

# Correlation of biofilm formation, virulence factors, and phylogenetic groups among *Escherichia coli* strains causing urinary tract infection: A global systematic review and meta-analysis

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**Background:** Different virulence factors are involved in the pathogenesis of urinary tract infection (UTI) caused by Uropathogenic *Escherichia coli* (UPEC); hence, this study aimed to study the prevalence of biofilm formation, virulence factors, and phylogenetic groups and their correlation with biofilm formation among UPEC isolates through a systematic review and meta-analysis. **Materials and Methods:** A literature search was conducted from 1, 2000, to the end of 2021 in different databases for studies that reported biofilm together with virulence genes or phylogenetic groups in UPEC isolates from patients with UTI according to PRISMA protocol. Data were analyzed by Comprehensive meta-analysis software. **Results:** The pooled prevalence of biofilm formers was 74.7%. The combined prevalence of phylogenetic Groups A, B1, B2, and D (s) were reported at 19.6%, 11%, 50.7%, and 20.5%, respectively. The most common virulence genes reported worldwide were *fimA*, *ecpA*, and *fimH*, with a combined prevalence of 90.3%, 86.6%, and 64.9%, respectively. The pooled prevalence of biofilm formation in UPEC isolates with phylogenetic Groups A, B1, B2, D, C, and F were 12.4%, 8.7%, 33.7%, 12.4%, 2.6%, and 2.65%, respectively. Several studies showed a correlation between biofilm production and virulence genes, or phylogenetic groups. **Conclusion:** Regarding data obtained, the high level of combined biofilm formation (74.7%) and the presence of a positive correlation between biofilm production and virulence genes, or phylogenetic groups as reported by the most studies included in the present review, indicates an important role of biofilm in the persistence of UPEC in the UTI.

**Key words:** Biofilms, *Escherichia coli*, phylogenetic groups, urinary tract infection, virulence factors

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## INTRODUCTION

Urinary tract infection (UTI) is widespread in the world today.<sup>[1,2]</sup> The disease affects both men and women but it is most common among women.<sup>[3]</sup> The disease can manifest in two forms, symptomatic

and asymptomatic.<sup>[4]</sup> The main manifestation of UTI is acute cystitis which is described as substantial bacteriuria-associated symptoms and includes about 95% of all symptomatic UTIs.<sup>[5]</sup>

Different types of bacteria, especially Gram-negative microorganisms of *Enterobacteriaceae* family cause UTI.<sup>[6]</sup>

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Of these, the major quota relates to uropathogenic *Escherichia coli* (UPEC), which accounts for up to 90% of UTIs.<sup>[7]</sup> About 80%–90% of community-acquired and 30%–50% of hospital-acquired UTIs are attributed to UPEC isolates.<sup>[8]</sup>

Different virulence factors are involved in the pathogenesis of this microorganism, among which we can mention the effective virulence factors in biofilm formation, colonization, adhesion, iron acquisition, and toxin production.<sup>[9]</sup>

Biofilms of bacteria produce a matrix composed of proteins, extracellular DNA, and polysaccharides, in fact, biofilms are a collection of microbial cells that form an irreversible adherence to solid surfaces and do not disappear with gentle washing.<sup>[10]</sup> The tendency of unattached cells makes phenotypic changes to adult biofilm surface (Planktonic) which has major consequences such as increased resistance to anti-inflammatory agents, antimicrobial, and host defense.<sup>[11]</sup> These phenotypic changes convert the cell from the unattached form to the attached form.<sup>[12]</sup> Biofilm affords an extra protective method by which the enclosed bacterial cells can avoid the damaging effect of antimicrobial agents besides extreme environmental circumstances.<sup>[13,14]</sup> Based on estimations, biofilm is responsible for 80% of all microbial infections and over 65% of hospital-acquired infections.<sup>[15,16]</sup> As well, biofilm plays a vital role in horizontal gene transfer which simplifies the movement of resistance genes and virulence factors, particularly under antibiotic selective pressure (s).<sup>[17,18]</sup>

UPEC has many virulence factors with a role in entering, adhering, colonizing, acquiring essential nutrients, multiplying in an antagonistic environment, and disseminating within the urinary tracts.<sup>[19,20]</sup> Therefore, persistence and biofilm formation cause pyelonephritis and even chronic and recurrent UTI. As a result, it leads to an increase in antimicrobial resistance and the severity of infection.<sup>[21]</sup>

Adherence plays an important role in the pathogenesis of UPEC by increasing bacterial adhesion, colonization, and facilitating bacterial interactions between this microorganism and the host cell matrix, and consequently biofilm formation.<sup>[8]</sup> The common adhesions in UPECs are type 1 fimbriae, P fimbriae, S fimbriae, F1C fimbriae, Dr. adhesins, and afimbrial adhesins.<sup>[22,23]</sup> The onset of infection depends on the primary attachment of the bacteria to the uroepithelial cells.<sup>[24]</sup> The attachment and the colonization of the upper respiratory tract to the renal vascular endothelium, and eventually pyelonephritis mediates through P-fimbriae.<sup>[24]</sup>

The relationship between a higher biofilm formation and several virulence genes such as P and type 1 fimbriae genes was previously described.<sup>[25,26]</sup>

*E. coli* strains are divided into four phylogenetic Groups (A, B1, B2, and D); of these, Group B2 have the most prevalent among UPEC isolates, and less frequently belong to Group D, while the most commensal isolates belong to phylogenetic A.<sup>[27]</sup>

A systematic review and meta-analysis of antibiotic resistance patterns, and the correlation between biofilm formations with virulence factors in UPEC isolated from UTIs conducted by Zhao *et al.*,<sup>[1]</sup> previously showed that most studies included in their review reported a significant relationship between biofilm with antibiotic resistance and virulence factors. The previous study first was reported locally only, while, our study will cover all over the world, second; they focused on the correlation between biofilm and antibiotic resistance gene and virulence factors, but we add a relationship between biofilm and phylogenetic groups, too. In total, due to the significant role of biofilm in the development of UTI caused by UPEC, and the fact that there is no comprehensive review of the correlation between biofilm formation and various virulence factors and phylogenetic groups, this review aimed to study the prevalence of biofilm formation, virulence factors, and phylogenetic groups and their correlation with biofilm formation among UPEC isolates through a systematic review and meta-analysis.

