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Research Article

Phenotypic and Molecular Detection of Virulence Markers in *Escherichia coli* from Human Urine

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ABSTRACT

Escherichia coli is a vast group of microorganisms. While the majority of varieties are harmless, certain strains become harmful by acquiring virulence traits that boost their disease-causing abilities. They have been linked to a variety of diarrheagenic and extraintestinal disorders, including septicemia, newborn meningitis, urinary tract infections (UTIs), cystitis, pyelonephritis, and traveler's diarrhoea. One hundred (100) urine samples randomly collected from out-patients attending the University of Teaching Hospital were examined in order to isolate *E. coli*. *E. coli* positive isolates were thereafter screened for the presence of virulence factors/genes like protease, hemolysin, colicin, curli fimbriae, heamagllutinatin, P fimbria (*pap*), *fimH*, hemolysin (*hly*), *aer*obactin (*aer*), cytotoxic necrotizing factor 1 (*cnf*1), and afimbrial adhesion I (afaI) using bacteriological, phenotypic tests and multiplex polymerase chain reaction (PCR). Fifty (50) of the 100 urine samples gave positive cultures on Eosine Methylene Blue agar. However, biochemical identification revealed only 31 (62%) of the 50 as positive wheereas only 23 (74%) of the 31were positive with molecular verified to be *E. coli*. The phenotypic and molecular testing revealed virulence factors/genes in 94% (n = 29/31) and 100% (n = 23/23) of the *E. coli* isolates, respectively. Colicin and *fimH* were the most common virulence traits/genes found using both approaches. The study found no evidence of Hemagglutinin, *hly*, or *cnf* expression. The presence of virulence indicators in *E. coli* isolated from urine samples of outpatients at the University of Port Harcourt Teaching Hospital in Rivers State, Nigeria, was found in our study. *E. coli* isolates have a variety of virulence factors that contribute to their pathogenicity in humans.

Keywords: *E. coli*, virulence traits, phenotypic tests, curli fimbriae, PCR, diarrheagenic and extraintestinal disorders.

Introduction

Escherichia coli are commonly found as commensal bacteria in the environment and the human intestine. Some strains, however, may acquire virulence characteristics, transforming them from commensal to pathogenic *E. coli*. Pathogenic *E. coli* possesses a diverse set of virulence factors that play an important role in pathogenesis. Furthermore, pathogenic strains show a higher incidence of virulence characteristics than commensal strains (Sobhy *et al*., 2020). Among the medical conditions created by *Escherichia coli* is urinary tract infection, which is responsible for 70-90% of all UTIs. Diarrhoea, neonatal meningitis, sepsis causing *E. coli* infection, and *E. coli* causing obstetric infection are among the additional disorders related with *E. coli* (Gurevich *et al*., 2016).

All pathogenic *E. coli* virulence factors are classified into two types: bacterial cell surface virulence factors and secreted virulence factors (Vagarali *et al*., 2008). Fimbriae adhesins (Type 1 fimbriae, P fimbriae, S fimbriae, and other fimbrial adhesins), iron-acquisition systems (siderophore and *aer*obactin), and secreted toxins (hemolysin and cytotoxic necrotizing factor) are examples of these factors (El-Sh*aer et al*. 2018). Fimbriae adhesins aid in host cell surface attachment and tissue penetration (both of which are significant in urinary tract pathogenesis) (Shah *et al*., 2019). Iron acquisition systems provide enough iron to bacteria in iron-deficient conditions, while hemolysin and cytotoxic necrotizinf factor generate toxins that cause cell lysis/apoptosis (Ochoa *et al*. 2016).

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Virulent *E. coli* can be categorised into three types based on where it exists: commensal, intestinal, and extra-intestinal *E. coli*. Commensal *E. coli* can also acquire a certain number/set of virulence genes, which can result in intestinal and extra-intestinal *E. coli* infections. Intestinal *E. coli* strains are classified as enterotoxigenic (ETEC), enteropathogenic (EPEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), or enteroaggregative (EaggEC) based on their virulence qualities. Furthermore, each of these pathotypes carry a unique set of virulence features that, when combined, are associated with their infectiousness and function in infection (Abd El-Baky *et al*., 2020). Colicins, for example, are antibacterial or bactericidal peptides produced by *E. coli* that prevent other *E. coli* strains from colonising their hosts (Abd El-Baky *et al*., 2020).

