STUDY OF EXTENDED-SPECTRUM BETA-LACTAMASES PRODUCING ENTEROBACTERIACEAE IN HOSPITAL-ACQUIRED URINARY TRACT INFECTION

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ABSTRACT

Urinary tract infection still remains one of the most common infections among hospital acquired infection. Due to increase prevalence of disease burden and rising antibiotic resistance among uropathogenic bacteria, it is essential to have local hospital-based information about organisms causing urinary tract infection and their antibiotic sensitivity pattern to choose correct and effective treatment regime. The study was conducted in Department of Urosurgical and Renal Medical Unit, Yangon Specialist Hospital and Department of Microbiology, University of Medicine 1, from September, 2017 to August, 2018. A total of 130 midstream urine samples were collected from patient with hospital-acquired UTI after getting informed consent. Then identification of causal bacteria and antibiotic susceptibility pattern of the isolated Enterobacteriaceae were detected by VITEK2 compact system and finally Extended-spectrum beta-lactamase production among isolated species was carried out by Double Disc Synergy Test. Of the 130 urine sample with significant bacteriuria (>10^5cfu/ml) was detected in 52 (40%). In culture positive samples, Enterobacteriaceae were 36 (28.5%), the majority of cases were aged more than 60 years 13 (36.1%), and male was more common 20 (55.6%). The most common underlying diseases were renal stone 37 (28.46%), ureteric stone 18 (13.84%), Pyonephrosis 16 (12.31 %). The common isolates were *Escherichia coli* 18 (34.62 %), *Klebsiella pneumoniae* 4 (7.69%), *Proteus mirabilis* 4 (7.69%), *Providentia rettgeri* 3 (5.77%). Among 36 isolated Enterobacteriaceae, four (11.1%) were ESBL producing type. Among them, three (75%) were *Escherichia coli* and one (25%) was *Klebsiella pneumoniae*. The isolated Enterobacteriaceae were sensitive to amikacin, meropenum, imipenem, ertapenem, nitrofurantoin, piperacillin/ tazobactam while they were highly resistance to cefazolin, ciprofloxacin, cefepime, ceftriaxone, gentamicin, aztreonam. In this study *Escherichia coli* was the most common uropathogen among patient with hospital-acquired UTI. Amikacin, meropenum, imipenem could therefore be recommended for treatment of drug resistance organism. To prevent the emergence of drug resistant pathogens, rational use of antibiotic according to culture and sensitivity results is encouraged.

Keywords: Extended-Spectrum Beta-lactamases, Enterobacteriaceae, hospital acquired urinary tract infection

INTRODUCTION

Hospital acquired or nosocomial infections are the infections that are acquired in hospitals and other healthcare associated facilities. These infections occurred from 48 to 72 hours after hospitalization or at the maximum 6 weeks after discharge from hospitals [1]. Urinary tract infection is the most common hospital-acquired

infections. In hospital-acquired urinary tract infections, the most likely infected organisms are *Escherichia coli, Klebsiella* species, *Proteus* species, *Staphylococci*, other *Enterobacteriaceae, Pseudomonas aeruginosa, enterococci, and Candida* species. Among them, Enterobacteriaceae are the most common pathogens causing UTI and result in the development of antibiotic resistance, which recently became a major problem worldwide [2]. This issue is challenging in low-income developing countries because of high prevalence in infections, irrational uses of antibiotics, over-the counter availability of antibiotics and poor infection prevention practices [3].

Extended-spectrum beta-lactamase are spreading among Enterobacteriaceae, interfering the routine treatment caused by these organisms[4]. High prevalence of Extended-spectrum beta-lactamase producing Enterobacteriaceae has been reported in Southeast and Southeast Asia countries. In Myanmar, Sapai-Myint [5] conducted a study at Yangon General Hospital showed that the prevalence of ESBL production among the gram-negative bacilli was 6.8 %.

