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Plasmid Mediated Resistance to Cephalosporin and Adhesion Properties in *E.Coli*

Salwa Oufrid[1, 2], El Mostapha Mliji[2], Hassan Latrache[3], Mustapha Mabrouki[3], Fatima Hamadi[3], Mustapha Talmi[1], Mohammed Timinouni [2]

[1]*Microbiology, Health and Environment Team, Department of Biology, School of Sciences,Chouaib Doukkali University, EL Jadida, Morocco*

[2] *Molecular* Bacteriology *Laboratories, Pasteur Institute of Morocco, Casablanca, Morocco*

[3] *Laboratory of development and food safety, Faculty of Science and Technology, Beni mellal, Morocco*

ABSTRACT

Introduction: The objective of this study is to evaluate the relationship between biofilm formation, surface characteristics and the presence of plasmid conferring resistance to cephalosporin

Methodology: The plasmid of resistance of *Salmonella 3349* was purified and transferred by electroporation to the *E. coli DH10B* originally incompetent to form biofilm. The physico-chemical surface properties of the three bacteria (*E. coli DH10B, Salmonella 3349* and its isogenic transformant *3519EC1*) were estimated and compared by the Microbial Adhesion to Solvents test (MAST) and angle contact measurement. Cellular densities of bacteria adhered to stainless supports were examined with a scanning electron microscope.

Results: The physicochemical properties of bacterial cell surface demonstrated that *E.coli DH10B* strain was hydrophilic, electron donating and weakly electron accepting than *Salmonella 3349* and its transformant *3519EC1* strains. Moreover, there was a weak correlation between the acid-base properties determined by the Microbial Adhesion to Solvents test and angle contact measurement. Analysis of microscopical images of bacterial adhesion indicated that *E.coli 3519EC1* and *Salmonella 3349* adhered to the stainless surface, whereas the *E.coli DH10B* does not adhere.

Conclusions: The results of this study suggest that the presences of the plasmid of resistance modify the microbial surface properties and biofilm formation.

Key words: Adhesion, biofilm, physicochemical properties, resistance plasmid.

I. INTRODUCTION

Biofilm formation is a complex process regulated by diverse characteristics of support, bacterial cell surface, growth medium and their interactions [1]. Bacteria possess surface properties, related to their charge, hydrophobicity and Lewis acid/base characteristics; that are involved in interactions between bacteria and their environment. These properties play a critical role in the attachment processes of microorganisms to surfaces [2].

The most difficult properties of bacterial biofilms are their extreme resistance to treatment with biocides and detergents and their high tolerance to prolonged antibiotic therapy in human infections [3,4].

it have been shown that Natural conjugative plasmids express factors that induce planktonic bacteria to form or enter biofilm communities, conditions which favor plasmid conjugation the infectious transfer of the plasmid. This parallel connection between conjugation and biofilms suggests that medically relevant plasmid-bearing bacterial strains are more likely to form biofilm. This may influence both the chances of biofilm-related infection risks and of conjugational spread of virulence factors [5].

Current research efforts have focused on the role of the presence of the plasmid of resistance in the microbial surface properties and biofilm formation.

II. METHODOLOGY

2.1 Bacterial strains and growth conditions:

Bacteria used in this study were *E.coli DH10B* and *Salmonella 3349*. plasmid of resistance was extracted from *salmonella 3349* and electroporated into *E.coli DH10B* to construct *3519EC1* as described in Sambrook et al., [6]).

Bacteria were routinely grown aerobically at 37°C in Luria Bertani (LB).

2.2 Microbial adhesion to solvents:

The Microbial adhesion to solvents (MATS) method, described by Bellon-Fontaine et al.[2] and based on the comparison of microbial cell affinity to a monopolar and an apolar solvent was used to determine the electron donor (basic) and the electron acceptor (acidic) properties of microbial cells.

Experimentally, bacteria were washed by a succession of three centrifugations (10 min with 4°C and 4000 G) and suspended to an optical density between 0.7 and 0.8 with 450nm (A 0) in plug PBS (pH = 7,2). 2.4 ml of each bacterial suspension was vortexed for 60 s with 0.8 ml of the solvent. The mixture was allowed to stand for 15min to ensure complete separation of the two phases. The percentage of affinity to the solvent was subsequently calculated by the following equation: %Affinité = $(1\text{-}A/A_{0)} \times 100)$.