Materials and Methods

Bacterial isolates

Urine samples were randomly obtained from out-patients at the University of Port Harcourt Teaching Hospital in Rivers State, Nigeria. In total, 100 urine samples were obtained for this study. Bacterial isolates were grown for 24 hours at 37° C on Eosine methylene blue (EMB) agar. Positive cultures were subcultured on nutrient agar to obtain pure colonies, which were then kept in slant bottles.

DNA extraction and Identification

Total DNA extraction was performed on each isolate using the PrestoTM Mini gDNA Bacteria-Kit (US) according to the manufacturer's protocol. Standard laboratory techniques were used to identify the isolates as *E. coli* (El-baz *et al*., 2022). Control strains were *E. coli* ATCC 25,922 and *E. coli* ATCC 35,218. As a confirmation marker, all biochemically confirmed isolates were screened for the uidA gene using polymerase chain reaction (PCR) (Ramirez Castillo *et al*., 2013).

Phenotypic detection of *E. coli* virulence traits

The procedure established by (Abeni *et al*., 2023) was utilised to phenotypically test urine *E. coli* isolates for virulence characteristics. Colicin, hemolysin, curli fimbriae, protease, and haemagglutination tests were among those performed.

Molecular detection of *E. coli* virulence genes

The prevalence of virulence genes among *E. coli* isolates were screened and amplified using PCR by adopting the primer sequences, band sizes in base pairs (bp) and specific annealing temperatures targeting adhesins: *fimH* (Farmer, 1999), P-fimbrial (*papC*) (Soto *et a*l., 2011), Afimbriae (afa) (Soto *et al*., 2011); toxins: hemolysin C (*hly*), cytotoxic necrotizing factor I (*cnf*1) (Licznar *et al*., 2003) and siderophore: *aer*obactin (*aer*) (Codruta *et al*., 2001) respectively, for the targeted genes. Polymerase chain reaction products were amplified in a DNA thermocycler using the following cycling conditions: initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at the specified temperature of each of the primers for 30 s, extension at 72 °C for 1 min, and final extension at 72 °C for 40 secs. PCR products were separated using agarose gel electrophoresis and examined under a UV transilluminator (Abd El-Baky, 2020).

Data Analysis

All data in this study were analyzed using the appropriate statistical method where needed. Statistical analysis was carried out using statistical Package for Social Sciences (SPSS) a P value of <0.05 was considered statistically significant.

Results

Result of the Prevalence of *E. coli* is shown in Figure 2. Out of 100 urine samples included in this study, 50 (50%) were positive culture on EMB. From the biochemical confirmation of positive cultures, the results revealed the prevalence of *E. coli* in the urine samples to be 62% (31/50) (Fig. 1).

Furthermore, the molecular screening of the 31 confirmed biochemical isolates for the uidA gene by PCR, gave a 74% (23/31) genotypic prevalence of *E. Coli* (Fig. 2)

The result of the phenotypic detection of virulence determinants of *E. coli* urine isolates is shown in Table 1. Colicin, protease, hemolysin, and curli fimbriae pathogenicity were found in 42%, 10%, 10%, and 36% of the isolates, respectively. However, all isolates tested negative for haemagglutination.

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Molecular detection of virulence genes among *E. coli* clinical isolates is expressed in Table 2. PCR analysis of the prevalence of virulence genes in *E. coli* isolates found the following: *FimH* was found in all 23 confirmed isolates (100%), A-fimbriae (afa) in 14 isolates (61%), *pap* in 8 isolates (35%), and *aer*, a siderophore, in 17 isolates (74%). The toxins *hly* and *cnf* were not present in the research (Table 2).

In terms of virulence factor co-occurrence distribution in phenotypic tests, more isolates (48%) were positive for only one virulence factor (Table 3). For the molecular distribution of combined genes, six patterns of gene arrangement were observed in the study with *fimH-aer-afaC* having the highest percentage (35%) (Table 4).

