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Research Article

Antibiotic resistance pattern and frequency of biofilm producing genes in *Escherichia coli* isolates from nosocomial infections in Tehran in 2023

Shaymaa Abdulrahman[1], Farokh Rokhbakhsh-Zamin[*2], Ashraf Kariminik [2,3]

1. Department of Microbiology, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Department of Microbiology, Kerman Branch, Islamic Azad University, Kerman, Iran
3. Food and Agricultural Safety Research Center, Kerman Branch, Islamic Azad University, Kerman, Iran

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ABSTRACT

Biofilm is a community of bacteria surrounded by extracellular polymeric substances, providing protection against harsh environmental conditions and serving as a major cause of various infections. The objective of this study was to evaluate the effects of various antibiotics on *Escherichia coli* biofilms obtained from nosocomial infections, as well as to explore the correlation between virulence factors and the formation of biofilms.A total of 140 *Escherichia coli* isolates from nosocomial infections were analyzed for antibiotic susceptibility using the Kirby-Bauer method. Biofilm formation was assessed using the crystal violet microplate technique, with a focus on identifying biofilm-associated genes such as *afa, pap, sfa, agg,* and *fimH*. Based on CLSI criteria, Imipenem exhibited the highest sensitivity at 99.28%, whereas Cotrimoxazole showed the highest resistance at 91.43%. Among the isolates, 41.43% did not form biofilms, while 21.43%, 19.29%, and 17.85% formed weak, moderate, and strong biofilms, respectively. A correlation was found between biofilm formation and antibiotic resistance. The frequency of *fimH, Pap, Sfa, afa,* and *agg* genes among the isolates was 59.28%, 26.42%, 17.85%, 8.57%, and 0.00%, respectively. The study suggests that high antibiotic resistance may be associated with strong or moderate biofilm production. The ability of *E. coli* strains to form biofilms may play a crucial role in managing nosocomial infections.

1. Introduction

Escherichia coli (*E. coli*) is a bacterium that typically resides in the gastrointestinal (GI) tract of healthy individuals and animals (Ramos et al., 2020). The specific *E. coli* strains present in the GI tract generally does not cause harm; in fact, it aids in food digestion (Ekici & Dümen, 2019). Numerous strains that cause infections possess the capability to adhere to cells and release toxins. (Bryan et al., 2015). Some harmful strains, if inadvertently ingested, can lead to symptoms such as watery diarrhea, stomach pain, and other digestive problems, collectively known as gastroenteritis (Bean et al., 2008). Several studies have provided evidence of the emergence of antimicrobial resistance in *E. coli*, with a concerning rise in resistance to commonly used antibiotics (Rossi et al., 2018). Recognizing the relationship between biofilm formation, the presence of virulence genes, and the distribution of antimicrobial resistance in *E. coli* strains are crucial for developing effective strategies and measures to prevent and manage infections

*Corresponding author: prof. Farokh Rokhbakhsh-Zamin
E-mail address: rokhbakhsh@gmail.com

(Naves et al., 2008). Infections caused by *E. coli* can be particularly challenging to eliminate due to the formation of biofilms. A biofilm refers to a community of microorganisms that live together in an aggregated form, often attaching to solid surfaces in moist environments. Within a biofilm, the microbes secrete a range of protective substances known as extracellular polymeric substances (EPS), which enhance their survival capabilities (Sharma et al., 2016). The presence of a biofilm makes it difficult for conventional antibiotics to penetrate and effectively target the bacterial cells within it. As a result, biofilm-associated cells become less susceptible to antibiotics (Qian et al., 2022). The *afa*, *pap, sfa, agg*, and *fimH* genes are all involved in biofilm formation in *E. coli* (Tajbakhsh et al., 2016). *afa* (a fimbrial adhesins) genes encode for adhesins that help *E. coli* attach to host cells and form biofilms (Naves et al., 2008). *pap* (pyelonephritis-associated pili) genes encode for pili that aid in bacterial adhesion and biofilm formation (Wahed et al., 2018). The *sfa* (S-fimbriae) genes encode for fimbriae that play a crucial role in helping *E. coli* adhere to specific receptors on host cells. Understanding the function of *sfa* genes and their associated fimbriae can provide valuable insights into the mechanisms of bacterial adhesion and biofilm formation, which may have implications for various fields, including microbiology and biomedicine (Boroumand et al., 2019). The *agg* (aggregative adherence fimbriae) genes encode for fimbriae that play a key role in promoting bacterial aggregation and biofilm formation. These genes are essential for the formation of biofilms, which are complex communities of bacteria embedded in a matrix of extracellular polymeric substances (Schiller et al., 2021). The *fimH* gene encodes for the fimH protein, which is a subunit of type 1 fimbriae. These fimbriae play a crucial role in bacterial adhesion to surfaces and biofilm formation (Yoshida et al., 2022). By understanding the function of these genes, researchers can gain valuable insights into the mechanisms of biofilm formation in *E. coli* (Öztürk et al., 2023). The objective of this study was to determine the prevalence *of afa, pap, sfa*, *agg*, and *fimH* genes associated with biofilm formation in *Escherichia coli* strains isolated from hospital-acquired infections.