## MATERIALS AND METHODS

### Literature search and search strategy

A literature search was performed from 1, 2000, to December 30, 2021, in PubMed, SCOPUS, Web of Sciences, and Google Scholar databases. The following terms have been used:

“Uropathogenic *Escherichia coli*,” OR “Uropathogenic *E. coli*,” OR “UPEC,” AND “Urinary Tract Infection,” OR “Urinary Tract Infections,” OR “UTI,” AND “Biofilm,” OR “Biofilms,” OR “Biofilm production,” OR “Biofilm formation,” AND “Virulence Factor,” OR “Pathogenicity Factor,” OR “Virulence Determinant,” OR “Virulence gene,” AND “Phylogenetic Groups,” OR “Phylogenetic Analysis,” OR “Phylogenetic Clustering,” OR “Molecular Phylogenetics.”

References from included articles or abstracts were checked through a manual search for additional data. The titles, abstracts, and full texts were reviewed independently by two authors (H. K. M and F. N) to determine if they met the eligibility criteria for inclusion. If they did not agree on something, they consulted with the third author (A. N) and reached an agreement, For example, the authors disagreed about the reference method to report the measurement of biofilm formation because several different methods were

used for this purpose in different studies, and the third reviewer was consulted and they reached a unit conclusion in this regard.

### Inclusion criteria

Studies were included if they clearly reported biofilm together with virulence genes or phylogenetic groups in UPEC isolates from patients with UTI. In addition, studies used standard molecular methods for the detection of virulence genes and phylogenetic grouping, and standard methods for assessing biofilm formation have been included.

### Exclusion criteria

Studies with no information regarding biofilm, virulence genes, or phylogenetic groups, studies with no standard molecular methods for the diagnosis of bacteria, detection of virulence genes, and phylogenetic grouping were excluded. Literature reviews (narrative review, systematic review, and meta-analysis), abstracts, meetings, conferences, editorials, letters to editors, case reports, and case series were also excluded. Studies identified in languages other than English and studies with unclear data were excluded.

### Screening and study selection

As shown in Figure 1, 1623 articles have been found by searching various databases. Then, the title and abstract of 1107 studies have been screened. About 428 duplicate studies were excluded. After that, 317 irrelevant records were excluded. Furthermore, 362 articles were further evaluated for eligibility. Studies ( $n = 342$ ) were excluded for reasons (unclear data, missed data, not reporting prevalence, and...). Finally, 20 articles were included in the current systematic review and meta-analysis.

### Data extraction

The following data have been extracted from each study by two reviewers as follows in data extraction forms, which included items such as the name of the first author, the time of the study, publication, location, UPEC, extended spectrum beta-lactamase, Multidrug-resistant, biofilm, methods for biofilm, molecular methods, and phylogenetic groups. If the researchers did not agree on an item to be included in the study, they would agree in consensus with a third investigator.

### Quality assessment of studies

The quality of the studies included in the present review was assessed by a checklist designed by the Joanna Briggs Institute.<sup>[28]</sup> Briefly, in this checklist, 9 questions (sample frame, sample size, study subjects, the setting, data analysis, methods, conditions, and so on) are asked to make a correct judgment of the quality of the conducted studies. Hence, the answer to each question is specified as yes, no, unclear, or

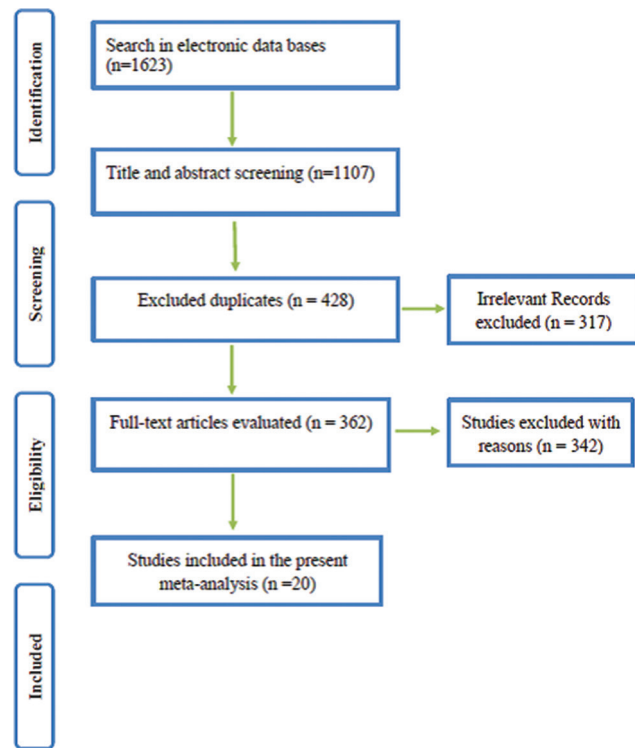


Figure 1: Flowchart for inclusion process of studies

not applicable. Overall, Studies are categorized into high, medium, and low quality; finally, low studies are excluded from the present review. The quality assessment of studies is abstracted in Supplementary Table 1.

### Statistical analysis

Comprehensive meta-analysis software was used to analyze the data. Due to the heterogeneity in the studies included, the random effect model was used. The between-study heterogeneity was checked using Cochran's Q and the  $I^2$  statistic. To assess publication bias, Egger's regression test was used, where  $P < 0.05$  was considered statistically significant publication bias.

