Jurnal Health Sains: p–ISSN: 2723-4339 e-ISSN: 2548-1398
Vol. 3, No.12, Desember 2022

Relationship Between Esbls Production And Virulence Factors Of Fima And Papc Gene In Uropathogenic Escherichia Coli Isolation From Private Hospital

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INFO ARTIKEL
Diterima
29 November 2022
Direvisi
12 December 2022
Disetujui
25 December 2022

Keywords:
ESBL, fimA, papC, Escherichia coli, virulence factors

ABSTRACT
Escherichia coli is one of the opportunistic pathogenic that occupies the highest position causing the incidence of UTI. Fimbriae, particularly type 1 and P fimbriae, are the most commonly implicated bacterial cell surface virulence factors. The production of ESBL and virulence factors in E.coli bacteria causes chronicity, persistence, and recurrence of infections that cause high morbidity and mortality. Therefore, this study was conducted to explain the relationship between ESBL production and its virulence factors in E.coli bacteria. The design of this research is analytic observational with a cross-sectional approach was conducted from March to May 2021. A total of 40 E. coli strains were isolated and collected from urine samples of UTI patients who were admitted to the hospital. in a private Hospital in Banyumas Region in Central Java, Indonesia. The HiChrome ESBL Agar Base media was used to screen for ESBL-Producing E. coli. Identification of firmA and ppC genes was performed by using the PCR method. All urine samples diagnosed with UTI were examined for ESBL production. As many as 25% of E.coli were ESBL-production. All isolates showing positive E.coli ESBL results were then analyzed for fimA and papC genes using the PCR method. The results obtained 100% fimA gene and 80% papC gene. The conclusion is that there is a strong relationship between ESBL production with fimA and papC genes.

Introduction

Infection of the urinary tract is caused by the proliferation of bacteria in the urinary system of humans (Andersen et al., 2022). Bacteria, viruses, and fungi can cause Urinary Tract Infections. The type of bacteria that causes UTI is anaerobic Gram-negative bacteria commonly found in the digestive tract (Enterobacteriaceae) (Terlizzi et al., 2017). Escherichia coli is opportunistic pathogenic. Enterobacteriaceae bacteria occupy the highest position, causing UTI incidence (Prasetya et al., 2019). E.coli can transform from flora in the intestine to pathogens in the urinary system, where they can flourish and persist. This pathogen has various virulence factors and tactics that allow them to infect and illness the urinary tract. This strain is a uropathogenic E.coli (UPEC) because it is persistently linked to uropathogenic infections (Shah et al., 2019).

How to cite: Ade Hasan Basri, Anang Kurniawan, Joko Setyono, Dwi Utami Anjarwati (2022). The Relationship Between Esbls Production And Virulence Factors Gene Fima And Papc In Uropathogenic Escherichia Coli Isolated From A Private Hospital In Banyumas Region In Central Java, Indonesia - A Cross Sectional Study. *Jurnal Health Sains, 3*(12).
https://doi.org/
E-ISSN: 2722-5356
Published by: Ridwan Institute

Fimbriae, especially type 1 and P fimbriae, are the most frequently implicated bacterial cell surface virulence factors (Emody et al., 2003). Fimbriae type 1 is an essential UPEC virulence factor that can stabilize bacteria's adhesion to different cell types in the urinary system (Parvez & Rahman, 2018). P fimbriae are connected to the carbohydrate complex alpha-D-Galp-(1-4)-beta-D-Galp and are pyelonephritogenic. They adhere tightly to Bowman's capsule, glomerulus, and endothelial cells that line blood channel walls in the kidney. PapC protein is the most significant protein with 80 KD, aiding this process by transporting subunits outside the cell (Wullt et al., 2000).

UTIs will be challenging to treat when experiencing antibiotic resistance problems. E.coli is one of the bacteria that can become resistant to antibiotic drugs in UTI because it can produce the Extended-Spectrum -lactamase (ESBL) enzyme, and E.coli is the highest producer of ESBL (Prasetya et al., 2019). Extended- Spectrum Beta-lactamase is an enzyme that breaks down Beta-lactams into ineffective. Beta-lactams are a class of antibiotics that work to inhibit and damage the cell walls of Gram-negative bacteria (Vickers, 2017). The production of ESBL and virulence factors in E.coli bacteria causes chronicity, persistence, and recurrence of infections that cause high morbidity and mortality (Dumaru et al., 2019). Understanding the relationship between virulence genes and ESBL production in E.coli is critical in developing successful UTI prevention and management strategies and actions, particularly for severe, recurrent, and complicated UTIs (Katongole et al., 2020). Therefore, this study was conducted to explain the relationship between ESBL production and its virulence factors in E.coli bacteria

Methods

This cross-sectional study was conducted from March to May 2021 in Central Java. E. coli isolates were isolated and collected from urine specimens of UTI patients admitted to a private Hospital in Banyumas Region in Central Java, Indonesia. The microorganisms were stored in TSB (Tryptic soy broth) containing 15% glycerol at -70°C (Fattahi et al., 2015).

