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The Pattern of Resistance of Antibiotics to *Escherichia Coli* Causes Urinary Tract Infection in East Java, Indonesia.

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ABSTRACT

Urinary Tract Infection (UTI) is an inflammatory reaction of the urothelium cells lining the urinary tract, as a form of defense of the body caused by bacteria entry into the urinary tract. Testing species of bacteria in the urine has an important role in relation with the antibiotic selection for the patient. People in Indonesia are often less orderly in taking antibiotics, so the antibiotic resistance pattern tends to change. The aim of the research is to know the pattern of antibiotic resistence to Escherichia coli causing urinary tract infections. This study was descriptive and carried out in Public Health Laboratory of Surabaya, from March to June 2013. The results show an antibiotic that has a pattern of resistance to Escherichia coli are trimethoprim sulfametoxazole (81.3%) and ciprofloxacin (76.5%), whereas Escherichia coli is sensitive to the antibiotic Fosfomycin, Meropenem (100%) and Amikacin (92.6%).

Keywords: urinary tract infections, antibiotic resistance pattern, Escherichia coli

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INTRODUCTION

Indonesia is a developing country with a high number of infectious diseases, dominated by respiratory tract infection followed by gastrointestinal infections, and other infections such as urinary tract infections, skin and even systemic infections (Wijaya, 2011). In the case of urinary tract infection, the bacteria are found in significant amounts (100,000 colony growth or more in 1 mL of urine). If the laboratory examination found three types of bacteria or more, it is probably caused by the way of taking and management of materials that are not perfect, although this can happen to patients with urinary tract infections using catheter (Jawetz, 2008). Chitraningtyas research (2014) indicates the type of bacteria causing UTIs in Surabaya, Indonesia, is a Gram-negative bacteria (78.7%) and most types of bacteria were Escherichia coli (44%). A similar research was also conducted in Ethiopia, the bacteria that cause UTI was 80.2% and Gram-negative bacterial species Escherichia coli was the most 55.11% (Getachew, 2010). In Iraq, the cause of UTI is 87% Gram-negative bacterial and the species Escherichia coli is the most 31% (Al-Jebouri, 2013). In Africa, Escherichia coli causing UTI is 45% (Tansarli, 2013), in Islamabad 46,98% of the cases of UTIs are caused by Escherichia coli (Bano, 2012) and in India 34.42% caused by Escherichia coli UTI (Rakesh, 2014). From the data of the number of UTI in the world, it can be concluded that most are caused by Gram-negative bacteria (+ 80%) and the type of the bacteria that cause UTIs is Escherichia coli (31-55%).

If the antibiotic treatment of UTI is not proper, the bacteria causing the infection can be resistant to the antibiotics. The selection of antibiotics should be appropriate to the type of the bacteria causing the infection and which are preferred to kill the bacteria, especially for patients with decreased body defense or after undergoing a heavy surgery. If the infection occurs in a tissue with poor blood circulation, the antibiotic with good penetration power is needed so that it can get into the infection site. (Lampiris and Maddix, 2001)

Resistance to antibiotics can occur through several mechanisms, among others, the process of selection during the exposure of the antibiotic, the antibiotic-sensitive bacteria will die while the bacteria which are resistant to microbes survive and continue to grow. Another mechanism is the occurrence of mutations in vital genes, so the antibiotics can not hit the right target. The transfer of genes encoding antibiotic-resistant (R factor) is often known as the Horizontal transfer of R gene. Gene encoding antibiotic-resistant of bacteria can be transfered to other bacteria through conjugation process, and there are also bacteria that are naturally resistant to certain antibiotics, such as Gram-negative bacteria tend to be resistant to glycopeptide class of antibiotics (Nasronudin et al., 2007)

Sensitivity testing of bacteria in the urine is essential as patients with suspected UTI using antibiotics for the long term can lead to bacterial resistance, although on the other side it can heal or reduce symptoms of UTI (Purnomo, 2003). The most important thing to be considered in the use of antibiotics is the dosage, interval of giving, and the time of usage must be correct. The dosage must be proper so that the drug levels in the blood may exceed the minimal inhibitory concentration (MIC) for bacterial causes of infection (Rahardja and Tjay, 2002). If the type of antibiotic given is inappropriate or less effective against certain bacteria or the dosage is less, then the bacteria will not die but undergo a mutation or form immunity against the antibiotic. The higher the antibiotic dosage is used, the higher the pressure of the selective process of evolution and the proliferation of resistant strains of microorganisms. Pathogenic microorganisms resistant to antibiotics is very difficult to be eliminated during the process of infection and can lead to death.