## RESULTS

### Study characteristics

Table 1 summarizes the most important characteristics of the selected articles. The publication of Studies included here was from 2000 to 2021. Finally, 20 articles were included in the current systematic review and meta-analysis. Studies have been conducted around the world, from countries such as Iran ( $n = 7$ ), Slovenia ( $n = 1$ ), Colombia ( $n = 1$ ), Poland ( $n = 1$ ), Uganda ( $n = 1$ ), Nepal ( $n = 1$ ), Egypt ( $n = 2$ ), India ( $n = 2$ ), Bulgaria ( $n = 1$ ), Spain ( $n = 1$ ), Italy ( $n = 1$ ), and Pakistan ( $n = 1$ ). Methods used for evaluating biofilm production were; microtiter plate method, Congo Red Agar, tube method, and tissue culture plate method. As well, molecular methods such as polymerase chain

**Table 1: Characteristics of included studies in the present review**

| Study  | Time study | Publication | Location | UPEC (n) | Biofilm (n) | Methods for biofilm | Molecular methods | Phylogenetic groups (n) |    |     |    |    |   |
|--|------------|-------------|----------|----------|-------------|---------------------|-------------------|-------------------------|----|-----|----|----|---|
|  |            |             |          |          |             |                     |                   | A                       | B1 | B2  | D  | C  | F |
| Rijavec <i>et al.</i> <sup>[29]</sup>        | 2000–2001  | 2008        | Slovenia | 105      | 55          | MTP                 | Multiplex PCR     | 16                      | 14 | 54  | 21 | -  | - |
| Baldiris-Avila <i>et al.</i> <sup>[30]</sup> | 2018       | 2020        | Colombia | 190      | 47          | CRA                 | PCR               | 21                      | 9  | 89  | 48 | 11 | 5 |
| Neamati <i>et al.</i> <sup>[31]</sup>        | 2014–2017  | 2019        | Iran     | 101      | 57          | MTP                 | PCR               | 32                      | 14 | 56  | 61 | -  | - |
| Kot <i>et al.</i> <sup>[32]</sup>            | 2007–2008  | 2016        | Poland   | 173      | 142         | -                   | Triplex PCR       | -                       | -  | -   | -  | -  | - |
| Katongole <i>et al.</i> <sup>[33]</sup>      | -          | 2020        | Uganda   | 200      | 125         | CRA                 | PCR               | -                       | -  | -   | -  | -  | - |
| Shrestha <i>et al.</i> <sup>[34]</sup>       | 2017       | 2019        | Nepal    | 159      | 86          | -                   | -                 | -                       | -  | -   | -  | -  | - |
| Tajbakhsh <i>et al.</i> <sup>[17]</sup>      | 2016–2017  | 2016        | Iran     | 130      | 80          | CRA                 | Multiplex PCR     | -                       | -  | -   | -  | -  | - |
| Kadry <i>et al.</i> <sup>[35]</sup>          | 2016–2017  | 2020        | Egypt    | 112      | 89          | MTP                 | PCR               | -                       | -  | -   | -  | -  | - |
| Agarwal <i>et al.</i> <sup>[36]</sup>        | 2010–2012  | 2013        | India    | 172      | 145         | MTP                 | -                 | 50                      | 22 | 81  | 19 | -  | - |
| Marhova <i>et al.</i> <sup>[37]</sup>        | -          | 2014        | Bulgaria | 50       | 12          | MTP                 | -                 | -                       | -  | -   | -  | -  | - |
| Soto <i>et al.</i> <sup>[38]</sup>           | -          | 2007        | Spain    | 151      | 69          | MTP                 | PCR               | 35                      | 7  | 88  | 21 | -  | - |
| Fattahi <i>et al.</i> <sup>[39]</sup>        | 2014       | 2015        | Iran     | 100      | 92          | MTP                 | PCR               | -                       | -  | -   | -  | -  | - |
| Nikzad <i>et al.</i> <sup>[40]</sup>         | 2017       | 2021        | Iran     | 64       | 55          | MTP                 | Duplex PCR        | -                       | -  | -   | -  | -  | - |
| Karam <i>et al.</i> <sup>[9]</sup>           | -          | 2018        | Iran     | 110      | 94          | MTP                 | PCR               | -                       | -  | -   | -  | -  | - |
| Elsayed Gawad <i>et al.</i> <sup>[41]</sup>  | 2014–2015  | 2018        | Egypt    | 175      | 134         | MTP                 | PCR               | 19                      | 10 | 113 | 33 | -  | - |
| Pompilio <i>et al.</i> <sup>[42]</sup>       | 2012–2014  | 2018        | Italy    | 37       | 29          | MTP, tissue culture | Triplex PCR       | 5                       | 26 | 2   | 3  | -  | - |
| Zamani and Salehzadeh <sup>[43]</sup>        | 2016       | 2018        | Iran     | 100      | 94          | MTP                 | PCR               | -                       | -  | -   | -  | -  | - |
| Naziri <i>et al.</i> <sup>[44]</sup>         | -          | 2021        | Iran     | 100      | 99          | MTP                 | Multiplex PCR     | -                       | -  | -   | -  | -  | - |
| Singh <i>et al.</i> <sup>[45]</sup>          | 2014       | 2016        | India    | 33       | 33          | MTP                 | -                 | -                       | -  | -   | -  | -  | - |
| Javed <i>et al.</i> <sup>[46]</sup>          | 2019       | 2020        | Pakistan | 50       | 50          | CRA, MTP            | Triplex PCR       | 15                      | 1  | 24  | 10 | -  | - |

Detection of biofilm-related virulence genes. UPEC=Uropathogenic *Escherichia coli*; MTP=Microtiter plat; CRA=Congo Red Agar; PCR=Polymerase chain reaction

reaction (PCR), Multiplex PCR, Triplex PCR, and Duplex PCR were also used in these studies to identify virulence genes.

## Overall effects

### Biofilm formation

As can be seen from the results in Figure 2 and Table 2, the prevalence of strains that were able to form biofilm varied between 24% and 99%. The pooled prevalence of biofilm formers was 74.7% (95% confidence interval [CI]: 65.1–82.4),  $Z = 4.6$ ,  $P = 0.00$ ,  $Q = 355.9$ , and  $I^2 = 94.6$ . Each line in the graphical display (Forest plot) denotes a study. The midpoint of the box signifies the point estimation of the effect (effect size), and its size (area) is proportionate to the weight of the study. Not all studies donate equally to the pooled consequences. Totally, studies that have a bigger N (number) afford more data and are as a result allocated larger weight. This is observed easily in most studies included in the present review. The diamond below the studies symbolizes the total combined effect (74.7%) from the involved studies. The width of the diamond displays the CI for the overall effect.

