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Research Article,

Detection of CTX-M Gene in *Escherichia coli* Producing Extended Spectrum Beta Lactamase (ESBL) Isolated from Patients with Urinary Tract Infection

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Abstrect:

Escherichia coli is a Gram-negative bacterium from Enterobacteriaceae family causes urinary tract infections (UTI). A major problem encountered in antibiotics therapy is Multiple Drug Resistant Organisms (MDROs). MDROs occur because of the presence of resistance coding genes such as CTX-M which causes bacteria to produce the Extended Spectrum Beta-Lactamase (ESBL) enzyme. This study aims to detect the presence of the CTX-M gene in Extended-Spectrum Beta Lactamase (ESBL) Escherichia coli isolated from UTI patients. The study was conducted at the Institute of Tropical Disease (ITD) Airlangga University, Jl. Mulyorejo Campus C Surabaya, Indonesia for polymerase chain reaction (PCR) examination. The isolation and identification of Escherichia coli bacteria were carried out at the Microbiology Laboratory Department in Poltekkes Kemenkes Surabaya, Jl. Karangmenjangan 18A, Surabaya, Indonesia. Conventional identification of Escherichia coli and the presence of the CTX-M gene were observed using the PCR method. The results showed that 18 of 30 samples (60%) were caused by Escherichia coli. Escherichia coli producing ESBL was found in 15 samples (83%), of which 12 samples (80%) showed the presence of the CTX-M gene.

Keywords: CTX-M gene, Extended-Spectrum Beta Lactamase (ESBL), Escherichia coli.

Introduction:

Urinary tract infection (UTI) is an infection caused by microorganisms in the urinary tract, which starts from infection in urinary tract, genital organs, until kidneys. UTI is the second most common infectious disease after respiratory tract infection, with 8.3 million cases reported every year. The prevalence of UTI in Indonesia is still quite high. According to data from the Ministry of Health of the Republic of Indonesia, the number of UTI patients in Indonesia is 90-100 cases per 100,000 population per year or around 180,000 new cases per year. Meanwhile, in the United States in 2002, deaths arising from UTI were estimated to be more than 13,000 or had a mortality rate of 2.3% (Kausuhe et al., 2017).

Currently, immediate rational empiric antibiotic therapy without delay in the management of infectious cases has been shown to reduce morbidity and save many lives. However, inappropriate use of antibiotics can increase the number of microorganisms that are resistant or even multi-resistant to antibiotics. The World Health Organization (WHO) defines antibiotic resistance as the process of mutation in microorganisms exposed to antibiotic drugs, thereby making the infection last and increasing the risk of spreading the disease to others (WHO, 2015). Antibiotic resistance often results in failure to treat the disease, resulting in disability and even death. If a patient is already resistant to antibiotics, various medical procedures such as organ transplants, chemotherapy, diabetes treatment, and major surgery become very risky. As a result, patients have to endure longer and more expensive treatments (RI Ministry of Health, 2018).

Data from hospitals and communities of patients with UTI in Canberra, Australia, provide an overview of the temporal trend of antimicrobial resistance over five years, from 2009 to 2013. In most cases of UTI, more than 95% of *Enterobacteriaceae* including *Escherichia coli* and *Enterococcus faecalis* are the main causative bacteria. A total of 15,022 positive cultures from 8724 patients were identified. The five-year highest resistance rates of antibiotics in hospital and community were to ampicillin (41.9%) and trimethoprim (20.7%). The lowest resistance rates were to meropenem (0.0%), nitrofurantoin (2.7%), piperacillin tazobactam (2.9%) and ciprofloxacin (6.5%). Resistance to amoxycillin clavulanate, cefazolin, gentamicin and piperacillin tazobactam was significantly higher in hospital than in community-acquired UTIs (9.3% vs 6.2%; 15.4% vs 9.7%; 5, respectively, 2% 3.7% and 5.2% versus 2.5%). The result of data analyses showed a significant trend of increasing resistance over five years to amoxicillin clavulanate, trimethoprim, ciprofloxacin, nitrofurantoin, trimethoprim sulfamethoxazole, cefazolin, ceftriaxone and gentamicin (Fasugba et al., 2016). Results for Amoxicillin Clavulanic Acid (100%), Ampicillin (100%), Cefasolin (99.7%), Astreonam (99.7%), Ceftasidime (99.7%). The prevalence of *Escherichia coli* in UTI patients was 27.5%, of which, *Escherichia coli* producing ESBL was found to be 72.5% (Ariana, Pestariati and Sasongkowati, 2019).

