**Supplementary Materials**

***Associations between virulence factors and resistance to antibiotics***

Resistance of more virulent bacteria could interfere with the effective treatment of infections due to them and increase the rate of morbidity and mortality. Investigation of the associations between the antimicrobial resistance and presence of the virulence genes showed that the presence of *papC* gene was associated with increased in Ceftriaxone resistance (*p* = 0.04) (Supplementary Table S1) and also the presence of *malX* gene was associated with increased in Amikacin (*p* = 0.007), Ciprofloxacin (*p* = 0.007), and Levofloxacin resistance (*p* = 0.02) (Supplementary Table S2). In the Study from Mongolia1, UPEC strains which harbored the *papC* gene showed the more resistance to Ampicillin and Trimethoprim-Sulfamethoxazole.

In contrast, the presence of *fimH* gene was associated with decreased in Cefixime (*p* = 0.03), Ceftazidime (*p* = 0.04), and Ciprofloxacin resistance (*p* = 0.04) (Supplementary Table S1). Presence of *hlyD* gene was associated with decreased in Cephalexin (*p* = 0.02), Ceftriaxone (*p* = 0.02), Ceftazidime (*p* = 0.002), Cefotaxime (*p* = 0.03), Levofloxacin (*p* = 0.02), and Tetracycline resistance (*p* = 0.03) (Supplementary Table S2). Further, the presence of *ibeA* gene was associated with decreased in Ceftazidime (*p* = 0.03), and Tetracycline resistance (*p* = 0.002) (Supplementary Table S2). Presence of *iss* gene was associated with decreased in Gentamicin resistance (*p* = 0.05) and also presence of *tsh* gene was associated with decreased in Nitrofurantoin resistance (*p* = 0.02) (Supplementary Table S2). Some articles revealed that the presence of virulence genes is negatively associated with resistance to some antibiotics, but results of some other articles were contradictory.2,3

