Original Article

STUDY FOR MONITORING OF ANTIMICROBIAL RESISTANCE TRENDS (SMART): A Surveillance of Gram-negative Bacilli Causing Urinary Tract Infections in Inpatients

Mohd Nazil Salleh[1], Siti Nur Lina Azman[1], Henkie Isahwan Ahmad Mulyadi Lai[1], Seri Ambal[2]

[1]*Medical Laboratory Science Unit, Department of Health Sciences, Faculty of Engineering and Life Sciences, Shah Alam City Campus, University Selangor, 40000, Shah Alam, Selangor, Malaysia.*

[2]*Microbiology Section, KPJ Lablink (M) Sdn. Bhd., Level 3, Pharmacare Building, 14(129) Jalan Pahang Barat, 53000, Kuala Lumpur, Malaysia.*

Background; **Urinary tract infection (UTI) is an infection of one or more structures in the urinary system, most of which is caused by gram-negative bacteria. Study for Monitoring Antimicrobial Resistance Trends (SMART), an ongoing global surveillance program, monitors the susceptibilities of gram-negative bacilli from inpatient with urinary tract infections (UTIs).** ***Materials and Methods*****; Hospitalised patient (n=200) with clinical features of UTI were evaluated using R/E urine culture and sensitivity test. Isolated pathogen was differentiated using Micronaut-E Identification system whereas determination of *in-vitro* antimicrobial susceptibility profile was performed using Micronaut-SB analysis.** ***Results;*** **Our results showed that the gram-negative bacilli from UTI isolates was *Escherichia coli* (52%), *Klebsiella pneumoniae* (23%), *Proteus mirabilis* (14%), *Acinetobacter spp* (5%) and *Morganella morganii* (5%). Sensitivity testing demonstrated that amikacin was the most effective antibiotic (95% susceptibility) followed by the carbapenem (imipenem) (86%), and ceftazidime (81%). The remaining susceptibility profile was cefaclor (57%), ciprofloxacin (62%) and cefixime (67%). Our findings also showed that the gram-negative bacilli had higher rate of resistance to amoxicillin/clavulanate acid and vancomycin. *E. coli* and *K. pneumoniae* are the most common pathogens for the development of UTI.** ***Conclusions;*** **Our findings also address the importance of continuing surveillance of gram-negative bacilli infection in UTI and monitoring the full trend in antimicrobial activities, new resistance mechanism(s), in order to implement effective infection control and ensure the reducing of the antimicrobial resistance.**

Key words:

Introduction

Urinary tract infection (UTI) is one of the most common hospital-associated bacterial infections [1]. The term UTI encompasses a variety of clinical entities, ranging from asymptomatic infection to cystitis, prostatitis and pyelonephritis [2]. Abnormalities of the urinary tract as well as several factors reduce its natural resistance to infection. These factors include sex, age, underlying disease, hospitalization and obstruction [2]. Due to the increasing antimicrobial resistance, the treatment for UTI with complicated infections has become more challenging period *E. coli* and *K. pneumoniae* strains isolated from UTIs have been reported to increasingly produce extended-spectrum β-lactamse (ESBL) [3,4]. Studies show that 75% cases of UTI were caused by normal bowel flora, principally *E. coli* and the family of Enterobacteriaceae, including *K. pneumoniae* and *P. mirabilis* [5, 6]. Worldwide, ESBL-producing Enterobacteriaceae, particularly *E. coli*

Received: April 01, 2017 Revised: June 20, 2017 Accepted: September 29, 2017
*Corresponding Author: Assoc. Prof. Dr. Mohd Nazil Salleh, Department of Health Sciences, University Selangor, Shah Alam City Campus, 40000 Shah Alam, Selangor, Malaysia.
Tel: (+603) – 5522 3471, Fax: (+603) – 5522 4323, Email: drnazil@unisel.com

was as a common cause of infection in UTI. The spectrum of activity of the drug against the known pathogen must be taken into consideration before the decision regarding antimicrobial selection and duration of therapy. Antimicrobial resistance varies by institution, country, and continent. Several studies showed that resistance to commonly used antimicrobials such as aminoglycosides, third-generation cephalosporins, carbapenems, and β-lactam/ β-lactamase inhibitor combinations were increasing [7,8]. Monitoring the antibacterial resistance plays an important role for health care providers in treating UTI. In addition, the patterns of antimicrobial resistance in gram-negative bacilli are increasing alarmingly worldwide. Epidemiological studies have shown that Asia has the highest prevalence of the monitoring antimicrobial resistance trends (SMART) [9]. In this study, we observed the trends in antimicrobial susceptibility pattern of isolates from UTI subjects in Malaysia. Susceptibility results of selected species of Enterobacteriaceae were compared using different MIC interpretive criteria recommended by the Clinical and Laboratory Standards Institute (CLSI). Several reports have described the prevalence of ESBLs in the Asian countries; however the numbers of Enterobacteriaceae isolates screened and contributed were not equal and limited. On other hand, sufficient scientific data on the epidemiology of ESBLs was unavailable particularly from private hospital in Malaysia [10]. To the best of our knowledge, this is the first study on the antimicrobial susceptibilities of ESBL-producing Enterobacteriaceae from inpatients from private hospital in the city.

