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Evaluation of the ability of some bacterial species isolated from UTI to form biofilm

Authors Names	ABSTRACT
<p>a. Dhuha Mahdi Jabir</p> <p>Article History Received on: Revised on: Accepted on:</p> <p>Keywords: UTI, biofilm, Congo red agar, tube method</p> <p>DOI: https://doi.org/10.29350/jops.2022.27.1.1492</p>	<p>Urinary tract infection is one of the most serious infectious diseases in the world, This study aimed to isolate and diagnose the bacteria that cause UTI and then evaluate its ability to form a biofilm. 100 urine samples were collected for a group of patients attending Al-Diwaniyah Teaching Hospital, who were confirmed to have UTIs at different ages. The results showed the presence of bacteria. <i>Escherichia coli</i> and <i>Proteus spp</i> in all samples(100%), while <i>Staphylococcus aureus</i> was found in 87% of samples, <i>Enterobacter spp</i> was isolated by 67%, and <i>Pseudomonas aeruginosa</i> by 65%. <i>Klebsiella pneumonia</i> at 50 % and <i>Staphylococcus epidermidis</i> and <i>Micrococcus spp</i> each with 30%. Regarding the ability of the isolated bacteria to form a biofilm, two methods used Congo-red agar and tube method, and the results were as follows 64% for <i>E.coli</i>, <i>K pneumonia</i> with 66.6% while <i>S. aureus</i> showed the ability of formation with 41.6%, and negative results for the rest by using tube method while Congo-red agar method results were 25% for 50% for <i>E.coli</i> and <i>S. aureus</i>. The study concluded that Congo red agar method is easy to perform and interpret, while the tube method is highly sensitive and specific.</p>

1.Introduction

Biofilms are defined as microbial communities whose cells are loosely bound to the surface. It is inverted and is embedded in an extracellular polymeric substance produced by its cells, and these cells appear with a different phenotype in terms of growth rate and genetic reproduction (Donlan, and Costerton 2002). (A biofilm is an assembly of bacterial cells attached to the surface of a non-reversible, easily removed surface and embedded in a polymeric material outside the cellular (Gunardi 2021)) Within the biofilm, there are non-cellular materials that depend on the environment in which it is formed, such as mineral crystals, sandy or clay particles, blood components, and erosion products)(Prasad., *et al* 2012) The microstructures contained within a biofilm differ from their counterparts in planktonic cells in that they have a lower growth rate and high resistance, and their gene expression is different, as it was found that movement genes are inhibited after the bacteria bind to the surface, and the exchange rate of genetic material within the biofilm exceeds that found in floating cells, allowing antibiotic resistance genes to spread (Murray., *et al* 2020) Biofilms can be formed on a wide range of surfaces, such as living tissues, implanted medical instruments, water pipes, drinking and natural aquatic ecosystems.

The researchers (Dunne and Jr 2002). indicated that there are three important components of biofilm, which are: bacteria, extracellular matter, and surface. If one of these components is missing, the biofilm will not be formed. The ability to form biofilm is one of the most prevalent and virulent factors in bacteria, and this ability can be found in bacteria living in the external environment or pathogens (Holla and Ruzicka 2011) Infections related to the presence of biofilms are considered a major problem because they counteract the host's immune defenses (. Petersen, and McLaughlin 2016) Because of the significant increase in the incidence of hospital-acquired infections in Iraq, which can be attributed to a variety of factors, including medical tools and the problems related to their use (Sampaio., et al 2016), especially those that are stuck in the body of the patient, like urinary catheters and the urinary tract infections related to their use (Hancock ., et al 2010), which is mainly due to their being biofilms that have a major role in pathogenesis as well as a lack of studies on this aspect (Holla, and Ruzick 2006).

Many studies have suggested tube and Congo red agar as efficient methods in the detection of biofilm formation as they depend on the improvement of the production of polysaccharides using rich nutritional media. Broth soy tryptic medium is used by the tube method and infusion heart-brain broth with the addition of 5% sucrose in the Congo red method. It was proved that the dye Congo red was chosen as the stain to show the presence of polysaccharides of gram-negative bacilli (Hassan., et al 2011)

This research focuses on the ability of some types of bacteria associated with UTIs to form biofilms in two different methods: the tube and Congo red agar methods.

2.Methodology

Specimen collection: urine samples were collected for 100 patients who attended Al-Diwaniyah Hospital after being confirmed to have UTI by cultivation of the samples on blood and MacConkey agar, for one month starting from May 2021 until June 2021 at different ages.