2. Materials and Methods

2.1. Sample collection

In this cross-sectional study conducted between September and December 2023, a total of 140 *E. coli* isolates from patients with nosocomial infections such as skin burns and urinary tract infections were collected from Motahari hospitals in Tehran, Iran. The identification of the bacterial isolates was conducted through Gram staining and biochemical tests, including catalase, oxidase, indole production, citrate utilization, Triple sugar iron (TSI) agar, Urease test and methyl red-Voges Proskauer tests (Fesseha et al., 2022). Subsequently, the bacteria were preserved in Brain heart infusion (BHI) broth (Merck, Germany) supplemented with glycerol 18% and stored at -70 ºC for future investigations.

2.2. Antibiotic resistance pattern

The antibiotic resistance pattern of the isolates was determined using the disk diffusion method following the CLSI (Clinical and Laboratory Standards Institute) protocol (Rai et al.,2023). The antibiotic disks (Padtan Teb. Co., Iran) used in this study included, Ampicillin (10 µg), Cefuroxime (30 µg), Amoxicillin/Clavulanic acid (10 µg), Gentamicin (10 µg), Cotrimoxazole (25 µg), Chloramphenicol (30 µg), Ceftriaxone (30 µg), Ceftazidime (10 µg), Imipenem (10 µg), Nalidixic acid (30 µg), and Nitrofurantoin (300 µg). Multidrug-resistant (MDR) *E. coli* was defined as an isolate exhibiting resistance to more than one antimicrobial agent in three or more antimicrobial categories. Following a 24-hour the incubation period at 37°C, the inhibition zone surrounding each disk was measured and recorded. Results were reported as sensitive (S), intermediate (I), or resistant (R) (Feßler et al., 2023).

2.3. Assessment of Biofilm formation

Biofilm formation was quantitatively evaluated using a modified colorimetric microtiter plate (MTP) assay (Xu et al., 2023). An overnight culture of *E. coli* was adjusted to the turbidity of a 1 McFarland standard and then diluted 1:100 in 200 µL of nutrient broth (Merck, Germany). The diluted suspensions were transferred to sterile flat-bottomed 96-well

polystyrene microplates (JET Biofil, Guangzhou, China) and incubated for 24 h at 37 °C. Subsequently, the wells were washed three times with sterile phosphate-buffered saline (PBS) (pH 7.1), and the adherent biofilms were fixed with 99% methanol for 15 min. After fixation, the biofilms were stained with 0.1% crystal violet (Sigma Chemical Co., St. Louis, MO, USA) for 5 min at room temperature. Following staining, the wells were rinsed with water and air-dried. The biofilms in each well were destained with 95% ethanol for 30 min, and the optical density (OD) of the destained solution was measured at 570 nm using a microtiter plate reader (BioTek, Bad Friedrichshall, Germany). All experiments were conducted in triplicate and repeated three times for accuracy. A cut-off value (ODc) was determined as three standard deviations (SD) above the mean OD of the negative control: ODC = average OD of negative control + (3 × SD of negative control). Based on the OD values obtained, the isolates were categorized into four groups:

1. OD ≤ OD control (ODc) = Negative, 2. ODc < OD ≤ 2ODc = Weak positive, 3. 2ODc < OD ≤ 4ODc = Positive and 4. 4OD < ODc = Strong positive.

