ANTIBIOTICS RESISTANCE PROFILE OF BACTERIAL STRAINS   PRODUCED OF BIOFILM ISOLATED FROM PATIENTS IN AL-DIWANIYA CITY, IRAQ

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Abstract- **Majority of the microorganisms develop different types of survival mechanisms such as growth regulation, heterogeneity in population, proteolytic systems etc. to adapt to stress conditions. Pathogenic microorganisms acquire the ability to sustain various host immunological responses, The study included collection of about 150 clinical samples from a patients and included samples (Urine, fecal and blood). And for both gender and of all, The necessary examinations and tests were performed for the bacterial isolates. The results were as follows presence of five bacterial genera and in percentage *Pseudomonas aeruginosa* 12 isolates (40%), *Escherichia coli* 10 isolates (33.3%), *Staphylococcus*   5 isolates (16.6%), 1 isolate (3.33%).   *bacillus* isolates (6.6%)2 and then *Proteus mirabilis***
Also using the Field Emission Scanning Electron Microscopy (FE-SEM) to detection of the biofilm, The isolates was tested to 12 antibiotic by kirby bauer method and the results was presence of high ratio of resistant. Where the bacterial strains showed high resistance against (Amoxicillin, Amikacin, Cefotaxime, Ciprofloxacin, Gentamicin, Nalidixic acid, Ceftazidime, Cefoxitin) while The remaining antibiotics show middle and low resistance.

Key words - **Biofilm; Antibiotics resistance, isolation of bacteria**

I.   INTRODUCTION

Biofilms defined as the well-organized cooperating communities of surface-associated microorganisms enclosed in extracellular matrix produce by themselves. It is a trait of microorganisms (bacteria) **(Hall *et al* ., (2004) ; Stewart *et al* ., (2008).** Biofilms are heterogeneoeus congregation for surface associated microorganisms encapsulated within a self-produced polymermatrix consisting of polysaccharide, protein and DNA. Several factors such as bacterial defenses mechanisms, suitable areas for colonization, the community cooperation related benefits and extraordinary mode of growth in their habitat accelerate biofilms formation **(Jefferson, 2004).** Bacteriae such as *S. aureus P. aeruginosa, Acinetobacter baumanniiand* other clinically important microorganismses that thrive on medical devices form biofilmes that confer them from resistance and tolerance to antibiocides as compared to their planktonice forms. About 80% of the pathogenic bacteria are associated with medical device related infections such as ortho dental prosthetics, contact lenses, cardiovascular valves, urinary catheters, pacemakersand breast implants **(Scott, 2009).** Bacteria residing in the biofilm are protected by the shelter and homeostasis as the EPS are produced having protein, nucleic acid, carbohydrate and other substances. It plays a key role in structure forming and functioning of biofilm communities in diverse environments   Antimicrobial agents and biofilm microbial community combat for dominancy where diffusion of molecules determine the success. Biofilm has the ability to develop a barrier of EPS against diffusion molecules of the antimicrobial agents.   *P. aeruginosa* forms biofilms on wet surfaces and in hospital environments such as sinks, humidifiers, and respiratory therapy equipment [Ruiz]. Therefore, the organism can be often isolated from a variety of sources in hospital environments, including respi-ratory therapy equipments, antiseptics, soaps, sinks, mops, medicines, and physiotherapy-hydrotherapy pools and feces of patients **(Shehabi *et al.,* (2005).** Microbial resistance to antimicrobial agents is due to either (i)

intrinsic properties (natural phenotypic traits) or (ii) the acquisition of resistance genes through mobile genetic elements, such as plasmids and transposons, or the mutation of indigenous genes **(European Food Safety Authority, (2012).**

II.   MATERIALS AND METHODS

The study was conducted over a period of six months, A total of 150   samples were collected from different ages of clinical patients who were visited different hospitals in Al-Diwaniyah city, Iraq, The ages of patients ranged from few days to 60 years old.

A. **Isolation and identification** The specimens that include (blood, fecal and urine) were transported immediately to the lab and inoculated on MaConckey and blood agar, and incubated aerobically at 37 °C for 24 h. Non-lactose fermenting pale colonies from MaConckey and large flat dark greenish colonies from blood agar (after sub-culturing on nutrient agar) were tested for conventional biochemical tests: oxidase test, catalase, citrate utilization, and oxidative fermentation. The isolates were further sub-cultured on to two nutrient agar plates and incubated separately. Based on colony morphology, gram stain, oxidase test, urease test, Simon-citrates test.

