Physicochemical surface characteristics in different pathogenic bacteria

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Abstract: Understanding physicochemical surface characteristics of microorganisms is vital in order to reduce or prevent bacterial adhesion as an important stage of pathogenicity. These characteristics are influenced by such factors as temperature, pH, or media determining the extent of bacterial adhesion. The present study adopted a simple technique, i.e. MATS (microbial adhesion to solvents) method, to better understand microbial cell surface characteristics in four different strains, namely Staphylococcus aureus, Listeria Monocytogenes, Escherichia coli, and Pseudomonas aeruginosa. For this aim, the effect of different concentrations of human blood serum in the media of these microorganisms on physicochemical characteristics of the microorganism was evaluated. The results revealed that the microorganisms possessed different characteristics at different doses of human blood serum. Some doses of human blood serum had no effect on hydrophobicity and electron donation and reception in the microorganisms whereas some doses could cause changes in these characteristics. These findings show that care should be taken when using different concentrations of antibiotics or other compounds such as rhamnolipids.

Subjects: Microbiology; Biotechnology; Food Microbiology

ABOUT THE AUTHOR
The authors have been mainly focusing on studies on applied microbiology, especially where microbiological considerations are taken into account in medical practices. The present study is in line with the authors’ previous studies where they have considered various aspects of microbiology to yield better results when working on various strains. Dr. Ailar Jamalli is one of the experienced faculty members in Department of Microbiology, School of Medicine, Golestan University of Medical Sciences, Gorgan, Iran and she has carried out extensive research on various strain and different areas related to microbiology. Dr. Teena Dadgar from Department of Biology, Gorgan Branch, Islamic Azad University, Gorgan, Iran, is also an experienced researcher in molecular microbiology. Ms. Fariba Farniya has fulfilled her master degree in Islamic Azad University, Gorgan, Iran at the Department of Biology.

PUBLIC INTEREST STATEMENT
The present study aimed to determine physicochemical surface characteristics in different pathogenic bacteria. The results of this study showed that four microorganisms possessed different characteristics at different doses of human blood serum. These results could be of great interest for public because they might gain a higher awareness of adhesion characteristics of various bacterial strains. Furthermore, they may use the results of this study to build up their knowledge in microbiological aspects of their own career as the results can be adopted in a wide range of disciplines.
Keywords: hydrophobicity; electron donation; electron reception; physicochemical surface characteristics

1. Introduction

Adhesion is the first and most important stage in bacterial pathogenicity after entering the body (Busscher & van der Mei, 2012). It is of great importance to study adhesion in the context of bacterial pathogenesis in medicine, industry, agriculture, and decomposition in the environment. Hydrophobicity and electron donation and reception are important factors in bacterial adhesion (Habimana, Semião, & Casey, 2014). Bacterial adhesion is brought about by the interactions between surface proteins of bacteria and surface receptors (Wizemann, Adamou, & Langermann, 1999), which are dependent on surface characteristics and environmental factors (e.g. serums and antibiotics) (Bardiau, Szalo, & Mainil, 2010). Bacterial adhesion is the most important stage in bacterial pathogenicity and biofilm formation (Busscher & van der Mei, 2012; Hori & Matsumoto, 2010). The biofilms may cause resistance to antibiotics, dysfunction in drug absorption, and change in the minimum inhibitory concentration of the drug (Tenke, Kovacs, Jäckel, & Nagy, 2006). Bacterial adhesion brings about problems in industry, medicine, and environment and may even claim lives (Hsu, Fang, Borca-Tasciuc, Worobo, & Moraru, 2013). Moreover, it may cause increase in expression of encoding genes for virulence factors of bacteria (Wizemann et al., 1999). Certain bacteria have secretion systems type 3, 4, and 6 by which they send protein effector to the host cell needing bacterial adhesion (Krachler & Orth, 2013). Electrostatic and hydrophobic interactions, hydrogen bonds, Van der Waals forces (Saralaya, Bhat, Kamath, & Shivananda, 2004), surface appendages such as flagella and pili (Pizarro-Cerdá and Cossart, 2006), and exopolysaccharide (Hori & Matsumoto, 2010) are some of the mechanisms of bacterial adhesion.