2.4. DNA Extraction and PCR

The amplification reactions were conducted in 25 µL volumes, including one microliter of target DNA (10 ng/µL), 12.5 µL of dye Master mix 2X (CinaGen, Iran), 1 µL of each Forward and Reverse primers (20 p/mol), and 9.5 µL of double-distilled water. Bacterial isolates were sub-cultured overnight in Brain heart infusion (BHI) broth medium (Merck, Germany) and genomic DNA was extracted from typical *E. coli* colonies using the Karmania Pars Gene kit (KPG, Iran) according to the instructions. Specific primers were used to detect some virulence factors (a*fa*, *pap, Sfa, agg*, and *fimH* genes) in *E. coli* isolates. Table 1 shows the primers used for the detection of the virulence genes (Katongole et al., 2020). The PCR temperature program for all genes followed a protocol of denaturation at 95 ºC for 5 min, followed by 40 cycles of 95 ºC for 40 seconds, annealing at 56 ºC for 1 min, extension at 72 ºC for 2 min, and a final extension at 72 ºC for 5 min. The amplified DNA products were subsequently analyzed using standard gel electrophoresis with ethidium bromide, utilizing 10 µL of the final reaction mixture on a 1.5% agarose gel in TBE buffer. The amplified DNA fragments of specific sizes were visualized under UV fluorescence. To determine the molecular size of the PCR products, a 50 bp ladder (Sinaclone, Iran) was employed.

Table 1. Sequence of specific primers related to the genes under investigation

Genes	Primer sequences (5'→3')	Product size (bp)
afa	F: 5' GCTGGGCAGCAAACTGATAACTCTC3' R: 5' CATCAAGCTGTTTGTTCGTCCGCCG 3'	750
pap	F: 5' GACGGCACTGCTGCAGGGTGTGGCG3' R: 5' ATATCCTTTCTGCAGGGATGCAATA 3'	328
sfa	F: 5' CTCCGGAGAACTGGGTGCATCTTAC 3' R: 5' CGGAGGAGTAATTACAAACCTGGCA 3'	410
fimH	F: 5' TGTACTGCTGATGGGCTGGTC 3' R: 5' GGGTAGTCCGGCAGAGTAACG 3'	564
agg	F: 5' CTAATTGTACAATCGATGTA3' R: 5' AGAGTCATCTCTTTGATAAG 3'	457

3. Results

A total of 140 distinct *E. coli* isolates were obtained from 400 clinical specimens. Analysis of *E. coli* distribution in clinical specimens indicated that the most isolates (n□=□97, 69.29%) were originated from female followed by male (n□=□43, 30.71%). Based on the CLSI interpretive criteria, the highest sensitivity was observed towards imipenem at 99.28%, while the highest resistance was noted towards cotrimoxazole at 91.43%. (Figure 1).

In case of phenotypic and genotypic biofilm in *E. coli* isolates, biofilm phenotypes accounted for 140 isolates, being distributed in the following categories: 17.85% (n□=□25) produced strong biofilm; 19.29% (n□=□27) produced moderate biofilm; 21.43 % (n□=□30) produced weak biofilm and 41.43 % (n□=□58) with no biofilm production. A high occurrence of biofilm-encoding genes was found. The frequency of *the fimH, Pap, Sfa, afa,* and *agg,* genes among the strains was 59.28%,26.42%, 17.85, 8.57% and 0.00%, respectively (Figure 2).