Material and Methods

Samples collection. Gram-negative bacilli from a total of 200 urine samples were obtained from both male and female hospital-associated patients with a clinical and microbiological diagnosis of UTIs. Each sample contained 20 ml of urine sample, which was collected aseptically from a midstream clean catch and put in a sterile mouthed glass bottles with screw cap tops. The samples were analyzed bacteriologically. *Bacterial Isolates.* All isolates deemed clinically significant ($>10^5$ CFU/mL) were considered as a causative agent. Only one isolate per species per patient has chosen to be analysed. Bacteria were initially identified by standard methods in the clinical microbiology laboratories including Gram staining. *Antimicrobial Susceptibility Testing.* Briefly, Antimicrobial resistance were determined by the standard broth microdilution method using the commercial Micronaut microtiter plates (Micronaut Merlin Diagnostika, Bornheim, Germany) containing a panel of dehydrated antimicrobial agent in two-fold dilutions in accordance with the Clinical and Laboratory Standard Institute interpretive criteria breakpoint (CLSI, 2012). The agents tested were amikacin, imipenem, ceftazidime, ciprofloxacin, cefixime, amoxicillin/clavulanate and vancomycin. *Micronaut-E system.* Biochemical Micronaut-E test results were evaluated and interpreted with the Micronaut Software by calculation algorithms that will lead to a positive or negative result of the respective reactions. Negative controls will assist a clear interpretation of the biochemical reactions according to the manufacturer's recommendation. *Quality and Performance Data***.** Quality control (QC) was performed each day using the CLSI-recommended QC strains: *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853 and *Enterococcus faecalis* ATCC 29212. Results were obtained from the data only when the corresponding quality control values were within the accepted range specified by the CLSI. *Statistical Analysis.* p-values were calculated with confidence intervals set to 95% Statistical significance was considered when p-values was less than 0.05. Data were analysed using PASW statistics for windows, version 18.0 (SPSS Inc. Chicago, IL, USA).

Results

In this study, 21 out of 200 (10.5%) of the hospital associated UTI patients were infected by Enterobacteriaceae. Among all isolated species, *E. coli* (52%) was the most common pathogen causing UTIs in this study, followed by *K. pneumoniae* (24%) and *P. mirabilis* (14%), whereas *Acinetobacter spp* and *M. morganii* represented 5% of the gram-negative bacilli respectively, as summarized in figure 1. The rates of susceptibility of the gram-negative pathogens causing UTIs to 8 antimicrobial agents, according to the CLSI, are presented in Table 1. *E. coli*, *K. pneumoniae*, and *P. mirabilis* showed the most susceptibility to amikacin, imipenem and ceftazidime. These isolates were less susceptible to ciprofloxacin, cefixime and cefepime (<70%). The less commonly isolated bacteria of *Acinetobacter spp* and *M. morganii* demonstrated resistance towards cefixime and vanomycin. The overall rates of susceptibility showed 95% of Enterobacteriaceae isolates were susceptible to amikacin, 86% were

susceptible to imipenem, and 81% were susceptible to ceftazidime. The least effective antimicrobial tested were amoxicillin-clavulanic acid and vancomycin (<30%) as shown in figure 2.

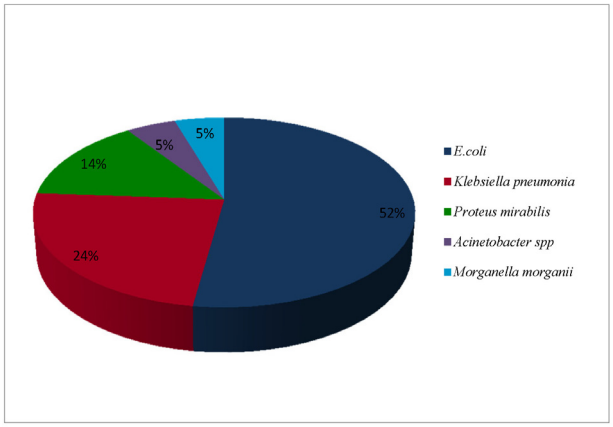

Fig.1 The percentage of gram-negative pathogens associated with UTI from inpatient urine specimen

