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The Study on the Susceptibility of *Enterococcus faecalis* and *Klebsiella ozaenae* Cause UTI to Antibiotics in East Java, Indonesia.

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ABSTRACT

Urinary Tract Infection (UTI) is caused by bacteria entry into the urinary tract. Testing the species of bacteria in the urine has an important role in relations with the antibiotic selection for the patient. The aim of the research is to know the susceptibility of *Enterococcus faecalis* and *Klebsiella ozaenae* cause of UTIs for each type of antibiotic. This study was descriptive and carried out in the Public Health Laboratory of Surabaya from March to June 2013. *Enterococcus faecalis* that caused the UTIs in East Java, Indonesia, had a high susceptibility to Ampicillin, Penicillin, Vancomycin, Teicoplanin, Fosfomycin and high resistance to Erythromycin. Whereas *Klebsiella ozaenae* that caused the UTIs in the same area had a high susceptibility to Meropenem and Cefoperazone sulbactam and high resistance against Trimethoprim, Sulfamethoxazole and Ciprofloxacin.

Keywords: *Enterococcus faecalis, Klebsiella ozaenae,* Urinary Tract infections, Antibiotics.

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INTRODUCTION

Urinary tract infection (UTI) is an infection caused by bacteria from the intestinal flora of patients. The incidence of the UTIs in Indonesia is still quite high, the prevalence of UTI in the community increased in line with the increasing of age. Peoplea at the age of 40-60 years have a prevalence rate of 3.2%, while people at the age of 65 or over have a UTI prevalence rate of 20%. Urinary tract infections can be suffered by men and women of all ages, children and adolescents, adults and the elderly. Approximately 15% of women experienced at least one attack of acute urinary tract infection during their lifetime. Most of UTIs occurred are asymptomatics, the number of events in pregnant women is 5% -6% and increased to 10% at high risk (Kim, 2011).

The incidence of the UTI in the town of Meerut, India has a prevalence of 53.82% in patients (Prakash, 2013). According to the report high prevalence of the UTI will affect up to 150 million people per year worldwide (Stamm, 1999).

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 the ISO/IEC 17025: 2005 accreditation number LP-399-IDN. Chitraningtyas et al (2014) obtain data on species of bacteria that cause urinary tract infections are *Escherichia coli* (44%), followed by *Enterococcus faecalis* (13.3%), *Acinetobacter sp*. (12%), *Klebsiella ozaenae* (8%), *Proteus vulgaris* (6.7%). This study aims to determine the susceptibility of *Enterococcus faecalis* and *Klebsiella ozaenae* cause of UTIs for each type of antibiotics.

MATERIALS AND METHODS

The research used descriptive method 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. From the isolated bacteria, 16 of the 75 urine specimens gave positive results containing *Klebsiella ozaenae* and *Enterococcus faecalis*. Colonies of *Klebsiella ozaenae* and *Enterococcus faecalis* isolated from urine, respectively, continued to test antibiotic sensitivity.

- Tools used: Bunsen, Petri Dish, 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, Amoxycillin Clavulanic Acid, Tetracycline, Ofloxacin, Fosfomycin, Penicillin, Tetracyclin, Chloramphenicol, Erythromycin, Vancomycin, Teicoplanin, Piperacillin, Ticarcillin Clavulanic Acid, Nalidixid acid, Cefotaxime, Ceftriaxone, Ceftazidime, Cefepime, Cefoperazone Sulbactam, Trimethropim sulfamethoxazole, Ciprofloxacin and Levofloxacin
- Standard strains of *Klebsiella ozaenae* (ATCC 11296) and *Enterococcus faecalis* (ATCC 29212)

Antibiotic Sensitivity Test of the Diffusion Method

The isolated bacteria were tested for antimicrobial susceptibility using 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 DISCUSSIONS

The result of the *Enterococcus faecalis* susceptibility test for some types of antibiotics can be seen in the table below

Table 1: Antibiotics Sensitivity Testing for *Enterococcus faecalis*

Antibiotics	∅ Zone (mm)	% sensitivity
Ampicillin	≥ 17	100,0
Penicilllin	≥ 15	100,0
Vancomycin	≥ 17	100,0
Teicoplanin	≥ 14	100,0
Fosfomycin	≥ 16	100,0
Ciprofloxacin	≥ 21	75,0
Levofloxacin	≥ 17	75,0
Chloramphenicol	≥ 18	66,7
Tetracycline	≥ 19	57,1
Erythromycin	≥ 23	10,0

The testing of 16 isolates shows the susceptibility of *E. faecalis* to antibiotics that have a high percentage of zones of Ampicillin, Penicillin, Vancomycin, Teicoplanin, Fosfomycin respectively 100%, ciprofloxacin and levofloxacin (75%). Similar results were obtained by Rudy (2004), in the Silesian Hospital, New York, Genaro (2005) from Brazil, and Rams (2013) of the USA discovered that all isolated strains of *E. faecalis* are 100% sensitive to glycopeptides (Vancomycin and Teicoplanin).

