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A systematic review and meta-analysis of antibiotic resistance patterns, and the correlation between biofilm formation with virulence factors in uropathogenic *E. coli* isolated from urinary tract infections

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ABSTRACT

Urinary tract infection (UTI) is caused by the invasion of the pathogen in the urinary system that can manifest as symptomatic or asymptomatic bacteriuria. This study was conducted to investigate antibiotic resistance patterns, and the correlation between biofilm formations with virulence factors in uropathogenic *E. coli* isolates retrieved from UTI.

We searched Scopus and Google Scholar, PubMed, Web of sciences for studies published in the English language between 1st 2005 to 31st December 2019. The Mesh terms and text words included "biofilms", OR "biofilm formation", AND "antibiotic resistance", OR "drug-resistance", OR "antimicrobial drug resistance", AND "urinary tract infections", OR "UTI", AND "biofilm related-genes", AND "virulence factors" AND "correlation", AND "Uropathogenic Escherichia coli", OR "Uropathogenic *E. coli*" AND "prevalence" AND "Iran".

Data analyzed using Comprehensive Meta-Analysis (CMA) software. The random-effects model was used to calculate the pooled prevalence with 95% confidence interval (CI).

The combined rates of biofilm formation in Uropathogenic *E. coli* (UPEC) isolates were achieved as 84.6% (95% CI: 72.7–91.9). Also, 24.8%, 26.1% and 44.6% of UPEC isolates were able to create strong, moderate and weak biofilm, respectively. The highest pooled antibiotic resistance was against Ampicillin followed by Tetracycline with resistance rates of 74.6% and 64.9%, respectively. Accordingly, some studies reported that biofilm production was significantly associated with antibiotic resistance and virulence genes ($p < 0.05$).

This study showed a high tendency among UPEC isolates to form biofilm (more than 84%), also, most studies included in the present review reported a significant correlation between biofilm formation with antibiotic resistance and virulence factors.

1. Introduction

Urinary tract infection (UTI) is caused by the invasion and proliferation of the pathogen in the urinary system, which disrupts kidney and urinary function and can manifest as symptomatic or asymptomatic bacteriuria [1], particularly among hospitalized patients with Catheter-associated UTIs (CA-UTI) [2]. The bacterial UTI is the second most serious health problem and causing morbidity. Approximately, between 50% and 80% of the population especially women experience UTI in their lifetime; Of course, 20%–50% of people will have recurrent or recurrent infections. About 150 million people are yearly diagnosed with UTI with costs more than $6 billion in all age groups worldwide [3]. UTI is often observed in communities and hospitals all over the world [4]. Up to 90% of community-acquired and 50% of nosocomial UTIs are caused by uropathogenic *E. coli* [5], followed by *Staphylococcus*, *Klebsiella, Enterobacter, Proteus,* and *Enterococci* spp [6].

Some factors such as age group, sex, geographical location and setting, sexual activity, diabetes mellitus, and previous history of UTI

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influence the type of UTI, causative agents and resistance rates [1].

One mechanism that helps the pathogenesis of the *E. coli*, And to help keep it in the urinary tract, and hinders its eradication is the biofilm formation [7]. These infections are difficult to treat and act as a reservoir of drug resistance [8]. Biofilms are communities of microorganisms and their microbial products assisting bacteria to attach to uroepithelial cells [9].

Some genes including *rpoS*, *sdiA*, and *rcsA* have a significant role in biofilm formation and obtain resistance to antibiotics [8]. Studies reported that the presence of adhesins (*fimH* and *mrkD*) are connected to biofilm formation [10].