Bacterial adhesion is mainly composed of a primary phase and a secondary phase (Katsikogianni & Missirlis, 2004). In the primary phase, physicochemical interactions are formed between the surface and bacteria providing the ground to enter the second phase (Katsikogianni & Missirlis, 2004). The whole process is reversible until the first phase (Habimana et al., 2014). In the second phase, however, molecular and cellular interactions strengthen the adhesion through selective bridging between polymeric structures on the bacterial surface such as pili and capsule and S-layer and surface (Katsikogianni & Missirlis, 2004), which leads to irreversible adhesion (Habimana et al., 2014). These two phases as well as different mechanisms of adhesion can be a purpose for vaccine production (Wizemann et al., 1999).

One of the most important determinants in bacterial adhesion is hydrophobicity. In general, bacteria with hydrophobic characteristics prefer hydrophobic surfaces for adhesion (Katsikogianni & Missirlis, 2004). Another important determinant of adhesion is bacterial surface charge, which is influenced by environmental factors such as pH, media, and ionic concentration (Habimana et al., 2014).

Bacterial adhesion to solid surfaces is an important stage in pathogenicity. It is a prominent factor in artificial implants in medicine as well as in food industry by its role in biofilm formation. Bacterial adhesion is based upon electrostatic interactions and Lewis acid/base interactions (Hori & Matsumoto, 2010). Therefore, regarding its undeniable importance in a wide range of fields, it is very important to study its different aspects. The findings of the current study could be useful in medical practices. There has been only one method to determine these characteristics based upon measurement of contact angle (Gallardo-Moreno, Navarro-Pérez, Vadillo-Rodriguez, Bruque, & González-Martín, 2011). However, the present study adopted a simple technique called MATS (Microbial Adhesion to Solvents) method, which is based on microbial cell affinity to polar and nonpolar solvent (Bellon-Fontaine, Rault, & Van Oss, 1996; Hori & Matsumoto, 2010; Zeraik & Nitschke, 2012). Therefore, physicochemical surface characteristics of four bacterial strains, i.e. Staphylococcus aureus, Listeria Monocytogenes, Escherichia coli, and Pseudomonas aeruginosa,
were determined using this new technique and the effect of different concentrations of human blood serum on these characteristics were evaluated.

2. Materials and methods

2.1. Separation and identification

Four isolated samples of *L. Monocytogenes* (ATCC19115), *E. coli* (ATCC2522), *S. aureus* (ATCC25923), and *P. aeruginosa* (PTCC1430) were purchased and cultured in nutrient agar plates (Merck, Germany) and then incubated for 24 h at 37°C. The strains were transferred to new environments weekly in order to remove the influence of the type and durability of medium on physicochemical characteristics (Bellon-Fontaine et al., 1996). After identification tests, the strains were transferred to tilted nutrient agar and kept at 4°C. Gram staining was carried out for all samples after incubation for 24 h. Microscopic observations revealed the existence of two gram-positive and two gram-negative samples. All the four samples were catalase positive. The samples were cultured on different media including OF (Oxidation & Fermentation; Merck, Germany), TSI (Triple Sugar Iron; Merck, Germany), mannitol salt agar, SIM (sulfide Indole Motility; Merck, Germany), EMB (Eosin Methylene Blue; Merck, Germany). Afterwards, single colonies, which were similar *E. coli* colonies, were used for identification tests (IMViC). These tests included indole, MR (Methyl Red), VP (Voges–Proskauer), and citrate. The identification test results are shown in Table 1. The characteristics evaluated to identify bacteria were sugar fermentation, gas formation, H₂S formation, motion, indole production, and MR/VP, and urea tests.

2.2. TSI (Triple Sugar Iron)

TSI dry powder (64.6 g) was blended with distilled water (1 L) and then the mixture was heated to yield a uniform solution (64.6 g/L). The solution was poured into a test tube followed by autoclave in tilted position. In this medium, fermentation is characterized by color change from red to yellow using phenol red and H₂S formation is identified by formation of black precipitates (Forbes, Sahm, & Weissfeld, 2007). If inside the medium is yellow (acidic) and the tilted area is red (alkaline), it can be inferred that glucose fermentation occurred; however, if both are yellow, lactose is fermented whereas if both are red, the strain is non-fermentative. *E.coli* usually causes yellow color in both areas.

