Ability of *Staphylococcus* spp. Isolated from Meningitis Patients to Biofilm Formation

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Abstract

A total 248 clinical samples of cerebrospinal fluid (CSF) were collected from meningitis patients, for the period of July to October 2018 from the Child Protection Teaching Hospital in the Medical City Compound in Baghdad. All isolates were identification depending on macroscopic, microscopic, biochemical tests and definite with Vitek-2 compact system. The results showed a growth in 42 samples. Sixteen isolates of *Staphylococcus* spp. were obtained from samples. The number (percentage) of isolates according to species as follow: 7/16) 44%) of *S. epidermidis*, 3/16 (19%) of *S. hominis*, 3/16) 19%) of *S. haemolyticus*, 2/16 (12%) of *S. aureus*, 1/16 (6%) of *S.warneri*. These isolates were tested for ability to biofilm formation by using Luria Broth and Tryptone Soya Broth (TSB) supplemented with 0.25% and 2% glucose. The ability of the isolates to form biofilm with Luria Broth, for *S.epidermidis*, *S. aureus, S. haemolyticus*, *S.hominis* and *S. warneri* were 7/7 (100%), 1/2 (50%), 2/3 (66.66%), 1/3 (33.33%),1/1 (100%) respectively not forming biofilm.As for using a TSB supplemented with 0.25% glucose, *S.epidermidis, S. aureus, S. haemolyticus* and *S.hominis* were 6/7 (85.71%), 2/2 (100%), 2/3 (66.66%) and 3/3 (100%) respectively were weakly biofilm formed and when using TSB supplemented with 2% glucose, showed that all *Staphylococcus* spp. (100%) were positive for the test..The study showed the effect of minimum inhibition concentration(8 µg/mL) and minimum bactericidal concentrationand (16µg/mL) of Erythromycin on biofilm of *Staphylococcus* spp. *Staphylococcus* spp. isolated from CSF showed weak ability to biofilm formation and depending on the host content of sugars . This study showed also weak biofilm forming of *Staphylococcus* spp. was resistence to erythromosin.

Keyword: *Staphylococcus spp., Meningitis, Biofilm Formation.*

Introduction

Biofilm is aggregation of bacterial cells that are attached to each other biological surface or abiotic via production of extracellular substances consist of sugars, proteins and extracellular DNA (eDNA)[1], biofilm starts with adhesion, proliferation and maturation of bacterial cells due to Quorum Sensing (QS), who responsible of biofilm formation as a result of aggregate of bacteria and sending signals[2,3]. [4,5] biofilm structure composed from adhesion of bacteria on surfaces, forming colonies through accumulation of bacteria with each other to form triple dimension structure of multi layer extracellular substances. [6] pointed to biofilm formation in *Staphylococcus* due to product of slime layer as well as capsule forming, and by operon *ica* that encode to polysaccharide intercellular adhesion (PIA). Several studies pointed to biofilm formation through stage of attachment, accumulation and maturation via (PIA)[7,8]. Accumulation associated protein (Aap) and Extracellular matrix binding protein (Embp)[9,10], while stage of separation of cells by Serine protease[11], surface-located fibronectin binding protein A, B (FnBPA, B)[12], Cysteine protease (EcpA) and Metalloprotease (SepA)[13]. [14,15] showed the role of phenol souluble modulins (PSms) and Surfactant peptides that Product by *Staphylococcus*

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in biofilm decomposition. Biofilm formation includes a group of compounds: PIA is one of the main saccharides in *S.epidermidis* that called Polysaccharide intracellular adhesion or poly N-acetyl glucosamine encoded by *ica* operone (*ica* A, D, B, C)[16] .Bap homologue protein Bap/ BhP One of the most virulent factors for *S.epidermidis* has a role in the formation of the biofilm also called biofilm associated protein (Bap), and contributes to adhesion and aggregation through biofilm formation[17], bap presence only in *S.epidermidis* and *S.aureus* isolated from Cows infected with mastitis[18]. Protein Bap is an equivalent protein called Bhp that presence at some strains of *S.epidermidis* ,but it does not contribute to the formation of the biofilm[19]. The current study aimed to know the ability of *Staphylococcus* spp. isolated from CSF of meningitis patients to biofilm formation ,and the effect of type of media on its ability of biofilm formation addition to resistance of biofilm to Erythromycin.

Materials and Method

Collection of Samples

Collect 248 clinical samples of cerebrospinal fluid (CSF) from meningitis patients in children, from the Child Protection Teaching Hospital in the Medical City Complex in Baghdad. For the period of July to October 2018. All clinical samples were collected and straight cultured on Blood agar, Chocholate agar and MacConkey agar plates then incubated at 37˚C for 24 hours. All isolates were identification depending on macroscopic, microscopic, biochemical tests and definite with Vitek-2 compact.

Ability of *Staphylococcus* spp.of biofilm formation test

The ability of bacterial isolates have been tested for biofilm formation according to [17], by using micro titer plate with,Trypton soy broth supplemented with (0.25%) glucose and Trypton soy broth supplemented with (2%) glucose. The calculations were performed according to[20].

Resistence of *Staphylococcus* spp. of Erythromycin

Biofilm resistance detection of *Staphylococcus*spp isolates carried out According to [17],by using micro titer plate,25 µl of bacteria was inoculated with 175µl of Trypton soy broth(2% glucose) and incubated at 37˚C for 24h ,decanted plate and washed with distilled water then put 200 µl of Mueller-Hinton broth in the first well ,100 µl of Mueller-Hinton broth and 100 µl of MIC of Erythromycin (8) µg/mL,and the third well 100 µl of Mueller-Hinton broth and100 µl ofMBC of Erythromycin (16)µg/mL ,plates were incubated at 37˚C for 24h.Optical density was measured at 600 nm using the Elisa device.

