biofilm formation of *Proteus* reached to (66.7%) after using of vinegar, while normally it reaches to (100%). Other types of gram negative bacteria show that the biofilm reaches to (0%) after using vinegar.

Table (8) Effect of vinegar (acetic acid) in biofilm formation in gram positive bacteria

Bacterial isolates (No.)	Biofilm after adding vinegar			
	Strong	Moderate	% of biofilm formation	Weak
S. aureus (6)	0%	4	66.7%	2(33.3%)
S. epidermidis (6)	0%	0%	0%	6(100%)
Streptococcus spp. (6)	0%	5	83.3%	1(16.7%)

Table (9) Effect of vinegar (acetic acid) in biofilm formation in gram negative bacteria

Bacterial isolates (No.)	Biofilm after adding vinegar			
	Strong	Moderate	% of biofilm formation	Weak
Proteus spp.(6)	0%	4	66.7%	2(33.3%)
Klebsiella pneumonia(6)	0%	0	0%	6(100%)
Pseudomonas aeroginosa(6)	0%	0	0%	6(100%)
*Enterobacter*spp.(6)	0%	0	0%	6(100%)
Serratiamarsc(6)	0%	0	0%	6(100%)
Acinetobacterbaumanii(6)	0%	0	0%	6(100%)
E. coli (1)	0%	0	0%	1(100%)

Upon passive diffusion of acetic in to the bacteria, where the pH is near or above neutrality and the acid will dissociate and lower the bacteria internal pH, leading to situation that will impair or stop the growth of bacteria. On the other hand, the acetic acid that cannot escape the bacteria in its dissociated from will accumulate within the bacteria and disrupt many metabolic functions leading to osmotic pressure increase incompatible with the survival of bacteria [31].

Thus from the results expressed above, the most common causation agent of otitis media was *S. epidermidis and* The all bacterial isolates form biofilm as strong and moderate form.

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