2.3. SIM (Sulfide, Indole, Motility)

It is a quasi-solid medium, in which bacteria with tryptophanase can produce indole by decomposing tryptophan. SIM dry powder (30 g) was mixed with distilled water (1 L) and then the mixture was heated to fully dissolve agar granules. The mixture (30 g/L) was subsequently transferred to test tube for autoclaving. This medium is also used for motility and production of H₂S. After bacteria culture and incubation for 24 h, the strain motility was registered and 5 drops of Kovacs reagent was introduced to the medium to conclude positive reaction if red color is detected.

2.4. MR/VP (Methyl Red Voges–Proskauer)

MR/VP dry powder (17 g) was dissolved in distilled water (1 L). After heating, the solution (17 g/L) was transferred into test tube and autoclaved. After strain culture, MR test was performed by introducing one drop of methylene red per ml. moreover, for VP test, 0.6 ml alpha-naphthol and 0.2 ml KOH per ml were added. The red color indicated the positive reaction (Forbes et al., 2007).

2.5. Simmons citrate

Simmons citrate dry powder (24.2 g) was dissolved in distilled water (1 L). After heating, the solution (24.2 g/L) was poured in test tube and autoclaved and then the tube was placed in tilted position. The strain able to use citrate as carbon and energy source and use ammonium as nitrogen source can turn the medium containing bromothymol blue from green to blue.
2.6. Urea medium
Urea test is one of the most important identification tests for E. coli. Urea suspension (40%) was prepared and added to 200 ml medium using a filtered syringe near flame. After thorough mixture with the medium, the mixture was transferred to sterile tubes and then it was placed in a tilted way.

2.7. MATS (microbial adhesion to solvents)
MATS is used to evaluate hydrophobicity and electron exchange. It is based upon surface affinity of strain to polar and nonpolar solvents. Polar solvent can be either acidic (electron receptor) or alkaline (electron donor). However, both should have similar Van der Waals forces. There is no cut-off value to be considered electron donor or acceptor; instead, if the affinity toward the electron donor solvent exceeds the affinity for the nonpolar solvent, the cell surface possesses electron acceptor features. Likewise, if the affinity for the electron acceptor solvent exceeds the affinity toward the nonpolar solvent, the cell surface can be considered to have electron donor characteristics. Higher affinity for hydrophobic solvents (decane and hexadecane) could be regarded as a sign of higher level of surface hydrophobicity of the cells (Prokopovich & Perni, 2009).

The solvents used were chloroform (electron receptor acidic solvent), ethyl acetate (electron donor alkaline solvent), hexadecane (hydrophobic nonpolar solvent), and decane (hydrophobic nonpolar solvent). Energy characteristics of the solvents are summarized in Table 2.

The total surface free energy $\gamma^{\text{TOT}}$ comprises two parts: $\gamma^{\text{TOT}} = \gamma^{\text{LW}} + \gamma^{\text{AB}}$. $\gamma^{\text{LW}}$ is the nonpolar constituent of the surface free energy related to Lifshitz–Van der Waals interactions, $\gamma^{\text{AB}}$ is the acid-base constituent of surface free energy. $\gamma^{\text{AB}}$ is obtained from the electron-donor ($\gamma^-$) and electron-acceptor ($\gamma^+$) molecular interactions (i.e. Lewis acid–base interactions) (Prokopovich & Perni, 2009).

An 18–20 h bacterial culture was prepared in BHI (Brain Heart Infusion) broth. After centrifuging at 3000 rpm for 15 min, the supernatant was removed and the precipitate was used to prepare bacterial suspension by using physiologic serum (0.09 M). The physiologic serum was prepared by blending 9 gr NaCl (Merck, Germany) in 100 ml distilled water followed by autoclaving. Afterwards, absorbance was taken to 0.08 ($A_0$) at 600 nm. Then, 2.4 ml of the suspension was added to 0.4 ml of the hydrocarbon solvents. The mixture was then placed at ambient temperature for 90 min until two water and organic phases are appear. The water phase was taken using a Pasteur pipette and its absorbance was read at 600 nm ($A_1$). Adhesion percentage, i.e. affinity of microorganisms to the solvents, was calculated as follows:

$$\text{Adhesion} \% = \left( \frac{1 - A_1}{A_0} \right) \times 100$$

BHI media containing 5%, 10%, and 15% human blood serum were prepared and all the four samples were cultured in these media to determine the influence of human blood serum (as a nutrient for bacteria) on electron reception and donation as well as their hydrophobicity (Bellon-Fontaine et al., 1996).