B. **Diagnosis of bacteria:** bacteria and colonies are diagnosed Developing depending on the phenotypic characteristics which include Forms of colony, color, surface of the colony and its structure Smell, transparency, and the pattern of hemolysis on medium   Blood and lactose fermentation on medium agar macConkey **(Winn *et al*., (2006).** It was also diagnosed microscopically, as samples were examined by taking Smear from the bacterial colonies growing on the media The implant was attached and stained with Gram stain for observation Forms and arrangement of bacterial cells and their interaction with dye, Biochemical tests were also performed **(Brown, 2007)**.

C. **direct detection of Biofilms via Field Emission Scanning Electron Microscopy (FE-SEM(** The biofilm formation ability of isolates were examined by FE-SEM. The surfaces were prepared with some modifications as described previously by Jahid *et al.,* (2013). The surfaces were fixed at room temperature for 4 h with 2.5% glutaraldehyde. The surfaces were then serially treated with ethanol (50% for 15 min, 60% for 15 min, 70% for 15 min, 80% for 15 min, 90% for 15 min, and twice with 100% for 15 min each time) and successively dehydrated by soaking in 33, 50, 66, and 100% hexamethyldisilazane in ethanol for 15 min each time. The dehydrated surfaces were sputter coated with platinum and visualized by FE-SEM (Hitachi/Baltec, S-4700). In this study.

D. **Indirect detection of biofilm by** Plate count Quantitative Culture plating to determine the number of colony forming units (CFU**). (González *et al* ., 2018).** Quantitative Cell counts from biofilms are calculated.

E. **Sensitivity test**
kirby bauer method: The drug sensitivity of bacterial isolates was tested in a manner Tablets depending on that include

transfer To bacterial isolates 4 colonies of bacteria-2 A test tube contains 5 ml of soy tryptone broth   Nourished and incubated at 37 ° C for 8 hours. Tempered   Growth using physiological salt solution, completed Comparing Growth in Tube with Macfarland Tube (0.5) Standard, cotton swab dipped in Trypton broth Cultivated soy, squeeze out the sides Interior of the tube, bacteria spread on the Muller Hinton medium Steel by way of planning more than once And in different directions for the purpose of making sure the bacteria are spread Equally tested their sensitivity, and the dishes were left 15 minutes At room temperature to ensure absorption Moisture, antibiotic tablets were placed   To the center of the other disc, the dishes were incubated at 37 °C For 18 hours for all types of antibiotics, then measured Inhibition diameters using Vernia and were compared with values Standard mentioned in **3** table.

III.   RESULTS and DISCUSSION

A general definition of antimicrobial resistance is the ability of an organism to resist the action of an antimicrobial agent to which it was previously susceptible. Where some of this bacterial genera considered as Nosocomial infections caused by antibiotic resistant *P. aeruginosa, E. coli, Staphylococcus sp* have emerged as major concern in clinical care settings as the increasing development of MDR strains (i.e. resistance to at least three antibiotics) **(Juan *et al*., (2005).** Samples were isolated from the   patients in Al-Diwaniyah city/Iraq. Blood, stool and urine isolates were randomly collected from infected patients and from all ages of (children to elderly) and for both sexes who showed symptoms of bacterium infection where the current study about 150 samples and the positive samples are 30 (20%) isolates, also this microorganisms can be present in soil **(Kadhim *et al* ., 2019) Table .1.**

Table 1: bacterial isolation Ratio in patients

Result	Total samples	No.	Percentage%
Positive samples	150	30	20%
Negative samples		120	80%

.and then isolates were identified by examination of morphology of colony on each culture media. isolates were differentiated to 30   *bacterial* strains include *(Pseudomonas aeruginosa 12 (40%) and Escherichia coli 10(33.3%) and Staphylococcus 5 (16.6%)   and Proteus mirabilis 2 (6.6%) and bacillus 1 (3.33%).*

Table 2: bacterial strains produced of biofilm and Gram stain

Genus	Gram stain	NO.isolates
Pseudomonas aeruginosa	*Gram negative*	)40%12(
Escherichia coli	*Gram negative*	*10 (33.3%)*
Staphylococcus	*Gram positive*	*5 (16.6%)*
Proteus mirabilis	*Gram negative*	*2 (6.6%)*

bacillus	*Gram positive*	*1 (3.33%)*
total		*30 (99.9%)*

The results showed the high resistance of bacterial strains towards antibiotic in amoxicillin and Amikacin , Cefotaxime , Gentamicin , Nalidixic acid , Ceftazidime , Cefoxitin ranging from (70%-99.4 %).where as the ampicillin and azithromycin , ciprofloxacin, chloramphenicol, tetracycline ranging from (28%-70%) show the low or mediated resistant .

Table 3. the Antibiotic resistance profile of *bacterial* strains produced biofilms based on inhibition zone diameter (mm).

