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RESEARCH ARTICLE

Ability of *Staphylococcus spp*. Isolated from Meningitis Patients to Adhesion

Abbas Hameed Al-Wandawy[1], Luma Abdulhady Zwain[2*]

1. *Directorate of Education of Kirkuk/ Ministry of Education/Iraq.*
2. *Department of Biology /Collage of Education Pure Science Ibn Al-Haitham/University of Baghdad/Iraq.*

***Corresponding Author: Luma Abdulhady Zwain**

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 of the 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. The ability of isolates on adhesion were studied and the results showed the use polystyrene plates, that the number of adherent cells of S. epidermidis was 230 cells / field, S. hominis was 62 cells / fields, whereas S. aureus was 3 cells / field, while in S. haemolyticus >300 cells / field, finally the number of cells in each field of S.warneri reached 40 cells / field. In use epithelial cells (Vero cell line), the results of our current study we observed that, the number of all Staphylococcus spp. in zero dilution were >300 cfu. and S. epidermidis, S .hominis, S. aureus, S. haemolyticus , S. warneri were (85,120,>300 , ,>300 ,>300)cfu respectively at 1:10 dilution, while at the dilution 1:100 the number were(9,30, >300, >300 ,292)cfu respectively.

Keywords: *Staphylococcus spp., Meningitis, Adhesion.*

Introduction

Staphylococcus spp. are spherical, single, pairs or short chains of 3-4 cells and are irregular clusters in the form of grape clusters, (1.5-0.5) µm in diameter, it is Gram positive, nonmotile, capsule-forming or limited formation, most staphylococcus strains grow with 10% NaCl and temperature 18-40 ° C, catalase positive and oxidase negative [1]. It exist naturally and mainly on the skin, glands and mucous membranes of warm-blooded animals, It can isolated from various sources including animal products (eg. meat, milk, cheese), from various environmental sources (soil, sand, dust, air, natural water, as well as clothing and stuff) [2].

Some types of *Staphylococcus* spp. as *S.aureus* are opportunistic caused by diseases such as boils, stomatosis, toxic lipolysis, pneumonia, osteomyelitis, inflammation of the heart, inflammation of the intestine, mastitis, cystitis, prostatitis, cervical inflammation, Meningitis, bacteremia, as well as internal toxins that cause food poisoning [3, 4]. *Staphylococcus* spp. have many of the structures that produces virulence factors including, Teichoic acid (TA) is found on the surface of the Gram positive bacteria [5], that essential in the adherence, colonization and inflammation [6, 7] and has role in adhesion to fibronectin [8]. Intracellular matrix- binding protein (Embp) is a giant protein [9], which are also involved in adhesion and biofilm formation [10], as well as possession of protein A [11]. Protein A is an important factor that protects bacteria from the immune system [12], and has roles in adhesion [13] and soft tissue [14].

There are many species of bacteria *Staphylococcus* spp.Including *Staphylococcus aureus*, which possesses many virulence factors, including microbial surface

component recognizing adhesive matrix molecules (MSCRAMMs) which acts on adhesion to cellular exogenous materials such as fibronectin, fibrinogen and collagen [11]. As well as having, an enzyme hyaluronidase and Clumping factor [15].

Meningitis is known as a disease that Infect the central nervous system (CNS), it is mainly caused by bacteria, mycobacteria, fungi, viruses and parasites that cause disease and death, bacterial infections constitute the highest percentage of the other species, with 56.6% and viruses 30.2% [16]. Robertson *et al* [17]. Recorded infect 2,907,146 people a year with meningitis all over the world. Current study aims to isolation *Staphylococcus* spp. from meningitis patients and their ability to adhesion.

Materials and Methods

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.

Adhesion Ability

Adhesion to Polystyrene petri dishes the procedures used for study adhesion depended on the modified [18], The *Staphylococcus* spp. was inoculated in TSB medium supplemented with 0.25% glucose and incubated at 37 ° C for 24 hours. Place 10 mL of the inculated medium in Polysterne petri dishes and incubate at 37 ° C for 1 hour, media were removed and then washed at least 3 times with phosphate-buffered saline (PBS), cells fixed with methanol and stained with Gram stain, bacterial cells were adherent observed by oil immersion 1000X and counted.

Adhesion to Epithelial Cells

Followed the method cited by Cucarella [19], modified and adopted by the Iraqi Center for Cancer Research and Medical Genetics**.** Transfer 200 µl of Vero cell line to tissue culture micro titer titer plate 96 wells.

Incubate at 37 ° C for 24 hours until the cells are attached to the wells, then remove the medium and add 200 µl of (RPMI-1640 without antibiotic) with *Staphylococcus* spp. (18×10^8 cfu) and incubated at 37 ° C for 2 hours, and media removed ,the cell monolayer were washed at least 3 times with PBS, add 200 µl of trypsin, Then a series of dilutions (0, 1:10 ,1:100) was performed and plated on blood agar was incubated at 37 ° C for 24 hours and according to the number of colonies per dish.

Results

All isolates were identified depending on the macroscopic, microscopic characteristics and confirm biochemical test. Identification of all *Staphylococcus* spp. isolates were definite with Vitek-2, the results showed a growth in 42 samples, 16(38%) isolates of *Staphylococcus* spp. were obtained from patients with meningitis.