The research was conducted at the Public Health Laboratory of Surabaya (PHLS). PHLS is a reference laboratory to the eastern part of Indonesia and as an accredited testing laboratory of ISO/IEC 17025: 2005 accreditation number LP-399-IDN. The research aims to analyze the pattern of antibiotic resistance to Escherichia coli causing UTI in East Java, Indonesia.

MATERIALS AND METHODS

The research used descriptive methode and the population covered all patients of suspected Urinary Tract Infection with checking parameter of urine culture and bacterial growth amounted to more than 100,000/mL /hour. The research was conducted from March to July 2013 as a non-probability sampling technique by purposive sampling. Of the isolated bacteria, 33 of the 75 urine specimens gave positive results containing Escherichia coli and colonies of Escherichia coli isolated from urine, respectively, continued to test antibiotic sensitivity.

- Tools used: Bunsen, PetriDish, Ose (10-3 ml volume), Incubators, Bacterial Counter
- Media used: Mac Conkey (MC), Cysteine Lactose Electrolyte Deficient (CLED), Mueller Hinton
- The following standard antibiotic discs were used for the isolates, ampicillin sulbactam, amikacin, gentamycin, meropenem, Tazobactam Piperacillin, ticarcillin clavulanic acid, cefotaxime, ceftriaxone, ceftazidime, Cefepime, trimethropim sulfamethoxazole, ciprofloxacin and levofloxacin
- Standard strains of Escherichia coli (ATCC 25922).

Antibiotic Sensitivity Test of the diffusion method

The isolated bacteria were tested for antimicrobial susceptibility testing by the standard Kirby Bauer's disc diffusion method. The standard inoculums adjusted to 0.5 McFarland was swabbed on Mueller Hinton agar and allowed to soak for 2 to 5 minutes. After the antibiotic disks were placed on the surface of the media and pressed gently, Mueller Hinton agar plates were then incubated at 37 ° C for 24 h. After 24 h the inhibition zones were measured and interpreted by the recommendations of clinical and laboratory standards/tables of the Clinical Laboratory Standards Institute (CLSI) in 2012 to determine the sensitive and resistant zone.

RESULTS AND DISCUSSION

The results shows the type of sensitive antibiotics for Escherichia coli

Table 1: Percentage of antibiotic sensitivity to Escherichia coli was causing UTI

Type of Antibiotics	Sensitivity
Meropenem	100,0%
Fosfomycin	100,0%
Amikacin	92,6%
Piperacillin Tazobactam	78,8%
Gentamycin	78,1%
Cefoperazone Sulbactam	62,5%
Cefepime	57,6%
Ceftazidime	54,5%
Cefixime	50,0%
Ceftriaxone	48,0%
Cefotaxime	47,8%
Nalidixid Acid	45,2%
Ofloxacin	40,0%
Amoxycillin Clavulanic Acid	39,4%
Ampicillin Sulbactam	35,5%
Cefuroxime	20,0%
Trimethoprim Sulfamethoxazole	18,8%
Ciprofloxacin	17,6%
Tetracycline	15,4%
Levofloxacin	12,5%
Ticarcillin Clavulanic Acid	11,1%

The appropriate antibiotic for Escherichia coli from the above table is the percentage of zones that have high sensitive pattern i.e. Fosfomycin, Meropenem (100%) and Amikacin (92.6%). The results of this study are similar to Tansarli (2013) that Escherichia coli causing UTI in Africa are sensitive to Fosfomycin (100%), and Imipenem (99%), carbapenems, Imipenem. Meropenem is a bactericidal antibiotic with a broader spectrum of target types inhibits bacterial cell wall synthesis (Joly Guillou, 2010). The research of Prakash (2013) reported

in Meerut City, India, Escherichia coli causing UTI is sensitive to Imipenem (98.45%) and Meropenem (95.45%). Al-jebouri (2013) reported Escherichia coli causing UTI in Tikrit City, Iraq, is sensitive to Amikacin and Ampicillin. Ampicillin has the same mechanism with Amikacin in the destruction of the wall peptidoglycan, but Ampicillin is able to penetrate Gram-positive and negative bacteria. This is due to the presence of the amino group of ampicillin, so as to penetrate the outer membrane (outer membrane) in Gram-negative bacteria (Mueller, 2004).

