Investigation of Biofilm Formation in Clinically Acquired *Escherichia coli* Strains

Klinik Kaynaklı *Escherichia coli* Suşlarında Biyofilm Oluşumunun Araştırılması

Research Article

Nermin Hande Avcıoğlu*, Gülcan Şahal and Işıl Seyis Bilkay
Hacettepe University, Faculty of Science, Department of Biology, Beytepe, Ankara, Turkey

ABSTRACT

In this study, investigation of biofilm formation levels of clinically acquired *Escherichia coli* strains and examination of each *E. coli* strains' clinical information such as clinical material and service units according to their biofilm formation results were determined. In this respect, according to our results; *E. coli* strains are grouped as 31% Strong Biofilm Former (SBF), 27% Intermediate Biofilm Former (IBF), 25 % Weak Biofilm Former (WBF) and 17% None Biofilm Former (NBF). In addition to this, clinical materials of wound and urine were found as the most frequent clinical materials from which strong biofilm Former *E. coli* strains isolated. Besides, urology and cardiology were found as the most SBF isolated service units. Apart from these, *E. coli* strains were mostly isolated from urinary tract infections and from women who are at the period of post-menopausal. Lastly, the antibiotic susceptibility patterns were investigated and the greatest susceptibility was observed against amikacin and the least susceptibility was observed against trimethoprim/sulfamethoxazole. Accordingly, NBF strains of *E. coli* were more susceptible to eight antibiotics than Strong Biofilm Former (SBF) strains.

Key Words
Biofilm Formation, *Escherichia coli*, antibiotic susceptibility.

ÖZET

Bu çalışmada, klinik materyallerden elde edilen *Escherichia coli* suşlarının biyofilm oluşturma yetenekleri araştırıldı ve her bir *E. coli* suşunun klinik materyal ve servis ünitesi bilgileri biyofilm oluşturma sonuçları ile bir arada değerlendirildi. Bu bağlamda çalışmamızın sonuçlarına göre *E. coli* suşlarının %31'i Yüksek Biyofilm Oluşturan (YBF), %27'si Ilımlı Biyofilm Oluşturan (IBO), %25'i Zayıf Biyofilm Oluşturan (ZBF) ve %17'si ise Biyofilm Oluşturmayan olarak gruplandırıldı. Buna ek olarak klinik materyallerden yara ve idrarın en yüksek sıklıkta izole edilen klinik materyaller olduğu saptandı. Ayrıca Yüksek Biyofilm Oluşturan (YBO) suşların en fazla üroloji ve kardiyoloji servislerinden izole edildiği gözlendi. Bunların yanı sıra, *E. coli* suşlarının post-menapozal dönemdeki üriner yol enfeksiyonu geçiren kadınlardan en fazla sıklıkta izole edildiği saptandı. Son olarak *E. coli* suşlarının antibiyotik hassaslık paternleri incelendiğinde en yüksek hassaslığın amikasin antibiyotiğine, en düşük hassaslığın ise trimetoprim sülfametaksazol antibiyotiğine karşı olduğu gözlendi. Bununla ilişkili olarak Biyofilm Oluşturmayan *E. coli* suşlarının çalışmada kullanılan 8 antibiyotiğe karşı Yüksek Biyofilm Oluşturan (YBF) *E. coli* suşlarına kıyasla daha hassas oldukları belirlendi.

Anahtar Kelimeler
Biyofilm oluşumu, *Escherichia coli*, antibiyotik hassasiyeti.

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Correspondence to: N.H. Avcıoğlu, Hacettepe University, Faculty of Science, Department of Biology, Beytepe, Ankara, Turkey.
Tel: +90 (312) 297 80 24 Fax: +90 (312) 299 20 28 E-Mail: hurkmez@hacettepe.edu.tr

INTRODUCTION

E. coli causes a variety of infections in peritoneum, blood, gastrointestinal and especially in urinary tract and also identified as one of the main common agents of gram negative acquired bacterial infections. Accordingly, *E. coli* strains are isolated up to 90% of community-acquired and approximately 50% of nosocomial UTIs [1,2]. The surfaces of medical devices such as implants and catheters triggered the formation of biofilms and can cause the spread of biofilm acquired infections. Following this, complicated treatment period of these infections may be affected by increasing antibiotic resistance of biofilm Former strains. Furthermore, by transferring horizontal genes between bacterial species, the spread of antibiotic resistance become a huge problem in medical case [3]. Especially, by means of biofilms, bacteria become more resistant than their planktonic forms to antibiotics, disinfectants, extreme temperatures, sanitizers and other extreme environmental [4]. Therefore, in this study; biofilm formation levels and antibiotic susceptibilities of *E. coli* strains were examined and these results were compared with the clinical prevalences of isolated *E. coli* strains in order to take precautions against biofilm acquired *E. coli* infections.