Where A_0 is the absorbance measured at 450nm of the bacterial suspension before mixing and A is the absorbance after mixing.

Measurements were made three times on independent cultures, and the average of 3 measurements was taken as the affinity for each solvent.

The following pairs of solvents were used: chloroform, an electron acceptor solvent, and hexadecane, an apolar solvent; and Ethyl acetate, a strong electron donor solvent, and decane, an apolar solvent. Due to the similar Lifshitz–van der Waals components of the surface tension in each pair of solvents, differences between the results obtained with chloroform and hexadecane, on one hand, and between Ethyl acetate and decane, on the other hand, would indicate the electron donor and electron acceptor character of the bacterial surface, respectively.

2.3 Contact angle measurements:

Measurements of contact angle and the energy properties of surface were carried out by using 3 solvents:

- Distilled water
- Diiodométhane (ALDRICH® 99%)
- Formamide (SIGMA® ~ 100%).

The Lifshitz van der Waals (γ^{Lw}) ,electrondonor $(\gamma\text{-})$, and electron-acceptor $(\gamma+)$, components of the surface tension of bacteria (B) were estimated from the approach proposed by van Oss et al. [7]. The pure liquid (L) contact angles (□) can be expressed as: $\cos\theta = -1+2(\gamma B\ LW.\gamma L\ LW)1/2/\gamma L +2(\gamma B+.\ \gamma L-)1/2/\gamma L+2\ (\gamma B-.\ \gamma L+)\ 1/2/\gamma L$

2.4 Scanning Electron Microscopy:

The samples with adhered cells was dried with free air, metalized and observed using scanning electron microscopy (SEM). All SEM images were processed with subroutine program developed in Mathlab□ to determine the percentage of glass surface covered by the cells. We use a development algorithm identifying the boundaries in image, based on some mathematical methods, exploring also image to detect edges and using statistical functions to calculate mean and standard deviation.

III. RESULT

3.1 Hydrophobicity:

We investigated the physicochemical surface properties of the three bacterial strains (*Salmonella 3349*, *DH10B* and transformant *3519EC1*) by the microbial adhesion to solvents test (MATS). The biofilm deficient *DH10B* strain was very hydrophilic due to its low affinity to apolar solvents, hexadecane and Decane (Fig 1). The *salmonella 3349* and the transformant *3519EC1* strains were hydrophobic because of their high affinity to apolar solvents (hexadecane and Decane). These results suggest that presence of the plasmid is responsible for the modification of the hydrophobicity of *E.coli DH10B*.

3.2 Electron donor / acceptor properties:

The higher affinity of cells surface for chloroform (acidic solvent) than for hexadecane (apolar solvents) indicates that the cell surface is electron donating. Based on this comparison; our results (Table 1 and Fig 2) show that the *DH10B* has a pronounced electron donor character. For *Salmonella 3349* and transformant *3519EC1*, the affinity to chloroform is slightly higher than to hexadecane.

Table1: Electron acceptor character of E. coli DH10B, Salmonella 3349 and 3519EC1 transformant

	Electron/donor character (%)		
Concentration (ul)	*E. coli DH10B*	*3519EC1transformant*	*Salmonella 3349*
100	68,94	38,88	43,31
300	72,25	27,27	21,25
500	75,94	1,68	6,69
700	76,97	0	0,64