2.8. Data analyses
Statistical analyses were performed by one-way analysis of variance (ANOVA). Differences between means were evaluated by Duncan test. All the statistical operations were performed in the Statistical Package for the Social Sciences (SPSS) version 22.0 (SPSS Inc., USA). Differences were considered significant at $p < 0.05$.

3. Results
Table 3 shows affinity of the strains (i.e. S. aureus, L. monocytogenes, P. aeruginosa, and E. coli) to different concentrations of chloroform, hexadecane, decane, and ethyl acetate in the presence of different concentrations of blood serum. According to the table, in BHI without human blood serum,
|                     | TSI | MR | VP | Citrate | Indole | Motility | SH2 | Gas |
|---------------------|-----|----|----|---------|--------|----------|-----|-----|
| S. aureus           | A/A | +  | -  | +       | +      | +        | +   | +   |
| L. monocytogenes    | A/A | -  | -  | -       | -      | -        | -   | -   |
| P. aeruginosa       | Alk/Alk | -/+ | -/+ | +       | -/+    | +/+       | -/+ | -/+ |
| E. coli             | A/A | -  | +  | -       | -      | -        | -   | -   |

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P. aeruginosa represents more hydrophobic and electron donor characteristics whereas S. aureus is more hydrophilic and electron receptor. Between the other two strains, L. monocytogenes was more hydrophobic and less electron donor than E. coli. However, in the BHI containing 5% human blood serum, E. coli showed the highest hydrophobic and electron donating characteristics. In BHI with 10% human blood serum, S. aureus had the lowest affinity to chloroform. Therefore, it can be claimed that this strain possesses very low electron donation characteristics in BHI with 10% human blood cell. The highest electron receptor characteristic in BHI with 10% human blood serum belonged to P. aeruginosa. Furthermore, L. monocytogenes, which showed the highest electron donor characteristic in this medium, did not have strong affinity to ethyl acetate; this is consistent with the results related to the high affinity of this strain to chloroform. The results revealed that the strains L. monocytogenes, E. coli, and P. aeruginosa have almost high hydrophobic characteristics while S. aureus showed weak hydrophobic characteristics. In BHI with 15% human blood serum, E. coli and L. monocytogenes had high level of electron donating characteristic. In this medium, S. aureus and E. coli showed the highest affinity to ethyl acetate and therefore, they have the highest electron reception characteristics. Nevertheless, L. monocytogenes and P. aeruginosa did not possess high level of electron receptor characteristic. E. coli had almost 80% affinity to hexadecane and therefore, showed hydrophobic characteristics in this medium while other strain lacked this characteristic.
The results of one-way analysis of variance revealed that there was no significant difference in the affinity of \textit{S. aureus} to chloroform, hexadecane, and decane in BHI without human blood serum \((p > 0.05)\) while the affinity of this strain to ethyl acetate is significantly higher than that to other solvents \((p < 0.05)\). Moreover, in this medium, the affinity of \textit{L. monocytogenes} to hexadecane and decane had no significant difference \((p > 0.05)\) while there were significant differences in affinity of this strain to chloroform and ethyl acetate \((p < 0.05)\). The results also indicated that there were no significant differences in affinity of \textit{E. coli} to decane and hexadecane \((p > 0.05)\) and to chloroform and ethyl acetate \((p > 0.05)\).

In BHI with 5\% human blood serum, there were significant differences in the affinity of \textit{S. aureus} to chloroform with that to other solvents \((p < 0.05)\) except with hexadecane \((p > 0.05)\). In addition, affinity of \textit{L. monocytogenes} to ethyl acetate was significantly higher than that to other solvents \((p < 0.05)\) while there were no significant differences in the affinity of this strain to other solvents \((p > 0.05)\). The affinity of \textit{E. coli} to ethyl acetate was significantly lower than that to other solvents \((p < 0.05)\) whereas no significant difference was detected in the affinity of this strain to other solvents \((p > 0.05)\). Moreover, there was no significant difference between the affinity of \textit{P. aeruginosa} to decane and chloroform \((p > 0.05)\) while the differences in the affinity of this strain in other cases were all significant \((p < 0.05)\).