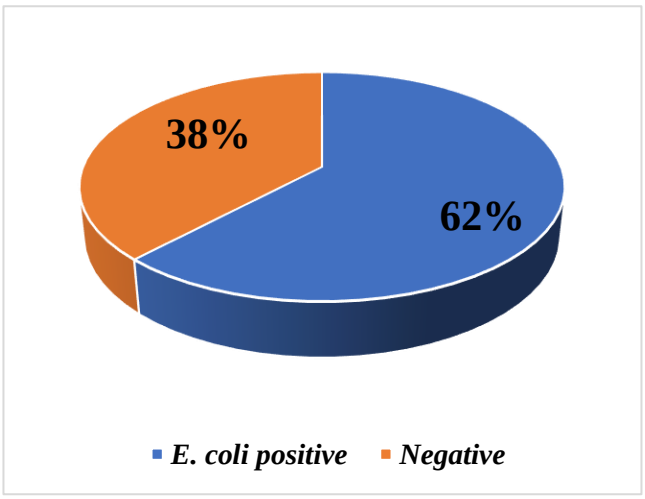

Figure 1: Phenotypic prevalence of *Escherichia coli*

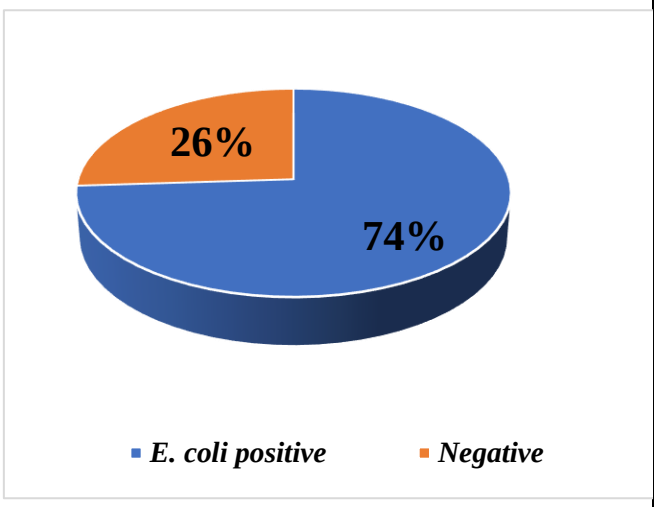

Figure 2: Genotypic prevalence of *Escherichia coli*

Table 1: Phenotypic virulence factors distribution of *E. coli* from urine samples

Virulence factors (n=31)	No Positive	Percentage (%)
Colicin	13	42
Hemolysin	3	10
Curli fimbriae	11	36
Protease	3	10
Haemagglutination	0	0

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Table 2: Molecular distribution of *E. coli* virulence genes isolated from urine

Virulence factors n=23	No Positive	Percentage (%)
fimH	**23**	**100**
aer	**17**	**74**
afaCc	**14**	**60**
hly	**0**	**0**
cnf	**0**	**0**
papC	**8**	**35**

Table 3: Co-occurrence of virulence factors of *E. coli* in the study

Virulence factors (n=31)	Frequency	Percentage (%)
0 virulence factor	**9**	**29**
One virulence factor	**15**	**48**
Two virulence factors	**7**	**23**
Three virulence factors	**0**	**0**
Four virulence factors	**0**	**0**
Five virulence factors	**0**	**0**

Table 4: Virulence profile of combined *E. coli* genes in the study

Virulence genes n=23	No Positive	Percentage (%)
fimH	4	17
fimH-aer-afaC	8	35
fimH-afaC-papC	2	9
fimH-aer-papC	2	9
fimH-aer-afaC-papC	4	17
fimH-aer	3	13

Discussion

E. coli are complex bacteria with numerous strains. While the majority of strains are non-pathogenic, those that have acquired virulence markers through natural processes or horizontal gene transfer are pathogenic and can cause a variety of disorders. They accomplish this with the assistance of particular virulence markers. These strains' ability to cause infection is dependent not only on their virulence factors, but also on risk factors that predispose their hosts to these diseases, such as old age, immunosuppression, prolonged urinary catheterization in hospitals, and antibiotic prolongation/misuse (Toval *et al*., 2014).

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In this study, we used phenotypic and molecular analyses to assay for *E. coli* and virulence markers in 100 urine samples. In the study, the molecular prevalence of *E. coli* was 74%. Dhanani et al. (2018), Sahoo *et al*. (2019), and Gajja *et al*. (2021) reported prevalence rates ranging from 74% to 65.3% to 20.95% respectively. Changes in isolation rates could be attributed to changes in sample size (as some studies concentrated on outpatients while others focused on inpatients), participants age, state of health/demographics of the patients studied, and solation methods used.

Colicin is a toxin that certain strains of *E. coli* can produce. It can cause disease by producing cell damage and inflammation. In our investigation, colicin was found in 42% of the people. Martin-Sampedro *et al*. (2014) and Gode *et al*. (2012) found a higher and lower colicin prevalence of 56% and 16.9% in patients with urinary tract infections, respectively, than in our study. The high prevalence of colicin may be attributable to widespread antibiotic use in the region or population studied, which may have resulted in the selection of resistance strains. The prevalence of *E. coli* harbouring colicin in urine may vary depending on sample size, influencing the results. Furthermore, different methodologies for detecting the colicin gene may have an effect on the prevalences.