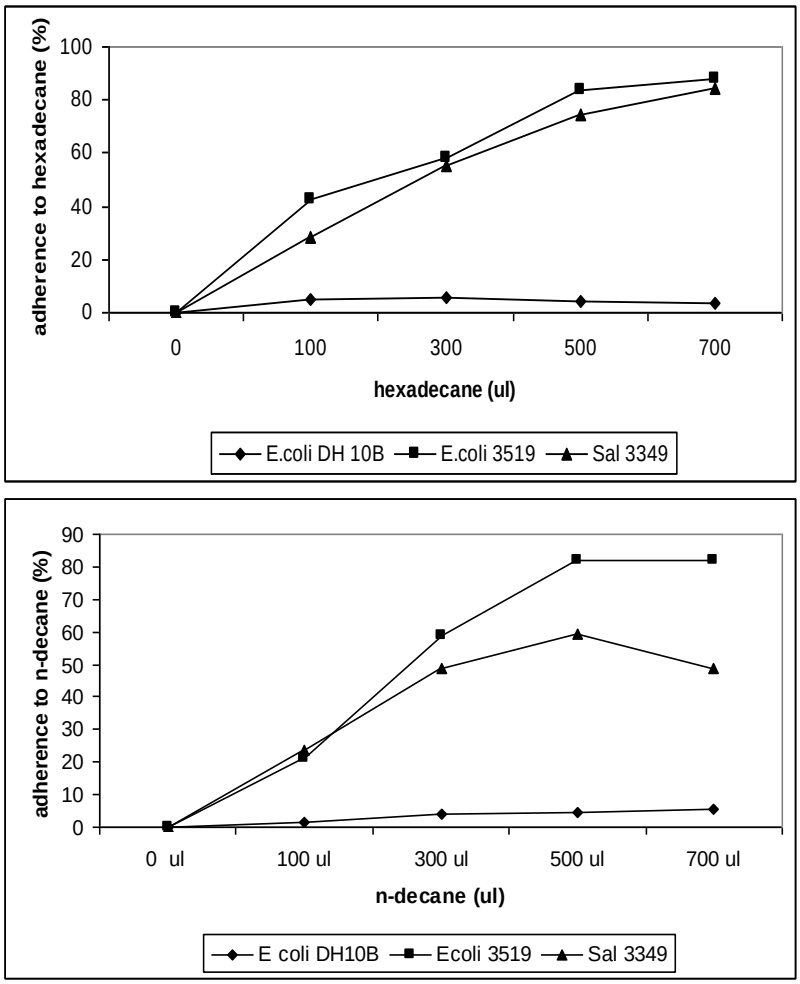
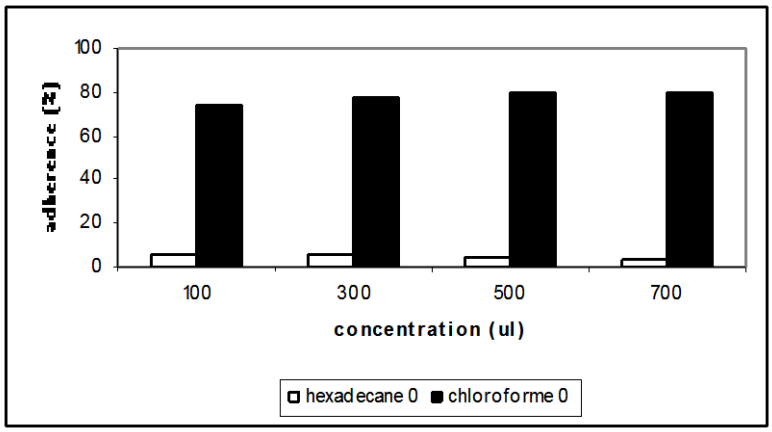


Figure 1: Cell surface hydrophobicity of *E.coli DH10B* strains, the transformant *3519EC1* and *Salmonella 3349*.

Table2: Electron acceptor character of E. coli DH10B, Salmonella 3349 and 3519EC1 transformant

	Electron/acceptor character (%)		
Concentration (ul)	*E. coli DH10B*	*3519EC1*	*Salmonella 3349*
100	34,9	15,86	10,54
300	22,6	0	0
500	21,4	0	0
700	20	0	0



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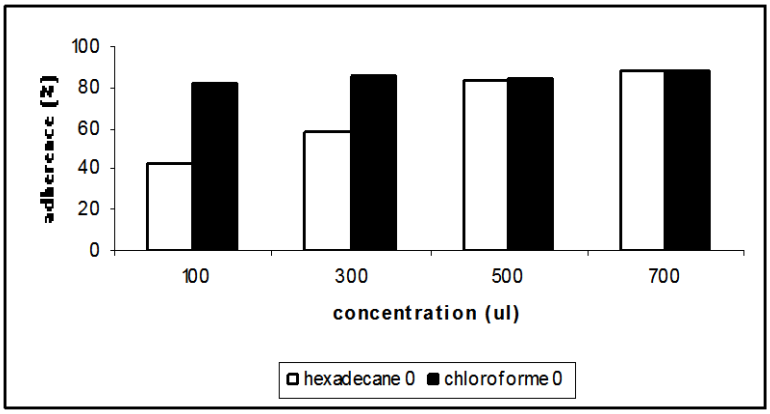