Isolation and Identification: Urine samples were cultured on the medium of blood and MacConkey agar, for 24 hours at 37C, the developing bacteria were diagnosed by using Gram stain classified into their different species using many biochemical tests. As indol, methyl red, vegas pros kour, citrate utilization, coagulase, oxidase and catalase tests which were done manually.

Investigating the ability of isolated bacteria to form biofilms

Congo red agar medium use: The isolated bacteria were inoculated on Congo red medium and incubated at 37°C for 24 hours, after the incubation period, the membrane-forming isolates were investigated vital in terms of the appearance of colonies with a dry or shiny black appearance, while non-membrane-forming isolates appear Wine, red, or vivid pink (Parsek ., et al 2003) .

Tube method: A full loop from the growing colonies of the studied bacteria were transferred to container glass test tubes. on broth soy tryptic medium with 1% glucose added and incubated at 37°C for 24 hours. after the incubation period, the samples were poured out and the tubes were washed with phosphate-buffered saline, then dried, and then dyed with crystal violet dye in concentration 1% for three minutes, then the excess dye was poured out and washed with deionized water. and then left the tubes to dry upside down to observe the formation of biofilms on the inner walls of tube-shaped violet (Parsek ., et al 2003)

3.Results & Discussion

The ability of bacteria to form a biofilm is one of the most important indicators of its pathogenicity and its resistance to antibiotics, In the current study, the bacteria causing urinary tract infection were isolated and diagnosed for a group of patients in Al-Diwaniyah Teaching Hospital, and then the ability of the isolated bacteria to form biofilm was investigated in two different ways. Table 1 shows the isolated bacteria and their percentages, Where it is clear that *E.coli* and *Proteus spp* were isolated from all samples, while the rest of the species, as the table shows, were isolated in varying proportions, These species are common types of UTI cases, according to the EUA guideline on urogenital infections which updated at 2018(Kumari .,et al 2017)

Table 1 the percentage of isolated bacteria from UTI patients

Bacteria	Percent of isolation
<i>Escherichia coli</i> and <i>proteus spp</i>	100%
<i>Staphylococcus aureus</i>	87%
<i>Enterobacter spp</i>	67%
<i>Pseudomonas aeruginosa</i>	65%
<i>Klebsiella pneumonia</i>	50%
<i>Staphylococcus epidermidis</i> and <i>Micrococcus spp</i>	30%

As regards biofilm formation using the tube method the results appear in terms of biofilms on the inner walls and bottom of the tubes are colored with a violet layer figure 1, table 2 show the results of biofilm formation using the tube method



Figure 1 the biofilm formation using the tube method

Table (2) number of positive and negative bacteria in biofilm formation by tube method

Bacterial species	percentage of positive samples	percentage of negative samples
<i>S.aureus</i>	41.6%	58.4%
<i>S.epidermidis</i>	0%	100%
<i>Micrococcus spp.</i>	0%	100%
<i>P.aeruginosa</i>	33.33%	66.66%
<i>E.coli</i>	64%	36%
<i>Klebsiella.pneumonia</i>	66.66%	33.33%
<i>Proteus spp</i>	28.57%	71.43%

The Congo red agar positive results appear as a dry or shiny black colony, while non-membrane-forming isolates appear to be black, vivid, pink, red, or wine-colored, figure (2), table No 3 explain the number and percentages of bacteria that form biofilm by Congo red agar method.

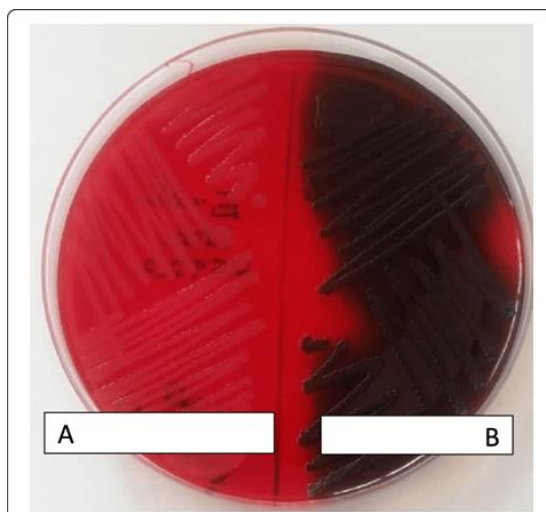


Figure 2 A: the bacteria which are unable to form biofilm formation on Congo red agar
B: the bacteria which can form biofilm formation on Congo red agar

