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Biofilm Formation by *Protues Mirabilis* Isolated from Patients Suffering from Urinary Tract Infections in Baqubah city

Shaymaa Majeed AL–Dahlaki

College of Science, University of Diyala, Iraq.

Shymamm226@gmail.com

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Abstract

Protues mirabilis has the ability to induce urinary tract infections primarily has to do with the formation of biofilms on catheter and UTI surfaces. This bacteria expresses a number of virulence factors essential to the creation of biofilms. Fifty urine specimens were taken from patients who were hospitalized and under treatment from the clinical laboratories of Baquba Teaching Hospital. Of those fifty specimens, fifteen clinical specimens were positive for isolates of *Protues mirabilis*. The results showed that all the *P. mirabilis* isolates under study were 100% resistant to the Gentamicin, Cefotaxime and the resistance of the isolates to Vancomysin and Azithromycin were 90%, Cefixime, Augmentin by 80%, and their resistance to Piperacillin-tazobactam by 70%. According to these percentages, the isolates were divided into two classes Extensively Drug Resistance (XDR) (90%), which were resistant to the seven antibiotics that were used in the study, and (10%) were Multi Drug Resistance (MDR) which were resistant to four antibiotic. Results showed that the *P. mirabilis* have the ability to produce biofilm, all isolates could produce biofilm but in deferent percentage 70% were strong ,30% were moderate, and the absorbency value was ranged from (0.353 – 0.159). The convention PCR analysis revealed that the gene (*Esp*), encoding the enzymes that cause Proteus mirabilis to form biofilm, accounted for 15 (40%) of the total.

Key words: Biofilm, Vancomysin, Absorbency value, Resistance.

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تكوين الأغشية الحيوية بواسطة بروتوس ميرابيليس المعزولة من مرضى يعانون من التهابات المسالك البولية في مدينة بعقوبة

شيماء مجيد الدهلكي

قسم العلوم الحياة – كلية العلوم – جامعة ديالى

الخلاصة

تمتلك بكتيريا *Proteus mirabilis* القدرة على إحداث التهابات المسالك البولية وذلك في المقام الأول من خلال تكوين الأغشية الحيوية على أسطح القسطرة والتهابات المسالك البولية. تعبر هذه البكتيريا عن عدد من عوامل الضراوة الأساسية لتكوين الأغشية الحيوية.تم جمع خمسين عينة بول من المرضى ، ومن بين تلك العينات ، أعطت 15 عينات سريرية نتيجة إيجابية لعزلات Protues mirabilis. أظهرت النتائج أن جميع عزلات P. Mirabilis قيد الدراسة كانت مقاومة بنسبة 100٪لل Gentamicinn و Cefotaxime، وكانت مقاومة العزلات لل Vancomysin و Azithromycin 90٪ ،و لل Cefixime و Augmentin بنسبة 80٪ ، ومقاومتها لعقار Piperacillin-tazobactam بنسبة 70٪. وبحسب هذه النسب فقد قسمت العزلات إلى فئتين90 ٪ (XDR) ، والتي كانت مقاومة للمضادات الحيوية السبعة التي استخدمت في الدراسة ، أما العزلات الباقية 10٪(MDR) والتي كانت مقاومة للمضادات الحيوية السبعة. مقاومة لأربعة مضادات حيوية. أظهرت النتائج أن P. mirabilis لديها القدرة على إنتاج غشاء حيوي ، ويمكن أن تنتج جميع العزلات غشاء حيوي ولكن بنسبة إرجاء 70٪ كانت قوية ، 30٪ كانت معتدلة ، وتراوحت قيمة الامتصاص من (0.353 - 0.159).اظهرت النتائج أن الجين (Esp) الذي يشفر الإنزيمات المسؤولة عن تكوين الأغشية الحيوية في Proteus mirabilis باستخدام تقنية PCR يمثل 15(40%).

الكلمات المفتاحية : غشاء حيوي، فانكومايسين ، قيمة الامتصاصية ، مقاومة.

Introduction

Urinary tract infections (UTIs) are the second greatest cause of infectious diseases, affecting more than 150 million individuals globally. When a pathogen enters the urinary tract and develops to 105 colonies per milliliter of urine, it causes UTIs[1]. However *Proteus* species are the third most common cause behind UTIs [2].The catheter associated urinary tract infections (CAUTIs) with use are frequently caused by *Proteus mirabilis*, this bacteria's ability to induce these infections primarily has to do with the formation of biofilms on catheter and UTI surfaces. *P. mirabilis* expresses a number of virulence factors essential to the creation of biofilms. Adhesion proteins, quorum sensing molecules, lipopolysaccharides, efflux pumps, and urease enzymes are a few examples of some of these variables. Urine retention and ascending UTIs

usually appear together with catheter encrustation and obstruction [3]. Because of their ureolytic biomineralization, *P. mirabilis* biofilms that build on catheter surfaces have a unique quality; these are crystalline. In recent years, *Protues mirabilis* has shown a growth in resistance to several antibiotics[4].In addition to enhancing antimicrobial treatments, antibiotic resistance has led to worse prognoses and an increase in hospitalized patient mortality [5].The objective of this work was to determine the potentially pathogenic of *P. mirabilis* virulence factors. To fulfill this goal, the following processes were used in this research:

Material and Methods

A-Collection

A total of fifty (50) clinical specimens of both genders and ages were taken from patients for the period from early September 2021 to early January 2022.