MATERIALS AND METHODS

Bacterial strains

In this study; *E. coli* strains which were isolated from a hospital in Ankara, Turkey and were obtained from three different clinical materials (urine, tracheal aspirate and wound) and from eight different services (urology, surgical intensive care unit, endocrinology, emergency, neurology, pediatrics, gynecology and cardiology) were used. All isolated strains were identified by phenotypical methods [5] and were inoculated in to the Brain Heart Infusion Broth media including 10% glycerol and stored at -20°C for later analysis.

Biofilm Formation

In order to investigate biofilm formation of *E. coli* strains, modified method of Crystal Violet Binding Assay described by O'Toole, was used [6]. In this method, *E. coli* strains were sub cultured in to the Brain Heart Infusion Broth at 37°C overnight. After the incubation period, these cultures were diluted to the proportion of 1:100 and transferred in to the 24-well polystyrene plates and incubated for 24 hours at 37°C. Then, the wells were washed and stained with 1% crystal violet. Following this, bound crystal violet in each well was solubilized by addition of ethanol (96%). Finally, the absorbance of solubilized crystal violet for each well was measured at 540 nm. The experiment was performed in triplicate. According to *E. coli* strains' biofilm formations, they were classified into four categories as follows: $0 \leq OD < 0.4$- None Biofilm Former (NBF), $0.4 \leq OD < 0.8$- Weak Biofilm Former (WBF), $0.8 \leq OD < 1.2$- Intermediate Biofilm Former (IBF) and $OD \geq 1.2$- Strong Biofilm Former (SBF).

Antibiotic Susceptibility Testing and Antibiotyping

In order to determine antibiotic susceptibilities of *E. coli* strains to eight different antibiotics (Tobramycin: 10 µg, Amikacin: 30 µg, Ceftazidime: 30 µg, Trimethoprim/sulfamethoxazole: 1.25 µg/23.75 µg, Ciprofloxacin: 5 µg, Gentamicin: 10 µg, Cefoperazone/Sulbactam: 75 µg/30 µg, Levofloxacin: 5 µg), Kirby-Bauer disc diffusion method was used. The strains were classified as Resistant (R), Intermediate (I) or Sensitive (S) according to the zone table which was proposed by CLSI (Clinical and Laboratory Standards Institute) and the percentage of antibiotic resistances of *E. coli* strains were calculated.

RESULTS AND DISCUSSION

Medically acquired biofilms, composed of embedded microbial communities in self-produced matrix and it is very difficult to eradicate with them because of their highly increased resistance towards antibiotics and some disinfectants [7,8]. Besides, biofilm Former species differ from their planktonic forms because of having different metabolic states, host immunity, resistances to conventional antibiotics and biocides [9-11]. In this respect, it is very important to identify biofilm Former medically acquired strains and investigate these strains' antibiotic resistance profiles in order to take precautions against bacterial infections. As well as being a predominant species of gastrointestinal tract, *E. coli* is also known