**Supplementary Table S1** Associations between the antibiotic resistance and presence of the adhesins a

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Antibiotics** | ***fimH*** | | ***papA*** | | | ***papC*** | | ***csgA*** | | ***afa*** | | ***tsh*** | |
| ***+***  **(n=57)** | ***-***  **(n=21)** | ***+***  **(n=27)** | ***-***  **(n=51)** | | ***+***  **(n=35)** | ***-***  **(n=43)** | ***+***  **(n=64)** | ***-***  **(n=14)** | ***+***  **(n=11)** | ***-***  **(n=67)** | ***+***  **(n=28)** | ***-***  **(n=50)** |
| **β- lactam antibiotics (Penicillins)** | | | | | | | |
| Penicillin | 57 (100) | 21 (100) | 27 (100) | 51 (100) | | 35 (100) | 43 (100) | 64 (100) | 14 (100) | 11 (100) | 67 (100) | 28 (100) | 50 (100) |
| Ampicillin | 57 (100) | 21 (100) | 27 (100) | 51 (100) | | 35 (100) | 43 (100) | 64 (100) | 14 (100) | 11 (100) | 67 (100) | 28 (100) | 50 (100) |
| Amoxicillin | 57 (100) | 21 (100) | 27 (100) | 51 (100) | | 35 (100) | 43 (100) | 64 (100) | 14 (100) | 11 (100) | 67 (100) | 28 (100) | 50 (100) |
| **β- lactam antibiotics (First generation cephalosporins)** | | | | |
| Cephalexin | 30 (52.6) | 14 (66.7) | 15 (55.6) | 29 (56.9) | | 21 (60.0) | 23 (53.5) | 36 (56.3) | 8 (57.1) | 6 (54.5) | 38 (56.7) | 13 (46.4) | 31 (62.0) |
| **β- lactam antibiotics (third generation cephalosporins)** | | | | |
| Cefixime | 27 (47.4) | 16 (76.2) | 17 (63.0) | 26 (51.0) | | 23 (65.7) | 20 (46.5) | 36 (56.3) | 7 (50.0) | 6 (54.5) | 37 (55.2) | 14 (50.0) | 29 (58.0) |
| Ceftriaxone | 27 (47.4) | 15 (71.4) | 16 (59.3) | 26 (51.0) | | 23 (65.7) | 19 (44.2) | 36 (56.3) | 6 (42.9) | 6 (54.5) | 36 (53.7) | 11 (39.3) | 31 (62.0) |
| Ceftazidime | 21 (36.8) | 13 (61.9) | 11 (40.7) | 23 (45.1) | | 16 (45.7) | 18 (41.9) | 28 (43.8) | 6 (42.9) | 4 (36.4) | 30 (44.8) | 9 (32.1) | 25 (50.0) |
| Cefotaxime | 28 (49.1) | 15 (71.4) | 15 (55.6) | 28 (54.9) | | 23 (65.7) | 20 (46.5) | 35 (54.7) | 8 (57.1) | 6 (54.5) | 37 (55.2) | 14 (50.0) | 29 (58.0) |
| **Aminoglycosides** |  | |  | | |  | |  | |  |  |  |  |
| Gentamicin | 7 (12.3) | 6 (28.6) | 5 (18.5) | 8 (15.7) | | 5 (14.3) | 8 (18.6) | 10 (15.6) | 3 (21.4) | 3 (27.3) | 10 (14.9) | 4 (14.3) | 9 (18.0) |
| Amikacin | 11 (19.3) | 6 (28.6) | 3 (11.1) | 14 (27.5) | | 10 (28.6) | 7 (16.3) | 13 (20.3) | 4 (28.6) | 4 (36.4) | 13 (19.4) | 8 (28.6) | 9 (18.0) |
| **Fluoroquinolones** | | |  | | |  | |  | |  |  |  |  |
| Ciprofloxacin | 31 (54.4) | 16 (76.2) | 14 (51.9) | 33 (64.7) | | 20 (57.1) | 27 (62.8) | 39 (60.9) | 8 (57.1) | 6 (54.5) | 41 (61.2) | 15 (53.6) | 32 (64.0) |
| Levofloxacin | 29 (50.9) | 15 (71.4) | 13 (48.1) | 31 (60.8) | | 17 (48.6) | 27 (62.8) | 35 (54.7) | 9 (64.3) | 6 (54.5) | 38 (56.7) | 16 (57.1) | 28 (56.0) |
| **Dihydrofolate reductase inhibitors** | | | | | | | |
| Trimethoprim- Sulfamethoxazole | 34 (59.6) | 16 (76.2) | 18 (66.7) | 32 (62.7) | | 21 (60.0) | 29 (67.4) | 39 (60.9) | 11 (78.6) | 7 (63.6) | 43 (64.2) | 19 (67.9) | 31 (62.0) |
| **Nitrofurans** |  | |  | | |  | |  | |  |  |  |  |
| Nitrofurantoin | 12 (21.1) | 6 (28.6) | 8 (29.6) | 10 (19.6) | | 7 (20.0) | 11 (25.6) | 16 (25.0) | 2 (14.3) | 1 (9.1) | 17 (25.4) | 3 (10.7) | 15 (30.0) |
| **Tetracyclines** |  | |  | | |  | |  | |  |  |  |  |
| Tetracycline | 33 (57.9) | 16 (76.2) | 19 (70.4) | 30 (58.8) | | 23 (65.7) | 26 (60.5) | 41 (64.1) | 8 (57.1) | 6 (54.5) | 43 (64.2) | 15 (53.6) | 34 (68.0) |

a Values were showed as No. (%).