In severe UTIs that caused by UPEC several virulence factors such as P fimbriae (*pap*), type1 fimbriae, afimbrial adhesin I (*afaI*), hemolysin (*hly*), cytotoxic necrotizing factor 1 (*cnf* 1), aerobactin (*aer*), S fimbriae (*sfa*), adhesins and fimbriae are contributed [11]. As well, other virulence genes including *kpsMT*, *ompT*, *usp*, *iroN*, *iha*, *set 1*, *astA*, group II capsule synthesis; *sfa/foc*, S and F1C fimbriae; *iutA*, *traT*, serum resistance; and *fimH* have the main role in its pathogenicity [12,13]. This organism possibly using mentioned virulence factors colonize host surfaces, avoids host defense mechanisms, invade host cells, and leading to clinical manifestations [14].

Regarding the high cost of UTIs treatment and the defect treatment of UTIs infections due to biofilm production and subsequently drug resistance, this infection has received global attention in the health care system and its policies. Since this issue is of great importance and its various aspects such as biofilm formation and drug resistance in Iran have not been investigated, this study was conducted to investigate antibiotic resistance pattern, and correlation between biofilm formation with virulence and biofilm-related factors in uropathogenic *E. coli* strains isolated from urinary tract infections through a systematic review and meta-analysis.

2. Materials and methods

2.1. Search strategy

We searched PubMed, Web of sciences, Scopus and Google Scholar for studies published in the English language between 1st 2005 to 31th December 2019. The MeSH terms and text words included “biofilms”, OR “biofilm formation”, AND “antibiotic resistance”, OR “drug resistance”, OR “antimicrobial drug resistance”, AND “urinary tract infections”, OR “UTI”, AND “biofilm related-genes”, AND “virulence factors” AND “correlation”, AND “Uropathogenic *Escherichia coli”*, OR “Uropathogenic *E. coli*” AND “prevalence” AND “Iran”.

For example, the search strategy in PubMed was as follows; (Biofilms [MeSH Terms] OR biofilm formation [Title/Abstract]) AND (Drug-Resistance [MeSH Terms] OR Antimicrobial Drug Resistance [MeSH Terms] OR Antibiotic Resistance [MeSH Terms]) AND (“urinary tract infections” [MeSH Terms]), OR UTI [Title/Abstract], AND (biofilm related-genes[Title/Abstract]), AND (virulence factors[Title/Abstract]) AND (correlation” [Title/Abstract]), AND (“Uropathogenic *Escherichia coli”* [MeSH Terms], OR “Uropathogenic *E. coli*” [Title/Abstract]) AND (“prevalence” [MeSH Terms]) AND “(Iran[MeSH Terms]).

2.2. Selection criteria

Two investigators (AH and KM) searched all titles and abstracts independently for eligibility. The studies for selection had to meet the following eligibility criteria: Cross-sectional studies in the English language that were conducted on the prevalence of Uropathogenic *E. coli*, the prevalence of biofilm-related genes, virulence factors and the rate of biofilm formation in *E. coli* isolates recovered from clinical samples of Iranian patients were considered.

2.2.1. Data extraction

Full texts of all included studies were considered by two reviewers (AK, and KM) independently and data extracted using an extract form. The data extracted were: First author, publication year, location, sample size (EPEC), mean age, genus (male and female), biofilm formation, biofilm type (strong, moderate, and weak), and the correlation between biofilm formation and biofilm related-genes and virulence factors.

2.2.2. Quality assessment

Selection bias was assessed with the Critical Appraisal Skills Programme (CASP) checklist for cross-sectional studies (www.casp-uk.net). We produced quality assessment charts based on a series of accurate questions was designed. If the relevant data was obtainable, the question was answered ‘yes’, and if there was any doubt or no matching data, the question was responded as ‘no’. Overall, 10 questions were designed and included in the scoring system. At last, scores were classified as weak (0–4), moderate (6–8), and robust (8–10).

2.3. Data synthesis and analysis

Statistical analyses conducted using according to PRISMA guidelines [15]. We calculated estimates of the combined prevalence of each variable by producing a forest plot. The rand-effects model was used to calculate the pooled prevalence with 95% confidence interval (CI). The heterogeneity was evaluated using Q and I^2 indices. *P* value less than 0.05 was considered as statistically significant heterogeneity. Finally, we created funnel plot to explore the possibility of publication bias.

