Trends antimicrobial resistance in urinary tract infections: a research at the Dr. Zainoel Abidin Teaching Hospital, Banda Aceh, Indonesia

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Abstract. The aim of this study was to identify the most common bacterial cause of urinary tract infections and the patterns of antimicrobial resistance. The study was conducted at Dr. Zainoel Abidin Teaching Hospital, Banda Aceh, Indonesia with descriptive retrospective study approach. The data was taken from medical records. One hundred and nineteen bacterials was identified, encountered gram-negative bacteria namely *Escherichia coli* (31.7%), *Klebsiella pneumoniae* (27.7%), *Acinetobacter* (12.6%), *Pseudomonas aeruginosa* (10.1%), *Enterobacter* (5.1%) and *Proteus mirabilis* (0.8%). Meanwhile encountered gram-positive bacteria were *Beta-hemolytic Streptococci* (9.2%) and *Staphylococcus aureus* (3.4%). Observations on the sensitivity profile suggested that the pathogenic gram-negative bacteria had a high resistance against ampicilin, ciprofloxacin, cefuroxime and cefotaxime, while meropenem, chloramphenicol, gentamicin and tobramycin have a high sensitivity level. Descriptive analysis also found pathogenic gram-positive bacteria showed high resistance to ceftriaxone, clindamycin, tetracycline and cephalotin and had a high sensitivity level to meropenem, vancomycin and oxacillin. Based on these results we concluded that the most common of urinary tract infections were caused by gram-negative bacteria species *Escherichia coli*. Encounters a high resistance pattern to 3rd generation cephalosporin antibiotics group, this was caused by the irrational use of antibiotics and the ability of bacteria to produce beta-lactamase enzymes which results in degradation of beta-lactam ring (β-lactam). We recommended the antibiotics group of chloramphenicol, gentamicin and vancomycin to use as empirical therapy in urinary tract infections. Antibiotic treatment should be prescribed only for as long as necessary to be effective. Recurrent urinary tract infections may be managed better by self initiated therapy or prophylaxis than by continuing to treat each case emergently. We also recommend health practitioners to use antibiotics rationally to prevent the resistances.

Key words: Urinary tract infection, antimicrobial resistance, antibiotic sensitivity.

Introduction

Urinary tract infection (UTI) is one of the most common medical problems. It was estimated that 150 million patients are diagnosed with a UTI yearly, resulting in at least $6 billion in health care expenditures. UTIs are difficult to diagnose; some cases respond to a short course of a specific antibiotic, while others require a longer course of a broad-spectrum antibiotic. Accurate diagnosis and treatment of a UTI is essential to limit associated morbidity and mortality and avoid prolonged or unnecessary use of antibiotics (Tanagho et al. 2008). Although susceptibility patterns have changed, the spectrum of agents that causing UTI has remained relatively constant. *Escherichia coli* accounts for 75% to 90% of cases; *Staphylococcus saprophyticus* accounts for 5% to 15% (particularly in younger women); and *enterococci* and non–*E. coli* aerobic gram negative rods, such as *Klebsiella* species and *Proteus mirabilis*, account for the remaining 5% to 10%. Although less well studied, the spectrum of agents causing uncomplicated pyelonephritis is similar to that causing acute cystitis (Gupta et al. 2001).

The prevalence of uropathogens resistant to Trimethoprim/sulfamethoxazole (TMP-SMZ), nitrofurantoin, and first-generation of cephalosporins are continuously rise. There are data that suggest overall resistance to TMP-SMZ is approximately 25% (range, 10-45%) based on the area of the country, and resistance to nitrofurantoin is slightly higher. Over the last decade, resistance to fluoroquinolones has been acceptably low in most studies; however, more recent microbiologic data showed that fluoroquinolone resistance, particularly in the West coast, may be an outbreaking problem (Killgore et al. 2004).

The purpose of this study was to identify the patterns of antimicrobial resistance (as the primary outcome) and the most common bacterial that caused urinary tract infections (as the secondary outcome) in patients who've done examination of urine culture and sensitivity tests at laboratory of microbiology Dr. Zainoel Abidin Teaching Hospital, Indonesia.