Curli fimbriae is a kind of fimbriae found in *Escherichia coli* and other Enterobacteriaceae that is involved in surface adherence, cell aggregation, and biofilm formation (Barnhart and Chapman, 2006). They are abundant in uropathogenic *Escherichia coli* (UPEC) and play a role in adherence to human bladder cell surfaces and biofilm formation. Curli aid in the attachment of bacteria to the extracellular matrix and different serum proteins (Barnhart and Chapman, 2006). Curli production was reported in 36% of our isolates in our investigation. Curli has been found to be more common in other research. Curli expression was identified in 89% of UPEC isolates from women with upper urinary tract symptoms and 74% of UPEC isolates from women with cystitis, respectively (Norinder *et al*., 2012). Curli production was found in 56 and 60% of uropathogenic *Escherichia coli* isolates from children with cystitis and pyelonephritis, respectively (Kudinha *et al*., 2013). Yano *et al*. (2016) discovered that patients with recurrent cystitis had 100% curli presence.

In the current investigation, PCR established that the confirmed *E. coli* strains possessed four of the six virulence genes tested. Fimbriae, *pap*, and afa are virulence indicators that increase bacterial adherence, allowing for entry and colonisation. Some adhesins also facilitate bacterial penetration of host cells. Mulvey (2002) believes they are the most critical virulence-associated molecules in the pathophysiology of urinary tract infection. The most common virulence gene was *fimH*, which was found in 100% of the isolates. The distribution of the *fimH* gene in our study is consistent with that reported by Yazdi *et al*., 2018 and Raeispour and Ranjbar, (2018), who reported 89% and 100% for *fimH*, respectively. The *hly* and *papC* genes were not detected at the molecular level in this study. These findings contradict with those of Yazdanpour *et al*., (2020) and Shahbazi et al., (2018), who discovered a 26% and 41.7% prevalence of a *hly* gene in *E. coli* isolated from urine, respectively. Hemolytic activity may contribute to *E. coli* pathogenicity via bloodstream infection and sepsis (Daga *et al*., 2019). Hemolysin is cytotoxic to many different types of cells and causes severe tissue damage during UTIs (Wiles and Mulvey, 2013). *Cnf*, on the other hand, is an abbreviation for cytotoxic necrotizing factor, a toxin generated by some strains of *E. coli*. It acts by causing urinary tract cell damage, which can lead to infection. Two studies on patients presenting with UTI in Jos, Nigeria (Maduagwu *et al*., 2019) and Singapore (Ang *et al*., 2019) discovered that the overall prevalence of *cnf* was 36.5% and 22.6%, respectively. *Aer*obactin is an iron-chelating siderophore generated by certain bacteria, notably *E. coli*. It aids bacteria in obtaining iron from their surroundings, which is necessary for their development and survival. *Aer*obactin in urine has been linked to urinary tract infections and other health concerns in people. In our investigation, we discovered a 74% prevalence of *aer*. Ours is higher than the 30% *aer* prevalence reported by Das *et al*. (2022) in UTI patients. Aliyu *et al*. (2019) found *aer* prevalence in *E. coli* isolates from asymptomatic and urinary tract infection patients to be 52.9% and 64.3%, respectively. The high rate of antibiotic use in Nigeria could explain the high prevalence of virulence genes. Antibiotics can upset the usual balance of bacteria in the urinary tract, resulting in an increase of bacteria carrying virulence genes. Furthermore, in some regions of Nigeria, poor sanitation and hygiene practises may lead to the spread of virulent bacteria.

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Pap pili are protein structures that aid in bacteria binding. They aid *E. coli* in adhering to the mucosal lining of the urinary system, which can result in urinary tract infections. The current analysis discovered a 35% frequency of the *pap* gene. Ajayi *et al*. (2020) and Adelakun *et al*. (2014) reported higher (53.5%) and lower (29.5%) prevalence than our study. The high prevalence of the *pap* gene in this study could be attributed to a number of causes. For starters, the study population was from a densely populated area, which could have contributed to the high incidence. Furthermore, the study employed more sophisticated methods for detecting the *pap* gene, which may have contributed to the higher incidence.