### Heterogeneity

If results of several studies constantly differ somewhat, studies are said to be heterogeneous when their fundamental target parameters vary. The magnitude of heterogeneity is assessed by the  $I^2$ . We observed the heterogeneity ( $Q = 355.9$ , and  $I^2 = 94.6$ ) between the studies included. These are results of outlying study results, bias in publication, differences in

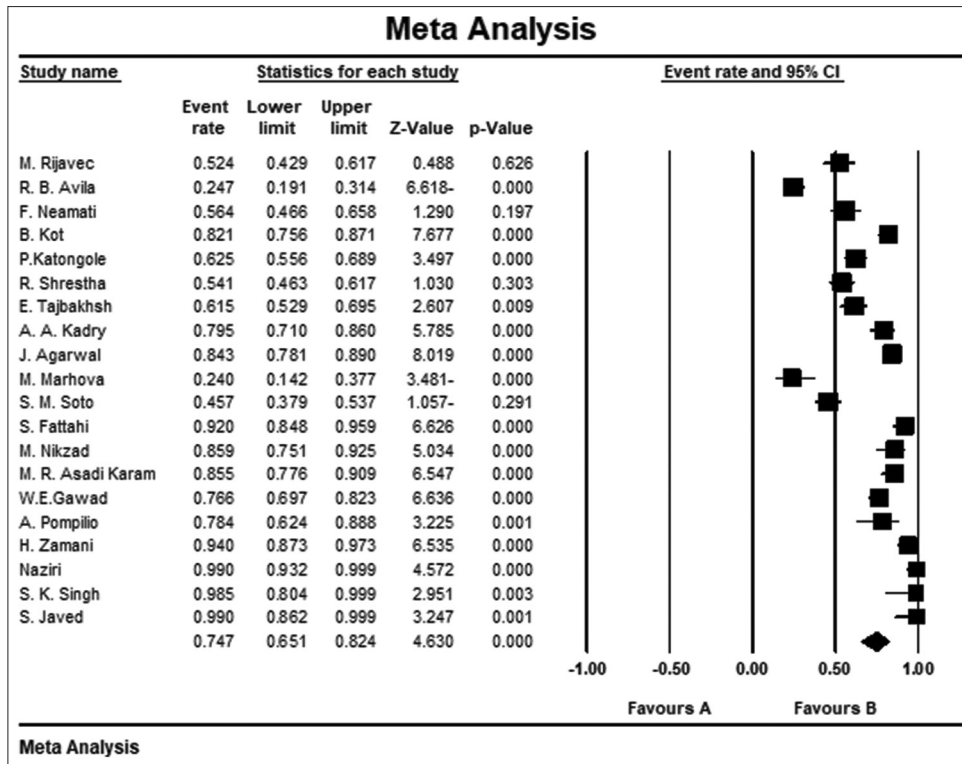
study methodologies, conditions used for measurement of variables, sampling, time of the study, study quality or geographical locations, and settings differences. Hence, for further study of heterogeneity, we analyzed through random effect model, evaluation of publication bias, assessment of sensitivity analysis, and performed subgroup analyses (based on the quality of studies, and time of the study, the prevalence of phylogenetic groups, and virulence genes).

### Publication bias

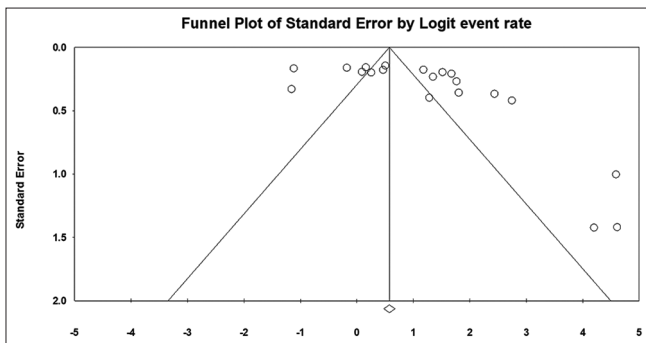
Publication bias is the most famous reporting bias. It results from the publication or non-publication of related articles, be contingent on the nature and direction of the findings. For example, a study is more probable to be published if the findings are significant. Due to the most studies are outside the funnel plot [Figure 3], indicating the presence of bias in the publication of studies included. For further evaluation, Egger's regression test was performed which showed no publication bias ( $P = 0.019$ ).

### Assessment of sensitivity analysis

We evaluated the sensitivity analysis by the exclusion of the studies with the biggest sample size (study conducted by Katongole *et al.*<sup>[39]</sup> sample size: 200), and the smallest sample size (Singh *et al.*<sup>[59]</sup> sample size: 33), or the study with the highest prevalence (Javed *et al.*<sup>[50]</sup> prevalence of 99%) from the analysis. The sensitivity analysis showed that there was no significant change in the target prevalence (73% [95% CI: 61.9–81.7]) versus combined prevalence (74.7% [95% CI: 65.1–82.4]).



**Figure 2:** Forest plots of studies reporting the frequency of biofilm producers of UPEC isolates recovered from patients with UTI. UPEC = Uropathogenic *Escherichia coli*; UTI = Urinary tract infection



**Figure 3:** Funnel plot of studies reporting the frequency of biofilm producers of UPEC isolates recovered from patients with UTI. UPEC = Uropathogenic *Escherichia coli*; UTI = Urinary tract infection

### Subgroup analyses based on the quality of studies

In the present review, only a study conducted by Marhova et al.,<sup>[40]</sup> has moderate quality, and the other ones have high quality. Results of the prevalence of biofilm formation in high-quality studies were (76.9% [95% CI: 67.7–84]) versus combined prevalence (both moderate and high-quality studies) 74.7% (95% CI: 65.1–82.4).