3. Results

3.1. Study characteristics

Here 702 Records identified through database searching. About 321 Records after duplicates removed. Then, 381 records screened (Fig. 1). The 210 records did not meet our eligibility criteria and removed. We assessed 171 full-text papers for eligibility, 164 papers did not meet our eligibility criteria for reasons. Thus, 7 records enrolled in the current study. The studies included in the current study covered several regions of Iran but studies were chiefly from North and north-west. Also, the mean age of participants was between 1 and 91 years old (Table 1).

3.2. Overall effects

3.2.1. Biofilm formation in uropathogenic E. coli

According to Table 1 and 6 out of 7 studies used of microtiter plate (MTP) for biofilm formation assay, and only a study conducted by Tajbakhsh et al. [11] used of Congo Red Agar (CRA) method for this matter. According order of studies listed in Table 1, 61.8%, 98.6%, 61.5%, 94%, 85.5%, 85%, and 93.7% of isolates were biofilm producers. As we can observe, all studies that used MTP have more sensitivity than the study conducted by Tajbakhsh et al. that used CRA for biofilm assay. As shown in Table 2 and Fig. 2, the combined rate of biofilm formation in UPEC isolates was achieved as 84.6% (95% CI: 72.7–91.9). Also, 24.8%, 26.1% and 44.6% of UPEC isolates were able to create strong, moderate and weak biofilm, respectively. Furthermore, only 9.3% of isolates didn't have the capability of biofilm production.

3.2.2. Heterogeneity and possible publication bias

Heterogeneity indices among the selected studies were as Q2 = 70.6, I^2 = 91.5, and t = 3.5. Visual assessment of the Funnel plot and also Egger's linear regression test showed publication bias (Fig. 3, p = 0.016).

3.2.3. Subgroup analysis for antibiotics resistance

Subgroups analysis for antibiotics resistance showed that the

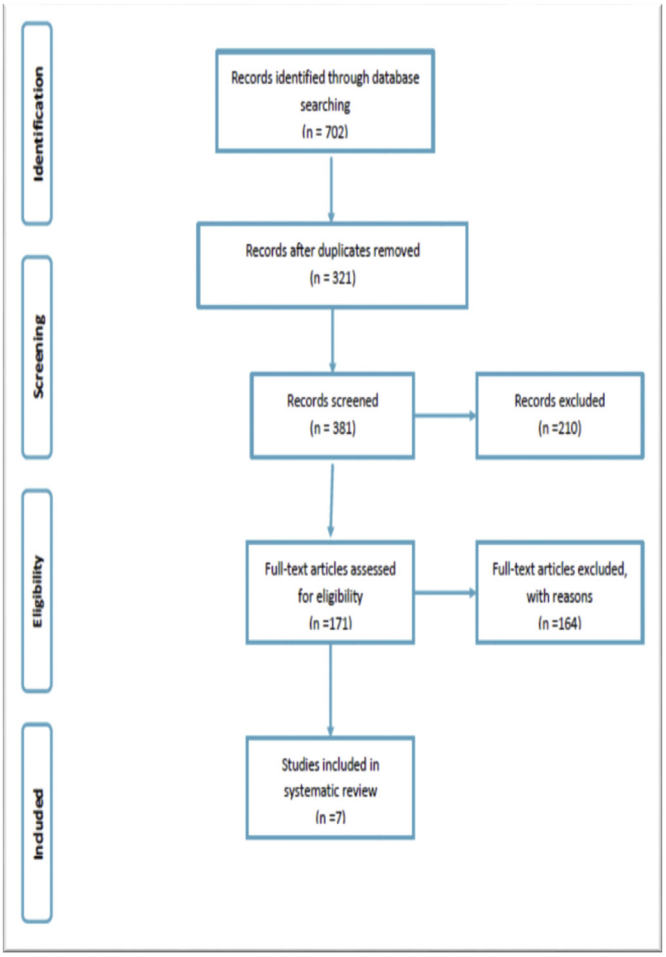

Fig. 1. Flow chart of inclusion process for studies.