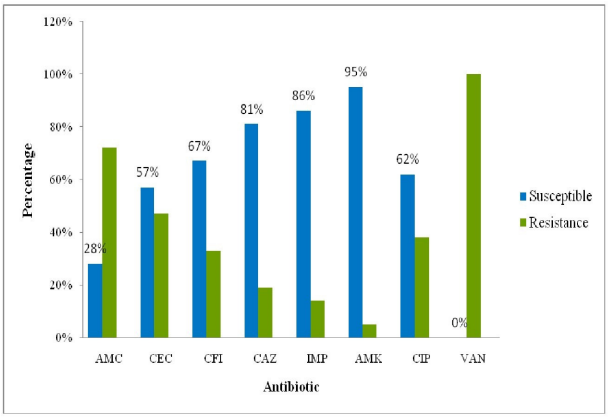

Fig.2 Overall rate of prevalence and antimicrobial resistance percentage of Gram-negative bacilli isolated from hospitalised patients in this study.
Abbreviations: AMC, amoxicillin-clavulanic acid; CEC, cefepime; CFI, cefixime; CAZ, ceftazidime; IMP, imipenem; AMK, amikacin; CIP, ciprofloxacin; VAN; vanomycin

Table 1 Prevalance and antimicrobial resistance of uropathogens isolated in this study. Abbreviations: AMC, amoxicilin-clavulanic acid; CEC, cefepime; CFI, cefixime; CAZ, ceftazidime; IMP, imipenem; AMK, amikacin; CIP, ciprofloxacin; VAN, vancomycin. R, resistance; S, susceptible; n/r, not reported.

Bacteria	No of isolates	% of isolates susceptible							
		AMC	CEC	CFI	CAZ	IMP	AMK	CIP	VAN
Escherichia coli	11	18	64	73	73	91	100	46	0
Klebsiella pneumonia	5	60	60	80	80	100	100	80	n/r
Proteus mirabilis	3	0	33	67	100	67	100	67	n/r
Acinetobacter spp	1	S	S	n/r	S	S	S	S	R
Morganella morganii	1	R	R	R	S	n/r	S	S	R

Discussion

The spectrum of bacteria isolated from hospitalised patients with UTIs is very broad and includes both gram-negative and gram-positive pathogen. Uropathogen identified in this study were similar to those of many other studies conducted in different countries [12]. A similar finding was reported in Asia-Pacific region [8,9], similarities and differences in the type and distribution of uropathogen have resulted from different environmental conditions and host factors. In this study, *E. coli* was the most common pathogen isolated followed by *K. pneumoniae,* consistent with other studies [14, 15]. In the Asia-Pacific region, antimicrobial resistance of ESBL-producing Enterobacteriaceae is a major problem. In this study, we found that, carbapenems and amikacin were very effective against UTI pathogens with susceptibility rate >80%, which was well supported in other populations [16]. Several studies have shown a great activity of carbapenems (imipenem) against gram-negative bacteria. Sensitivity to imipenem indicated that *E. coli* and other gram-negative bacteria isolates was metallo-β-lactamases negative [17,18]. Carbapenems are currently the preferred agents for treatment of serious infections caused by ESBL - producing Enterobacteriacease. Carbapenems are highly stable to β-lactamase hydrolysis, and porin penetration is facilitated by their general size and structure [19]. The susceptibility of most strains of Enterobacteriaceae makes them generally useful as treatment for multidrug-resistant organisms [20]. In view of the low

susceptibility rates to the third and fourth-generation cephalosporin, it would be helpful for physicians to be aware of the risk that in UTI cases involving ESBL producers of fluoroquinolone-resistant bacteria when prescribing empirical antimicrobial therapy clinically. In complicated UTIs, delayed effective therapy may jeopardise the patient's health and life, the drugs with high susceptibility rates such as carbapenems or amikacin will improve the patients' health. According to the 2012 CLSI M100-S22 criteria, ceftazidime (33.4% susceptible) was the most effective agent against Enterobacteriaceae isolates among the third and fourth-generation cephalosporins tested. However, in this study, it is found to be the third active agent against UTI after amikacin and imipenem. The limitation of this study was only identification of the known species, not including unidentified microorganisms. In summary, different diagnosis may require different diagnostic criteria and optimising the appropriateness of empirical antimicrobial therapy, continuing resistance surveillance and also identifying the cause of the infection and provide isolates for biochemical testing and anti-microbial susceptibility in different clinical settings.

Conclusion

The results from the present study demonstrated that gram-negative bacilli particularly *E. coli* and *K. pneumoniae* are the most common pathogens for the development of UTI. Regular surveillance of antimicrobial susceptibility and the proper treatment guidelines are recommended to help achieve optimal therapy for patients with UTI specifically. Our findings provide a better understanding of resistance to antimicrobial agents and also addressed the importance to continue surveillance and monitor the development of efficient and targeted intervention strategies.

Acknowledgment

The author would like to thank KPJ LabLink Hospital for the opportunity to use their analysis equipments.

Disclosure statement

The authors declare that they have no competing interests.