Methods
Study design and sampling strategy
This research was descriptive study with retrospective approach by taking a cross-sectional data. Samples in this study were the result of urine culture examination that take from medical records between January until December 2010 on the laboratory of microbiology Dr. Zainoel Abidin Teaching Hospital Banda Aceh, Indonesia, which fulfill the inclusion criteria (data encountered bacterial pathogen cultured in urine samples and has a sensitivity test). The simple were the patients that have been hospitalized and diagnosed as UTI both in symptomatic or asymptomatic.

A patient with UTI was defined as a patient who diagnosed with UTI (as stated on the laboratory referral documents) and already had an urine culture yielding $\geq 10^2$ colonies per mL of urine with presumptively identified as bacteria pathogen.

Urine specimen collection and microbiological methods
All urine specimens data obtained from patients daily, preserved with glicerol 15%, and stored at -80°C until the testing. Urine specimens were cultured by standard methods of the American Society for Microbiology. All susceptibility testing was interpreted according to Clinical and Laboratory Standards Institute (Pezzlo et al. 2004).

Results and Discussion
We retrospectively viewed the medical records of 119 patients with UTIs between January to December 2010. *Escherichia coli, Klepsiella pnemoniae, Acinetobacter* were the most common pathogens gram-negative bacteria isolated from patients with community - acquired urinary tract infections that have been hospitalized, the isolated gram positive bacteria was; *Beta-hemolytic streptococci* and *Staphylococcus aureus* which is 9.2%, and 3.4% (table 1).

This study provides current information regarding to etiologic agents that cause community-acquired UTI. The frequency distribution of the etiologic organisms within this study was quite consistent with the findings of previous study, who have been evaluated from three hundred patients that attending Dalhatu Araf Specialist Hospital, Lafia Nasarawa State, Nigeria. Results showed the most common organisms were *Escherichia coli* (Kolawole et al. 2009). This finding was also similar to other reports which indicated that gram negative bacteria, particularly *E. coli*, was the commonest pathogen isolated in patients with UTI (Burbige et al. 1984; Akinyemi et al. 1997; Ebie et al. 2001).

Not all bacteria are capable to adhering and infecting the urinary tract. Any strains of *Escherichia coli*, belong to a limited number of O, K, and H serogroups. They had increased the adherence properties to uroepithelial cells and the resistance to the bactericidal activity of human serum, hemolysin, and the increased expression of K capsular antigen. The ability of *E. coli* to adhere the epithelial cells is mediated by ligands that located on the tips of the bacterial fimbriae (pili) (Tanagho et al. 2008).

Recently, it has been observed that many bacteria such as *E. coli* have the ability to invade into the host cells, act as opportunistic intracellular pathogens (Bower et al, 2005). Cytotoxic necrotizing factor, Afa/Dr adhesions and type 1 pili have been shown to promote the invasion into the host cells. The intracellular bacteria mature into biofilms, creating pod-like bulges on the urothelial surface (Anderson et al. 2003). The pods contain bacteria encased in a polysaccharide-rich matrix surrounded by a protective shell of uroplakin. The ability of the uropathogenic bacteria to transiently invade, survive, and multiply within the host cells and to create biofilms on genitourinary tract tissues may provide a mechanism for the persistence and recurrence of UTIs (Tanagho et al. 2008).

Among the 37 *E. coli* isolates, the prevalence of resistance to ampicilin, ciprofloxacin, and cefotaxime was 91.9%, 83.7%, and 67.6%. No trends in the prevalence of resistance to meropenem, chloramphenicol, gentamicin, and tobramycin were detected.

Ampicilin sensitivity test against pathogenic bacteria that cause UTIs showed high levels of resistance against *Enterobacter* and *Klepsiella pnemoniae,* which is 100%. The 3rd generation cephalosporin class (cefotaxime) have also showed high levels of resistance to the bacteria *E. coli, Acinetobacter, Pseudomonas aeruginosa and beta-hemolytic streptococci* (these bacteria tested with the ceftriaxone) was 67.6%, 80%, 83.3%, and 81.8% (Table 2).