Results and Discussion

The results of the present study varied in the ability of isolates to form the biofilm from Luria broth medium, all isolates of *S.epidermidis* were not biofilm formation 7/7 (100%), while 1/2 (50%) of *S.aureus* were not biofilm formation and 1/2 (50%) weak biofilm formation. *S.haemolyticus* 2/3 (66.67%) were not biofilm formation and 3/1 (33.33%) weak biofilm formation. While *S.hominis* was 3/1 (33.33%) not biofilm forming and 2/3 (66.67%) weak biofilm forming.*S.warneri* was 1/1 (100%) not biofilm forming (Table 1). While the result of current study on biofilm formation by using tryptone soy broth with 0.25% glucose was 1/7 (14.29%) of *S.epidermidis* not forming biofilm and 6/7 (85.71%) weak biofilm forming. *S.aureus* and *S.hominis* were 2/2 (100%), 3/3 (100%) weak biofilm forming respectively. *S.haemolyticus* was 1/3 (33.33%) not biofilm forming and 2/3 (66.67%) weak biofilm forming. *S.warneri* 1/1(100%) was not biofilm forming (Table 2). The results showed the ability of bacterial isolates with Luria broth were 12/16 (75%) not biofilm forming and 4/16 (25%) weak ability of biofilm formation. *S.epidermidis* was 3/16 (18.75%) unable to biofilm formation and 13/16 (18.75%) weak ability of biofilm formation in TSB supplemented with (0.25%) glucose. All isolates (100%) of *S.epidermidis* had weak ability of biofilm formation in TSB (2%) glucose (Table 3). Felipe *et al.* (2017)[21] pointed to the ratio of *S.aureus* ability to biofilm formation 35% was strong and 45% moderate while CoNS bacteria 51%, 29% strong and moderate respectively. [22] the ability of *Staphylococcus* spp. to biofilm formation were 5.6%, 24.3%, 70.1%, strong ,moderate and weak or un able to biofilm formation respectively, at the same study using TSB was 2.5% of *S.aureus* and 7.9% of CoNS showed strong ability to biofilm formation respectively, while increased in TSB supplemented with (1%) glucose was 19% for *S.aureus*

and 16.4% for CoNS bacteria. [6] showed that the gene icaA had role in *Staphylococcus* pathogenesis that isolated of clinical samples led to forming Slime substance that forming biofilm on medical surfaces. [23] pointed out that the difference in the formation of the biofilm is due to bacterial strain type,operon *ica*ADBC and expression of enzymes encoding for intercellular adhesion sugars that depend on environmental conditions. Studies have shown a relationship between a gene *ica*A and biofilm formation of clinical isolates of *Staphylococcus*[24,25]. [26]a hundred percent of *S.aureus* possess *ica* gene and unable to form biofilm , that due to *Staphylococcus* has the ability to adapt to conditions and the ability to change the phenotypic and genetic characteristics when exposed to concentrations of antibiotics. [27] the ability of *S.epidermidis* to biofilm formation due to possess *S.epidermidis* Surface protein C (SesC), while[28] referred to the ability of *S.epidermidis* to biofilm formatin due to possess *ica*A gene, its rise in the isolates that it possesses *S.epidermidis* surface protein I (SesI) that consider it's virulence factor, and refers to studies have shown *S. epidermidis* isolated from healthy people no possession (SesI) making it unable to biofilm formation, and there are no deference of the ability of *S.epidermidis* to biofilm formation when growing it on TSB and TSB supplemented with (1%) glucose. *Staphylococcus* spp. isolated from CSF showed weak ability to biofilm formation and depending on the host content of sugars

Biofilm forming *Staphylococcus* spp. resistance to Erythromycin

The effect of MIC of (8) µg/ml Erythromycin against biofilm forming *Staphylococcus* spp. In current study, the results showed that Erythromycin had inhibition effectiveness against biofilm formation, that refer to the biofilm was sensitive to the antibiotic in proportions that differ according to the bacterial species and strain, may be due to the biofilm formation was weak (Figure 1). The MBC was at concentration (16) µg/ml for Erythromycin showed increase at inhibition rate due to high concentration of Erythromycin[17], as well as a weak biofilm formation by *Staphylococcus* spp. the results also show that the rate of MIC and MBC, It did not reach the minimum limits and this is due to the ability of the biofilm to protect the bacterial of antibiotics figure (3). The results showed that the inhibition rates (depending on absorbance) differ according to species and strains, and this may be due to their resistance to antibiotics [29], and possess or do not possess the genes responsible for the composition of the biofilm formation, as well as surface proteins possessed by *Staphylococcus* spp. which are responsible to biofilm formation. This study showed weak biofilm forming of *Staphylococcus* was resistence to erythromosin. We conclude from the present study the ability of biofilm formation of Staphylococcus spp. isolated from CSF of meningitis patients, is weakly by using a medium containing 2% glucose,As well as weak biofilm formation was resistence to erythromycin when using MIC and MBC concentrations.

Abbreviations:

Se= *S.epidermidis*, Sa= *S.aureus*, Sh= *S.hominis*, Sha= *S.haemolyticus*, Sw= *S.warneri*, MIC= Minimum inhibition concentration, MBC= Minimumbactericidal concentration.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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