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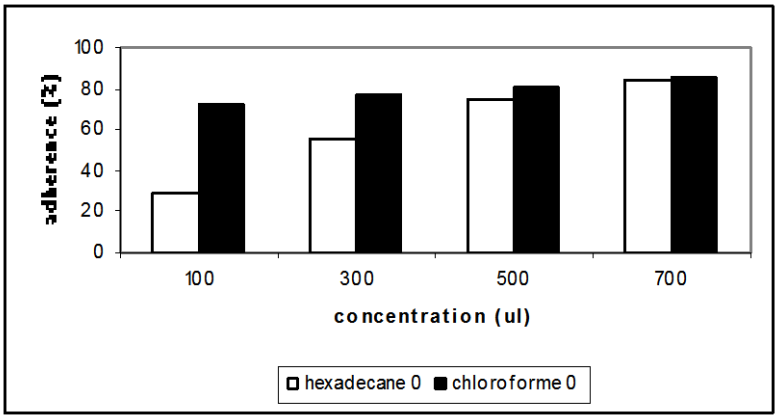

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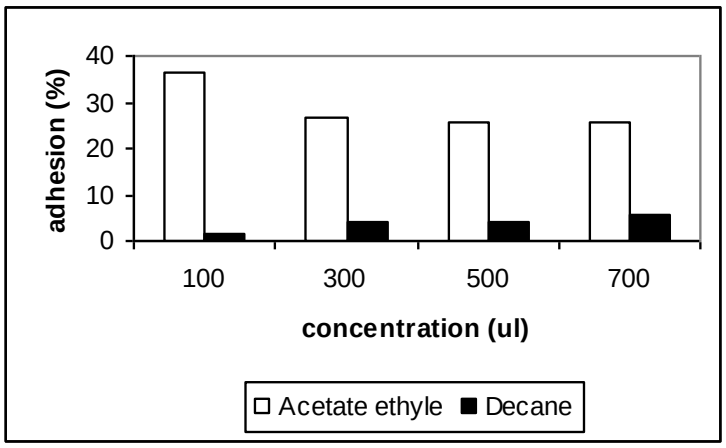
Figure 2: Comparison of the adhesion of chloroform (acidic solvent) with hexadecane (apolar solvents), for *E.coli DH10B* strains (2-1), *E.coli 3519EC1* transformant (2-2) and *Salmonella 3349* (2-3).

The Results of the electron acceptor properties of cell surface of *E. coli DH10B*, *Salmonella 3349* and transformant *3519EC1* are presented in Table 2 and Fig 3. The *E.coli DH10B* strain has an electron acceptor character, demonstrated by a greater affinity to ethyl acetate (basic solvent) than to decane (apolar solvents). It is noted that the electron-donor character of the DH10B is much higher than the electron-acceptor character.

At concentration 100 ul, the affinity of the two strains *Salmonella 3349* and transformant *3519EC1* is slightly higher with decane than with ethyl acetate. This shows that the two strains were electron acceptor at this concentration. For other concentration no electron acceptor character was

observed on the two strains. The difference between the result observed on *E coli DH10B*, *Salmonella 3349* and transformant *3519EC1*, confirms that the presence of the plasmid of resistance modifies the properties of adhesion of the bacteria.



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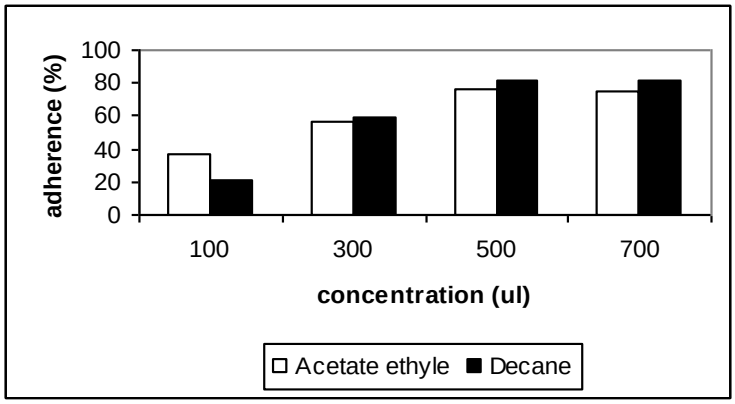