### Subgroup analyses based on the time of study

Here, we divide studies into study time groups (from 2000 to 2012, and 2013–2021), Combined prevalence of biofilm formation in the studies conducted ( $n = 7$ ) from 2000 to 2012 was 65% (95% CI: 48.5–78.5), and in studies ( $n = 13$ ) from 2013 to 2021 was 63.4% (95% CI: 50–75).

### Prevalence of phylogenetic groups

The prevalence of phylogenetic groups varied in studies included in the present review [Table 1]. Groups C and F only reported in one study each, for this reason, we deleted them from the meta-analysis. The combined prevalence of phylogenetic Groups A, B1, B2, and D was reported at 19.6% (95% CI: 14–26.8), 11% (95% CI: 5.1–22.1), 50.7% (95% CI: 43.2–58.1), and 20.5% (95% CI: 12.3–32), respectively.

### Prevalence of virulence genes

The most common virulence genes reported worldwide were *fimA*, *ecpA*, and *fimH*, with a combined prevalence of 90.3% (95% CI: 86.3–93.2), 86.6% (95% CI: 55.4–97.1), and 64.9% (95% CI: 45.8–80.2), respectively. In addition, the lowest prevalence related to genes *Hly* and *cnf1* with a prevalence of 8.7% (95% CI: 3.1–21.9), and 19.6% (95% CI: 0.073–0.43), respectively. Data regarding other virulence genes are abstracted in Table 2.

### Pooled prevalence of biofilm formation associated with uropathogenic *Escherichia coli* phylogenetic groups

The pooled prevalence of biofilm formation in UPEC isolates with phylogenetic Groups A, B1, B2, D, C, and F were 12.4%, 8.7%, 33.7%, 12.4%, 2.6%, and 2.65%, respectively. In addition, the combined prevalence of strong biofilm formation associated with UPEC phylogenetic Groups A, B1, B2, D, C, and F were 2.4, 3, 20.9, and 4.6%, respectively. Phylogenetic Groups C and F did not form strong biofilm.

**Table 2: Subgroups analysis for different variables in the present review**

| Subgroups           | Number studies | Heterogeneity test      |      |       | Egger's test |       | Random model   |       |       |
|---------------------|----------------|-------------------------|------|-------|--------------|-------|----------------|-------|-------|
|                     |                | Prevalence (95% CI) (%) | Z    | P     | Q            | P     | I <sup>2</sup> | T     | P     |
| Biofilm formation   | 20             | 74.7 (65.1–82.4)        | 4.6  | 0.00  | 355.9        | 0.019 | 94.6           | 2.56  | 0.00  |
| Phylogenetic groups |                |                         |      |       |              |       |                |       |       |
| A                   | 8              | 19.6 (14–26.8)          | 6.8  | 0.00  | 40.6         | 0.00  | 82.7           | 6     | 0.32  |
| B1                  | 8              | 11 (5.1–22.1)           | 4.9  | 0.00  | 89           | 0.00  | 92.1           | 0.4   | 0.69  |
| B2                  | 8              | 50.7 (43.2–58.1)        | 0.17 | 0.8   | 33.4         | 0.00  | 79.3           | 1.8   | 0.11  |
| D                   | 8              | 20.5 (12.3–32)          | 4.42 | 0.00  | 91.1         | 0.00  | 92.3           | 0.91  | 0.39  |
| Virulence genes     |                |                         |      |       |              |       |                |       |       |
| <i>cnf1</i>         | 6              | 19.6 (0.073–0.43)       | 2.44 | 0.01  | 139.2        | 0.00  | 96.4           | 1.1   | 0.29  |
| <i>ecpA</i>         | 2              | 86.6 (55.4–97.1)        | 2.2  | 0.027 | 5            | 0.025 | 80             | -     | -     |
| <i>fimA</i>         | 3              | 90.3 (86.3–93.2)        | 11.2 | 0.00  | 2.6          | 0.26  | 25.6           | 5.3   | 0.11  |
| <i>fimH</i>         | 7              | 85.6 (72.8–93)          | 4.3  | 0.00  | 115          | 0.00  | 94.7           | 6     | 0.001 |
| <i>fyuA</i>         | 6              | 64.9 (45.8–80.2)        | 1.5  | 0.12  | 125.2        | 0.00  | 96             | 1     | 0.35  |
| <i>Hly</i>          | 4              | 8.7 (3.1–21.9)          | 4.2  | 0.00  | 25.2         | 0.00  | 88.1           | 3.1   | 0.08  |
| <i>HlyA</i>         | 5              | 26.3 (12.8–46.5)        | 2.2  | 0.023 | 58           | 0.00  | 93.1           | 0.55  | 0.62  |
| <i>lha</i>          | 3              | 31.2 (17.5–49.4)        | 2    | 0.043 | 19.5         | 0.00  | 89.7           | 2.5   | 0.23  |
| <i>iroN</i>         | 3              | 44.2 (25.6–64.6)        | 0.54 | 0.58  | 25.4         | 0.00  | 92.1           | 5.6   | 0.11  |
| <i>iutA</i>         | 4              | 51.7 (29.4–73.4)        | 0.14 | 0.88  | 79           | 0.00  | 96.2           | 0.86  | 0.47  |
| <i>kpsMTII</i>      | 2              | 67 (60.6–72.8)          | 5    | 0.00  | 0.007        | 0.93  | 0.00           | -     | -     |
| <i>PAI</i>          | 2              | 50.9 (45.1–56.8)        | 0.31 | 0.75  | 12.1         | 0.00  | 91.7           | -     | -     |
| <i>pap</i>          | 3              | 36 (20.8–54.8)          | 1.4  | 0.14  | 32.3         | 0.00  | 93.8           | 0.07  | 0.95  |
| <i>papA</i>         | 2              | 33.2 (19.5–50.5)        | 1.9  | 0.057 | 9.3          | 0.002 | 89.2           | -     | -     |
| <i>papAH</i>        | 3              | 27.7 (16.1–43.2)        | 2.7  | 0.006 | 12.3         | 0.002 | 83.7           | 0.48  | 0.71  |
| <i>papC</i>         | 5              | 62.2 (37.4–81.9)        | 0.96 | 0.33  | 86.4         | 0.00  | 95.3           | 0.79  | 0.48  |
| <i>papG</i>         | 3              | 41.8 (15.4–73.9)        | 0.47 | 0.63  | 40.8         | 0.00  | 95             | 0.017 | 0.98  |
| <i>sfafoc</i>       | 11             | 20.4 (12.6–31.5)        | 4.5  | 0.00  | 152.7        | 0.00  | 93.4           | 2.4   | 0.035 |
| <i>traT</i>         | 3              | 65 (54.4–74.3)          | 2.7  | 0.006 | 14.5         | 0.002 | 79.3           | 0.45  | 0.69  |
| <i>usp</i>          | 3              | 21.2 (4.7–59.3)         | 1.5  | 0.12  | 46.8         | 0.00  | 95.7           | 0.70  | 0.60  |