B-Separation and identification of *Proteus mirabilis* isolates

The clinical specimens were cultured in blood agar and mackongy agar media for 24h under temperature 37ºC. Gram-stain was used to identify the isolates.

C- Vitek

The Vitek 2-GN system was used to validate the isolates' identity based on their pattern of biochemical profiles [6].

Testing for antibiotic susceptibility:

An antibiotic test in line with Weinstein [7] has been carried out on every isolate to identify the potential resistance of *P. mirabilis* isolates against seven antibiotics belonging to distinct classes.The following antibiotics were administered: Cefotaxime (30 µg), Augmentin (30 µg), Vancomysin (30 µg), Cefixime (5 µg), and Ceftriaxone (30 µg).

Biofilm formation (Quantitative Biofilm Production Assay):

Overnight bacterial culture, 20 µl was used to inoculate wells of flat-bottom microtiter, containing 180 µl of Brain Heart infusion broth with 2% sucrose. Control wells contained only 200µl of Brain heart infusion broth with the 2% sucrose (0.5 gm sucrose for each 25ml of brain heart broth). The microtiter plate was covered and sealed with Parafilm for a 24-hour incubation period at 37ºC. Three rounds washings for the wells were done using of normal saline pH = 7.2

to remove the detached bacterial cells. After 15 minutes of room temperature drying, 200µl of 0.1% crystal violet solution was added to each well and left for another 15 minutes.

After getting a crystal violet solution, each well was washed three times with distal water to remove the unbounded dye before it was allowed to dry at ambient temperature. Extract with 200µl of 95% ethanol. Using an ELISA reader, each well's absorbance was calculated at 630 nm [8] **.** Results were categorized based on absorbance. Optical Density (OD) and Optical Density Control (ODc) into 3 categories: strong "2 x ODc < OD", moderate "ODc < OD ≤ 2 x ODc" and weak OD" ≤ "OD" [9].

DNA Extraction and polymerase chain reaction (PCR) amplification

Following the manufacturer's instructions, genomic DNA was recovered from bacterial growth using the kite (ABIO pure) extraction and purification process. All 15 genomic extracts of isolates were screened by conventional polymerase chain reaction using a specific primer to *Esp* gene with F\ TTGCTAATGCTAGTCCACGACC and R\ GCGTCAACACTTGCATTGCCGAA and the annealing 61, product length 955bp. The PCR reaction tubes were put into a heat cycler and set up with the following settings: 5 mints of initial denaturation at 95°C (30 sec. at 940C, 30 sec. at 55°C, and 30 sec. at 72°C), followed by 5 mints of final extension at 72°C. Agarose gel electrophoresis was used to identify the amplified PCR products.

Results and Discussion

Isolation of *Proteus mirabilis*

The present study's findings demonstrated that a total of 15(30%) isolates of *Proteus mirabilis* were obtained from (50) clinical urine specimens including urinary tract infections from both male and female of different ages and from, diverse local regions, 35(70%) from 50 specimens had been given positive growth while 15 specimens showed no growth . *P. mirabilis* is easily isolated in typical cultures but it is relatively nonreactive in many biochemical tests that are usually used to distinguish among gram-negative bacteria [10].

Results of antibiotic susceptibility test:

All isolates of *P. mirabilis* were tested for antibiotic sensitivity tests according to the disc diffusion test against 7 different antibiotics previously mentioned The results of this study are

very similar to what was mentioned by Allawi and Motaweq [12], where the percentage of resistance to Azithromycin and Tetracycline were 97.5%, and it disagree with the results of antibiotic resistance Cefoxitin and Gentamicin 7.5% to each. The percentage of resistance to the antibiotics used in Fig**. 1.**

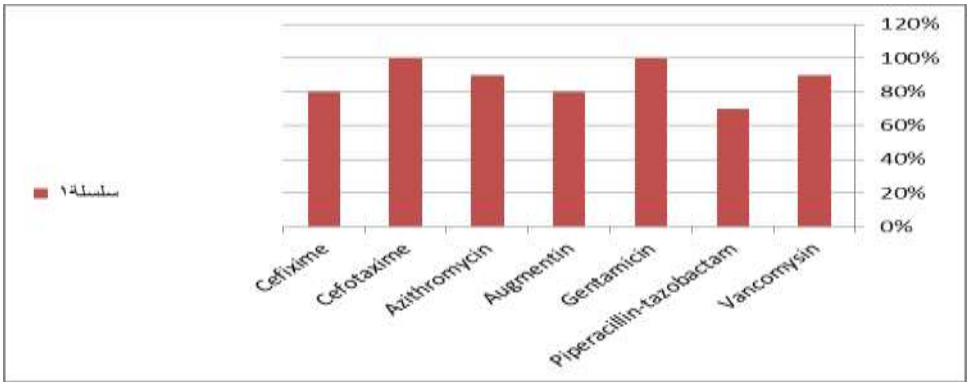

Figure 1: the antibiotic resistance to seven different antibiotics

Biofilm formation in *P. mirabilis*:

All isolates included in this study could produce biofilm but in deferent percentage 70% were strong, while30% were moderate, and the absorbency value was ranged from (0.353 – 0.159). It can be suggested that 100 percent higher resistance in positive strain had the ability to a stronger producing for biofilm [11]. Regarding the MDR, the current findings are consistent with the MDR *P. mirabilis* isolates found in Iranian UTI patients; these isolates were all capable of forming biofilms and exhibited significant antibiotic resistance [12].