Escherichia coli can undergo a mutation that enables it to produce Extended-Spectrum Beta Lactamase (ESBL), an enzyme that hydrolyses most beta-lactam antibiotics. Gene producing ESBL can be passed down from one bacterium to their offsprings or transferred from one bacterium to another, the process of occurrence of this kind of resistance is called acquired resistance (Sutandhio Alimsardjono and Lusida, 2015). Resistance to cephalosporins can be caused by the ability of Enterobacteriaceae bacteria to secrete the enzyme beta-lactamase. This enzyme is encoded by the R gene (resistance) on bacterial plasmids. The first gene mediated by beta lactamase was TEM, this gene can spread to various bacterial species through conjugation, then other genes also appear such as SHV and CTX-M which can lead to multi-resistant (Bogner et al., 2016). This study aims to detect the presence of the CTX-M gene in *Escherichia coli* producing Extended-Spectrum Beta Lactamase (ESBL) isolated from UTI patients.

Methods and Materials:

This research is a quantitative descriptive research, conducted at the Institute of Tropical Disease (ITD) Universitas Airlangga, Jl. Mulyorejo Campus C Surabaya, Indonesia for examination using Polymerase Chain Reaction (PCR), while for the isolation and identification of Escherichia coli bacteria was carried out at the Microbiology Laboratory of RS Dr. Soetomo Surabaya, Indonesia.

The sample used for this study was an isolate of Escherichia coli producing Extended-Spectrum Beta Lactamase (ESBL) isolated from UTI patients at RS Dr. Soetomo Surabaya and the meda used for urine culture was Dar agar, Brolacin agar (can be replaced with purple lactose agar, MacConkey agar, crystal violet agar or eosin methylene-blue agar), and CLED agar. For the isolation and identification of bacteria, we used Selenite broth fertilizing media, Nutrient Agar Slant (NAS) media, and Eosyn Methylene Blue (EMB) media. Sterile distilled water and sterile PZ (0.9% NaCl) were used as solvents. To examine ESBL antibiotic sensitivity, we used Mouller Hinton Medium and an antibiotic dish. Furthermore, we used Polymerase Chain Reaction (PCR) to identify the presence or absence of the CTX-M gene by genotype.

Results and Discussion:

According to the study conducted in early August 2020 at the Microbiology Laboratory in RSUD Dr. Soetomo Surabaya, the following results are obtained from urine sample of patients with UTI after identification and isolation had been performed:

A total of 15 samples showed the presence of Escherichia coli with positive ESBL (Table 1). Negative results for ESBL were found in 1 sample of Enterobacter cloaceae; 2 samples of Klebsiella pneumoniae; 1 sample of Proteus mirabilis; 1 sample of Pseudomonas aeruginosa; 2 samples of Eschericchia coli; 1 sample of Corinebacterium; 1 sample of Candida tropicalis, and in 5 sterile samples.

Table1: Results of Urine Isolation and Identification of Patients with UTI at RSUD Dr. Soetomo Surabaya

No.	ID	Initials	Age	Gender	Referred from	Results	
						Isolation and Identification	**ESBL**
1	11173	Sumiatun	62	Female	Outpatients	*Escherichia coli*	ESBL
2	11436	Md	56	Male	Outpatients	*Escherichia coli*	ESBL
3	11754	Wtn	57	Male	Surgical Dept. Aster	*Escherichia coli*	ESBL
4	11896	Br	6	Male	Pediatrics Dept.	*Escherichia coli*	ESBL
5	11165	Lkm	40	Male	Pandan 1	Sterile	Negative
6	11896	Br	6	Male	Pediatrics Dept.	*Escherichia coli*	ESBL
7	11992	Yosef	57	Male	Surgical Dept. D	*Escherichia coli*	ESBL
8	12016	Zaskia	7	Female	Bona 2	*Enterobacter*	
9	12020	Dwida	20	Male	RIK 1	*cloaceae*	Negative
10	12026	Ahmad	8	Male	IMU	*Klebsiela pneumonia*	Negative
11	12027	Smy	58	Male	Urological surgery	*Proteus mirabilis*	Negative
12	12031	Swj	64	Male	Cardiovascular Dept.	*Escherichia coli*	ESBL
13	12054	yasmin	2	Female	Urological surgery	*Escherichia coli*	ESBL
14	12145	Sit M	67	Female	Palm 1	*Escherichia coli*	ESBL
15	12158	Isnaningsih	66	Female	RIK 1	Steril	Negative
16	12200	Alfi	53	Female	BU	Steril	Negative
17	12235	Tauw	80	Female	Internal medicine for Female	*Escherichia coli*	ESBL
18	12236	Miftakhul	30	Female	Rosela 2	*Pseudomonas aeroginosa*	Negative
19	12255	Juwariah	65	Female	Merak	*Escherichia coli*	ESBL
20	12310	Novia	52	Female	BU	*Escherichia coli*	Negative
21	12357	Umar	72	Male	BU	*Escherichia coli*	ESBL
22	12362	Sum0no	25	Male	ER	*Steril*	Negative
23	12370	Pwt	55	Male	RIK	*Escherichia coli*	ESBL
24	12374	Susiati	35	Male	Merak	*Eschericia coli*	Negative
25	12444	Tj	65	Male	Pandanwangi	*Eschericia coli*	ESBL
26	12482	Juliati	70	Female	BU	*Coryne bacterium*	Negative
27	12506	Moisi	42	Female	Internal medicine for Female	Steril	Negative
28	12514	Syn	47	Male	BU	*Eschericia coli*	ESBL
29	12798	Mb	56	Male	RIK	*Eschericia coli*	Negative
30	12874	BKT	57	Male	HCU 2	*Candida tropicalis*	Negative