CI=Confidence interval

Combined strong and moderate biofilm formation in UPEC phylogenetic Group B2 was higher than that of the other phylogenetic groups, while the pooled prevalence of weak biofilm producers was higher in phylogenetic group D than that of the other phylogenetic groups [Table 3].

### Correlation between biofilm formation, virulence factors, and phylogenetic groups

Our findings in Table 4 showed that several studies showed a relationship between biofilm production and virulence genes or a correlation between biofilm formation and phylogenetic groups.

## DISCUSSION

In our review, the prevalence of strains that were able to form biofilm varied between 24% and 99%. The pooled prevalence of biofilm formers was 74.7%. Bacterial biofilm is of big concern owing to host immunological defences and also, antibiotic treatment failure.<sup>[47]</sup> This high biofilm level indicates the high importance of biofilm formation in UPEC strains causing UTI.<sup>[1]</sup> The capability of microorganisms to produce biofilms on medical devices, for example, catheters, is supposed to play a key role in the growth of

hospital-acquired infections such as catheter-associated UTI.<sup>[48,49]</sup> For successful biofilm growth, the significant stage is the adherence to the surfaces leading to accumulation, colonization, and finally biofilm production.<sup>[50]</sup> Therefore, in the present review, we expect that there will be a significant correlation between adhesins and attachment factors and biofilm formation. The same relationship was shown by the present review, as the highest prevalence of virulence factors belonged to genes involved in attachment and colonization (*fimA*, *ecpA*, and *fimH*, with a prevalence of 90.3%, 86.6%, and 64.9%, respectively). Our findings showed that several studies showed a relationship between biofilm production and virulence genes or a correlation between biofilm formation and phylogenetic groups. These results demonstrate that biofilm-formers are more pathogenic than the planktonic form in UTI and that biofilm production causes increasing the pathogenicity of UPEC isolates, and also the severity of disease, making biofilm-associated UTI very hard to treat.<sup>[39,51]</sup>

In a study conducted by Baldiris-Avila *et al.* virulence genetic profiles *fimH*, *fyuA*, *ompT*, *traT*, and *kpsMTII* were associated with strong biofilm formation.<sup>[30]</sup> Tajbakhsh *et al.* reported that the biofilm was significantly correlated

**Table 3: Prevalence of biofilm formation associated with uropathogenic *Escherichia coli* phylogenetic groups**

| Study                     | UPEC<br>(n) | Biofilm                    | Phylogenetic groups (%) |     |      |      |     |     | P   |
|---------------------------|-------------|----------------------------|-------------------------|-----|------|------|-----|-----|---|
|                           |             |                            | A                       | B1  | B2   | D    | C   | F   |   |
| Saima Javed, 2020         | 50          | Strong                     | 2                       | 1   | 15   | 2    | -   | -   | <0.001  |
|                           |             | Moderate                   | 4                       | 0   | 8    | 7    | -   | -   |   |
|                           |             | Weak                       | 9                       | 0   | 1    | 1    | -   | -   |   |
| Rosa Baldiris-Avila       | 190         | Strong                     | 0                       | 6   | 28   | 9    | 0   | 0   | 0.025   |
|                           |             | Moderate                   | 14                      | 0   | 41   | 14   | 5   | 5   |   |
|                           |             | Weak                       | 4                       | 0   | 10   | 25   | 0   | 0   |   |
| Soto                      | 151         | Strong                     | 10                      | 3   | 48   | 8    | -   | -   | Biofilm with B2 (P=0.009).<br>biofilm with B1 and<br>D (P=0.87 and 0.45,<br>respectively) |
|                           |             | Moderate                   |                         |     |      |      |     |     |   |
|                           |             | Weak                       |                         |     |      |      |     |     |   |
| Arianna Pompilio,<br>2018 | 37          | Strong                     | 1                       | -   | -    | -    | -   | -   | 0.026   |
|                           |             | Moderate                   | 2                       | 7   | 2    | -    | -   | -   |   |
|                           |             | Weak                       | 1                       | 24  | 1    | 2    | -   | -   |   |
| All studies               | Total       | Combined biofilm formation | 12.4                    | 8.7 | 33.7 | 12.4 | 2.6 | 2.6 | -   |
|                           |             | Combined strong biofilm    | 2.4                     | 3   | 20.9 | 4.6  | -   | -   |   |
|                           |             | Combined moderate biofilm  | 7.3                     | 2.2 | 19.4 | 9.7  | 2.6 | 2.6 |   |
|                           |             | Combined weak biofilm      | 5.2                     | 4   | 4.6  | 7.1  | -   | -   |   |

Due to the lack of data related to the correlation between biofilm formation and phylogenetic groups, meta-analysis was not performed. UPEC=Uropathogenic *Escherichia coli*

with *fimH*, *pap*, *afa*, and *sfa* virulence genes.<sup>[17]</sup> Similarly, in a study conducted by Agarwal *et al.*, *papA* and *malX* genes were found significantly higher in biofilm formers, while virulence factors scores did not differ meaningfully by the intensity of biofilm formation.<sup>[36]</sup> Another study reported a significant correlation between the existence of the *papC* gene and biofilm, but no statistically significant correlation was reported between the presence of *fimA* and *hly* genes and biofilm production.<sup>[39]</sup>

On the contrary, Neamati *et al.*,<sup>[31]</sup> Kot *et al.*,<sup>[32]</sup> Katongole *et al.*,<sup>[33]</sup> Marhova *et al.*,<sup>[37]</sup> and Rijavec *et al.*<sup>[29]</sup> reported no statistical correlation between biofilm production and different virulence factors in UPEC strains.