Determination of ESBl-producing E. coli isolates

The screening of ESBL-producing E. coli was performed using the HiCromeTM ESBL Agar Base media. Inoculate related samples directly on the plate and incubate for 18-24 hours in aerobic conditions at 35-37 °C. Pink to purple colonies showed a positive result, namely E.coli producing ESBL (Grohs et al., 2013).

DNA extraction and PCR method

The DNA extraction kit was used to extract total genomic DNA from ten E. coli isolates. The DNA of the fimA gene in the chromosome was extracted using Presto Mini gDNA Bacteria Kit Geneid and PapC gene. DNA in Plasmid was extracted using Presto Mini Plasmid Kit Geneid according to the manufacturer's directions. Specific primers were used for amplification of the fimA and papC genes (Table 1).

Table 1.
PCR primers

Gene	Primers (5'-3')	Size of product (bp)
fimA	F:GTTGTTCTGTCGGCTCTGTC R:ATGGTGTTGGTTCCGTTATCC	400
papC	F:GACGGCACTGCTGCAGGGTGTGGCG R:ATATCCTTTCTGCAGGGATGCAATA	328

PCR primers adapted from Zamani and Salehzadeh (Zamani & Salehzadeh, 2018) papC temperature 63°C for 30 seconds; elongation at 72°C for 1 minute and repeated 30 cycles; final elongation performed at 72°C for 5 minutes. The reaction was stopped at 4°C (Zamani & Salehzadeh, 2018).

Results and discussion

All urine samples from patients diagnosed with UTI were 40 samples. The samples were then tested for ESBL production. The results obtained were 10 isolates of *E.coli* ESBL (Figure 1) or 25% of *E.coli* ESBL, 18% other than E.coli (Figure 2). All isolates showing positive *E.coli* ESBL were then identified with fimA and papC genes using the PCR method. The results were 10 isolates (100%) positive for fimA gene (Figure 3), and papC gene 8 isolates (80%) positive, 2 isolates (20%) negative (Figure 4). Based on statistical tests to see the relationship between ESBL with fimA and papC genes, the results were $p < 0.01$ for the fimA gene, $p < 0.02$ for the papC gene (Table 2). These results indicate that there is a strong relationship between ESBL production with fimA and papC genes.

Statistical analysis

SPSS program for Windows, version 16, was used for statistical analysis (SPSS 16.0). The association between the variables was assessed using the Chi-square or Fisher's exact test. The level of significance at $p<0.05$.