Antibiotic	*Pseudomonas aeruginosa*	*Escherichia coli*	*Staphylococcus*	*Proteus mirabilis*	*bacillus*
Amoxicillin	70	82.1	85	70	91.1
Ampicillin	28	40	30.4	31.3	33.9
Amikacin	88	89	90	90.2	92
Cefotaxime	89.5	89	84	81	93
Azithromycin	60	79	66	68.2	70
Ciprofloxacin	70.1	69.3	67.3	77.3	80.5
Choramphenico	50	40	44	53	34
Tetracycline	70	60	74	58	40
Gentamicin	90	87	54.2	77.9	85
Nalidixic acid	81	92	85	75	90
Ceftazidime	86	90	89	91	77
Cefoxitin	98	99.4	90	91.5	97.7

The described tolerance mechanisms, all contribute to the persistence of biofilms, which provide a fertile ground for the emergence of antibiotic-resistant mutants. In planktonic cultures, it has been reported that tolerance precedes the occurrence of resistance (**Levin-Reisman *et al*., (2017).**
In study by **Yayan *et al* ., (2015)** similar resistance pattern was seen with ciprofloxacin (35.2% vs. 24.0%; 70.4% vs. 61.5%), ceftazidime (15.9% vs. 30.9; 33.3% vs. 61.5%), gentamicin (26.4% vs. 18.2%; 44.4% vs. 21.4%), and meropenem (20.2% vs. 20.3%; 42.3% vs. 50.0%). An elevated resistance of *P. aeruginosa* and MDR *P. aeruginosa* was found for amikacin (10.2% vs. 9.1%; 27.3% vs. 9.1%). While in other study by **Telling *et al* ., (2018)** showed the resistance ratio of isolates was of Ceftazidime, Gentamicin ,Ciprofloxacin, Amikacin (26.1% , 19.6%, 46.7% , 7.6%) and this no identicle to the present study .
In study by **Pormohammad, *et al* (2019)** showed that *E. coli* strains isolated have the lowest and highest resistance rates were for tetracycline (60%,),.resistance to, cefotaxime,

and ceftazidime was 1% (95%–14.5%) and for nalidixic acid 53%. and this identical to the present study, while in other study by **Naiyf *et al* ., (2019)** showed that More than 50%of the *E. coli* isolates obtained from wound infections were resistant to, ampicillin, ciprofloxacin, and tetracycline;
In Germany study by **Fiedler *et al* ., (2019)** showed that *Bacillus* strains showed resistance against the β-lactam antibiotics such as cefotaxim (100%), as well as amoxicillin and ampicillin (99.3%). Most strains were susceptible to ciprofloxacin (99.3%), chloramphenicol (98.6%), amikacin (98.0%), imipenem (93.9%), gentamicin (88.4%), tetracycline (76.2%) and this no identical to the current study.
Study by **Gulcan Sahal Isil Seyis Bilkay, (2017)** showed that the Antibiotic sensitivity testing of *P. mirabilis* strains against 15 different antibiotics revealed that all 15 strains tested were sensitive to third generation cephalosporins, also were resistant to ampicillin and ceftazidime among β-lactam antibiotics, and gentamicin among aminoglycoside antibiotics. *P. mirabilis* strains were the only strains showing resistance to ciprofloxacin .

.in Algeria study by **Achek, *et al*., (2018)** The antimicrobial resistance rates were higher in clinical isolates. The resistance rates of *S. aureus* to tetracycline were 48.7% *Staphylococcus aureus* showed considerable resistance (52.4%) to gentamycin. While other study by **Oladipo *et al* ., (2019)** was solates were resistant to amoxycillin (91.7%), ciprofloxacin (84.0%), azithromycin (78.0%), ceftazidime (76.0%), gentamycin (75.0%), and study by **Wang *et al* ., (2019)** resistant of tetracycline was common *S. aureus* about (31.5%). Resistance gentamicin (9.0%), and this results was no identical to the present study.

The Antibiotics resistance implies mutations in resistance genetics determinants lead to increase antibiotic minimal inhibitory concentrations for bacterial cells disrupted from biofilm and it is accepted as a side-effect of prolonged maintenance antibiotic therapy. In contrast to the planktonic, fast-dividing cells that are traditionally used to study antibiotic resistance development in shaking cultures, biofilm-grown bacteria encounter gradients of nutrients and oxygen which lead to a heterogeneous bacterial population including slow-growing or non-dividing cells **(Stewart, 2015; Stewart *et al*., 2016).**

IV. CONCLUSION

The study included isolation of bacteria producing Biofilms, as these bacteria are distinguished by their multiple resistance to antibiotics, and this may be due to their possession of other mechanisms that lead to this resistance, as these Biofilms are not affected by sterilization and disinfection, and therefore the presence of resistant strains and the difficulty of accepting these bacteria for treatment.

V. ACKNOWLEDGEMENT

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