UTIs are typically treated empirically particularly uncomplicated ones, which is accompanied by Excessive use of some antibiotics and the misuse of certain antibiotics.<sup>[52]</sup> This leads to the widespread prevalence of resistant strains,<sup>[52]</sup> which has made the treatment of infections caused by these strains difficult and has caused serious concern for the health system worldwide.<sup>[53]</sup>

The prevalence of phylogenetic groups varied in different studies included from worldwide, the combined prevalence of phylogenetic groups A, B1, B2, and D were reported at 19.6%, 11%, 50.7%, and 20.5%, respectively. Therefore, according to data obtained in this review, *E. coli* strains to cause UTI predominantly belonged to phylogenetic groups B2 and D. A study reported that specific chromosomal background, only partly consistent with the phylogenetic background could lead to mutation to antibiotic resistance.<sup>[54]</sup> These phylogenetic groups

(B2 and D) show a distinct trait owing to their high content of virulence factors making them pathogenic clinical strains and hard to treat.<sup>[55]</sup> In contrast, phylogenetic group A had a lower number of virulence factors, and isolates with phylogenetic group A probably are commensal which can lead to UTIs and obtain horizontally-transferred virulence genes in the gastrointestinal tract, thus permitting them to colonize the urinary tract.<sup>[55-57]</sup> Baldiris-Avila *et al.* showed a direct correlation between the virulence genes, and phylogenetic groups A and B2.<sup>[30]</sup> This difference reported from numerous studies in terms of variations in phylogenetic groups is due to factors such as geographical region, antibiotic resistance pattern, site of infection,<sup>[58]</sup> environmental and social conditions, dietary and host genetic factors, the health status of the host, and difference in sampling regions.<sup>[59]</sup>

In the present systematic review and meta-analysis, the pooled prevalence of biofilm formation in UPEC isolates with phylogenetic groups A, B1, B2, D, C, and F were 12.4%, 8.7%, 33.7%, 12.4%, 2.6%, and 2.65, respectively. In addition, the combined prevalence of strong biofilm formation associated with UPEC phylogenetic groups A, B1, B2, D, C, and F were 2.4, 3, 20.9, and 4.6%, respectively. Phylogenetic groups C and F did not form strong biofilm. Hence, combined strong and moderate biofilm formation in UPEC phylogenetic group B2 were higher than that of the other phylogenetic groups, while the pooled prevalence of weak biofilm producers was higher in phylogenetic group D than that of the other phylogenetic groups. There was a direct association between the virulence genes and phylogenetic groups A and B2,<sup>[30]</sup> and there is a higher biofilm formation among phylogenetic groups B2 and D as shown by Javed

**Table 4: Correlation between biofilm formation, virulence factors, and phylogenetic groups**

| Study             | Publication | Location | Explanations   |
|-------------------|-------------|----------|--|
| M. Rijavec        | 2008        | Slovenia | None of the virulence factors correlated with biofilm formation<br>Biofilm formation was related to phylogroups B1 and D   |
| R. B.-Avila       | 2020        | Colombia | There was a direct association between the virulence genes and phylogenetic Group A and B2<br>The correlation was observed between strong biofilm, multidrug resistance, and virulence genetic profiles <i>fimH</i> , <i>fyuA</i> , <i>ompT</i> , <i>traT</i> , and <i>kpsMTII</i>                               |
| F. Neamati        | 2019        | Iran     | No significant correlation was reported between resistance and virulence genes   |
| B. Kot            | 2016        | Poland   | No relationship was found between biofilm production and adhesin genes and expression of the mannose-resistant or mannose-sensitive fimbriae, or phylogenetic groups, except the <i>aerobactin</i> gene  |
| P. Katongole      | 2020        | Uganda   | Biofilm formation was not significantly correlated with the presence of the virulence genes  |
| R. Shrestha       | 2019        | Nepal    | -  |
| E. Tajbaksh       | 2016        | Iran     | A significant correlation between biofilm formation and <i>fimH</i> , <i>pap</i> , <i>afa</i> , and <i>sfa</i> virulence genes was confirmed ( $P<0.05$ )  |
| A. A Kadry        | 2020        | Egypt    | Strong biofilm formation in non-MDR strains were higher than MDR strains, while the percentage of MDR isolates tended to form weak biofilm was higher than non-MDR isolates  |
| J. Agarwal        | 2013        | India    | No significant correlation was observed in the intensity of biofilm production among different phylogroups or virulence scores<br><i>papA</i> and <i>malX</i> genes were found significantly higher in biofilm formers, while virulence factors scores did not differ meaningfully in terms of biofilm intensity |
| M. Marhova        | 2014        | Bulgaria |  |
| S. M. Soto        | 2007        | Spain    | Biofilm production in phylogenetic Group A was lower than that of the other phylogenetic groups<br>Biofilm formation was higher in phylogenetic Group B2 compared to the other phylogenetic groups<br>No relationship of biofilm production was seen with phylogenetic Groups B1 and D                           |
| S. Fattahi        | 2015        | Iran     | A significant correlation was confirmed between <i>papC</i> gene and biofilm ( $P<0.01$ ), no statistically significant correlation was found between <i>fimA</i> and <i>hly</i> genes and biofilm production ( $P<0.072$ , $P<0.104$ )  |
| Uma B. Maheswari  | 2013        | India    | -  |
| M. Nikzad         | 2021        | Iran     | -  |
| M. R. Asadi Karam | 2018        | Iran     | -  |
| W.E. Gawad        | 2018        | Egypt    | There was a significant relationship between biofilm production and multidrug resistance ( $P=0.00$ )  |
| A. Pompilio       | 2018        | Italy    | Data did not report statistically significant difference among phylogenetic groups of UPEC strains in biofilm production capacity. In APN isolates, Isolates with higher biofilm formation contained <i>iha</i> , but <i>iroN</i> and <i>KpSMT-K1</i> were seen in strains formed weak biofilm                   |
| H. Zamani         | 2018        | Iran     | UPEC isolates with a moderate to strong biofilm ability had a higher amount of three AFGs<br>No significant association was seen between existence of <i>fimA</i> , <i>fimH</i> , <i>papC</i> , and <i>papEF</i> and biofilm formers   |
| Naziri            | 2021        | Iran     | A significant correlation was found between <i>sfa/focDE</i> gene with moderate and strong biofilm production  |
| S. K. Singh       | 2016        | India    | -  |
| S. Javed          | 2020        | Pakistan | Strong and moderate biofilm producers were seen in strains contained phylogenetic Groups B2 and D<br>All of isolates with phylogenetic group, A were low biofilm producer  |