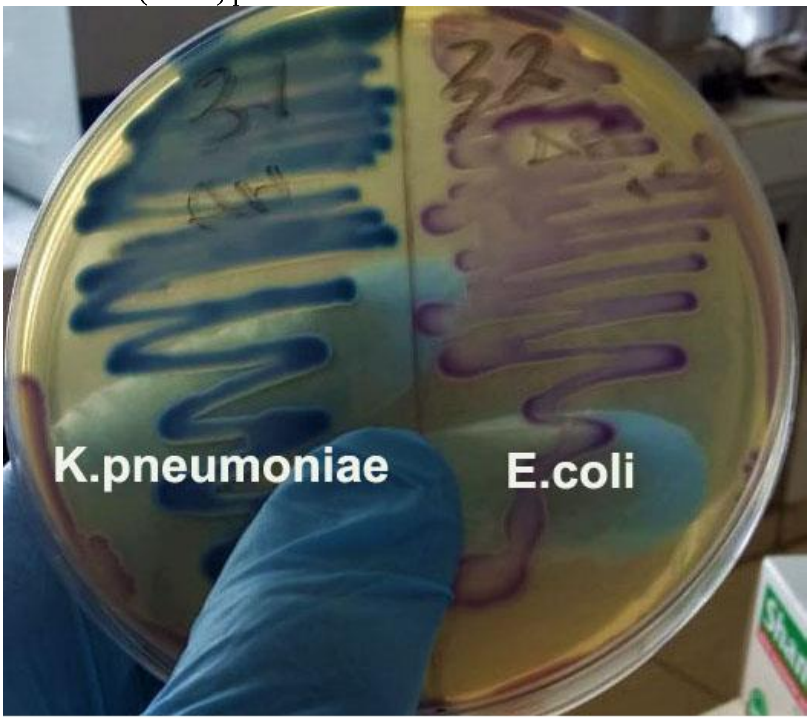

Figure 1. Isolated on HiCrome™ ESBL Agar Base media

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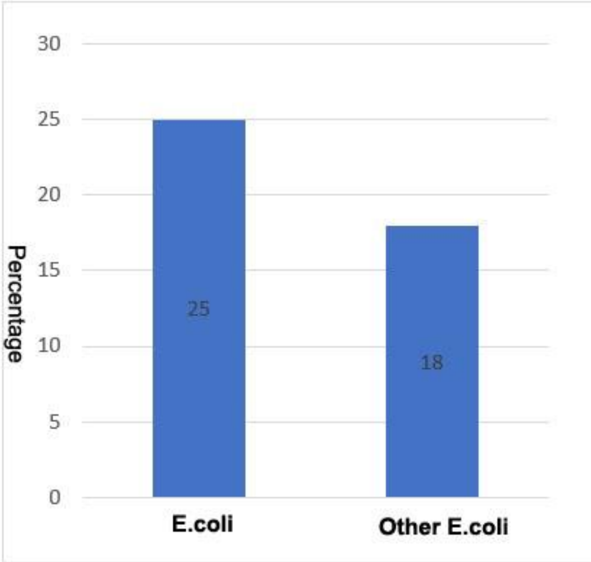