Table (3) number of positive and negative bacteria in biofilm formation by Congo Red agar method

Bacterial species	percentage of positive samples	No. and percentage of negative samples
<i>S.aureus</i>	25%	75%
<i>S.epidermidis</i>	0%	100%
<i>Micrococcus spp.</i>	0%	100%
<i>P.aeruginosa</i>	22.22%	77.77%
<i>E.coli</i>	50%	50%
<i>Klebsiella pneumonia.</i>	33.33%	66.66%
<i>Proteus spp</i>	14.28%	85.7%

Several studies indicated that the change in color by the method of Congo red agar occurs in the final stages of bacterial growth due to the presence of secondary metabolites and the use of sucrose or glucose at 5% gave similar results as an essential factor for determining the production of an exogenous layer of the biofilm (Khan., *et al* 2011)

From the results of the current study, it is noted that there is a discrepancy in the ability of bacteria to form biofilms in tube and Congo red agar methods, and this disparity is due to the difference in the specialization and sensitivity of both methods; this has been confirmed by many studies [17]. The Congo red agar method is easy to perform and interpret, while the tube method is highly sensitive and specific. Many studies mentioned that the specificity of the method of sticking to the tubes was calculated based on the presence of *ica* genes using the PCR technique. The results showed 100% sensitivity and 100% specificity for the tube method when performed on CNS coagulase-negative staphylococci, while the Congo red method showed 89% sensitivity and 100% specificity compared to PCR.

The obtaining results in the current study are partially agree with Havaei, and his group (2010) results. in terms of the role of *staphylococci* in urinary tract infection and their ability to form a biofilm, also Hasan., *et al* (2011) isolated the bacteria that can form biofilm from urinary catheters by the Congo Red agar method. with a percentage of 48.33% for *E.coli* of isolates and *S.aureus* percentage was 35.8%.

While the findings of this study do not agree with those of Gunardi *et al.* (2021). His findings revealed that when using the tube method, 7.64% of the isolates were *E. coli* bacteria, 33.5% were non-forming to them, and *P. aeruginosa* isolates were unable to form biofilms.

The results of researcher Hola and his group (2010) showed that the bacterium *P. aeruginosa* gave 5.74%. The biofilm was strong, 1.14% in medium, 4.9% weak, and 2% were non-forming, while the results of the bacterium *P. vulgaris* showed that 4 isolates formed the biofilm strongly and 2 on an average. 3% were considered able to form it strongly, 3% were considered medium, and 3% were non-forming.

As for *E. coli*, It gave 7.34% strong biofilm formation and 3.42% medium composition. 1.21% is weak and 9.1% is not. As for the bacterium *S. aureus*, it was found that 100% of its isolates were vigorously biofilm-forming. While the results of researcher Khan and his group (2011) on 262 isolates were selected to test their ability to form biofilm by the tube method, 35 isolates (3.133%) are strongly positive, and the number of The largest of the isolates is positive, medium, 3.50 % and 2.36 %. No biofilm was shown.

When conducting the Congo red method, it was found that 182 isolates gave black colonies and in 125 isolates of them 7.47 (%) black colonies with a dry crystalline appearance gave an indication of biofilm formation, while 75 isolates gave black colonies, but they were neither dry nor crystalline, and these were considered negative isolates to form the membrane of the biofilm. Eighty isolates (5.30%) gave pink colonies that were negative for biofilm formation.

The researchers, Hola and Ruzicka (2011), mentioned that a higher percentage of species are positive for biofilm formation.

The strength is *E. faecalis* with a percentage of 95% and *P. mirabilis* with a percentage of 94%, while the yeast *Candida* accounted for 91%, while the weak percentage of *E. coli* isolates was 35%.

The researchers demonstrated that biofilm infections from common problems such as urinary tract infections,

catheter infections, middle ear infections, plaque formation on teeth, gingivitis, contact lens injuries, and less commonly but more serious endocarditis, cystic fibrosis, and permanent injuries to medical devices such as artificial joints and heart valves.

Conclusion

In general, it is noted from the results of the current study that there is a discrepancy in the ability of pathogenic microbes to form biofilms and that most of the studied isolates were composed of them, and this may help protect other germs incapable of forming biofilms from the host's defenses. The action of antibiotics enhances the formation of communities of biofilms of multiple microbial species that benefit from coexistence, as is the case with the production of β -lactamases and the secretion of urease and other virulence factors..

Conflict of Interest: The authors declare that they have no conflict of interest.

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