Lipoteichoic Acid as Antibiofilm against *Staphylococcus aureus*

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Abstract

Background: It is a polysaccharide substance produced by a group of microorganisms that helps it adhere to living and non-living surfaces and it is responsible for many diseases, as it has a role in staphylococcal diseases such as pneumonia, urinary tract infection and endocarditis. Although lipoteichoic acid is a component of biofilm. However, it has a role in preventing biofilm formation

Materials and Methods: The methods of this study included isolated and identification of bacteria from UTI samples and identified by morphology characters and biochemical test to identify *S.aureus*. Then discover their ability to formbiofilm and Use lipoteichoic acid in different concentration as antibiofilm

Results: The results of identification of bacterial isolatesshowed the colonies grown on blood agar causing the typical B hemolytic state, these samples not grown on MacConkey agar and under the microscope showed gram positive cocci, a cluster like grape, these samples were identified as *Staphylococcus* when grown on mannitol medium converted it to yellowish color and appeared round, smooth, raised, mucous and gelatinous. The *staphylococcusaureus* formed biofilms with different concentrations, where the fourth isolate was the lowest percentage (0.065), while the second and fifth isolates were given (0.120 and 0.153) and the first isolation was given (0.220),however the highest percentage was given by the third isolation (0.357).So this study showed that the concentrations of LTA (200) µg/mlare MIC.The results of this work showed that after 24 hours of treatment with LTA it impeded the formation of biofilms of *Staphylococcus aureus* and the result after treatment was the concentration 50 and 100µg/ml gave less percentage of inhibition, while 500µg/ml appeared 100% of inhibition, so (200,300 and 400 gave 61%, 80% and90% inhibition to biofilm respectively.

Keyword: *Biofilm, MIC, gram positive and UTI*

Introduction

Staphylococcus aureus is a gram-positive bacterium that has the ability to adapt to living in various non-living environments. This bacterium is characterized by being an important factor in causing a variedcollection of clinical infections bacteremia and infectious endocarditis, osteoarticular, skin and soft tissue, pleuropulmonary, and device-related infections[1]. These bacteria are distinguished by their ability to attach to non-living materials by producing a polysaccharide matrix, which is a virulence factor that gives bacteria the ability to resist antibiotics and the immune systemin hospital; this leads to the emergence of antibiotic resistance[2]. This leads to chronic and destructive infections, as these membranes allow them to adhere to tissues such as heart valves and bones, and cause infective endocarditis and osteomyelitis, or on implanted medical devices such as catheters, artificial joints, artificial heart valves, and bone implants in hospitalized patients in the healthcare environment[3].The first step is to create a biofilm at the place where the cells adhere to surfaces and reinforce

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several mechanisms of irreversible adhesion[4].

Lipoteichoic acids (LTA),are connected with the membraneof the cell through a glycolipid anchorin a Gram-positive bacterium such as *S.aureus*, enterococci, *Listeriamonocytogenes*, *Streptococcus pneumoniae*, and *Bacillus subtilis* .LTA is an anionic 1,3-glycerolphosphate holding polymer attached to the cell wall[5].

It has an important role in bacterial growth, cell wall working, membrane stability, and virulence[6]. Anti-biofilmcauses were developed as replacements to antibiotics to protect against infection associated with biofilms to prevent these membranes from developing without bacterial resistance.Bacteria-derived amphiphilic have been largely studied for their anti-biofilm properties[7].

Materials and Methods

Collecting and diagnosing specimens

50 specimens of bacteria were collected from women suffering from urinary tract infection andwho did not take any antibiotics, in Al-Sadr Hospital in AL-Najaf Governorate. samples were cultured in pre-prepared media including (MacConkey agar, blood agar, and mannitol salt agar) thenincubated at 37 c° for 24 h. the bacterium was diagnosed based on the phenotypic properties using biochemical tests,where was staphylococcus aureus diagnosed by gram staining, catalase after growing on mannitol salt agar medium, coagulase test[8], in order to check the diagnosis.

Preparation of lipoteichoic acid

It was the ready prepared solution (according to Sigma Aldrich, Germany) and supplied in a glass vial (5 mg/ml) provided with water (1ml) as a stock solution. The stock solution was diluted by adding distilled water inproportion to100 µl of lipoteichoic acid: 900 µl D.W.

Minimum inhibitory concentration (mic) oflipoteichoic acid

The minimum inhibitory concentration (MIC) of lipoteichoic acid was definedas the microdilution broth method. Briefly, serial concentrations of lipoteichoic acid (50,100,200,300,400 and 500 µg/ml) were set with tryptic soy broth. The lowest concentration that inhibits the growth of bacteria is considered as the MIC[9].

Biofilm formation

The biofilm assay definedby[10], with some alterations: 10 ml of trypticase soy broth (TSB) with 1% glucose was inoculated with a loopful of test bacterium from an overnight culture on nutrient agar. The flat lowest tissue culture plates (96 wells) were filled with 200µl of diluted cultures separately. uninoculated sterile broth assisted as blank. The control bacteria were also diluted and incubated. The culture plates were incubated at 37˚C for 24 hours. After incubation, kind tapping of the plates was finished. The bores were washed with 200 µl of Normal saline four times to eliminate free-floating bacteria. Biofilms that remained adherent to the walls and the bottoms of the wells stained with 0.1% crystal violet for 10 min. another stain was washed with Normal saline and plates were dried properly then adding 200 µl of the destaining solution (95% ethanol) for 10 min. lastly, 200µl from each well was moved to a new microtiter plate and measured at 570 nm by a microplate reader. The biofilm degree was calculated as follows: Biofilm degree=Mean OD570of tested bacteria- Mean OD570 of control.