Table 1. The Distribution of pathogenic bacteria that cause urinary tract infections, results of urinary tract isolates from January, 1 until December, 31, 2010 in the microbiology laboratorium Dr. Zainoel Abidin Teaching Hospital Banda Aceh, Indonesia

No	Type of bacteria	Percentage (%)
	Gram-negative bacteria	
1	*Escherichia coli*	31.7
2	*Klepsiella pnemoniae*	27.7
3	*Acinetobacter*	12.6
4	*Pseudomonas aeruginosa*	10.1
5	*Enterobacter*	5.1
6	*Proteus mirabilis*	0.8
	Gram-positive bacteria	
1	*Beta-hemolytic Streptococci*	9.2
2	*Staphylococcus aureus*	3.4

Table 2. The patterns of antibiotic resistance in urinary tract infections that caused by Gram-negative bacteria which are isolated in urinary tract through the antibiotic sensitivity test

Bacteria	Antibiotic							
	Ampicilin (%)	Cefota-xime (%)	Genta-micine (%)	Chloram-phenicol (%)	Merope-nem (%)	Ciproflo-xacin (%)	Cefuroxi-me (%)	Tobramycin (%)
E. coli								
Resistant	91.9%	67.6%	19%	32.4%	0	83.7%	73%	19%
Sensitive	8.1%	32.4%	81%	67.6%	100%	16.3%	27%	81%
Kepsiella pneumoniae								
Resistant	100%	21.3%	30.1	12.1%	0	84.8%	91%	21.3%
Sensitive	0	78.7%	69.9%	87.9%	100%	15.2%	8%	78.7%
Acinetobacter								
Resistant	Not tested	80%	40%	86.7%	6.7%	73.3%	Not tested	60%
Sensitive		20%	60%	13.3%	73.3%	6.7%		40%
Enterobacter								
Resistant	100%	Not tested	33.3%	16.7%	0	50%	83.3%	Not tested
Sensitive	0		66.7%	83.3%	100%	50%	16.7%	
Proteus mirabilis								
Resistant	Not tested	100%	Not tested	Not tested	0	Not tested	100%	Not tested
Sensitive		0			100%		0	
Pseudomonas aeruginosa								
Resistant	Not tested	83.3%	83.3%	Not tested	33.4	50%	Not tested	Not tested
Sensitive		16.7%	16.7%		66.6%	50%		

Ampicilin, ciprofloxacin and cefotaxime has a high level resistance, these more likely caused by the irrational use of antibiotics. Irrational use of antibiotics is caused by the procurement and indiscriminate use.

Antibiotic resistance in *enterobacter*, one of the most common pathogens in UTIs, has been encountered worldwide (Nys et al. 2008). The emergence of multidrug resistance (MDR) in *E. coli*, such as s extended-spectrum β-lactamase (ESBL)-producing strains. The overexpression of efflux pumps was reported to contribute to MDR of *E. coli.* Almost all Gram-negative bacteria have genes for efflux pumps to the resistance nodulation division (RND) family, and seven homologous RND-type pumps are known in *E. coli*. Five of these (AcrB, AcrF, MdtB, MdtC, and YhiV) are known to expel many kinds of drugs. The overexpression of this pump often associated with the MDR phenotype in *E. coli*. A previous report showed that a point mutation in yhiV, coding for YhiV, was associated with the differences in the MDR phenotype. The overexpression of efflux pump genes such as marA, yhiU, yhiV, and mdfA is related to antibiotic resistance, especially fluoroquinolones (Yasufuku et al. 2010).

The characterization of efflux pumps could lead to the development of new weapons against antibiotic resistance, namely, efflux pump inhibitors (EPIs) (Page`s et al. 2009). EPIs efficiently inhibit the major AcrAB-TolC pump that responsible for MDR in *E. coli* (Page`s et al. 2009) and reduce the resistances to antibiotics in bacterial isolates (Yasufuku et al. 2010).

One study investigated the correlation of antibiotic susceptibilities with the expression of the efflux pump genes in *E. coli* of UTI patients by quantitative real-time reverse transcription-PCR (qRT-PCR) and with risk factors for antibiotic resistance. The increasing expression of efflux pump genes such as marA and mdfA can lead to fluoroquinolone resistance in *E. coli*. These results contribute to the efflux system and raise the possibility of developing new agents, such as EPIs, to antibiotic-resistant *E. coli* (Yasufuku et al. 2010)*.*

The low rate of ciprofloxacin (3.7%) resistance is noteworthy given its more than 13 years of continued use in the United States. However, resistance was slightly higher (2.2%) than reported in previous North American surveillance studies (Gupta et al. 1999; Jones et al. 1999). Ciprofloxacin has maintained a high level of activity against UTI isolates of *E. coli* compared with other commonly used agents, such as ampicillin and trimethoprim sulfamethoxazole (SXT) (Gupta et al. 1999). The current data demonstrated that a ciprofloxacin-resistant phenotype without concurrent resistance to other classes of antimicrobials is rare (1.8%) (Sahm et al. 2001). Previous study performed by Canadian investigators described a correlation between resistance of ampicillin and resistance to SXT and ciprofloxacin in *E. coli* but did not describe MDR phenotypes or patient demographics (Zhanel et al 2000). Specifically, this study found the increasing rates of ampicillin resistance (91.9%) and ciprofloxacin- resistant (83.7%) for *E. coli* isolates.