**Supplementary Table S2.** Associations between the antibiotic resistance and presence of some virulence genes a

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Antibiotics** | ***hlyD*** | | ***ibeA*** | | ***iss*** | | ***sitA*** | | ***malX*** | |
| ***+***  **(n=19)** | ***-***  **(n=59)** | ***+***  **(n=11)** | ***-***  **(n=67)** | ***+***  **(n=18)** | ***-***  **(n=60)** | ***+***  **(n=57)** | ***-***  **(n=21)** | ***+***  **(n=21)** | ***-***  **(n=57)** |
| **β- lactam antibiotics (Penicillins)** | | | | | | |
| Penicillin | 19 (100) | 59 (100) | 11 (100) | 67 (100) | 18 (100) | 60 (100) | 57 (100) | 21 (100) | 21 (100) | 57 (100) |
| Ampicillin | 19 (100) | 59 (100) | 11 (100) | 67 (100) | 18 (100) | 60 (100) | 57 (100) | 21 (100) | 21 (100) | 57 (100) |
| Amoxicillin | 19 (100) | 59 (100) | 11 (100) | 67 (100) | 18 (100) | 60 (100) | 57 (100) | 21 (100) | 21 (100) | 57 (100) |
| **β- lactam antibiotics (First generation cephalosporins)** | | | | |
| Cephalexin | 6 (31.6) | 38 (64.4) | 4 (36.4) | 40 (59.7) | 8 (44.4) | 36 (60.0) | 33 (57.9) | 11 (52.4) | 14 (66.7) | 30 (52.6) |
| **β- lactam antibiotics (third generation cephalosporins)** | | | | |
| Cefixime | 8 (42.1) | 35 (59.3) | 4 (36.4) | 39 (58.2) | 8 (44.4) | 35 (58.3) | 33 (57.9) | 10 (47.6) | 15 (71.4) | 28 (49.1) |
| Ceftriaxone | 6 (31.6) | 36 (61.0) | 5 (45.5) | 37 (55.2) | 9 (50.0) | 33 (55.0) | 30 (52.6) | 12 (57.1) | 14 (66.7)) | 28 (49.1) |
| Ceftazidime | 4 (21.1) | 30 (50.8) | 1 (9.1) | 33 (49.3) | 7 (38.9) | 27 (45.0) | 24 (42.1) | 10 (47.6) | 10 (47.6) | 24 (42.1) |
| Cefotaxime | 8 (42.1) | 35 (59.3) | 3 (27.3) | 40 (59.7) | 8 (44.4) | 35 (58.3) | 32 (56.1) | 11 (52.4) | 15 (71.4) | 28 (49.1) |
| **Aminoglycosides** |  | |  | |  | |  | |  |  |
| Gentamicin | 2 (10.5) | 11 (18.6) | 1 (9.1) | 12 (17.9) | 0 (0.0) | 13 (21.7) | 9 (15.8) | 4 (19.0) | 4 (19.0) | 9 (15.8) |
| Amikacin | 3 (15.8) | 14 (23.7) | 4 (36.4) | 13 (19.4) | 3 (16.7) | 14 (23.3) | 14 (24.6) | 3 (14.3) | 9 (42.9) | 8 (14.0) |
| **Fluoroquinolones** | | |  | |  | |  | |  |  |
| Ciprofloxacin | 9 (47.4) | 38 (64.4) | 4 (36.4) | 43 (64.2) | 8 (44.4) | 39 (65.0) | 32 (56.1) | 15 (71.4) | 18 (85.7) | 29 (50.9) |
| Levofloxacin | 6 (31.6) | 38 (64.4) | 4 (36.4) | 40 (59.7) | 7 (38.9) | 37 (61.7) | 29 (50.9) | 15 (71.4) | 17 (81.0) | 27 (47.4) |
| **Dihydrofolate reductase inhibitors** | | | | | | | | | | |
| Trimethoprim- Sulfamethoxazole | 11 (57.9) | 39 (66.1) | 5 (45.5) | 45 (67.2) | 9 (50.0) | 41 (68.3) | 37 (64.9) | 13 (61.9) | 15 (71.4) | 35 (61.4) |
| **Nitrofurans** |  | |  | |  | |  | |  |  |
| Nitrofurantoin | 2 (10.5) | 16 (27.1) | 0 (0.0) | 18 (26.9) | 4 (22.2) | 14 (23.3) | 11 (19.3) | 7 (33.3) | 4 (19.0) | 14 (24.6) |
| **Tetracyclines** |  | |  | |  | |  | |  |  |
| Tetracycline | 8 (42.1) | 41 (69.5) | 2 (18.2) | 47 (70.1) | 12 (66.7) | 37 (61.7) | 36 (63.2) | 13 (61.9) | 15 (71.4) | 34 (59.6) |

a Values were showed as No. (%)

***Associations between the presences of different virulence genes***

Significant associations were found among the presences of the tested adhesins, as presence of *fimH* gene was associated with *papA* gene (*p* = 0.02), and presence of *papC* gene was associated with *csgA* gene (*p* = 0.01) (Supplementary Table S3). Likewise Derakhshandeh et al study4 also revealed a significant association (*p* < 0.05) between the presence of *fimH* and *papA* genes.

Additionally significant associations were found between the presence of other virulence genes in our study, as the presence of *afa* gene was associated with *malX* gene (*p* = 0.007), the presence of *tsh* gene was associated with *ibeA* (*p* = 0.04) and *sitA* genes (*p* = 0.001), and finally, the presence of *hlyD* gene was associated with *sitA* gene (*p* = 0.002) (Supplementary Tables S3-S4).