highest pooled antibiotic resistance was against Ampicillin followed by Tetracycline and Cefotaxime **with resistance rates of** 74.6% (95% CI: 68.3–80.1)**,** 64.9% (95% CI: 55.5–73.2)**, and** 62.9% (95% CI: 35.8–83.8), respectively. **Also, the best antibiotics against** UPEC **strains were** nitrofurantoin and Chloramphenicol with respective resistance rates of 6.2% and 8.7% (Table 2). Accordingly, three studies reported that biofilm production was significantly associated with antibiotic resistance and virulence genes. Only one study didn't show any association ($p > 0.05$). Also, other studies included in the present meta-analysis didn't address this subject at all.

4. Discussion

Our results showed that the combined rate of biofilm formation in UPEC isolates was 87.9%. Also, 26.3%, 26%, and 47.1% of UPEC isolates were able to create strong, moderate and weak biofilms, respectively. The distribution of UTI in male and female were 45% and 79.5%, respectively. As well, the highest pooled antibiotic resistance was reported against Ampicillin followed by Tetracycline **with resistance rates of** 74.6% and 64.9%**,** respectively. **Also, we showed that the best antibiotics against** UPEC **strains were** Nitrofurantoin and Chloramphenicol with respective resistance rates of 6.2% and 8.7%. Besides, some studies reported that biofilm production was significantly associated with virulence genes ($p < 0.05$).

The sex distribution in the present study is consistent with other studies conducted in other parts of the world, showing statistically a significant frequency of females with UTI (75%) [16–19]. Where we similarly reported the pooled UTI prevalence in males and females with respective rates of 45% and 79.5%. This difference in UTI prevalence in males and females referred to the difference in anatomy and microflora of their genitourinary system, and also physical factors [20]. One of the major concerns about urinary tract infections is the high resistance of uropathogenic *E. coli* isolates. One of the resistance mechanisms which this microorganism follows is the biofilm formation. As in the present study, about 84.6% of the isolates were capable of producing biofilms. This is comparable with the results reported by Sharma et al. [21], Soto et al. [22], and Meshram et al. [23] where they reported that the biofilm formation was higher in the UPEC than commensal isolates. In contrast, other studies conducted by Neupane et al. [24] reported a lower prevalence of biofilm in the UPEC isolates. The same study showed that the antibiotic resistance in biofilm-producing *E. coli* was found significantly higher than that of biofilm non-producing *E. coli* [24]. Comparable findings were stated by Abdallah et al. (2011); who presented a statistically significant difference in antibiotic susceptibility between planktonic form and biofilm form of the same microorganism [18]. biofilm protects bacteria from host defense mechanisms and antibiotics, too [25]. Due to intrinsically resistance of microorganisms growing in a biofilm to most antibiotics, a high antimicrobial concentration is necessary to overcome them [26,27].

Amikacin and nitrofurantoin could be considered as selective antibiotics for the treatment of biofilm structures [28]. Accordingly, our review showed that the most effective antibiotics **against** UPEC **strains were** Nitrofurantoin and Chloramphenicol. In contrary to our findings, in a study conducted by Ponnusamy et al., the rates of antibiotic resistance of biofilm-producing *E. coli* isolates were found to be 100% for chloramphenicol and amoxiclav (amoxicillin and clavulanic acid), 86% for gentamicin and cefotaxime, 84% for ceftazidime, 83% for cotrimoxazole and piperacillin/tazobactam, 75% for tetracycline and 70% for Amikacin [29]. In their review, this is owing to the dissemination of MDRs (strains of bacteria with acquired resistance to at least one agent in three or more antibiotic classes) in hospital settings among the biofilm-producing uropathogenic *E. coli* isolates [29].