With the spread of ESBL producing strains the adverse effects such as healthcare costs, longer hospital stays, and higher mortality became problematic in addition to difficulty in choosing appropriate antibiotic for treatment of urinary tract infections. For appropriate therapy, it is essential to get current knowledge on antibiotic resistance status of these organisms in local area. By doing this research, we can get the drug resistance information about current prescribing drugs which is essential for changing treatment trends. Guidelines for antibiotic therapy can be helpful for clinicians to select more appropriate antibiotics for effective treatment. The aims of this study were to screen and isolate the Enterobacteriaceae causing hospital-acquired urinary tract infection by using VITEK2 automatic system and to determine the antibiotic susceptibility patterns of the Enterobacteriaceae and confirmation of ESBL production by phenotypic methods.

MATERIALS AND METHODS

The study was a cross-sectional descriptive study and carried out in Urosurgical and Renal Medical Unit, Yangon Specialist Hospital and Microbiology Department, University of Medicine 1, Yangon. A total of 130 midstream urine samples from patient with hospital-acquired UTI were collected after getting consent from patients. After that the urine samples were delivered to the laboratory within 2 hours after collection. In laboratory, all samples were inoculated onto CLED agar (Oxoid) and blood agar (Oxoid) plates and incubated at 37ºC for 24 hour. Urine specimens with bacterial growth of $>10^5$cfu/ml were regarded as significant bacteriuria [6].

All Gram-negative bacteria were identified and antibiotic susceptibility testing was performed by automated VITEK 2 compact system (boiMerieux, France). The results were interpreted according to the guide lines of the Clinical and Laboratory Standards Institute, microscopic examination, isolation on culture, identification and antibiotic sensitivity tests by VITEK 2 was done. Then detection of ESBL producing strain by Double disc diffusion phenotypic method was performed.

Detection of ESBL

Isolates that indicate the zone diameter of ceftazidime (≤22mm), cefotaxime (≤27mm) and ceftriaxone (≤ 25mm) will be presumably ESBL producer, and testing of ESBL production by phenotypic screening and confirmatory methods was carried.

Phenotypic confirmatory test

Cephalosporin/clavulanate combination disc methods

Mueller Hinton agar plate was seeded with standardized inoculum of the test organism (corresponding to 0.5 McFarland tube). Discs containing ceftazidime, ceftazidime plus clavulanic acid and cefotaxime, cefotaxime plus clavulanic acid was placed on Mueller Hinton agar, center to center at least 25 mm apart. After 16-18 hours incubation at 37°C, a difference of ≥ 5 mm between the zone diameter of either of the cephalosporin discs and their respective cephalo-sporin/clavulanic disc was taken to be phenotypic confirmation of ESBL production [6].

Data Analysis

Data collected were checked for completeness, errors and inconsistencies before data entry and analysis was done by Statistical Package for Social Science (SPSS) version 18.0.

RESULTS

UTI is one of the common causes of hospital acquired infection with high morbidity. In this study from September, 2017 to August, 2018, a total of 130 patients with hospital-acquired urinary tract infection, 52 (40%) was culture positive while 78(60%) yielded no significant growth. Concerning underlying diseases, renal stone 37 (28.46%) was the major cause of hospital acquired urinary infection followed by ureteric stone 18 (13.84%) and pyonephrosis 16 (12.31%).

Among 130 study population, 52 samples were culture positive and 51(98%) were gram negative bacteria. Among these gram negative bacteria, Enterobacteriaceae was detected in 36 cases (28.5%). Out of 36 Enterobacteriaceae culture positive samples with significant bacteriuria, 20 (55.6%) were male and 16 (44.4%) were female. It was observed that infection was more common in patient of above 60 years old 13(36.1%).

In isolated Enterobacteriaceae, the commonest organism was *Escherichia coli* 18(50%), followed by *Klebsiella pneumoniae* 4 (11.1%), *Proteus mirabilis* 4 (11.1%) and *Providentia rettgeri* 3 (8.3%) respectively. Among 36 Enterobacteriaceae positive samples, 4 (11.1%) were ESBL producing bacteria while 32 (88.9%) were non ESBL producer. In these ESBL producing samples, *Escherichia coli* 3 (75%), followed by *Klebsiella pneumoniae* 1 (25%).