The number of the 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.warner*i. The ability of these isolates on adhesion were studied and the results showed, in two ways, the first polystyrene plates, The result was obtained after examining the dishes under the microscope on the X1000, The number of cells adhering to each isolate on polystyrene was shown in Table (1).

Table 1: Number of *Staphylococcus* spp. attached to Polystyrene plates

Bacterial species	Average number of cells /field
S. epidermidis	*230*
S .hominis	*62*
S. aureus	*3*
S. haemolyticus	*300>*
S. warneri	40

The results of the study showed that the number of adherent cells of *S. epidermidis* was 230 cells / field, *S. hominis* was 62 cells / fields, whereas *S. aureus* was 3 cells / field, While in *S. haemolyticus* more than 300 cells / field, Finally, the number of cells in each field of *S.warneri* reached 40 cells / field. Another ways epithelial cells (Vero cell line)

Table 2: Number of *Staphylococcus* spp. attached by the Vero cell line

Bacterial Species	Dilutions		
	0	1:10	1:100
S. epidermidis	>300	85	9
S .hominis	>300	120	30
S. aureus	>300	>300	>300
S. haemolyticus	>300	>300	>300
S. warneri	>300	>300	292

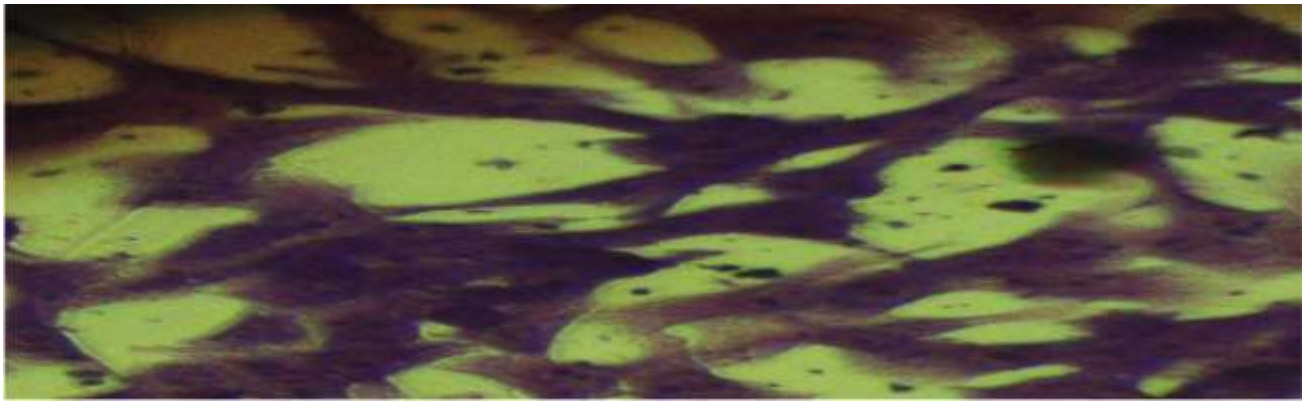

Figure 1: Adhesion of *Staphylococcus* spp. On epithelial cells

The results of our current study we observed that, the number of all *Staphylococcus* spp. in zero dilution were >300 cfu. And *S. epidermidis, S .hominis*, *S. aureus*, *S. haemolyticus*, *S. warneri*, were (85, 120, >300, >300, >300) cfu respectively at 1:10 dilution. While at the dilution 1:100, the number were (9, 30, >300, >300, 292) cfu respectively (Table 2) (Figure 1). Brescó *et al* [20]. And Ghasemian *et al* [21] pointed to the ability of *S.aureus* and *S. epidermidis* adhesion to fibrinogen and fibronectin, its multiple proteins covering the host tissue. MSCRAMMS mediate *Staphylococcal* adherence to component of the matrix of the host, while Otto [22] suggests that adhesion is attributable to PIA. Cucarella *et al* [18].

Attributed the ability of *S. aureus* to adhesion to host, due to possess bap gene its synthesis of the protein contributing to the formation of the biofilm associated protein, which is responsible for adhesion and the formation of the biofilm .Hussain *et al* [8].Found the role of Teichoic acid in the adhesion *of S. epidermidis* to host surfaces coated with fibronectin. As Otto [22] suggests that bacterial adhesion to surfaces can be reduced by altering the surface layer.

Qi *et al* [23] indicated that *S. epidermidis* can bind to host surface is attributable to *S. epidermidis* surface protein 1 (SesI). While Becker *et al* [24].And Büttner *et al* [25].The ability of *S. epidermidis* to adhesion to host, attributable to their possession serine - aspartate, repeat protein (SdrG), AtIE, Ebps and Embp. Cucarella *et al* [26].Pointed out that the bacterial isolates that possessed the bap gene did not show their adhesion to the host surface compared with the bacterial isolates that did not possess the gene, this is not attributable to the low level of gene expression of MSCRAMM proteins that play a large role in the adhesion of bacteria to the host surface,

But the presence of Bap proteins that interfere with MSCRAMM and inhibit the adhesion process as well as decrease the production of polysaccharides important external adhesion process, *Staphylococcus* spp. The adhesion varies depending on the isolates themselves and the different species As well as the difference depending on the nature of the surface used for the study and this may be due to several factors due to the possession of the same cell for different types of proteins that help the adhesion.

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