In Pakistan, Farhan (2006) found out the *E. faecalis* susceptibility to the antibiotic Vancomycin and Teicoplanin amounted to 99.1% (99.1%), followed by Nitrofurantoin (97.3%), Fosfomycin (91.0%) but slightly different sensitivity to Chloramphenicol (78.5%). In India, Chaudahary's research (2007) showed the susceptibility of Vancomycin (98%), followed by Teicoplanin (88%), Linezolid (79%), Novobiocin (78%), Spectinomycin (55%), and Doxycycline (51%).

The result of some kind of antibiotic resistance against *Enterococcus faecalis* can be seen in the table below:

Table 2: Antibiotics Resistance Testing for Enterococcus faecalis

Antibiotics	**∅ Zone (mm)**	**% Resistence**
Erythromycin	≤ 13	50,0
Tetracycline	≤ 14	42,9
Ciprofloxacin	≤ 15	25,0
Levofloxacin	≤ 13	25,0
Chloramphenicol	≤ 12	22,2
Vancomycin	≤ 14	0
Teicoplanin	≤ 10	0
Fosfomycin	≤ 12	0
Ampicillin	≤ 16	0
Penicilllin	≤ 14	0

The testing of the 16 isolates showed that *Enterococcus faecalis* most resistant to antibiotics Erythromycin (50%) located in group antibiotics Macrolide class of antibiotics that inhibit protein synthesis specific for Gram positive cocci. According to Farhan (2006) the antibiotic resistance to *Enterococcus faecalis* is Pipericillin-Tazobactam (72.3%), Ampicillin (54.4%), Meropenem (45.5%) and Ciprofloxacin (35.7%). Koo, (2013) mentioned that *E. faecalis* isolates were resistant to Tetracycline (58.3%), Erythromycin (11.1%) and Nitrofurantoin (2.8%). Rams (2013), found out that isolates of *E. faecalis* resistant to Tetracycline at 53.2%, Erythromycin (80.8%), Clindamycin (100%, a dose of 2 mg / mL), and Metronidazole (100%, a dose of 4 mg / mL).

Enterococci is an important nosocomial pathogen bacteria since it contains penicillin - binding protein (PBP) capapability to enable it to tolerate beta-lactam antibiotics to synthesize some of the components of the cell wall. Enterococci is resistant to Penicillins, Cephalosporins, Nalidixic acid, Aztreonam, Macrolides. Enterococci used the formed-folic acid that enable the synthesis of the cell - wall (Courvalin, 2006). Long (2005) reported that *E. faecalis* and *E. faecium* are resistant to lipopeptide antibiotic Daptomycin working on bacterial cell membranes.

The table of zone diameter and the percentage of antibiotic sensitivity of bacteria *Klebsiella ozaenae* can be seen as follows:

Table 3: Antibiotic susceptibility testing for bacteria *Klebsiella ozaenae*

Antibiotics	∅ Zone (mm)	% Sensitivity
Cefoperazone Sulbactam	≥ 21	100,0
Meropenem	≥ 23	100,0
Amikacin	≥ 17	83,3
Ampicillin Sulbactam	≥ 15	80,0
Piperacillin Tazobactam	≥ 21	80,0
Fosfomycin	≥ 16	80,0
Cefotaxime	≥ 26	80,0
Ceftriaxone	≥ 23	75,0
Ceftazidime	≥ 21	66,7
Cefepime	≥ 18	66,7
Gentamycin	≥ 15	66,7
Amoxycillin Clavulanic Acid	≥ 18	66,7
Tetracycline	≥ 15	50,0
Ticarcillin Clavulanic Acid	≥ 20	50,0
Ofloxacin	≥ 16	50,0
Nalidixid Acid	≥ 19	50,0
Ciprofloxacin	≥ 21	20,0
Trimethoprim Sulfamethoxazole	≥ 16	0

The the testing of the 16 isolates showed that Klebsiella ozaenae that has antibiotic-sensitive zones with the highest percentage are Meropenem and Cefoperazone sulbactam (100%), antibiotics in combination of Trimethroprim with Sulfametaxazole (TMP-SMZ) and the class of quinolones.