Table 2: Percentage of antibiotic resistence to Escherichia coli was causing UTI

Type of antibiotics	Resistence
Tetracycline	84.60%
Trimethoprim Sulfamethoxazole	81.30%
Ciprofloxacin	76.50%
Levofloxacin	75.00%
Cefuroxime	60.00%
Ofloxacin	60.00%
Ticarcillin Clavulanic Acid	55.60%
Ampicillin Sulbactam	54.80%
Nalidixid Acid	51.60%
Cefixime	50.00%
Amoxycillin Clavulanic Acid	48.50%
Ceftriaxone	48.00%
Ceftazidime	42.40%
Cefepime	42.40%
Cefotaxime	39.10%
Cefoperazone Sulbactam	37.50%
Gentamycin	21.90%
Piperacillin Tazobactam	9.10%
Amikacin	7.40%
Meropenem	0.00%
Fosfomycin	0.00%

Antibiotics which have a high resistance for Escherichia coli are tetracyclin (84,60%), sulfametoxazole trimethoprim (81.3%) and ciprofloxacin (76.5%). Different results were reported by Getachew (2010). Escherichia coli as a source of intestinal flora and the main etiology of urinary tract infection is a bacterial population that is most likely to receive antibiotic therapy (Sjahrurrachman et al, 2004). Resistant antibiotics mentioned above are in the class of beta-lactamase, cephalosporins, trimethoprim combination with sulfametaxazole (TMP-SMZ) and the class of quinolones). The results also showed high resistance of Escherichia coli to ampicillin sulbactam (54.8%), ticarcillin clavulanic acid (55.6%). Febriy Firizky (2014) examined in UPTD Health Laboratory Lampung province on cephalosporin antibiotic sensitivity pattern of steadily declining from 2008 to 2012, in the range of 25.4 to 40%,

Beta-lactam inhibits bacterial growth by binding to the DD-transpeptidase enzymes that mediate bacterial peptidoglycan wall, and thus would weaken the cell wall of bacteria This resulted in cytolysis due to osmotic pressure imbalance, as well as the activation of hydrolases and autolysins that digest the peptidoglycan wall preconceived . Beta-lactam (penicillin and) only effective against Gram-positive bacteria, because the presence of the outer membrane (outer membrane) were found in Gram-negative bacteria makes it unable to penetrate the wall peptidoglycan. Gram-negative bacteria such as Escherichia coli produces

plasmid can transfer resistance genes and also produce beta-lactamase enzymes that can inhibit the mechanism of action of beta-lactam class of antibiotics (Sjahrurachman et al., 2004).

Escherichia coli is able to produce DHFR (dihydrofolate Reductase) in excess to block the actions of Trimethoprim. Trimetophrim is antimetabolic with a mechanism to inhibit the formation of DNA and protein. Trimetophrim will inhibit the reductase enzyme dihidrofolate should be required to change the dihydrofolate (DHF) to tetrahydrofolate (THF), therefore the resistance of Escherichia coli has over 80% of the Trimetophrim (Mueller, 2004).

Different results were reported by Prakash (2013) in Meerut, India, UTI causing Escherichia coli resistant to tobramycin (96.91%), nalidixic acid (90.91%) and Cefotaxime (87.88%). Nalidixic acid is a bactericidal antibiotic which has the same mechanism of action of the quinolone, nalidixic acid but is widely used for typhoid fever (Mueller, 2014) and therefore Escherichia coli resistant to nalidixic acid with the percentage of more than 50%.

Escherichia coli is the cause of UTI in Ethiopia, resistant to Amoxiciline-clavulanic acid (96%), tetracycline (66.9%) and ampicillin (64.7%). Tetracycline is an antibiotic bakteriostatis that binds to the 16S-30S ribosomal subunit and preventing the binding of aminoacyl-tRNA from the A site of the ribosome, thereby inhibiting protein translation, but this type antibiotics have side effects that cause the teeth to become stained and its impact on the kidneys and liver. Bacteria can alter penicillin-binding proteins (PBPs/Penicillin Binding Proteins) as an example of Methicillin-Resistant Staphylococcus Aureus (MRSA) is a change in the ß lactam PBPs that cannot bind to components of the bacterial cell. It is also common on Vancomycin Resistant Enterococci can change the bonding area of the D-ala D-ala to D-ala L-lys. This bond can be recognized by the bacterial transpeptidase enzyme but not recognized by Vancomycin, another example is the erythromycin resistance through changes 50s ribosomal subunit.

Reduced antibiotic pathway toward the target cells so that bacteria can change their outer membrane proteins, such as Porin, who used antibiotics for bacterial entry into the cell which results in cells prevents entry of antibiotics, although antibiotics are still able to go through other channels, such as resistance to penicillin is due to the changes in the permeability of penicillin. This causes the dose to handle infections is increasing.

CONCLUSION

Giving the type of antibiotic and the proper dosage according to the species of bacteria that cause UTIs will cure patients from infectious diseases. Antibiotic resistance and sensitivity to Escherichia coli that cause UTIs highly variable allows different results if conducted in other locations.

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