Effects of lipoteichoic acid on biofilm formation

The method described by[11]wasaccepted to investigate the effect of LPT on biofilm formation. An overnight bacterial culture (in trypton soya broth) was attunedwith McFarland standard No. 0.5. Tryptone soya broth containing MIC of LPT was inoculated with a before prepared bacterial suspension and incubated for 24 hours at 37°C. An amount of 200 µl of the culture was transferred in triplicate into the vertical rows of a polystyrene microtiter plate well for each isolate and served as control. A volume of 200 µl of culture containing the MIC concentration of the LPT was transferred into another three wells. All plates were incubated at a temperature of 37°C for 24 hours. Subsequently, the

biofilm formation protocol was followed as mentioned earlier. Percentage of biofilm inhibition was calculated following the equation: percentage of inhibition of biofilm formation = 1 – (O.D of treatment / O.D of control) × 100

Results and Discussion

Collecting and diagnosing specimens

50 samples were collected from women suffering from urinary tract infection and the results of the bacterial culture showed 5 samples grown on blood agar causing the typical B hemolytic state, these samples not grown on MacConkey agar and under the microscope showed gram positive cocci, a cluster like grape, these samples were identified as Staphylococcus when grown on mannitol medium converted it to yellowish color and appeared round, smooth, raised, mucous and gelatinous[8]. The results of the current study showed that all mannitol fermenters 5(10%) were coagulant and catalase test positive, and therefore they were considered *S. aureus*.

Biofilm formation

In this study, the result was that the five isolates of staphylococcusaureus formed biofilms with different concentrations, where the fourth isolate was the lowest percentage (0.065), while the second and fifth isolates were given (0.120 and 0.153) and the first isolation was given (0.220),however, the highest percentage was given by the third isolation (0.357) which was used in the subsequent experiments,(table -1).

Table-1) show the percentage of the biofilm formed by *S. aureus*

No of isolate	Biofilm formation
1	0.220
2	0.153
3	0.357
4	0.065
5	0.120

3.3 minimum inhibitory concentration (mic) oflipoteichoic acid

The result of this study showed that the concentrations (500) µg/ml were lethal to *Staph aureus*, while (200) µg/ml were inhibitor and the concentrations (50,100 µg/ml) gavewere not effective, (table-2).

(Table-2)minimum inhibitory concentration (mic) of lipoteichoic acid

Con of lipoteichoic acid	MIC
50	0%
100	0%
200	20%
300	40%
400	70%
500	100%

3.4. Effects of lipoteichoic acid on biofilm formation

The results of this work showed that after 24 hours of treatment with LTA it impeded the formation of biofilms of *Staphylococcus aureus* and the result after treatment was the concentration50 and 100µg/ml gave less percentage of inhibition, while 500µg/ml appeared 100% of inhibition, so (200,300 and 400 gave 61%, 80% and90% inhibition to biofilm respectively, (table-3)

(Table-3) Explain the influence of LTA on biofilm formation

Lipoteichoic acid concentration	Biofilm inhibition %
50	4%
100	7%
200	61%
300	80%
400	90%
500	100%

[12] found that the total 355 urine samples of UTI suspected patients were screened 119 (33.52%) sample showed grow out of which *Staphylococcus aureus*, while[13] found the *Staph aureus*(1.9%) from UTI.[14] found the effect of Lactobacillus plantarum lipoteichoic acid on developed biofilm, *E. faecalis* was grownup on glass-bottom plate's treatment with Lp.LTA for 24 h. The 3-week-old *E. faecalis* biofilm was condensed by Lp.LTA in a dose-dependent manner. In addition, [7] appeared that *Lactobacillus plantarum* LTA could inhibit *S. aureus* biofilm development.

*S.aureus*is a bacterium that causes many inflammatory infections and biofilm development is closely related to antibiotic resistance and adhesion to surfaces. The inhibitory influence on the development of *Staph aureus* biofilm is a communal characteristic of LTA of gram-positive bacteria including *S. aureus, S. pneumoniae, S. gordonii, E. faecalis*, and *B. subtilis*[7]. D-Alanine moieties in the LTA play an important role in inhibiting biofilm development, and many explanations have been found for this inhibition. First, the presence of the positive charge resulting from the presence of de-alanine has an inhibitory role as the positive charge prevents biofilms from forming. For example, an increase in the degree of N-deacetylation causing an increased positive charge[15]. Second, D-amino acids interfere with the formation of biofilms as they break down biofilms. For example, *S. aureus* biofilm development was inhibited by D-phenylalanine, D-proline, and D-tyrosine[16]. The inhibitory influence of LTA on *S. aureus* biofilm development is not only due to competition with bacterial cell membrane-anchored LTA, but is also due to bacterial sensing and signaling in response to LTA because LTA could finish the pre-formed biofilm[17].

Conclusion

The aim of this study was to investigate antibacterial activities and disruption of biofilm structure by Lipoteichoic acid. Staph aureus was chosen because its ability to form biofilm on surfaces makes the cells impervious to therapeutic concentrations.

Conflict of Interest: Nill

Source of Funding: Self

Ethical Clearance: Taken from Hospital Ethical Committee at Medical, Collage, Kufa

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