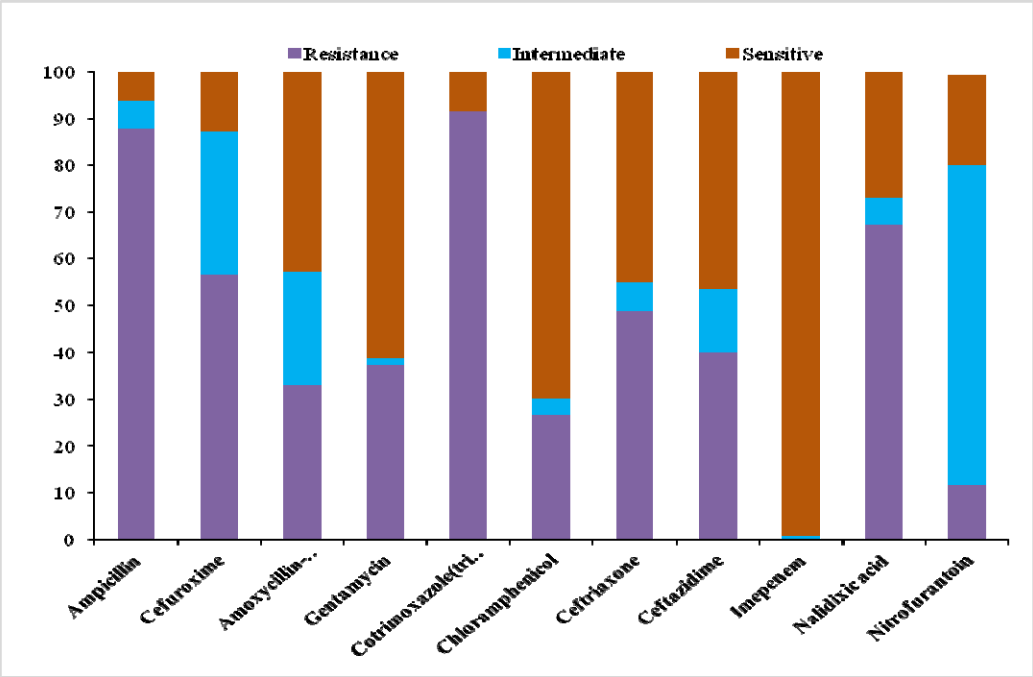

Figure 1. Antimicrobial sensitivity pattern of E. coli isolates

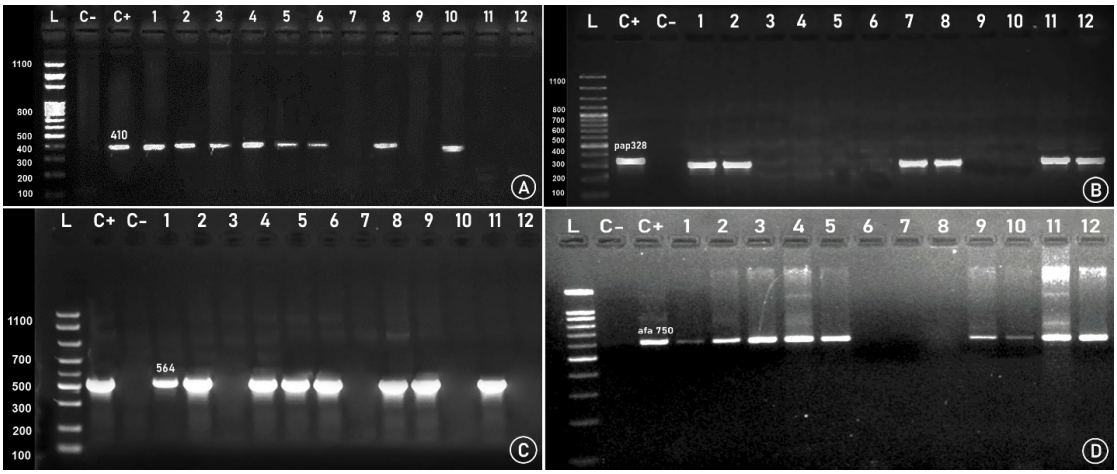

Figure 2. Agarose gel electrophoresis of PCR amplified biofilm genes. L: ladder. A: sfa gene appear at 410 bp, B: pap gene appear at 328 bp, C: fimH gene appear at 564 bp, D: afa gene appear at 750 bp.

4. Discussion

Indeed, biofilm-associated infections pose a significant challenge in healthcare due to their resistance to conventional therapies. The matrix surrounding biofilms, along with antimicrobial tolerance mechanisms expressed within them,

creates a formidable barrier to treatment efficacy. This resilience enables biofilms to develop high levels of resistance to antibiotics and evade the immune system, making them difficult to eradicate. Understanding the mechanisms underlying biofilm formation and resistance is crucial for developing effective strategies to combat biofilm-associated infections and improve patient outcomes (Sharma et al., 2023).