In BHI with 10\% human blood serum, no significant difference was detected in the affinity of \textit{S. aureus} to different solvents \((p > 0.05)\). Furthermore, the affinity of \textit{L. monocytogenes} to ethyl acetate is significantly lower than that to other solvents \((p < 0.05)\) while there was no significant difference between the affinity of this strain to other solvents \((p > 0.05)\). Moreover, in the case of \textit{E. coli}, the only significant difference in the affinity to solvents was detected between affinity to hexadecane and decane \((p < 0.05)\). No significant difference was found in the affinity of \textit{P. aeruginosa} to different solvents \((p > 0.05)\).

In BHI with 15\% human blood serum, there were no significant differences in the affinity to \textit{S. aureus} to hexadecane and decane and also between the affinity to decane and ethyl acetate \((p > 0.05)\) while significant differences were detected between other samples \((p < 0.05)\). Furthermore, the affinity of \textit{L. monocytogenes} to chloroform in this medium is significantly higher than that to other solvents \((p < 0.05)\). Moreover, there were significant differences between the affinity of \textit{E. coli} to the solvents \((p < 0.05)\) except between affinity to chloroform and ethyl acetate \((p > 0.05)\). The affinity of \textit{P. aeruginosa} to decane was significantly higher than that to other solvents \((p < 0.05)\). No significant difference was detected between the affinity of this strain to chloroform and hexadecane \((p > 0.05)\).

Additionally, the results of one-way analysis of variance indicated that the affinity of \textit{S. aureus} to chloroform in BHI with 10\% human blood serum was significantly lower than that in other media \((p < 0.05)\). Furthermore, there was no significant difference between the affinity of this strain to hexadecane and decane in BHI with 10\% and without human blood serum \((p > 0.05)\). The affinity to ethyl acetate in this strain in all the media showed significant differences \((p < 0.05)\).

There was no significant difference between the affinity of \textit{L. monocytogenes} to chloroform in BHI without human blood serum and with 5\% human blood serum \((p > 0.05)\). Furthermore, no significant difference was detected between the affinity of this strain to chloroform in BHI media with 10\% and 15\% human blood serum \((p > 0.05)\). Moreover, no significant difference was seen in the affinity of this strain to decane and hexadecane in media without and with 10\% human blood serum \((p > 0.05)\) and between the affinity to these solvents in media with 5\% and 15\% human blood serum \((p > 0.05)\). The affinity of this strain to ethyl acetate in BHI with 5\% human blood serum was significantly higher than that in other media \((p < 0.05)\).

There were significant differences between the affinity of \textit{E. coli} to chloroform in all the media \((p < 0.05)\) except between BHI without and with 15\% human blood serum \((p > 0.05)\). The affinity to
decane and hexadecane in BHI without human blood serum was significantly lower than that in other media (p < 0.05). There was no significant difference between the affinity of this strain to ethyl acetate in BHI without and with 10% human blood serum (p > 0.05) and between that in BHI with 5% and 15% human blood serum (p > 0.05).

The affinity of *P. aeruginosa* to chloroform in BHI without human blood serum was significantly higher than that in other media (p < 0.05). In addition, the affinity of this strain to chloroform in BHI with 15% human blood serum was significantly lower than that in other media (p < 0.05). The affinity of this strain to hexadecane in BHI without human blood serum was significantly higher than that in other media (p < 0.05) whereas no significant difference was seen between the affinity of this strain to hexadecane in BHI with 5% and 15% human blood serum (p > 0.05). The affinity of *P. aeruginosa* to decane in BHI without human blood serum and with 15% human blood serum was significantly lower than that in the other two media (p < 0.05). Finally, the affinity of this strain to ethyl acetate in BHI with 10% human blood serum is significantly higher than that in other media (p < 0.05).

