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Biofilm production by *E. Coli* Isolated from Filter Water System

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Abstract

E. Coli is recognized as part of humans and animals microbiota and also an important opportunistic pathogen, responsible for a variety of diseases. A total of 10 *E. coli* were isolated from filter water system at different sites from Baghdad city and investigated for their ability to form biofilm by using 96-well microtiterplate. The result showed strains with high coherent to PVC. The antimicrobial resistant of four antibiotic against *E. Coli* were determined in vitro and the highest resistant was to β-lactam antibiotics. The biofilm in environment, are vital for the existence of many microorganisms, and provided pathogenic microorganisms with a ways of persisting outside of the host to represent means for susceptible population to be repeated infection.

Keywords: *Biofilm, Water, E. Coli.*

Introduction

A biofilm is a one or group of microorganisms found in structural community surrounded by slime layer of exopolysaccharides which gave it the ability to adhere to living and nonliving surface. Many surface in the environment are susceptible for bacterial biofilm like stones surfaces, small rock in a stream, swimming boll, showers and the most observable example of biofilm blockage water distribution pipes that consider an important problem in industry and in the domestic [1,2].

E. Coli is prevalent pathogen belong to family *Enterobacteriaceae* and found in the environment as well as mucosal surfaces of humans and other mammals [3,4]. *E. Coli* strains possess many cell surface components that facilitate to biofilm formation which contribute infection and survival in the host, many studies suggest that ability of *E. coli* isolates to form biofilms in vitro under a variety of growth conditions, so formation of biofilm may also be an considerable virulence factor for *E. Coli*, also it has been revealed bacterial growth with the biofilms are high resistance to treatment with antibiotic [5].

The study investigated formation of biofilm by *E. Coli* that isolated from different sites of water in Iraq/ Baghdad, also the ability of these isolates resistant to antibiotic was demonstrated.

Material's and Methods

Isolates of E. Coli

A total of 10 isolates of *E. Coli* were analyzed. Samples were collected by using sterilized swaps, the collected samples tacked from different sites then transport to the laboratory for culturing and analysis, by using Macconkey agar (HiMedia) and EMB agar, then *E. Coli* isolates were tested by API20E system of gram negative bacteria to confirm the results (BioMrrieux, France).

Antimicrobial susceptibility tests

The antibiotic resistance of. The isolates of *E. Coli* was tested for their ability to resistant of selected antibiotic through the dissemination of antimicrobial

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agents on Mueller- Hinton agar according to Kirby and Bauer, (1966) and the recommendations of the clinical laboratory standards of the national committee [6].The antibiotic discs were gotten from (Bioanalyse/Turkey). The following antimicrobial discs (concentrations in µg) were used: Ceftriaxone (CRO) (30), Ampicillin(25), Amikacin (AK) (30), and Ciprofloxacin (CIP) (5)

Formation of biofilm in vitro

Biofilm Formation in 96-hole microtiterplate

The capability to form biofilm by the isolates of *E. Coli* in 96 well microtiterplate (the basic component is PVC) was determined and depending on O'Toole procedure [7,8]. The biofilm examines were achieved by using minimal medium (M63) complemented with CAA, glucose, and MgSO4, then ELISA plate reader was used to read the plate (Beckman coulter,Austria). Isolates were considered as efficient biofilm-forming when absorbance at 595 nm after crystal violet staining was equal to or more than 0.15.

Results

In this study 10 isolates of *E. Coli* exhibited highest resistant to ampicillin reach to 100% followed by piperacillin+ tazobactam 75% while these isolates had highest susceptibility to gentamicin and Ciprofloxacin as show in Table1.

The bacteria showed highly adherent to PVC microtiterplate and this indicated formation of biofilm, the result was confirmed after staining of adherent bacteria then quantified and measuring the OD of the accumulated mass of biofilm. In microtiterplate 4 of 10 strains were produce biofilm and range between (0.63-0.4) (Figure2).