Concerning antimicrobial susceptib-ility patterns of Enterobacteriaceae, sensitivity rates with decreasing orders were amikacin 28 (77.8%), meropenem 22 (61.1%), imipenem 21 (58.38%), ertapenem 22 (61.1%), nitrofurantoin 18 (27.8%). Highest resistance rate with decreasing order were ampicillin 34 (94.4%), cefazolin 33 (91.7%), Levofloxacin 30 (83.3%), ceftriaxone 30 (83.3%), ciprofloxacin 29 (80.6%), cefepime 29 (80.6%), aztreonam 28 (77.8%), gentamicin 24 (66.7%) respectively (Figure 1).

ESBL producing bacteria were 100% sensitive to amikacin, imipenem,

ertapenem, nitrofurantoin, 75% sensitive to meropenem, 50% sensitive to trimethoprim/sulfamethosazole and piperazolin/tazobactum. They were 100% resistant to ampicillin, cefazolin, ceftriaxone, cefepime, aztreonam, ciprofloxacin, levofloxacin (Figure 2).

DISCUSSION

Even though the widespread availability of antimicrobial agents, UTI remains the most common bacterial infections in human population in many countries [7]. Effective management of patients suffering from UTI commonly depends on the identification of causal organisms and selection of effective antibiotic to the organisms.

The prevalence of urinary tract infection in this study was 40%. This is closely related to the previous studies done by Khin Khin Wai[8] and Mya Mya Aye [9] who recorded that the prevalence rate was 43.3% and 48% respectively. A study conducted in northwestern Libya from 2012 to 2013, the occurrence was only 20.7%. The difference in occurrence of urinary tract infection might be due to differences in sample size, underlying medical conditions and in the criteria of selecting urine samples.

In this study, urinary tract infection was more common in male (55.6%) while in female (44.4%). Similar findings were reported by Khin Khin Wai [8], study on UTI cases in Yangon General Hospital, and Kaung Htet [10], carried out research in No.2 Military Hospital, Yangon. They found out that male were more common in developing urinary tract infection. In this study, the fact that male was more common than female may be due to study population including more male patients and unequal distribution of study population.

Concerning age, (36.1%) were above 60 years, which was similar to the study of Khin Khin Wai [8], in which 32.3% was also above 60 years. This may be due to the use of urinary catherization, duration of hospital stay and higher prevalence of prostate enlargement and retention of urine.

Among 130 study population, 52 samples were culture positive and 51(98%) were gram negative bacteria. In these gram negative bacteria, Enterobacteriaceae was detected in 36 cases (28.5%). Some study described that Enterobacteriaceae prevalence were more than this study in Khawcharoenporn [12] and Abujnah [13] in which Enterobacteriaceae were 88% and 91% respectively. This finding is similar to Khin Khin Wai[8] and Kaung Htet[10] where the Enterobacteriaceae were 26.2% and 33.3%. The findings are different due to different study site, study population and detection methods. Source of infection comes from patient's own bowel flora or from hospital environment. The hospital environment plays an important role in dissemination of organisms involved in urinary tract infection.

Among the Enterobacteriaceae isolates, Gram negative organisms constituted 98% while Gram positive organisms accounted for only 2%. The commonest organism was *Escherichia coli* (50%), followed by *Klebsiella pneumoniae* (11.1%), *Proteus mirabilis* (11.1%) and *Providentia rettgeri* (8.3%) respectively. This finding was in agreement with study of Otajevwo[14].