In this study we also compared the effectiveness of clindamycin against *Beta-hemolytic streptococci and Staphylococcus aureus*. From the test result of this group antibiotic resistance rates were 90.9% and 75% (table 3).

Among the 12 *Beta-hemolytic streptococci* isolates, the prevalence of resistance to meropenem and vancomycin was 0%, and 18.2%. No trends in the prevalence of resistance to meroponem, and vancomycin were detected. The pathogenic bacteria *Staphylococcus aureus* show the level of resistance to vancomycin and oxacillin is 0%. No profile resistance to vancomycin and oxacillin.

The most useful antibiotics in this study were chloramphenicol, gentamicin, and vancomycin (in gram-positives) because they inhibit the most commonly isolated UTI pathogens. These drugs are relatively expensive compared to the most antibiotics frequently used. This probably restricted their procurement and indiscriminate use, making the organisms susceptible to it. This is different to other reports where quinolones are the most effective (susceptible) (Mbata. 2007). Ampicilin, ciprofloxacin and cefotaxime which are commonly used antibiotics were poorly effective against majority of the isolated organisms in this study. This is similar to other reports where ampicilin, ciprofloxacin and cefotaxime were poorly effective against majority of the organisms that caused UTI (Zhanel et al. 2000). The difference may be due to the practices of self medication and indiscriminate use of these antibiotics in this part of Nigeria and the acquiring of plasmid-encoded resistance gene (Kolawole et al. 2009).

The results of this study and those of others may be not representative for the general population. UTI are often treated empirically and susceptibility tests are often carried out only when the patient has failed once or in more courses of antibiotics. Even though the susceptibility pattern shown by this study buttressed the need for in-vitro sensitivity reports before antibiotics therapy initiation, however, it should be borne in mind that in-vitro antimicrobial sensitivity is only a guide and that conditions in-vivo may be quite different (Kolawole et al. 2009).

Finally, the findings have no doubt in highlighted the need for constant monitoring of susceptibility of specific pathogens in different populations to commonly used anti-microbial agents. These data may be used to determine the trends in antimicrobial susceptibilities, formúlate the local antibiotic policies, compare to local and national data and to assist clinicians in the rational choice of antibiotic therapy to prevent misuse or overuse of antibiotics.

Table 3. The patterns of antibiotic resistance in UTIs that caused by Gram-positive bacteria which are isolated in urinary tract through the antibiotic sensitivity test

Bacteria	Antibiotic						
	Ceftriaxone (%)	Clindamycin (%)	Tetracycline (%)	Cephalotin (%)	Meropenem (%)	Vancomycin (%)	Oxacillin (%)
Beta-hemolytic Streptococci							
Resistant	81.8%	90.9%	81.8%	Not tested	Not tested	18.2%	Not
Sensitive	18.2%	9.1%	18.2%			81.8%	tested
Staphylococcus aureus							
Resistant	Not tested	75%	Not tested	25%	0	100%	0
Sensitive		25%		75%	100%	0	100%

Conclusions

Based on these results, we concluded that UTIs more commonly caused by gram-negative bacteria species such us *Escherichia coli*. Encounters a high resistance pattern to 3rd generation cephalosporin antibiotics group, this is caused by the majority of irrational therapy for UTIs and urinary tract pathogens are demonstrating increasing antimicrobial resistance due to the ability to produce ESBL-producing strains. Antibiotics group chloramphenicol, gentamicin and vancomycin we recommend that for use as empirical therapy in urinary tract infection. Health practitioners to use antibiotic treatment should be prescribed only necessary to be effective. Continuously update the data on antimicrobial susceptibility patterns would be beneficial to guide empiric treatment.