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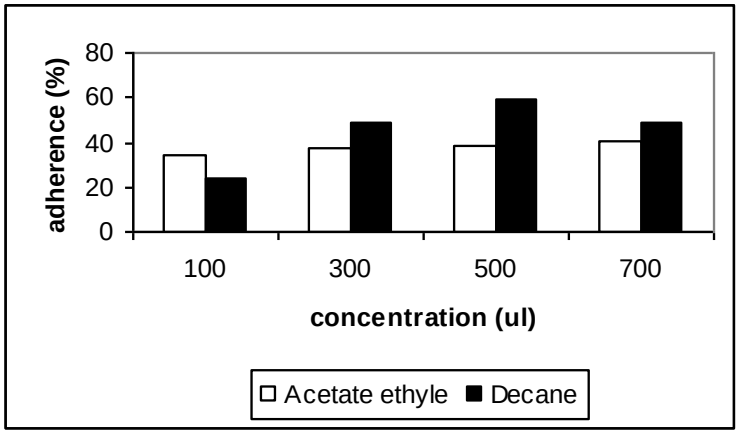


(3-3)

Figure 3: Comparison of the adhesion of Ethyl acetate (basic solvent) with Decane (apolar solvents), for *E.coli DH10B* strains (3-1), the *transformant 3519EC1* (3-2) and *Salmonella 3349* (3-3).

3.3 Contact angle measurements

The values of the contact angles with the different liquids and surface energy components for the *E coli DH10B, Salmonella 3349* and transformant 3519EC1 are presented in Table3.

The contact angle measurements show that the *E coli DH10B* surface is hydrophilic while the *Salmonella 3349* and transformant *3519EC1* are hydrophobic. This hydrophobicity is in accordance with the higher adhesion to hexadecane of these strains.

It is noted that the electron-donor component of the *DH10B* is much higher than the electron-acceptor component.

Table 3: Contact angles (in degrees) and surface energy components (in millijoules per square meter) of E. coli DH10B, Salmonella 3349 and 3519EC1 in water, formamide and diiodomethane

Bacteria	Contact angles (◦)			Surface energy components mJ/m2				
	Water	Formamide	Diiodomethane	γ^{LW}	γ^{AB}	γ^{+}	γ^{-}	γ^{Total}
E. coli DH10B	22,13±0,8	40,37 ±2,2	85,7 ±1,4	14,7	38,3	5,8	62,8	53
3519EC1	34,97±1,2	57,24 ±2,4	72,3 ±0,7	21,6	8,2	0,2	69,3	29,8
Salmonella 3349	19,24 ±1,2	55,92 ±0,8	67,43± 0,6	24,3	0,7	0	88,6	25

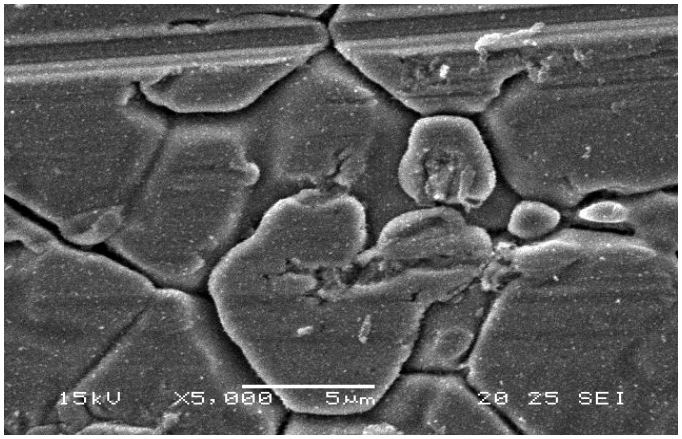
γ^{LW}: the Lifshitz-van der waals component. γ^{AB}: the Lewis acid-base component. γ^{-} : the Lewis electron donor. γ^{+}: the Lewis electron acceptor. γ^{Total}: the total surface energy

3.4 Scanning Electron Microscopy:

The images obtained by Scanning Electron Microscope (Figure 4) showed that the transformant (*E.coli 3519EC1*) and *Salmonella 3349* adhered to the stainless surface, but the *E.coli DH10B* do not adhere. The results obtained by our program are shown in Table 4 confirming the observation made on the images in Fig 4.