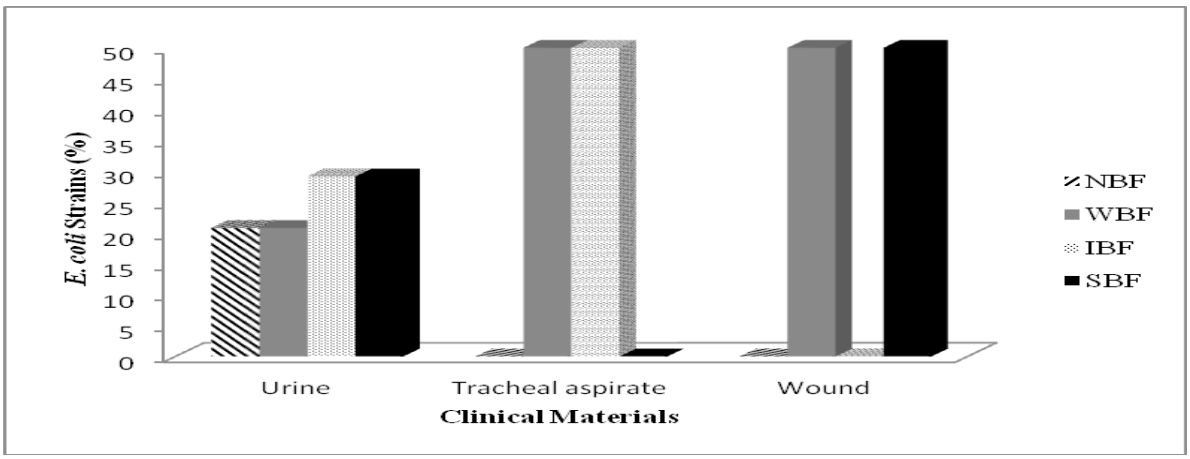

Figure 1. Percentage of *E. coli* strains in different clinical materials according to their biofilm formation levels. (*NBF: None Biofilm Former, WBF: Weak Biofilm Formers, IBF: Intermediate Biofilm Formers, SBF: Strong Biofilm Formers*).

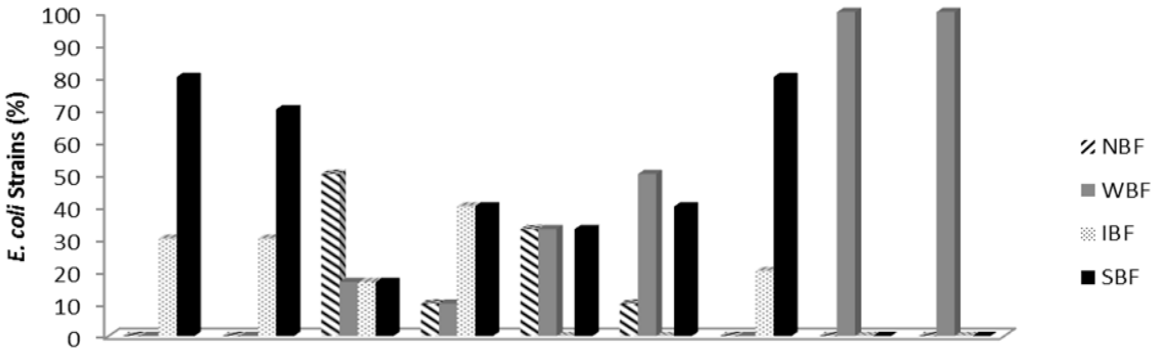

Figure 2. Percentage of *E. coli* strains in different service units according to their biofilm formation levels. *(URO: Urology, S.I.C.: Surgical Intensive Care, END.: Endocrinology, EM: Emergency, IM: Internal Medicine, GYN.: Gynecology, CAR.: Cardiology, NEU.: Neurology, PED.: Pediatry, NBF: None Biofilm Former, WBF: Weak Biofilm Formers, IBF: Intermediate Biofilm Formers, SBF: Strong Biofilm Formers).*

as an important opportunistic and nosocomial pathogen in a variety of infections [12]. In this respect, biofilm formation abilities of different *E. coli* strains were investigated in different researches and it is found that this bacterium is able to form biofilms both in vivo and in vitro conditions [9, 13-16]. Accordingly, in this study in vitro biofilm formation of clinical acquired *E. coli* strains were investigated and *E. coli* strains are categorized according to their biofilm formation abilities as 31% SBF, 27% IBF, 25% WBF and 17% NBF.

E. coli strains are known as causative agents of both community and nosocomial infections among gram negative pathogens especially in urinary tract infections (UTI) which is responsible for more than 40% of all cases in acute-care units [8,17]. Additionally; infected urinary, cardiovascular catheters and prostheses are served as biofilm Former surfaces. By means of its capability of Former and colonizing on catheters, *E. coli* colonization is comprehensive and also it is the most isolated bacteria in UTI's owing to the long term usage of urethral catheters in hospitalized patients [11,18]. Additionally, if biofilm is formed on the surface of a medical device, treatment becomes harder and it is very difficult to diagnose microorganism in this sessile bacterial community. In that case, by sampling different clinical materials such as bones, blood, swabs or soft tissues, identification may occur [19]. Accordingly, in the literature it is also found that 90% of all bloodstream infections are also related with catheter contaminations by biofilm Former species [11]. In this respect, this research assessed biofilm formation abilities of *E. coli* strains which were isolated from different

Table 1. Percentage of *E. coli* strains according to their isolated patients' age and sex.