UPEC=Uropathogenic *Escherichia coli*; MDR=Multidrug-resistant; APN=Acute pyelonephritis; AFGs=Adhesion factor genes

*et al.*,<sup>[46]</sup> and Soto *et al.*<sup>[38]</sup> On the contrary, other studies included in the present review did not report statistically significant differences among phylogenetic groups of UPEC strains in biofilm production capacity.<sup>[29,32,36,42]</sup>

In general, the presence of different phylogenetic groups causes genetic diversity in UPEC strains. Moreover, these isolates are the repository of genes that encode the factors virulence that can be transmitted horizontally to other bacterial species or they increase and strengthen the genetic background or the acquisition of new genetic information for possible transmission.<sup>[30]</sup>

The exclusion of unpublished studies and studies published in languages other than English, and not contacting the authors in case of a question are the most important limitations of the present study.

## CONCLUSIONS

According to data obtained in the present systematic review and meta-analysis, the highest combined biofilm formation was reported in UPEC strains. Furthermore, several studies showed a statistically significant correlation between biofilm production with virulence genes and phylogenetic groups.



This high level of biofilm production and the presence of a positive correlation indicates the important role biofilm plays in UTI caused by UPEC isolates. Subsequently, biofilm causes the persistence of UPEC in the urinary tract and indwelling devices (such as urinary catheters), increasing the recurrence, severity, and antibiotic treatment failure. Moreover, the detection of virulence factors and phylogenetic groups that cause biofilm production of UPEC strains is important in developing valuable preventive and therapeutic methods.

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#### Conflicts of interest

There are no conflicts of interest.

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**Supplementary Table 1: The Joanna Briggs institute prevalence critical appraisal tool**

| Study             | 1. Was the sample frame appropriate to address the target population? | 2. Were participants sampled in an appropriate way? | 3. Was the sample size adequate? | 4. Were the study subjects and the setting described in detail? | 5. Was the data analysis conducted with sufficient coverage of the identified sample? | 6. Were valid methods used for the identification of the condition? | 7. Was the condition measured in a standard, reliable way for all participants? | 8. Was there appropriate statistical analysis? | 9. Was the response rate adequate, and if not, was the low response rate managed appropriately? | Overall  |
|-------------------|---|---|----------------------------------|---|---|---|---|--|---|----------|
| M. Rijavec        | Yes   | Yes   | Yes                              | Yes   | Yes   | Yes   | Yes   | Yes  | Yes   | Included |
| R. B. Avila       | Yes   | Yes   | Yes                              | Yes   | Yes   | Yes   | Yes   | Yes  | Yes   | Included |
| F. Neamati        | Yes   | Yes   | Yes                              | Yes   | Yes   | Yes   | Yes   | Yes  | Yes   | Included |
| B. Kot            | Yes   | Yes   | Yes                              | Yes   | Yes   | Yes   | Yes   | Yes  | Yes   | Included |
| P.Katongole       | Yes   | Yes   | Yes                              | Yes   | Yes   | Yes   | Yes   | Yes  | Yes   | Included |
| R. Shrestha       | Yes   | Yes   | Yes                              | Yes   | Yes   | Yes   | Yes   | Yes  | Yes   | Included |
| E. Tajbakhsh      | Yes   | Yes   | Yes                              | Yes   | Yes   | Yes   | Yes   | Yes  | Yes   | Included |
| A. A. Kadry       | Yes   | Yes   | Yes                              | Yes   | Yes   | Yes   | Yes   | Yes  | Yes   | Included |
| J. Agarwal        | Yes   | Yes   | Yes                              | Yes   | Yes   | Yes   | Yes   | Yes  | Yes   | Included |
| M. Marhova        | Yes   | Yes   | No                               | Yes   | No  | Yes   | Yes   | Yes  | Yes   | Included |
| S. M. Soto        | Yes   | Yes   | Yes                              | Yes   | Yes   | Yes   | Yes   | Yes  | Yes   | Included |
| S. Fattahi        | Yes   | Yes   | Yes                              | Yes   | Yes   | Yes   | Yes   | Yes  | Yes   | Included |
| M. Nikzad         | Yes   | Yes   | No                               | Yes   | Yes   | Yes   | Yes   | Yes  | Yes   | Included |
| M. R. Asadi Karam | Yes   | Yes   | Yes                              | Yes   | Yes   | Yes   | Yes   | Yes  | Yes   | Included |
| W.E.Gawad         | Yes   | Yes   | Yes                              | Yes   | Yes   | Yes   | Yes   | Yes  | Yes   | Included |
| A. Pompilio       | Yes   | Yes   | Yes                              | Yes   | Yes   | Yes   | Yes   | Yes  | Yes   | Included |
| H. Zamani Naziri  | Yes   | Yes   | Yes                              | Yes   | Yes   | Yes   | Yes   | Yes  | Yes   | Included |
| S. K. Singh       | Yes   | Yes   | Yes                              | Yes   | Yes   | Yes   | Yes   | Yes  | Yes   | Included |
| S. Javed          | Yes   | Yes   | Yes                              | Yes   | Yes   | Yes   | Yes   | No   | Yes   | Included |

U=Unclear; N/A=Not applicable