Our findings showed that Chloramphenicol seems to be a suitable antibiotic for UTI since it has not been used for many years, and up to 90% of isolates were susceptible to this antimicrobial agent presently. It

Table 1
Characteristics of studies included in the present review.

First author	Publication	Location	UPEC	Genus(n)		Age(n)		Biofilm formation (n)	Biofilm type(n)			
				Male	Female		Method		Strong	Moderate	Weak	No biofilm
Samet [41]	2013	Gorgan	170	–	–	–	MTP	105	36	17	52	-
Taghadosi [42]	2015	Kerman	35	-	-	-	MTP	35	10	16	9	-
Tajbakhsh [11]	2016	–	130	–	–	–	CRA	80	15	20	45	–
Zamani [43]	2018	Rasht	100	–	–	–	MTP	94	36	48	10	6
Asadi Karam [39]	2018	Tehran	110		110	20–60	MTP	93	24	41	28	–
Noie Oskouie [38]	2019	Tabriz	120	84	36	1–91	MTP	102	18	14	70	15
Davari Abad [37]	2019	Maragheh	79	–	–	–	MTP	74	10	5	59	5

Note; MTP: Microtiter plate, CRA: Congo-red Agar method.

Table 2
Subgroups analysis for different variables in the present review.

Subgroups	No. studies	Heterogeneity test			Egger's test		Random model		
		Prevalence (95% CI) (%)	Z	P	Q	P	I^2	T	P
Biofilm formation	7	84.6(72.7–91.9)	4.6	0.00	70.6	0.00	91.5	3.5	0.016
Biofilm type									
Strong	7	24.8(18.5–32.5)	5.5	0.00	22.4	0.001	73.1	2	0.1
Moderate	7	26.1(15.3–40.8)	3.04	0.002	67.3	0.00	91	2	0.09
Weak	7	44.6(27.5–63.2)	0.5	0.5	98.8	0.00	93.9	0.9	0.36
No biofilm	3	9.3(5–16.5)	6.7	0.00	4.6	0.09	56.7	4.5	0.13
Antibiotic resistance rate									
Tetracycline	3	64.9(55.5–73.2)	3	00.2	5.7	0.055	65.4	1	0.016
Ciprofloxacin	3	49.7(39.7–59.7)	0.05	0.95	6.7	0.35	70.2	1	0.037
Ceftazidime	3	51.4(23.8–78.2)	0.08	0.92	47	0.00	95.7	1.5	0.36
Ceftriaxone	2	38.8(32.4–45.5)	3.2	0.001	0.22	0.63	0.00	–	–
Tobramycin	2	10.8(2.7–34.1)	2.8	0.004	7	0.008	85.7	–	–
Gentamicin	3	21.8(12.3–35.6)	3.6	0.000	12.7	0.002	84.2	1.5	0.36
Nitrofurantoin	4	6.2(3.8–10)	10.3	0.000	4.7	0.19	36.9	3.4	0.075
Amikacin	4	17.1(8–32.9)	3.5	0.000	33.2	0.00	90.9	5.1	0.036
Ampicillin	2	74.6(68.3–80.1)	6.7	0.000	0.1	0.00	0.00	–	–
Chloramphenicol	2	8.7(5.5–13.6)	9.2	0.000	0.8	0.00	0.00	–	–
Imipenem	3	10(2.4–34.1)	2.8	0.005	19.1	0.00	89.5	1	0.21
Nalidixic Acid	2	59.1(35.7–79)	0.75	0.45	10.8	0.001	90.7	–	–
Cefotaxime	2	62.9(35.8–83.8)	0.93	0.35	15.9	0.00	93.7	–	–
Norfloxacin	2	47.1(35.5–59)	0.47	0.63	35	0.061	71.5	–	–