According to Table 1, of 30 samples tested in August, we detected 15 (50%) samples from positive Escherichia coli producing ESBL, meaning that 50% of urine samples from UTI patients had Escherichia coli. Meanwhile, negative cultures that did not produce ESBL were found in 1 sample of Enterobacter cloaceae; 2 samples of Klebsiella pneumoniae; 1 sample of Proteus mirabilis; 1 sample of Pseudomonas aeruginosa; 2 samples of Escherichia coli; 1 sample of Corinebacterium; 1 sample of Candida tropicalis; and sterile (no bacteria were found: 5 samples). These ESBL positive findings were detected using the Disk Diffusion Test method on Mueller Hinton Agar medium. The Escherichia coli producing ESBL will form an inhibitory zone from cephalosporins towards the disc clavulanic acid with an increase in the inhibition zone around the disc with a difference of 5 mm (Afifah et al., 2017). This means that the 15 positive results of Escherichia coli bacteria producing ESBL cause resistance to beta-lactam antibiotics of the penicillin group, cephalosporins first, second, and third generation which belong to the antibiotics to treat infections in hospitals. The ESBL will break down the structure of antibiotics by breaking down the lactam ring, changing the structure of the drug and blocking the binding of penicillin binding protein (PBS).

In the study conducted by Arsal et al (2018) with the title of "Deteksi Pola Kepekaan Antibiotik Pada Extended Spectrum Beta Lactamase (ESBL) Escherichia coli dari Sampel Urin Petugas Kesehatan Di Rumah Sakit Ibnu Sina Makassar

Tahun 2018" (Detection of Antibiotic Sensitivity Patterns in Extended Spectrum Beta Lactamase (ESBL) Escherichia coli from Urine Samples of Health Officers at Ibnu Sina Hospital Makassar in 2018) stated that of 23 ER urine samples, Escherichia coli was found in 7 samples (30.4%) all of which (100%) were resistant to beta lactam antibiotics. This ESBL process causes cell wall synthesis to continue and then inactivates the drug (Biutifasari, Verna, 2018). ESBL is mostly produced by Enterobacteriaceae, especially Escherichia coli and Klebsiella pneumoniae (Yadav et al., 2015). Escherichia coli producing ESBL causes the selection of antibiotic therapy to be limited, especially for elderly and immunocompromised individuals (Prasetya, 2017).

The increasing number of Escherichia coli producing ESBL occurs because the ESBL gene is located on the plasmid, which is often obtained through the transfer of genetic information from one bacterium to another. In the US, ESBL-producing bacteria was firstly observed in 1988 with a prevalence between 0-25%. Since then, the average Escherichia coli resistance varied from 5% in Korea to 23.3% in Indonesia (Wartanegara & Ety, 2014).

The study results were furtherly analysed at the molecular level to detect whether the ESBL produced by the Escherichia coli isolate had the CTX-M gene using the PCR method presented in the form of DNA bands. The marker on the DNA band in this study shows the number of 593 bp, meaning that if the DNA band of the sample is aligned with or above the marker, it means that the Escherichia coli isolate producing ESBL is the CTX-M type ESBL.