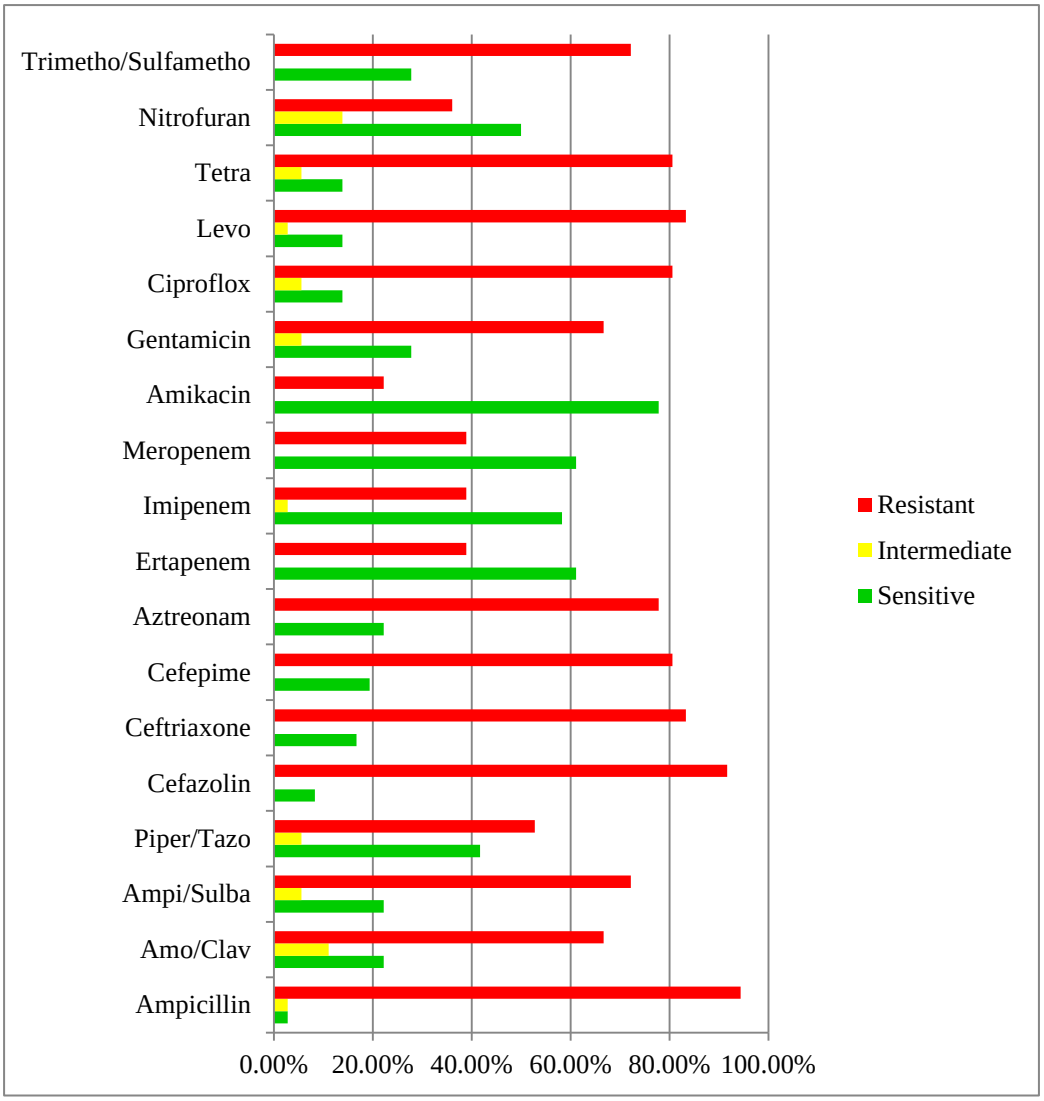

Figure (1) Antimicrobial sensitivity pattern of Enterobacteriaceae isolated from urinary tract infection

Since older day, uropathogenic *Escherichia coli* was the most common pathogen in the study of Barnett [15]. This may be due to the fact that causal organism of urinary tract infection comes from the lower gastrointestinal tract, predisposed by poor hygiene and instrumentation.

Gram negative organisms responsible for urinary tract infection have the ability to produce ESBL enzyme in large amount, so the prevalence of ESBL producing bacteria has increased worldwide over last decade. In this study, among 36 Enterobacteriaceae positive samples, 4 (11.1%) were ESBL producing bacteria while 32 (88.9%) were non ESBL producer. In these ESBL producing samples *Escherichia coli* 3 (75%), followed by *Klebsiella pneumoniae* 1 (25%). The ESBL detection rate of this study was less than in other study of Khin Thawtar Shein [16], in which the prevalence of ESBL producing Enterobacteriaceae was 51.7% in Mandalay General Hospital, the prevalence of commonest ESBL producing was *Escherichia coli*, 77.4% *Klebsiella pneumoniae* was 3.2%. Similarly, Yousef [17] stated that Highest resistance rate producing Entero-

bacteriaceae were 43.5%, and among these *Escherichia coli*, 73.6 % *Klebsiella pneumonia*, 26.4%. The results showed there was significant variation among countries, hospitals and wards due to difference in the antibiotic guideline, underlying exposure to antibiotic were found.