Detection of the *Esp* gene, which genes for the enzymes that cause *Proteus mirabilis* to produce biofilms:

Using a PCR approach, the *Esp* gene that codes for the enzymes involved for Biofilm generation in *Proteus mirabilis* represented 15 (40%) of the participants these results disagreed with the researcher Sayal [13].who discovered (50%) were favorable. According to the current findings, 40% of the examined *P. mirabilis* isolates lacked detectable levels of the *Esp* gene, as demonstrated in Fi 2. This could due to a variety of colonizing stimuli, *P. mirabilis* creates mannose-resistant Proteus-like (MR/P) pili after first attachment. These pili are essential for catheter-associated biofilm formation and help with biofilm production and colonization of the bladder and kidneys. The results also showed that the isolates that possessed the gene were

multidrug resistant to antibiotics has been showed by susceptibility test [14,15]. antimicrobial and the concentrations required to eradicate biofilm producing bacteria are higher than those required to eradicate strains that did not produce biofilm. Also [16] explained this issue as bacterial populations produces persister cells that neither grow nor die in the presence of antibiotics and that persisters are largely responsible for high levels of biofilm tolerance to antimicrobials. So that, the process of biofilm formation is particularly relevant for clinician because biofilm associated microorganisms exhibit dramatically decreased susceptibility to antimicrobial agents [17]. Biofilms and outer membrane lipoproteins can play an important role in protect bacteria from drugs exposure when compared with other bacteria don't has these virulence factors, therefore there was strong relationship between resistance of antibiotics and prevalence of virulence factors in bacteria [18]. Bacteria with biofilm forming are generally more resistant to many antibiotics; biofilms and outer membrane lipoproteins act as biodegradable effect on beta-lactamases antibiotics through act of beta-lactamases enzymes can secreted maintain their activity inside of biofilm matrix, decomposing beta-lactam antibiotics before these antibiotics reach the bacterial cells [19]. Antimicrobial resistance is an innate feature of bacterial biofilms that, in addition to the increasing rates of reported antimicrobial resistance amongst clinical strains, may further complicate patient treatment [20].

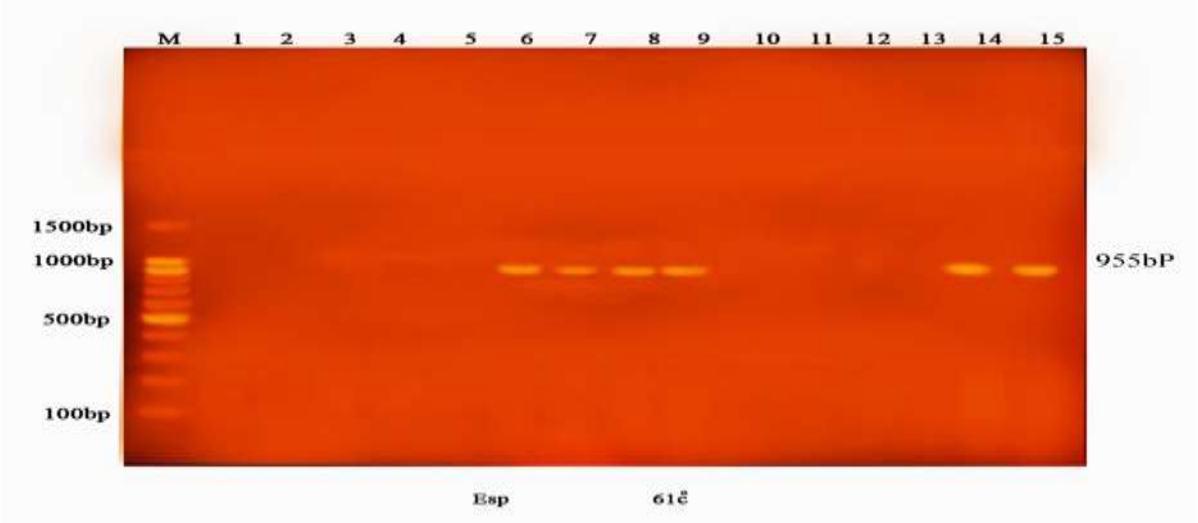

Figure 2: Proteus samples fractionated on 1.5% agarose gel electrophoresis at7volt /cm for 1 hour and stained with Eth.Br. M: 100 bp ladder marker were used for the amplification of the *Esp* gene. Lanes 1–15 resemble PCR products with 955 bp.

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