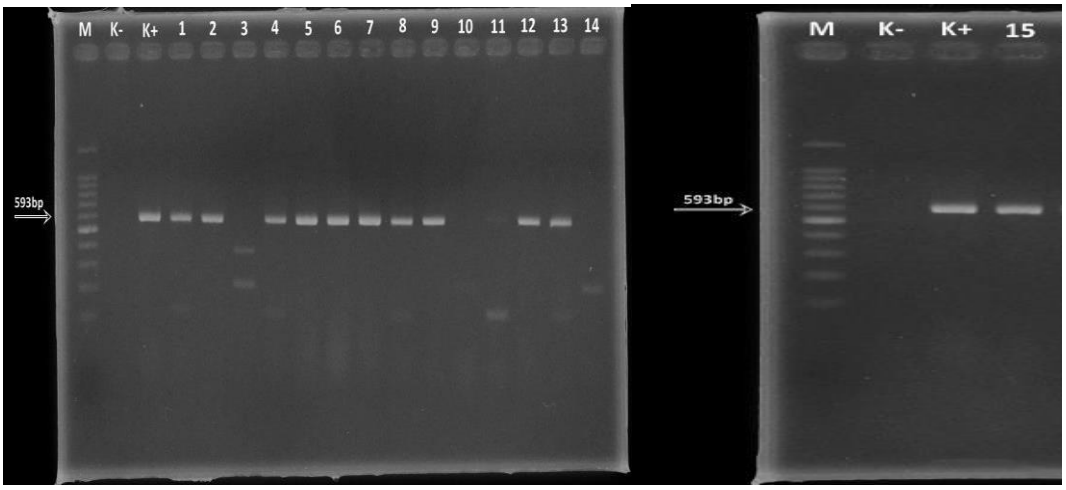

Figure1: *PCR results from isolates of Escherichia coli producing ESBL*

In Figure 1, K- (negative control) did not show DNA band, meaning that the negative control did not have the CTX-M gene; K+ (positive control) showed that the DNA band appeared above the DNA M band (marker) at a size of 593bp, meaning that the positive control has the CTX-M gene. In the samples of Escherichia coli isolate number 1, 2, 4, 5, 6, 7, 8, 9, 12, 13,15, the DNA bands were aligned with K+ (positive control), meaning that the CTX-M gene was detected on the isolates of Escherichia coli producing ESBL. However, the DNA bands from sample number 3, 11, and 14 were below the DNA band of K+ (positive control), meaning that the isolates of Escherichia coli producing ESBL do not have the CTX-M gene. The sample number 10 does not have a CTX-M band amplicon, meaning that the isolate of Escherichia coli producing ESBL is negative for the CTX-M gene. This result indicates that it is possible that the isolate of Escherichia coli produced the ESBL from other genes such as the SHV, TEM and OXA genes, because these three genes were not examined in this study (Linosefa et al., 2020). In a study conducted by Sikome et al., (2018) with the title of "Isolasi dan Identifikasi Secara Biomolekuler Bakteri Penyebab Penyakit Infeksi Saluran Kemih Yang Resisten Terhadap Antibiotik Ciprofloxacin di RSUP Prof.Dr.R.D.Kandou Manado" (Biomolecular Isolation and Identification of Bacteria Causing Urinary Tract Infections that are Resistant to Ciprofloxacin Antibiotics at Prof. Dr.

RD Kandou Hospital Manado), Klebsiella pneumoniae isolates had been identified to have the highest resistance against the antibiotic ciprofloxacin with a resistance percentage of 47.06% and a sensitivity of 52.94%. However, another study stated that Escherichia coli was the main organism causing UTI, and this study detected Escherichia coli in 18 of 30 samples; 15 of them produced ESBL, of which 11 samples were positive for the CTX-M gene.

Conclusions:

- A total of 18 (60%) of 30 urine samples of UTI patients were found to contain *Escherichia coli* bacteria.
- A total of 15 (50%) of 30 urine samples from UTI patients that were positive for *Escherichia coli* bacteria produced the Extended-Spectrum Beta Lactamase (ESBL) enzyme.
- A total of 11 (73%) of 15 urine samples from UTI patients were positive for Escherichia coli producing Extended-Spectrum Beta Lactamase (ESBL) having the CTX-M gene, 3 samples did not have the CTX-M gene, and 1 sample might have other ESBL-producing genes, such as the SHV, TEM, and OXA genes.

Suggestion:

Further detection of other genes in ESBL-positive Escherichia coli is needed, especially for the SHV, TEM and OXA genes which often cause beta-lactam antibiotic resistance.

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