References

1. Wagenlehner FME, Cek M, Naber KG, Kiyota H, Bjerklund-Johansen TE. Treatment and prevention of healthcare-associated urinary tract infections. *World J Urol* 2012; **30**: 59-67.
2. Stamm, WE, Norrby, SR. Urinary tract infections: disease panorama and challenges. *J Infect Dis* 2001; **183**: 1-4.
3. Al Yousef SA,Younis S, Farrag E, Moussa H, Bayoumi FS, Ali AM. Clinical and laboratory profile of urinary tract infections associated with extended spectrum beta-lactamase producing Escherichia coli and klebsiella pneumonia. *Ann Clin Lab Sci* , 2016; **46** : 393-400.
4. Caccamo M, Perilli M, Celenza G, Bonfiglio G, Tempera G, Amicosante G. Occurrence of extended spectrum beta-lactamses among isolates of Enterobacteriaceae from urinary tract infections in southern Italy. *Microb Drug Resist.* 2006; **12**: 257-264.
5. Alhambra A, Cuadros JA, Cacho J, Gomez-Garces JL, Alos JI. In vitro susceptibility of recent antibiotics-resistant urinary pathogens to ertapenem and 12 other antibiotics. *J Antimicrob Chemother* 2004; **53**: 1090-1094.
6. Mathai D, Jones RN, Pfaller MA. The SENTRY Participant Group North America. Epidemiology and frequency of resistance among pathogens causing urinary tract infection in 1,510 hospitalized patients: a report from the SENTRY Antimicrobial Surveillance Program (North America). *Diagn Microbial Infect Dis* 2001; **40**: 129-136.
7. Meier S, Weber R, Zbinden R, Ruef C, Hasse B. Extended-spectrum β-lactamase-producing Gram-negative pathogens in community-acquired urinary tract infection: an increasing challenge for antimicrobial therapy. *Infection* 2006; **39**: 333-340.
8. Wallace PKM, Endimiani A, Taracila A.M, Bonomo AR. Carbapenems: Past, Present and Future. *J Antimicrob Chemother* 2011; **55 (**11): 4943-4960.
9. JH, Tang HJ, Yu KW, Chen YS, Chuang YC, Xu Y, Ni Y, Chen YH, Hsueh PR. Epidemiology and antimicrobial susceptibility profiles of Gram-negative bacteria causing urinary tract infections in the Asia-Pacific region: 2009-2010 result from the Study for Monitoring Antimicrobial Resistance Trends (SMART). Ho. *Int J Antimicrob Agents* 2012; **40**: 37-43.
10. Ministry of Health Malaysia, Protocol on Antimicrobial Stewardship Program in Healthcare Facilities. 1st Ed. Kuala Lumpur: 2014.
11. Meier S, Weber R, Zbinden R, Ruef C, Hasse B. Extended-spectrum β-lactamase-producing Gram-negative pathogens in community-acquired urinary tract infection: an increasing challenge for antimicrobial therapy. *Infection* 2006; **39**: 333-340.
12. Wayne PA. Clinical and Laboratory Standard Institute (CLSI). Performance standard for antimicrobial susceptibility testing: *21st informational supplement*

2012: 100-122.
13. Astal ZY, Shariff F. Relationship of demographic characteristics and community acquired urinary tract infection. *East Mediterr Health J* 2002; **8**: 164-171.
14. Hsueh PR, Hoban DJ, Carmeli Y, Chen SY, Desikan S, Alejandria M, Ko WC, Binh TQ. Consensus review of the epidemiology and appropriate antimicrobial therapy of complicated urinary tract infections in Asia-Pacific region. *J Infect* 2011; **63**: 114-123.
15. Teppa RJ, Roberts JM. The uriscreen test to detect significant asymptomatic bacteriuria during pregnancy. *J Soc Gynecol Investig* 2005; **12**: 50-53.
16. Fatima N, Inshrat S. Frequency and risk factors of asymptomatic bacteriuria during pregnancy. *J Coll Physicians Surg* 2006; **16**: 273-275.
17. Yogeesha BKV, Kumari A, Kumar A, Raghu KKR.Role of Imipenem-resistant metallo-betalactamase positive pseudomonas aeruginosa carries in nosocomial infections. *J Nat. Sci. Biol. Med*. 2013; 4(1**)**: 181-186.
18. Kothari, SV. Antibiotic resistance in pathogens causing community-acquired urinary tract infections in India: a multicenter study. *J Infect Dev Ctries* 2008; **2**: 354-358.
19. Poirel L, Pitout JD, Nordmann P. Carbapenemases: Molecular diversity and clinical consequences. *Future Microbiol*. 2007; **2** (5): 501-512.
20. Meletis G. Carbapenem resistance: overview of the problem and future perspectives. *Ther Adv Infect Dis* 2016; **3**(1): 15-21.