Study	**Explanations**
Tajbakhsh [11]	Biofilm production was significantly associated with *fimH*, *pap*, *afa* and *sfa* virulence genes ($p < 0.05$).
Asadi Karam [39]	They reported a significant correlation between the high biofilm production and resistance to ceftazidime and norfloxacin
Davari Abad [37]	There was no significant correlation between them; however, the strongest and the weakest had related to *sfa* and *afa* genes, respectively. Of note, *afa* even has reduced ability of biofilm formation in combination with other genes.
Oskouei [38]	Biofilm production was significantly associated with the *sdiA*, *rcsA*, and *rpoS* genes ($p < 0.05$).

should be noted, however, in our review, only three included studies used this antibiotic. Because of safety concerns, Chloramphenicol is no longer a first-line antibiotic for the treatment of any infection in developed countries [30]. Chloramphenicol can bind to human serum albumin [31], and causes bone marrow toxicity and reversible form of bone marrow suppression, and also a fatal form of aplastic anemia [30]. As well, another antibiotic is tetracycline that should be prescribed with caution by physician in patients with liver impairment and those with kidney failure [32]. Besides, the breakdown products of tetracycline are toxic and affect proximal tubular function of the kidney nephrons and can result in deadly Fanconi syndrome [32,33].

This should be noted; however, as the antibiotics tested in the different studies were not always the same, and some antibiotics reported in two or three studies, so reported combined resistance rate will not naturally be as accurate as the antibiotics mentioned in several studies, which can create a bias in the interpretation of the results.

We showed that 6 out of 7 studies used of microtiter plate(MTP) for biofilm formation assay, and only a study conducted by Tajbakhsh et al. [11] used of Congo Red Agar (CRA) method for this matter. Accordingly, biofilm formation rate varied 61.8%–98.6% among studies used

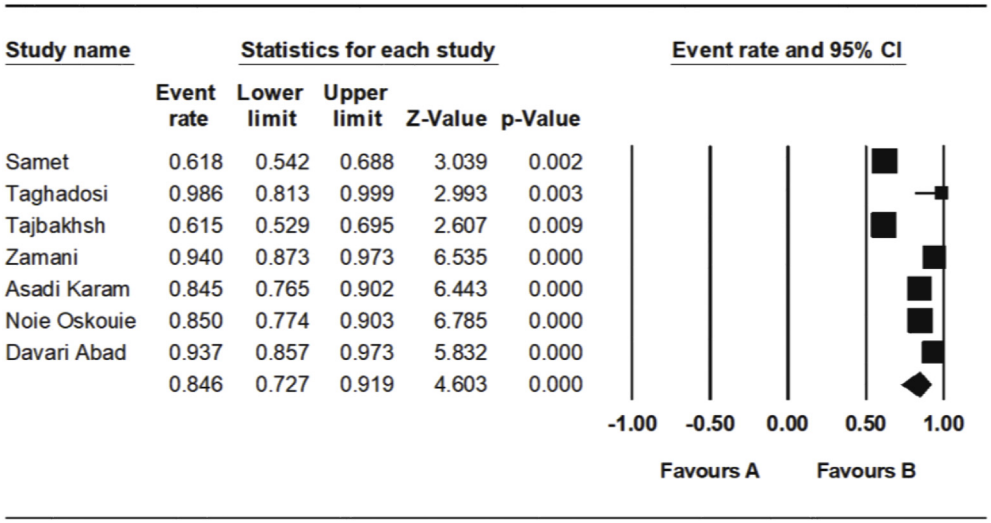

Fig. 2. Forest plot of the meta-analysis of biofilm formation rate in uropathogenic *E. coli* isolates recovered from patients suffered from UTI.