### 4. Discussion

Surface hydrophobicity of bacteria is one of the most important factors that determine physicochemical surface characteristics in relation with air-water interface, oil-water interface, biomaterials, teeth, animal cells, active sludge, and solid surfaces (Habimana et al., 2014). Furthermore, one of other surface physicochemical characteristics of bacteria is electron donation and reception. MATS is a technique based upon the affinity of microbes to monopolar and/or nonpolar solvents. The monopolar solvent may be acidic (electron receptor) or alkaline (electron donor) but both solvents should represent identical surface tensions. The present study was an attempt to determine two surface characteristics (hydrophobicity and electron donation/reception) in four different bacterial strains (i.e. *S. aureus*, *L. monocytogenes*, *E. coli*, and *P. aeruginosa*) in BHI without, with 5%, 10%, and 15% human blood serum via MATS.

The findings of the present study showed that *S. aureus* showed low hydrophobicity and electron donation in the medium without nutrient. According to the results of this study, although low hydrophobicity and electron donation of this strain were detected in all the media, addition of nutrients and increase in nutrient concentration caused significant changes in the affinity of this strain to different solvents. This indicates that external factors such as the presence of nutrients in the medium of this strain could cause changes in surface physicochemical characteristics, which is in line with the findings of Hamadi et al. (2004). They stated that changes in the media of this strain could bring about changes in hydrophobicity of this strain. They also uttered that this strain has low level of hydrophobicity and electron donation. Moreover, the results of this study are in line with the results of Kustos, Kustos, Kilár, Rappai, and Kocsis (2003), who concluded that hydrophobicity of *S. aureus* could be influenced by several factors such as nutrients and antibiotics.

Electron donation/reception and hydrophobicity of *L. monocytogenes* was influenced by presence of nutrients in solvent. When nutrient was present, physicochemical characteristics of this strain could be improved. This is very important especially in food science factories. *L. monocytogenes* is gram-positive pathogen in food products and is known as one of the main concerns in food industry. Acidic surfaces in food factories caused by fermentation and subsequent formation of lactic acid influence adhesion properties of microbial cells. The first stage of bacterial adhesion to surfaces is governed by physicochemical interactions. These interactions between surfaces and cells include Van der Waals reactions, electron donation/reception, and electrostatic interactions as well as surface nature and bacterial surface characteristics (Briandet et al., 1999; Giovannacci, Ermel, Salvat, Vendeuvre, & Bellon-Fontaine, 2000). The results of the present study on *L. monocytogenes* revealed that affinity of this strain to ethyl acetate in BHI with 5% human blood serum is significantly higher than that in other media (p < 0.05). Moreover, there was no significant different in the affinity of this strain to this solvent in BHI with 10% and 15% human blood serum (p > 0.05). These results show that presence of nutrient could bring about changes in surface
The results of the present study revealed that in BHI without human blood cell, *P. aeruginosa* showed that highest electron donation and hydrophobicity, which was exposed to a dramatic change after adding human blood serum as a nutrient. Overall, the best surface physicochemical characteristics of this strain was detected in the medium without nutrient. This is in agreement with the results of Bruisma et al. (2001). They claimed that higher hydrophobicity of this strain could indicate that it can be widely used in different areas such as adhesion to contact lenses.

Bacterial cell surface plays a pivotal role in adhesion to surfaces. Infection might spread on medical prostheses, urinal catheters, or contact lenses leading to prosthesis displacement and irreversible damage to patients. Such infections are often caused by *P. aeruginosa*. Surface physicochemical characteristics of bacteria attached to lenses surfaces are interfered by being exposed to antibiotics in lenses preservation solutions. Such solutions reduce adhesion capacity in *P. aeruginosa* (Bruisma et al., 2001).

In addition to nutrient in the medium, other factors might influence hydrophobicity and electron donation/reception by bacterial strains. One of these factors is the presence of antibiotics. Kustos et al. (2003) stated that presence of antibiotics could cause changes in hydrophobicity in studied strains. They mentioned that the presence of antibiotic in fact reduced hydrophobicity of the strains studied with concentration. However, the results of the present study showed that *P. aeruginosa* had significantly higher affinity to chloroform in BHI without nutrient (*p* < 0.05). Furthermore, the affinity of this strain to chloroform in the medium containing 15% human blood serum was significantly higher than that in other media (*p* < 0.05). These results indicate that different concentration of nutrients in media could cause change in hydrophobicity and electron donation/reception of this strain.