Figure 2. positive ESBL percentage

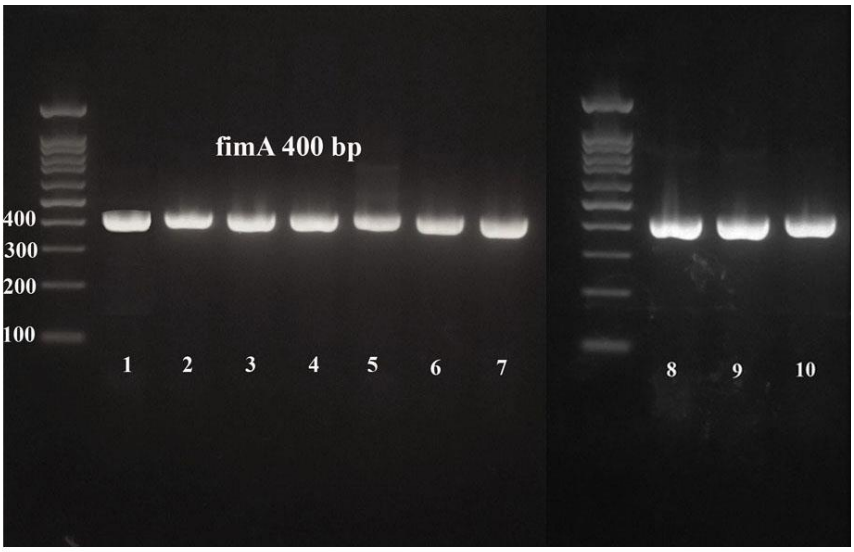

Figure 3. fimA gene PCR results

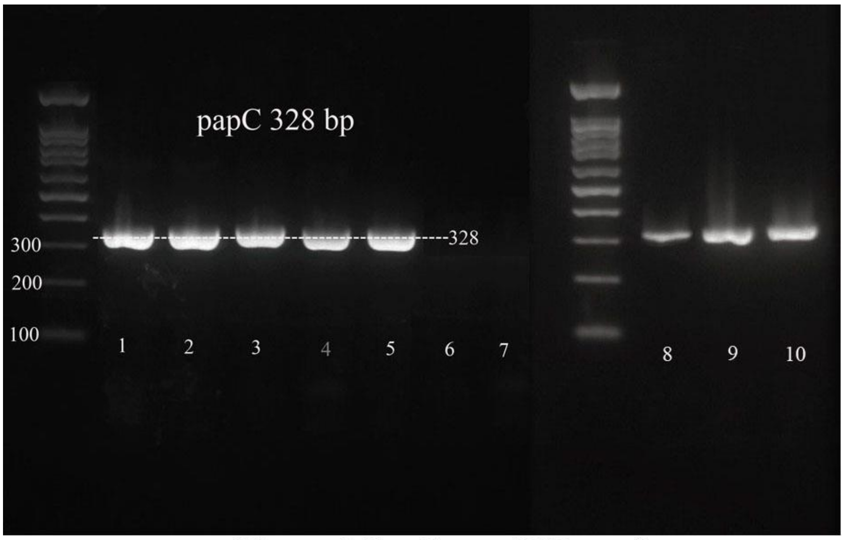

Figure 4. PapC gene PCR results

Table 2.
Relationship between ESBL production with fimA and papC genes

Gene	Positive	Negative	ESBL	P values
fimA	10	0	10	0.01
papC	8	2	10	0.02

One of the most frequent bacterial illnesses is urinary tract infection (UTI), and UPEC is the culprit that causes more than half of nosocomial UTIs. The virulence factors of UPEC strains can cause an inflammatory response in UTI (Bien et al., 2012). This study aimed to determine the relationship between ESBL production and its virulence factors in *E.coli* bacteria. In this study, out of 40 isolated urine samples, E. coli was the highest producer of ESBL, namely 10 isolates (25%). The possible reason was *E.coli* has a plasmid that can encode resistance genetic mutation factors. The mechanism of ESBL resistance in *E.coli* is genetically inherited by new intrinsically resistant strains (Grohs et al., 2013). The results of this study are in accordance with the study of (Prasetya et al., 2019) in East Java, which explained that *E.coli* is one of the opportunistic pathogenic Enterobacteriaceae bacteria that occupy the highest position causing the incidence of UTIs and is the highest ESBLproducing bacteria. The results of a study conducted by (Shrestha et al., 2019) in Nepal showed the same results, namely *E.coli*, which was positive for ESBL more than 20% of the total sample examined

The fimA and papC genes appeared in isolate *E. coli* ESBL. The result indicates that fimA and papC genes can occur in all *E.coli* strains (Bien et al., 2012). fimA is the most abundant protein produced by type 1 fimbriae and functions at the time of adhesin (Parvez & Rahman, 2018). PapC genes were detected to be associated with pyelonephritis and were found in 60% of *E.coli* strains. The ability to colonize the urinary tract epithelium is known to be linked to the presence of this gene. The papC gene produces an outer membrane protein that regulates the development of P fimbriae (Winberg, 1984). Most infections caused by *E.coli* are closely related to virulence factors with the pathogenicity of *E.coli* in urinary tract infections. Several essential virulent genes of the UPEC strain which is associated with severe urinary tract infections are afimbrial adhesin (afal), hemolysin (HLY), cytotoxic necrotizing factor (cnf 1), aerobactin (aer), S fimbriae (sfa), P fimbriae (pap), type 1 fimbriae (fimA) (Winberg, 1984).

The results of this study indicate that there is a strong relationship between ESBL production and the virulence factors of the fimA and papC genes. These results are consistent with the study of (Shah et al., 2019), which explains that there is a significant relationship between virulence factors and ESBL resistance.

In the urinary system, virulence factors play several roles in the development and colonization process (Winberg, 1984). The ability to colonize depends on the expression of other fimbrial adhesins. The virulence factors involved in the Adhesin process are type 1 fimbriae which are essential during the attachment process (Emody et al., 2003). They produce erythrocyte hemagglutination when they enter the urinary system and cause bacteriuria. They also enable bacteria to overcome the epithelial barrier to enter the circulation (Connell et al., 1996). For entry into urinary tract host cells, type 1 fimbriae play a significant role. Fimbriae type 1 is a highly versatile UPEC virulence factor that can stabilize bacterial attachment to various cell types throughout the urinary tract (Al-Amiery et al., 2016). UPEC strain 99% can encode genes present in type 1 fimbriae (Vigil et al., 2011), consisting mainly of the protein FimA along with FimF, FimG, and FimH (Klemm & Schembri, 2000). Another virulence factor is P fimbriae, which E. coli expresses. They produce erythrocyte hemagglutination when they enter the urinary system and cause bacteriuria. They also enable bacteria to overcome the epithelial barrier to enter the circulation (Riegman et al., 1988). This type of fimbriae is encoded by the pap gene (Wullt et al., 2000). The pap gene cluster contains at least nine genes, each with two restriction sites on each end. Another papC protein, the largest at 80 KD, assists in this process by transporting subunits outside the cell (Collinson et al., 1992).

ESBL production is a common resistance mechanism of UPEC (Talbot et al., 2006). UTIs caused by ESBL-producing *E.coli* are becoming more widespread, and ESBL-producing *E.coli* are found in various Asian nations (Heffernan et al., 2009). Multidrug resistance makes selecting an antibiotic agent difficult. There is a growing link between the creation of ESBLs and multidrug resistance. The emergence of multidrug-resistant UPEC poses a serious threat to managing UTIs as medical costs increase (Neupane et al., 2016). UPEC strains that acquire potential virulence factors can improve their ability to adapt to novel environments, colonize and invade host tissues, elude immune responses, and collect resources from the host (Köhler & Dobrindt, 2011).

Conclusions

The conclusion of this study is that a strong association between ESBL production and the virulence factors of the fimA and papC genes. These findings will undoubtedly aid in understanding the pathogenicity of UTIs and their effective management, thereby reducing the inappropriate use of antibiotics. Therefore, increased physician vigilance and increased testing with Laboratory tests are needed to reduce treatment failure and prevent the spread of ESBL-producing *E.coli*.

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