Indeed, biofilm-associated infections caused by *E. coli* pose a significant concern due to their role in recurrent infections, such as urinary tract infections. The complexity of biofilm formation and regulation plays a crucial role in the persistence and resistance of these infections to treatment. Understanding the mechanisms involved in biofilm formation is essential for developing effective strategies to prevent or disrupt biofilm formation, ultimately improving the management and treatment of biofilm-associated infections caused by *E. coli*.

Identifying new therapeutic targets for the development of effective strategies against *E. coli* biofilms is crucial in eradicating mature biofilms or preventing their formation by inhibiting adhesion to surfaces and between cells. The distribution of *E. coli*, with a higher prevalence in women compared to men, as observed in the present study, aligns with previous reports (Alghamdi et al., 2023). Factors such as a shorter urinary tract, proximity to the urethra, and anatomical characteristics of the female reproductive system contribute to women's increased susceptibility to bacterial growth in the urinary tract, particularly in the context of urinary tract infections. Understanding these factors can aid in developing targeted interventions to address the specific vulnerabilities faced by women in combating *E. coli* infections in the urinary tract (Mancuso et al., 2023). Based on the data obtained from the microtiter plate method and phenotypic measurement, it was observed that most of the examined *E. coli* strains showed slight variations in their ability to form biofilms in weak, moderate, and strong forms. This minimal variation suggests a close relationship between the presence or absence of certain influential genes in the studied strains. The study identified the presence *of fimH, pap, sfa, afa,* and *agg* genes in more resistant isolates, indicating a potential association between these virulence genes and antibiotic resistance. A notable correlation was identified between the presence of virulence genes and resistance to antibiotics like Ceftriaxone, Cefoxitin, Ciprofloxacin, and Nalidixic acid. The observation that biofilm-forming isolates from catheter-associated urinary tract infections (UTIs) exhibit drug resistance to multiple antibiotics is consistent with findings reported in various studies. Biofilm formation can provide a protective environment for bacteria, making them more resistant to antibiotic treatments. This phenomenon underscores the importance of understanding the mechanisms of biofilm formation and developing alternative strategies to combat biofilm-associated infections effectively (SarojGolia et al., 2012). The data suggests that the antibiotic resistance pattern was directly linked to the presence of the *fimH* gene, with subsequent priorities given to the pap and *sfa* genes in relation to resistance to various antibiotics across different groups. The study observed a significant relationship between biofilm-forming strains and resistance to ampicillin and cotrimoxazole, with a notable number of resistant strains identified. Additionally, biofilm formation was found to be significantly associated with the virulence genes *fimH* and pap, but not with other virulence genes. The findings of the current research align with previous studies that have reported the abundance of *fimH* and afa genes in *E. coli* strains. This consistency in research findings suggests a common prevalence of these genes across different *E. coli* strains, highlighting their importance in bacterial adhesion and biofilm formation processes. The presence of *fimH* and *afa* genes in *E. coli* strains underscores their significance in understanding the mechanisms underlying bacterial adhesion and biofilm formation, contributing to the broader scientific understanding of these processes (Ebrahimi et al., 2023).

Conclusion

In summary, this study has shed light on the complex interplay between biofilm formation and resistance in clinical isolates of *E. coli*, resolving previously debated issues. The findings underscore the notable correlation between multidrug resistance (MDR) and the ability to form biofilms. Therefore, forthcoming

research endeavors should investigate the impact of bacterial origin, environmental conditions, antibiotic resistance, and virulence factors on biofilm formation. A comprehensive comprehension of these diverse factors will aid in the development of novel therapeutic approaches that specifically target biofilm-related resistance mechanisms. Understanding the biofilm genes in *E. coli* can help in the development of strategies to prevent biofilm formation on medical devices, improve infection control measures, and enhance patient outcomes. So, by determining the biofilm genes in *E. coli*, researchers can identify specific genetic markers associated with antibiotic resistance, which can aid in the development of targeted therapies and the selection of appropriate antibiotics.

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Conflict of interest

None

Refereces

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