The results of this study on *E. coli* showed that addition of nutrient to the medium caused many changes in hydrophobicity and electron exchange in this strain. For example, addition of 5% human blood serum to the medium of this strain increased electron donation considerably compared to that in the medium without nutrient. Moreover, addition of nutrient increased hydrophobicity dramatically in this strain. The results of this study also revealed that affinity to decane and hexadecane in the medium without human blood serum was significantly lower than that in other media (*p* < 0.05). Therefore, with regard to the importance of this strain in different areas, special attention should be given to growth environment of this strain as well as existence of certain substances in these environments. The results of this study were not consistent with the results of Hamadi et al. (2004). They concluded that regardless of ionic concentration, *E. coli* could be considered a hydrophilic strain whereas the results of this study revealed that this strain showed high level of hydrophobicity, especially at the presence of nutrient.

The results of the present study are important in different areas. For instance, *S. aureus* are responsible for different diseases such as endocarditis, osteomyelitis, and external infections (Habimana et al., 2014). The strain is also engaged in infections related to biofilms, which are generally associated with resistance to antibiotics and immune reactions in the host. This is particularly important since surface hydrophobicity and primary adhesion of microorganisms to a given surface are associated with bacterial surface. Adhesion of *S. aureus* to a biotic surface is carried out by proteins related to cell wall such as adhesive matrix molecules.

5. Conclusion
Surface physicochemical characteristics of bacterial strains including their hydrophobicity and electron exchange properties are very important in different areas of medicine and para-medicine to food science and chemical engineering. Information on these characteristics and
their changes in different environments have yielded extensive progress in different fields of science and prevented from many problems caused by different pathogenic strains. The present study determined hydrophobicity and electron donation/reception in four bacterial strains (S. aureus, L. monocytogenes, E. coli, and P. aeruginosa) in BHI medium at four different conditions: without, with 5%, 10%, and 15% human blood serum. The results of this study revealed that different bacterial strains in BHI showed different hydrophobicity and electron exchange characteristics. Furthermore, the results revealed that addition of different concentrations of human blood serum to the media of these strains changed their surface physicochemical characteristics. The findings of this study are of great importance since they can be adopted in variety of decisions made in medical practices. The results obtained from this study regarding bacterial adhesion and surface characteristics through the affinity to variety of solvents can pave the way for making logical decisions in applied microbiology and in treatment operations. These results can be taken into account in different conditions such as determination of pathogens and the ways of cure, evaluation of microbial contaminations in food factories, and in different industries such as chemical and polymer industries. Overall, it can be said that more information on surface physicochemical characteristics of bacteria can be considered a big step toward curing microbial diseases in humans and animals as well as enhancing health level in factories.