The significant association (*p* < 0.05) between the presence of *hly* and P fimbriae was reported in several studies which conducted on *E. coli* isolates from UTIs.3,4,5 In Derakhshandeh et al study4 in Shiraz, Iran, significant associations (*p* < 0.05) was seen between the presence of *papA* gene and presence of *sitA* or *tsh* genes and also the presence of *hlyD* gene with *papA*, *sitA*, and *tsh* genes. These results indicate that a single *E. coli* strain could have multiple adhesins and also the combination of various VFs could present in a single *E. coli* in association with each other’s.

**Supplementary Table S3** Associations between the virulence genes and presence of the adhesins a

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Virulence gene** | ***fimH*** | | ***papA*** | | ***papC*** | | ***csgA*** | | ***afa*** | | ***tsh*** | |
| ***+***  **(n=57)** | ***-***  **(n=21)** | ***+***  **(n=27)** | ***-***  **(n=51)** | ***+***  **(n=35)** | ***-***  **(n=43)** | ***+***  **(n=64)** | ***-***  **(n=14)** | ***+***  **(n=11)** | ***-***  **(n=67)** | ***+***  **(n=28)** | ***-***  **(n=50)** |
| **Adhesin** |  |  |  |  |  |  |  |  |  |  |  |  |
| *fimH* (+) | \* | \* | 24 (88.9) | 33 (64.7) | 28 (80.0) | 29 (67.4) | 48 (75.0) | 9 (64.3) | 8 (72.7) | 49 (73.1) | 22 (78.6) | 35 (70.0) |
| *papA* (+) | 24 (42.1) | 3 (14.3) | \* | \* | 15 (42.9) | 12 (27.9) | 23 (35.9) | 4 (28.6) | 3 (27.3) | 24 (35.8) | 11 (39.3) | 16 (32.0) |
| *papC* (+) | 28 (49.1) | 7 (33.3) | 15 (55.6) | 20 (39.2) | \* | \* | 33 (51.6) | 2 (14.3) | 7 (63.6) | 28 (41.8) | 13 (46.4) | 22 (44.0) |
| *csgA* (+) | 48 (84.2) | 16 (76.2) | 23 (85.2) | 41 (80.4) | 33 (94.3) | 31 (72.1) | \* | \* | 10 (90.9) | 54 (80.6) | 20 (71.4) | 44 (88.0) |
| *afa* (+) | 8 (14.0) | 3 (14.3) | 3 (11.1) | 8 (15.7) | 7 (20.0) | 4 (9.3) | 10 (15.6) | 1 (7.1) | \* | \* | 6 (21.4) | 5 (10.0) |
| *tsh* (+) | 22 (38.6) | 6 (28.6) | 11 (40.7) | 17 (33.3) | 13 (37.1) | 15 (34.9) | 20 (31.3) | 8 (57.1) | 6 (54.5) | 22 (32.8) | \* | \* |
| **Toxin** |  |  |  |  |  |  |  |  |  |  |  |  |
| *hlyD* (+) | 16 (28.1) | 3 (14.3) | 8 (29.6) | 11 (21.6) | 11 (31.4) | 8 (18.6) | 17 (26.6) | 2 (14.3) | 3 (27.3) | 16 (23.9) | 10 (35.7) | 9 (18.0) |
| **Invasin** |  |  |  |  |  |  |  |  |  |  |  |  |
| *ibeA* (+) | 10 (17.5) | 1 (50.0) | 4 (14.8) | 7 (13.7) | 6 (17.1) | 5 (11.6) | 9 (14.1) | 2 (14.3) | 2 (18.2) | 9 (13.4) | 7 (25.0) | 4 (8.0) |
| **Protectin** |  |  |  |  |  |  |  |  |  |  |  |  |
| *iss* (+) | 14 (24.6) | 4 (19.0) | 7 (25.9) | 11 (21.6) | 11 (31.4) | 7 (16.3) | 17 (26.6) | 1 (7.1) | 4 (36.4) | 14 (20.9) | 7 (25.0) | 11 (22.0) |
| **Siderophore** |  |  |  |  |  |  |  |  |  |  |  |  |
| *sitA* (+) | 44 (77.2) | 13 (61.9) | 22 (81.5) | 35 (68.6) | 29 (82.9) | 28 (65.1) | 46 (71.9) | 11 (78.6) | 8 (72.7) | 49 (73.1) | 27 (96.4) | 30 (60.0) |
| **Miscellaneous** |  |  |  |  |  |  |  |  |  |  |  |  |
| *malX* (+) | 16 (28.1) | 5 (23.8) | 10 (37.0) | 11 (21.6) | 12 (34.3) | 9 (20.9) | 18 (28.1) | 3 (21.4) | 7 (63.6) | 14 (20.9) | 11 (39.3) | 10 (20.0) |