In conclusion, this investigation discovered that urine *E. coli* isolates include a number of significant fimbriae adhesion associated virulence genes, particularly *papC*, *afaC*, and *fimH*. The *aer* siderophore gene was also found with a high frequency. The *hly* and *cnf* toxin encoding genes were both not found in this study.

The presence of *Escherichia coli* virulence indicators in urine in this study is regarded as a potential health risk because it could lead to the emergence of a number of disorders. Furthermore, more extensive investigations in diverse places are required to identify the prevalent virulence genes of pathogenic *E. coli* in order to put mechanisms in place to checkmate these genes.

References

Abd El-Baky, R. M., Ibrahim, R. A., Mohamed, D. S., Ahmed, E. F. and Hashem, Z. S. (2020). Prevalence of virulence genes and their association with anti-microbial resistance among pathogenic *E. coli* isolated from Egyptian patients with different clinical infections. *Infection andDrug Resistance*. *13*: 1221–1236.

Abeni, B. A., Frank-Peterside, N., and Otokunefor, K. (2023). Analysis of virulence traits in *Escherichia coli* from human and poultry faeces. *International Journal of Microbiologyand Applied Sciences*. *2*: 121 - 128.

Adelakun, O., Atolagbe, O., Atolagbe, A., and Abubakar, I. (2014). Prevalence and antibiotic resistance of uropathogenic *Escherichia coli* in Ilorin, Nigeria. *Nigerian Journal ofMedicine*. *23(3)*: 211-216.

Ajayi, S. O., Adeleke, A. A., and Oladejo, M. O. (2020). antibiotic resistance pattern of uropathogenic *Escherichia coli*. *World Journal of Pharmacy and Pharmaceutical Sciences*. *14(4)*: 33-39.

Al-Dulaimi, T. H., Bunyan, I. A., and Banimuslem, T. A. (2022). Genotypic assay to determine some virulence factors of Uropathogenic *E. coli* (UPEC) isolates. International Journal of Health Sciences, 6(S4), 1593–1604. https://doi.org/10.53730/ijhsv6nS4.6289

Aliyu, M. I., Musa, S. B., Salihu, A. U., and Garba, A. A. (2019). Prevalence of *fimH*, *pap*, *aerobactin*, *hly*, *cnf*, *eaeA* and *ehxA* virulence genes in *Escherichia coli* isolates from asymptomatic and urinary tract infection patients in Nigeria. *African Journal of Microbiology Research*. *13(26)*: 2411–2418.

Ang, S. H., Chan, K. W., Li, C. Y., Wong, S. K., Li, R. W., Loh, C. S., Loo, S. Y., and Low, G. H. (2018). Characterization of uropathogenic *Escherichia coli* and *Klebsiella pneumonia* isolates from urinary tract infection patients in Singapore. *Journal of Microbiology, Immunology and Infection*. *51(3)*: 322-327.

Barnhart, M. M., and Chapman, M. R. (2006). Curli biogenesis and function. *Annual Reviews in Microbiology*. *60*: 131-147.

Das, R., Strahl, D., Rahfeld, B., Prade, T., Heiss, K., Ficht, T., and Schaper, H.. (2022). Prevalence of *fimH*, *aerobactin*, and biofilm-forming abilities among uropathogenic *Escherichia coli* isolated from patients in Germany. *Journal of Clinical Microbiology*. *60(7)*:e02593-21.

Dhanani, R. H., Falk, S. I., Tucker, J. D., Johnson, C. S., Grund, D. A., and Kristich, A. M. (2018) (2018). Core and accessory genes of *Escherichia coli* causing urinary tract infections in adults. *Infection and Immunity*. *86*: e01306-17.

El-baz, R., Said, H. S., Abdelmegeed, E. S. and Barwa, R. (2022). Characterization of virulence determinants and phylogenetic background of multiple and extensively drug resistant *Escherichia coli* isolated from different clinical sources in Egypt. *Applied Microbiology and Biotechnology*. *106*: 1279–1298.

Citation: Abeni *et al*. (2023b). Phenotypic and molecular detection of virulence markers in *Escherichia coli* from Human urine. *International Journal of Microbiology and Applied Sciences*. *2*: 155 - 161.

Gajja, P., Bhanu, B., Sahu, S. K., Radha, R., Prasad, N., and Singh, B. P. (2021). Prevalence and Characterization of Extended-Spectrum Beta-Lactamase (ESBL) and Carbapenemase Producing *Escherichia coli* from Outpatient Urine Samples in a Rural Indian Hospital. *Antimicrobial Resistance and Infection Control*. *10*: 66-70.