Figure1: *E. Coli* isolates on eosin methylene blue

Figure2: *E. Coli* isolates forming biofilm in microtiter plate

Table.1 Susceptibility of the 10 isolates of *E. Coli* to 4 antibiotics.

No.	Antibiotics	Resistant (%)
1	Ampicillin	100%
2	Amikacin	0%
3	Ciprofloxacin	0%
4	Piperacillin/ tazobactam	75%

Discussion

E. Coli is gram(-ve) bacteria which is found ubiquitously in the environment. *E. Coli* bacteria are an etiological factor in many infections, the most frequent are those of the urinary tract infection that can lead to kidney failure, septic infections and severe human gastrointestinal disease with diarrhea due to production of a toxin from pathogenic strains [9].

The virulence of certain *E. Coli* strains is caused by some structural antigens of the cell, including the fimbriae or pili, nonfimbrial adhesion factors, toxins, lipopolysaccharides, factors of acquisition iron and polysaccharide capsules [10,11].

E. coli is consider a normal flora or indigenous microbiota of the small intestine. Some strains of *E. coli* are pathogens that cause many disease. The ability of *E. Coli* to colonize the gastrointestinal tract of human rely on adherent factor like pili that found in both pathogenic and normal flora in the human gut that enable the bacteria to attached to mucosal surfaces then may *E. coli* produce biofilm to protect themselves from stresses that encounter in the gastrointestinal tract [12].

The results microtitration assay cleared the ability of isolated strain to production biofilm. A number of research studies define assembling of microorganisms as biofilms that can develop inside water system tubes like pipes and another surfaces and have revealed that *E. coli* which is a part of coliform bacteria can be an important contributor in biofilm formation with microbial populations that can propagate in the distribution drinking water systems [13,14,15].

Also Murthy and Venkatesan [15] reported that biofilms are omnipresent in industrial and distribution systems of drinking water and biofilms contribute as a major source of to the fouling of pipes and underwater surfaces in industrial water systems.

The ability of *E. Coli* to make biofilm that consider significant virulence factor and presence of this bacteria in water enabled the colonization on abiotic and biotic surfaces, So the capability of *E. Coli* to stick on abiotic surfaces particularly the water system tubes was examined by using (PVC) polyvinyl material which is the basic material of water tubes and the result revealed that the isolates was able to production biofilm. Collectedly biofilm production, existence of bacteria in water, may have virulence factor as well as antibiotic resistant made the bacteria made it frightening sources of pollution and must alarming the community about it with taking special steps to control it.

Also in this study we investigated *E. Coli* resistance to antibiotics and results showed highest resistance to β-Lactam antibiotic. The excessive use of antibiotics caused increasing resistance of bacteria to antimicrobial agents in worldwide, one of the reasons of this resistance that spreading of resistant strain to the environment like agricultural areas via sewage water, treatment plants and receiving water [16]. In the environment, bacteria may exchange genetic material and this resulted in appearance of new resistant strains. The bacteria found in water which are from diverse origins like animal, environmental and human are develops resistance to antibiotic because they able to mix. So the existence of bacteria that resist antibiotic in surface water and sewage

and may transferred to drinking water is consider an increasing public health problem [17].

It is also community-acquired *E. Coli* is particular concern because the appearance of extended spectrum β-lactamase (ESBL)-producing strains. It was reported many strain with increased antimicrobial resistance had been isolated as consequences of repeating antimicrobial courses which may promote the development of antimicrobial resistant *E coli* in the gut flora and this may lead to dissemination of antimicrobial resistant *E coli* to the environment especially water [18]. So it must take steps to control their development and spread to the environment.

Conclusions

The biofilm in environment, are vital for the existence of many microorganisms, and provided pathogenic microorganisms with a ways of persisting outside of the host to represent means for susceptible population to be repeated infection. The ability of *E. Coli* to make biofilm and resistance to antibiotics is particular concern because of dissemination of antimicrobial resistant *E coli* to the environment especially water. So it must take steps to control their development and spread to the environment.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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