Acknowledgements

References

Akinyemi K.O., Alabi S.A., Taiwo M.A., Omonigbehin E.A. 1997. Antimicrobial Susceptibility Pattern and Plasmid profiles of Pathogenic Bacteria isolated from Subjects with Urinary Tract Infections in Lagos, Nigeria. Niger. Qtr. J. Hosp. Med. 1: 7-11.

Anderson G.G., Palermo J.J., Schilling J.D., Roth R., Heuser R., Hultgren S.J. 2003. Intracellular bacterial biofilm-like pods in urinary tract infections, Science. pp. 105–7.

Bower J.M., Eto D.S., Mulvey M.A. 2005 Covert operations of uropathogenic *Escherichia coli* within the urinary tract. Traffic, pp.951–4.

Burbige K.A., Retic A.B., Colony A., Bauer S.B., Lebowitz R. 1984. UTI in boys. J. Urol. 132: 541-542

Ebie M., Olukemi K., Tanyigna A.J. 2001. UTI infections in a Nigerian Military Hospital. Niger. J. Microbiol. 15(1): 31-37.

Gupta, K.A., Scholes D., Stamm W.E. 1999. Increasing prevalence of antimicrobial resistance among uropathogens causing acute uncomplicated cystitis in women. JAMA 281:736–738.

Gupta K., Hooton T.M., Stamm W.E. 2001. Increasing Antimicrobial Resistance and the Management of Uncomplicated Community-Acquired Urinary Tract Infections. Ann Intern Med, 135:41-50.

Jones, R.N., Kugler K.C., Pfaller M.A., Winokur W.L. 1999. Characteristics of pathogens causing urinary tract infections in hospitals in North America: results from the SENTRY antimicrobial surveillance program, 1997. Diagn. Microbiol. Infect. Dis. 35:55–63.

Killgore K.M., March K.L., Guglielmo B.J. 2004. Risk factors for community-acquired ciprofloxacin-resistant Escherichia coli urinary tract infection. Ann Pharmacother, 38;(7-8):1148-52

Kolawole A.S., Kolawole O.M., Olukemi, Y.T., Babatunde S.K., Durowade K.A., Kolawole C.F. 2009. Prevalence of urinary tract infections (UTI) among patients attending Dalhatu Araf Specialist Hospital, Lafia, Nasarawa State, Nigeria. Int Journal of Medicine and Medical Sciences, pp.163-167.

Mbata T.I. 2007. Prevalence and Antibiogram of UTIs Among Prisons Inmates in Nigeria, J. Microbiol pp.3 (2).

Nys S., Terporten P.H., Korstanje H.J.A., Stobberingh E.E. 2008. Trends in antimicrobial susceptibility of Escherichia coli isolates from urology services in The Netherlands (1998–2005). Pp.62:126–132.

Page`s J.M., Amaral L. 2009. Mechanisms of drug efflux and strategies to combat them: challenging the efflux pump of Gram-negative bacteria. Biochim. Biophys. Acta 1794:826–833

Pezzlo YM. 2004. Urine cultures. In: Isenberg H, ed. Clinical microbiology procedures handbook. 2nd ed. Washington, DC: ASM Press.

Sahm D.F., Thornsberry C., Mayfield D.C., Jones M.E., Karlowsky J.A. 2001. Multidrug-Resistant Urinary Tract Isolates of Escherichia coli: Prevalence and Patient Demographics in the United States in 2000. p. 1402–1406

Tanagho E.A., McAninch J.W. 2008. Smith's general urology 17th edition. In: Nguyen H.T. (eds), bacterial infections of the genitourinary tract, pp 193-219, mc graw hill medical.

Yasufuku T., Shigemura K., Shirakawa T., Matsumoto M., Nakano Y., Tanaka K., et al. 2010. Correlation of Overexpression of Efflux Pump Genes with Antibiotic Resistance in Escherichia coli Strains Clinically Isolated from Urinary Tract Infection Patients, J. of clin microbiology. pp. 189–194.

Zhanel, G.G., Karlowsky J.A., Harding G.K.M., Mazzulli A.C.T, Low D.E. Hoban D.J. 2000. A Canadian national surveillance study of urinary tract isolates from outpatients: comparison of the activities of trimethoprim-sulfamethoxazole, ampicillin, mecillinam, nitrofurantoin, and ciprofloxacin. Antimicrob. Agents Chemother. 44:1089–1092.