Table 4: Results of subroutine program in Mathlab giving a number of bacterial and occupied surfaces by E. coli DH10B, Salmonella 3349 and 3519EC1

MEB Image	Number of adhering bacteria	Surface of bacteria	Total surface	% of surface microorganism occupation
E.coli DH10B	0	0	75012	0%
Transformant3519EC1	86	20640	75012	27,51%
Salmonella 3349	110	33000	75012	44%



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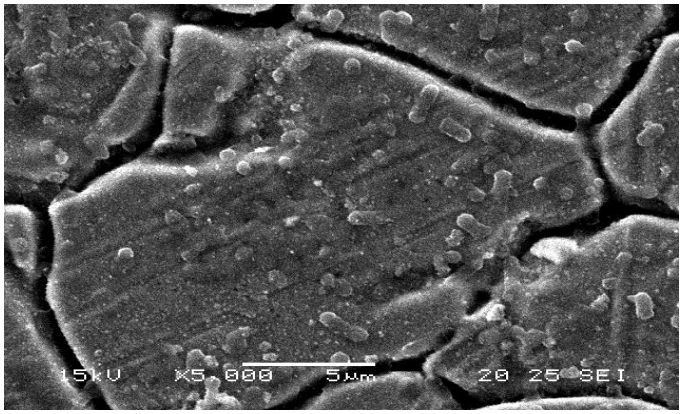

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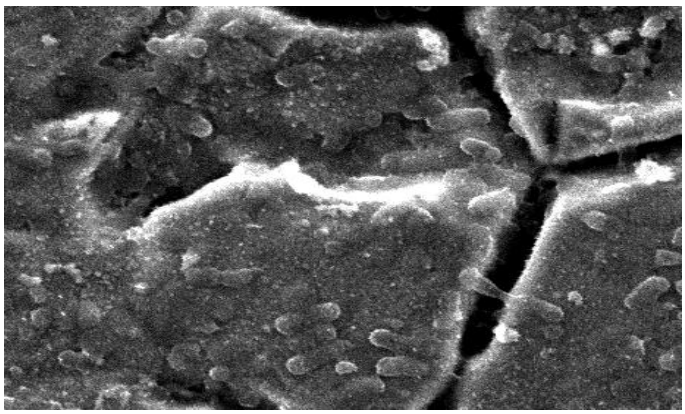

(4-3)

Figure 4: Scanning electron photomicrograph of *E.coli DH10B* (4-1), the *transformant* 3519EC1 (4-2) and *Salmonella 3349* (4-3).

IV. DISCUSSION

Microbial surface properties are considered to play a major role in interactions between bacteria and their environment, especially in the field of adhesion to a substrate [8]. This phenomenon depends on electrostatic, van der Waals and Lewis acid/base characters of both substrates and cells [9]. The present study of the modifications induced in the Microbial surface properties shows that this parameter is altered in bacteria with plasmid conferring resistance to cephalosporin.

According to the result obtained by the microbial adhesion to solvents test, The *E.coli DH10B* was very hydrophilic. This hydrophilic property of *E.coli* has previously been obtained by other reports using different methods [10-15].

Surface hydrophobicity of bacteria with or without plasmids showed that the presence of the R-plasmid modify the hydrophobicity. Ferreiros & Criado [16] found that different R-plasmids can induce significant variations that depend on the

carrier bacteria and on the method employed by measuring hydrophobicity. Similar results were obtained with Shinz,[17]. He found that some R-plasmids produce significant variations in adherence and/or hydrophobicity but that these variations show no quantitative or qualitative correlation [17].

Scanning electron microscopy photographs revealed that the presence of the R-plasmid altered the adhesion capacity of the transformant (*E.coli 3519EC1*); this strain showed more adherence characteristics than the *E.coli DH10B*. Many works showed that strains containing plasmids adhered better than similar strains without plasmids[18,19]. Previous studies have shown that the presence of ESBL-encoding plasmids alters the basal adhesion capacity of the recipient strain, and cured strains adhered more than the parental strains [20]. Gallant et al. noted that the presence of a TEM-1-encoding plasmid causes defects in adherence and biofilm formation by *Pseudomonas aeruginosa* [21].

The mechanisms by which R-plasmids alter hydrophobicity and adherence are not clear, but they may code for the production of different surface components on bacteria.

In this study, the correlation between acid-base properties determined by MATS and CAM was very weak. This result was in agreement with the previous works of Fatima Hamadi [22], it showed

that there are no general rules correlating MATS with water contact angle methods.

In conclusion, this work demonstrates that the presence of the R-plasmid modifies the microbial surface properties. Consequently, the adhesion process and biofilm formation may be affected.

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