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References
Bardiau, M., Szolo, M., & Maini, J. G. (2010). Initial adherence of EPEC, EHEC and VTEC to host cells. Veterinary Research. 41(5), 57. doi:10.1051/vetres/2010029
Bellon-Fontaine, M. N., Roult, J., & Vass, C. J. (1996). Microbial adhesion to solvents: A novel method to determine the electron-donor/electron-acceptor or Lewis acid-base properties of microbial cells. Colloids and Surfaces B: Biointerfaces, 7(1–2), 47–53. doi:10.1016/0927-7765(96)00172-6
Brandt, R., Leriche, V., Carpentier, B., & Bellon-Fontaine, M. N. (1999). Effects of the growth procedure on the surface hydrophobicity of Listeria monocytogenes cells and their adhesion to stainless steel. Journal of Food Protection, 62 (9), 994–998. doi:10.4315/0362-028X-62.9.994
Bruinsma, G. M., Van der Mei, H. C., & Busscher, H. J. (2001). Bacterial adhesion to surface hydrophilic and hydrophobic contact lenses. Biomaterials, 22(24), 3217–3224.
Busscher, H. J., & van der Mei, H. C. (2012). How do bacteria know they are on a surface and regulate their response to an adhering state? PLoS Pathogens, 8(1), e1002440. doi:10.1371/journal.ppat.1002440
Forbes, B. A., Sahm, D. F., & Weissfeld, A. S. (2007). Study guide for Bailey & Scott's diagnostic microbiology (12 ed.). St. Louis: Mosby, Elsevier.
Gallardo-Moreno, A. M., Navarro-Pérez, M. L., Vadillo-Rodriguez, V., Brrique, J. M., & González-Martin, M. L. (2011). Insights into bacterial contact angles: Difficulties in defining hydrophobicity and surface Gibbs energy. Colloids and Surfaces B: Biointerfaces, 88(1), 373–380. doi:10.1016/j.colsurfb.2011.07.016
Giovannacci, I., Ermel, G., Salvat, G., Vendeuvre, J. L., & Bellon-Fontaine, M. N. (2000). Physicochemical surface properties of five Listeria monocytogenes strains from a pork-processing environment in relation to serotypes, genotypes and growth temperature. Journal of Applied Microbiology, 88(6), 992–1000. doi:10.1046/j.1365-2672.2000.01057.x
Habimana, O., Semindo, A. J. C., & Casey, E. (2014). The role of cell-surface interactions in bacterial initial adhesion and consequent biofilm formation on nanofiltration/reverse osmosis membranes. Journal of Membrane Science, 454, 82–96. doi:10.1016/j.memsci.2013.11.043
Hamadi, F., Latrache, H., El Ghmari, A., Elouaifi, M., Mabrouki, M., & Kourider, N. (2004). Effect of pH and ionic strength on hydrophobicity and electron donor and acceptor characteristics of Escherichia coli and Staphylococcus aureus. Annals of Microbiology, 54, 213–226.
Hori, K., & Matsumoto, S. (2010). Bacterial adhesion: From mechanism to control. Biochemical Engineering Journal, 48(3), 424–434. doi:10.1016/j.bej.2009.11.014
Hsu, L. C., Fang, J., Borca-Tasciuc, D. A., Worobo, R. W., & Moraru, C. I. (2011). Effect of micro- and nanoscale topography on the adhesion of bacterial cells to solid surfaces. Applied and Environmental Microbiology, 79(8), 2703–2712. doi:10.1128/AEM.03436-12
Katsikogianni, M., & Missiliris, Y. F. (2004). Concise review of mechanisms of bacterial adhesion to biomaterials and of techniques used in estimating bacteria-material interactions. European Cells & Materials, 8(3), 37–57. doi:10.22203/ecm
Krachler, A. M., & Orth, K. (2013). Targeting the bacteria-Host interface: Strategies in anti-adhesion therapy. Virulence, 4(4), 284–294. doi:10.4161/viru.24530
Kustos, T., Kustos, J., Kilár, F., Rappai, G., & Kocsis, B. (2003). Effect of antibiotics on cell surface
hydrophobicity of bacteria causing orthopedic wound infections. Chemotherapy, 49(5), 237–242. doi:10.1159/000072447
Pizarro-Cerdá, J., & Cossart, P. (2006). Bacterial adhesion and entry into host cells. Cell, 124(4), 715–727. doi:10.1016/j.cell.2006.02.012
Prokopovich, P., & Perni, S. (2009). An investigation of microbial adhesion to natural and synthetic polysaccharide-based films and its relationship with the surface energy components. Journal of Materials Science: Materials in Medicine, 20, 195–202.
Saralaya, V., Bhat, G., Kamath, A., & Shivananda, P. G. (2004). Effect of trace elements on surface hydrophobicity and adherence of Escherichia coli to uroepithelial cells. Indian Journal of Experimental Biology, 42, 681–685.
Tenke, P., Kovacs, B., Jäckel, M., & Nagy, E. (2006). The role of biofilm infection in urology. World Journal of Urology, 24(1), 13–20. doi:10.1007/s00345-006-0100-4
Wizemann, T. M., Adamou, J. E., & Longermann, S. (1999). Adhesins as targets for vaccine development. Emerging Infectious Diseases, 5(3), 395–403. doi:10.3201/eid0503.990310
Zeraik, A. E., & Nitschke, M. (2012). Influence of growth media and temperature on bacterial adhesion to polystyrene surfaces. Brazilian Archives of Biology and Technology, 55(4), 569–576. doi:10.1590/S1516-89132012000400012