Gode, M. G., Kolhe, M. G., Jadhav, M. N., Kshirsagar, D. V., and Shetye, S. N. (2012). Characterization of urinary tract infection-causing *Escherichia coli* strains and their multidrug resistance patterns in Pune city. *International Journal of Medical Microbiology*. *12*: 467-472.

Gurevich, E., Tchernin, D., Schreyber, R., Muller, R., and Leibovitz, E. (2016). Follow-up after infants younger than 2 months of age with urinary tract infection in southern Israel: epidemiologic, microbiologic and disease recurrence characteristics. *Braz. J. Infects Dis. 20*: 19–25. 10.1016/j.bjid.2015.09.003.

Hufnagel, D. A., Depas, W. H., and Chapman, M. R. (2015). The biology of the *Escherichia coli* extracellular matrix. *Microbiol. Spectr. 3*: 249–273. doi: 10.1128/microbiolspec. MB-0014-2014.

Kudinha, T., Johnson, J. R., Andrew, S. D., Kong, F., Anderson, P., and Gilbert, G. L. (2013). Genotypic and phenotypic characterization of *Escherichia coli* isolates from children with urinary tract infection and from healthy carriers. *Pediatr. Infect. Dis. J. 32*: 543 548.

Maduagwu, J. C., Duruaku, A. P., Ayanlade, J. S., and Onyebueke, F. (2019). Prevalence and risk factors associated with uropathogenic *Escherichia coli* producing extended spectrum beta lactamases (ESBL), cytotoxic necrotizing factor and *aer*obactin among children in Jos, Nigeria. *BMC Research Note*. *15*: 1.

Martin-Sampedro, J. C., Blázquez, J. M., Quintana, A., Sanz, M. P. and Hernández-Allés, P. (2014).Prevalence of antibiotic-resistant and virulence genes in *Escherichia coli* isolates from urinary tract infections in Spain. *Journal Microbial Pathogenesis*. *4*: 220-223.

Mulvey, M. A. (2002). Adhesion and entry of uropathogenic *Escherichia coli*. *Cell Microbiol*. *4*: 257-271.

Norinder, B. S., Koves, B., Yadav, M., Brauner, A., and Svanborg, C. (2012). Do *Escherichia coli* strains causing acute cystitis have a distinct virulence repertoire? *Microb. Pathog. 52*: 10–16. doi: 10.1016/j. micpath.2011.08.005

Ochoa, S. A., Cruz-Córdova, A., Luna-Pineda, V. M., Reyes-Grajeda, J. P., Cázares-Domínguez,V., Escalona, G., Sepúlveda-González, M. E., López-Montiel, F.Arellano-Galindo, J., López Martínez, B., *et al*. (2016). Multidrug-and extensively drug-resistant uropathogenic *Escherichia coli* clinical strains: phylogenetic groups widely associatedwith integrons maintain high genetic diversity. *Front Microbiol*. *7*: 2042.

Sahoo, S., Singh, G., Bhargava, G., Singh, N. K., and Sahu, N. (2019). Characterization of urinary *Escherichia coli* isolates with emphasis on prevalence and antibiotic resistance genes fromfour hospitals in Madhya Pradesh, India. *PLoS ONE*. *14*: e0217435.

Shah, C., Baral, R., Bartaula, B., and Shrestha, L. B. (2019). Virulence factors of uropathogenic *Escherichia coli* (UPEC) and correlation with antimicrobial resistance. *BMC Microbiol*. *19*: 204.

Toval, F., Köhler, C-D., Vogel, U., Wagenlehner, F., Mellmann, A., and Fruth, A, (2014). Characterization of *Escherichia coli* isolates from hospital inpatients or outpatients withurinary tract infection. *J Clin Microbiol*. *52(2)*: 407–418.

Vagarali, M. A., Karadesai, S. G., Patil, C. S., Metgud, S. C., and Mutnal, M. B. (2008).Haemagglutination and siderophore production as the urovirulence markers of uropathogenic *Escherichia coli*. *Indian J Med Microbiol*. *26(1)*: 68–70.

Yano, T., Milanez, G. P., Werle, C. H., and Cordeiro, M. A. (2016). Curli fimbria: An Escherichia coli adhesin associated with human cystitis. *Brazilian Journal of Microbiology 47*: 414 - 416.

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