a Values were showed as No. (%).

**Supplementary Table S4** Associations of the virulence genes with each other a

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Virulence gene** | ***hlyD*** | | ***ibeA*** | | ***iss*** | | ***sitA*** | | ***malX*** | |
| ***+***  **(n=19)** | ***-***  **(n=59)** | ***+***  **(n=11)** | ***-***  **(n=67)** | ***+***  **(n=18)** | ***-***  **(n=60)** | ***+***  **(n=57)** | ***-***  **(n=21)** | ***+***  **(n=21)** | ***-***  **(n=57)** |
| **Adhesin** |  |  |  |  |  |  |  |  |  |  |
| *fimH* (+) | 16 (84.2) | 41 (69.5) | 10 (90.9) | 47 (70.1) | 14 (77.8) | 43 (71.7) | 44 (77.2) | 13 (61.9) | 16 (76.2) | 41 (71.9) |
| *papA* (+) | 8 (42.1) | 19 (32.2) | 4 (36.4) | 23 (34.3) | 7 (38.9) | 20 (33.3) | 22 (38.6) | 5 (23.8) | 10 (47.6) | 17 (29.8) |
| *papC* (+) | 11 (57.9) | 24 (40.7) | 6 (54.5) | 29 (43.3) | 11 (61.1) | 24 (40.0) | 29 (50.9) | 6 (28.6) | 12 (57.1) | 23 (40.4) |
| *csgA* (+) | 17 (89.5) | 47 (79.7) | 9 (81.8) | 55 (82.1) | 17 (94.4) | 47 (78.3) | 46 (80.7) | 18 (85.7) | 18 (85.7) | 46 (80.7) |
| *afa* (+) | 3 (15.8) | 8 (93.2) | 2 (18.2) | 9 (13.4) | 4 (22.2) | 7 (11.7) | 8 (14.0) | 3 (14.3) | 7 (33.3) | 4 (7.0) |
| *tsh* (+) | 10 (52.6) | 18 (30.5) | 7 (63.6) | 21 (31.3) | 7 (38.9) | 21 (35.0) | 27 (47.4) | 1 (4.8) | 11 (52.4) | 17 (29.8) |
| **Toxin** |  |  |  |  |  |  |  |  |  |  |
| *hlyD* (+) | \* | \* | 4 (36.4) | 15 (22.4) | 2 (11.1) | 17 (28.3) | 19 (33.3) | 0 (0.0) | 5 (23.8) | 14 (24.6) |
| **Invasin** |  |  |  |  |  |  |  |  |  |  |
| *ibeA* (+) | 4 (21.1) | 7 (11.9) | \* | \* | 4 (22.2) | 7 (11.7) | 10 (17.5) | 1 (4.8) | 5 (23.8) | 6 (10.5) |
| **Protectin** |  |  |  |  |  |  |  |  |  |  |
| *iss* (+) | 2 (10.5) | 16 (27.1) | 4 (36.4) | 14 (20.9) | \* | \* | 11 (19.3) | 7 (33.3) | 7 (33.3) | 11 (19.3) |
| **Siderophore** |  |  |  |  |  |  |  |  |  |  |
| *sitA* (+) | 19 (100) | 38 (64.4) | 10 (90.9) | 47 (70.1) | 11 (61.1) | 46 (76.7) | \* | \* | 17 (81.0) | 40 (70.2) |
| **Miscellaneous** |  |  |  |  |  |  |  |  |  |  |
| *malX* (+) | 5 (26.3) | 16 (27.1) | 5 (45.5) | 16 (23.9) | 7 (38.9) | 14 (23.3) | 17 (29.8) | 4 (19.0) | \* | \* |

a Values were showed as No. (%).

# References

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