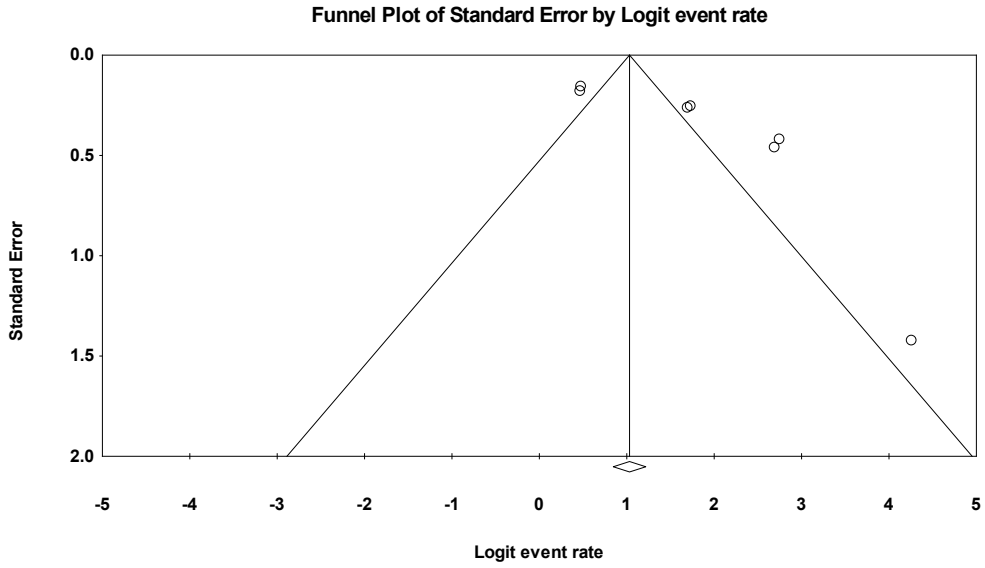

Fig. 3. Funnel plot of meta-analysis of biofilm formation rate in uropathogenic *E. coli* recovered from patients suffered from UTI.

of MTP compared to the study conducted by Tajbakhsh et al. [11] used CRA for biofilm assay with biofilm formation rate of 61.5%. This showing the higher sensitivity of MTP other than that of CRA in the measurement of biofilm production. In accordance with our results, in a study conducted by Ruzicka et al. [34] reported that out of 147 isolates of *S. epidermidis*, biofilm formation rates through Tube method (TM) and CRA methods were 53.7% and 43.5%, respectively. Also, Baqaiet al.20 tested CRA to detect biofilm formation among uropathogens. Their findings indicated that with the CRA method, only 11% of isolates were biofilm producing [35].

The severity of UTI caused by *E. coli* depends on bacterial biofilm-related genes and virulence factors and host-related factors that may be involved in attachment, colonization, biofilm formation, antibiotic resistance, and pathogenicity [36]. A study conducted by Davari Abad et al. [37] included in the present review did not find any correlation between biofilm production and virulence genes. But other studies reported such correlation. In a study conducted by Tajbakhsh et al. biofilm production was significantly associated with *fimH*, *pap*, *afa,* and *sfa* virulence genes [11]. Similarly, a study conducted by Oskouei et al. that found a significant correlation between biofilm production and the *sdiA*, *rcsA*, and, *rpoS* genes [38]. Also, Asadi Karam et al. reported a significant correlation between high biofilm production and resistance to both antibiotics(ceftazidime and norfloxacin) [39].

As the correlation between biofilm-producing capability and UTIs has been proven previously [40], improvement of novel therapeutic or prophylactic strategies against uropathogenic *E. coli* needs more knowledge about biofilm formation, virulence factors, biofilm related genes, drug resistance, and their interrelationships.

Regarding our results, UPEC isolates recovered from Iranian patients showed a high ability to produce biofilm (more than 84%), and most studies included in the present review described a significant correlation between biofilm formation with antibiotic resistance and virulence factors, indicating these bacteria are much more resistant to conventional antimicrobial agents. Also, biofilm formation increases the chronicity of urinary tract infection. Therefore, the improvement of novel therapeutic strategies against this pathogen requires more knowledge about the correlation between biofilm formation, and virulence factors, biofilm-related genes, and consequently antibiotic resistance patterns.

Declaration of competing interest

The authors declare that they have no competing interests.

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