Sex	≤15	16-30	31-45	46-60	61-75	76-90	All
Famale	9%	17%	13%	17%	30%	14%	93%
Male	40%	17%	13%	17%	30%	20%	7%

Table 2. Percentage of *E. coli* strains displaying susceptibility to 8 different antibiotics according to their biofilm formation levels.

Antibiotics	NBF	WBF	IBF	SBF	All Strains
NN	100%	85.72%	75%	50%	75%
AN	100%	100%	100%	100%	100%
CAZ	100%	100%	87.5%	75%	85.72%
SXT	80%	71.43%	62.5%	50%	62.29%
CIP	100%	71.43%	62.5%	50%	64.29%
GN	100%	100%	75%	62.5%	82.15%
SCF	100%	100%	87.5%	75%	89.29%
LEVO	100%	85.72%	75%	75%	82.15%

(NN: Tobramycin, AN: Amikacin, CAZ: Ceftazidime, SXT: Trimethoprim/sulfamethoxazole, CIP: Ciprofloxacin, GN: Gentamicin, SCF: Cefoperazone: Sulbactam, LEVO: Levofloxacin, NBF: None Biofilm Former, WBF: Weak Biofilm Formers, IBF: Intermediate Biofilm Formers, SBF: Strong Biofilm Formers).

clinical materials and it is found that wound and urine are the most SBF isolated clinical materials respectively among all SBF *E. coli* strains (Figure 1). Also, biofilm formation levels in collected services of *E. coli* were also investigated and it is found that urology and cardiology are the most SBF isolated service units respectively (Figure 2). Accordingly, in this research all SBF strains were obtained from hospitalized patients and especially in cardiology and urology units, so in the view of these results they may be occurred by blood or urinary catheters and seen as nosocomial.

By means of hormonally changes in the pH values of vaginal flora, UTIs are mostly common in the post-menopausal period of women. Before menopause, estrogens secreted in different levels and this secretion encouraged *Lactobacillus* which produces lactic acid and decrease pH value of vagina, so vaginal flora protected from colonization of uropathogens. But in post-menopausal period, hormonal changes occur and by increasing pH value of vagina, *Enterobacteriaceae* family especially *E. coli* are being colonized and UTIs are mostly seen in woman which are at the stage of menopause [20]. Besides, 40% of each woman are also encountered with UTIs in their lives and approximately the incidence of UTI was 0.5 – 0.7 per person/year and also sexually active woman are being at risk of UTIs [21-23]. In the view of these results, similarly *E. coli* strains were also isolated mostly from women whom are especially sexually active and at the post-menopausal period in this study (Table 1).

Bacteria in biofilms differs from their sessile forms because of having extraordinary resistance to antimicrobial agents and also causing mostly chronically infections which are difficult to treat when compared with non-biofilm formers' [9, 24]. This antibiotic resistance may be directly related to the inability of antibiotic penetration to the biofilm layer, decreased growth rate of bacteria in biofilm, expression of different factors in biofilm state or physiological changes in bacteria [22]. Also, treatment period is mostly difficult especially in hospitalized patients and unfortunately these infections may cause morbidity and mortality in immunocompromised patients. In this respect, this research was investigated biofilm formation levels of clinically obtained *E. coli* strains and it is found that SBF strains are more resistant than none biofilm Former ones (Table 2). Among 8 antibiotics, the least susceptibility was observed against amikacin (Table 2) similarly in some other researches such as 98.7% [25], 98% [26],

99.1% [27], 96.9% [28]. Additionally, the greatest resistance was observed against trimethoprim/sulfamethoxazole and when compared with literature it is found that trimethoprim/sulfamethoxazole resistance increased among *E. coli* strains [29, 30].

In brief, according to our results, the most SBF isolated strains of *E. coli* was isolated from the samples of wound and urine respectively, and also from the service units of urology and cardiology. Additionally, clinical information of patients was also investigated and it was observed that all SBF strains of *E. coli* were isolated from hospitalized patients. Women were the most SBF isolated sex among patients and in the view of these results it is very important to take precautions against urinary tract infections especially for women who are in the period of post-menopausal. At last, antibiotic susceptibility profiles were investigated and amikacin was found to be as the most effective antibiotic in the treatment of *E. coli* acquired infections among other antibiotics. Accordingly, it is observed that, NBF strains were more susceptible than SBF ones so it is very important to identify biofilm Former strains in order to prevent biofilm acquired infections.

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