Table 4: Test of antibiotic resistance to bacteria Klebsiella ozaenae

Antibiotics	∅ Zone (mm)	% Resistence
Trimethoprim Sulfamethoxazole	≤ 10	100,0
Ciprofloxacin	≤ 15	60,0
Ticarcillin Clavulanic Acid	≤ 14	50,0
Ofloxacin	≤ 12	50,0
Tetracycline	≤ 11	50,0
Nalidixid Acid	≤ 13	50,0
Ceftazidime	≤ 17	33,3
Cefepime	≤ 14	33,3
Amoxycillin Clavulanic Acid	≤ 13	33,3
Gentamycin	≤ 12	33,3
Ceftriaxone	≤ 19	25,0
Ampicillin Sulbactam	≤ 11	20,0
Cefotaxime	≤ 22	20,0
Piperacillin Tazobactam	≤ 17	20,0
Meropenem	≤ 19	0,0
Amikacin	≤ 14	0,0
Fosfomycin	≤ 12	0,0
Cefoperazone Sulbactam	≤ 15	0,0

The testing 0f the 16 isolates of *Klebsiella ozaenae* showed that antibiotics which are resistant to *Klebsiella ozaenae* zone with the highest percentage are Trimethoprim sulfamethoxazole (100%) and Ciprofloxacin (60%). Goldstein (1978) performed antibiotic susceptibility testing by agar dilution method in 21 isolates. Ninety-five percent of the 21 isolates were susceptible to Cephalothin, 90% of the 20 isolates were susceptible to Gentamicin, 90% of the 9 isolates were susceptible to Amikacin and 88% of the 8 isolates were susceptible to Kanamycin. Only 26% of 19 isolates were susceptible to Ampicillin and 21% of the 14 isolates were susceptible to Tetracycline. All three isolates were susceptible to Chloramphenicol and one of the two isolates were susceptible to Tobramycin. Murray (1981) studied 16 strains of *K. ozaenae*, all susceptible to Cephalothin, Chloramphenicol, Tetracycline, Gentamicin, Streptomycin, Kanamycin and Amikacin. Less than 20% were susceptible to Ampicillin and Carbenicillin. *K. ozaenae* susceptible to Cefotaxime in the isolates tested by Strampfer (1987) and Ciprofloxacin in the isolates tested by Chowdhury (1992). Interestingly, the first reports of plasmid-mediated resistance to broad-spectrum Cephalosporins is an isolate *K. ozaenae* in Germany analyzed in 1983 (Kliebe (1985), Podbielski (1990).

Isolates of *Klebsiella ozaenae* is sensitive to Ceftazidime, Ciprofloxacin, Chloramphenicol, Gentamicin and Sulfamethoxazole-trimethoprim but resistant to Ampicillin. *Klebsiella ozaenae* responds well to 4 weeks iv Ceftazidime and i.v. Amoxicillin-clavulanic acid. (Ng, 2009).

Ampicillin, Vancomycin, Teicoplanin, Linezolid, Penicillin, Ampicillin Sulbactam, Sulbactam Cefoperazone, Cefotaxime, Ceftriaxone, Ceftazidime, Cefixime, Cefepime, Amikacin, Gentamycin, Chloramphenicol, Ofloxacin, Levofloxacin, Meropenem, Piperacillin Tazobactam, Fosfomycin and Clindamycin are the most effective antibiotics in the study. The research data showed that antibiotics are capable of producing a good clinical response.

According to Setiabudy (2007) the first-generation of Cephalosporin antibiotics has been long in use and often used to treat urinary tract infections that can lead to resistance. Its second generation is effective mainly against Gram-negative bacteria, while the third generation is active and has a broad spectrum against Enterobacteriaceae, including Penicillinase-producing strains or strains that are resistant to second-generation cephalosporins. However, with the various mechanisms of bacteria in causing resistance, these bacteria may become resistant.

According to the literature, the bacteria that cause urinary tract infections are still sensitive to Quinolones (eg, Ciprofloxacin), but along with the increased use of quinolones in hospitals has resulted in an increase of the resistant bacteria to quinolones (Schaeffer, 2007). The data in the Arifin Ahmad Hospitals show quinolones are used as the primary antibiotic therapy Urinary Tract Infection, while the results of the research conducted at the Laboratory of Microbiology Faculty of Medicine University of Indobesia in 2004 showed bacterial resistance to quinolones by 50% (Noorhamdani et al, 1996), 60% to *Klebsiela* and 25% to *Enterococci.* The resistance of Quiolon bacterial is a growing problem now in some European countries (Schaeffer, 2007).

Urinary tract infections that are mostly caused by bacteria require antibiotics for treatment. But, since the population of bacteria often give the pictures with different sensitivity to antibiotics depending on the time and place of the occurrence, the antibiotics used for treatment should meet the local isolates sensitivity picture. In addition, in order to achieve bacterial eradication in the treatment of urinary tract the records of the etiology patterns and its resistance should be continuously updated (Sjarurrachman et al, 2004).

CONCLUSIONS

The Enterococcus faecalis that caused of the UTIs in East Java, Indonesia, has a high susceptibility to Ampicillin, Penicillin, Vancomycin, Teicoplanin, fosfomycin and high resistance to Erythromycin. The Klebsiella ozaenae that caused of the UTIs in East Java, Indonesia, have a high susceptibility to Meropenem and Cefoperazone sulbactam and high resistance against Trimethoprim Sulfamethoxazole and Ciprofloxacin.

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