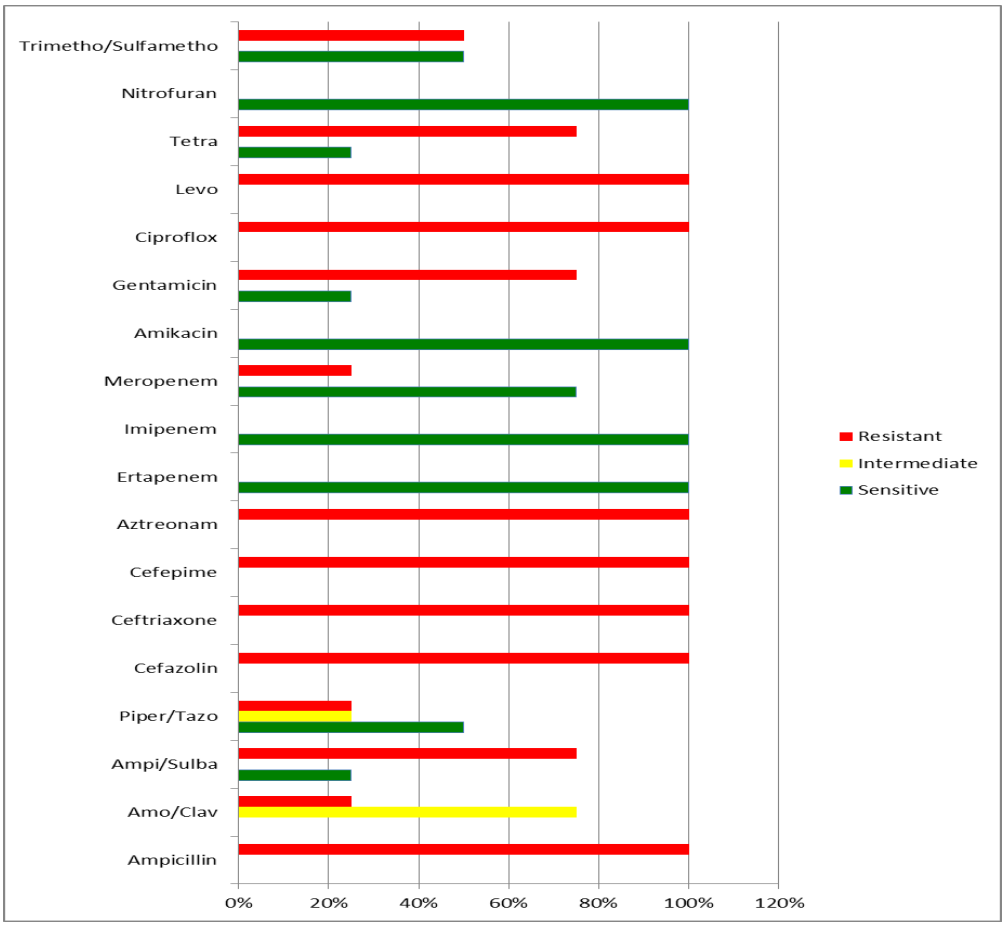

Figure 2. Antimicrobial sensitivity pattern of ESBL producing Gram negative bacteria from urinary tract infection.

Although the type of pathological bacteria isolated from urine of patients remained unchanged over the past few decades, there have been significant changes in their antibiotic sensitivity patterns in many countries. In this study, figure (1) showed antimicrobial susceptibility patterns of Enterobacteriaceae. among ampicillin (94.4%), cefazolin (91.7%), levofloxacin (83.3%), ceftriaxone (83.3%), cipro-floxacin (80.6%), cefepime (80.6%), gentamicin (66.7%) respectively. Figure 2 showed the antimicrobial sensitivity pattern of ESBL producing Entero-bacteriaceae and *Escherichia coli* showed that they were 100% resistant to ampicillin, cefazolin, ceftriaxone, cefepime, aztreonam, ciprofloxacillin, levofloxacin. In Urosurgical Unit, Yangon General Hospital, most of the uncomplicated urinary tract infections were treated with levofloxacin, cefuroxime and for symptomatic patients, levofloxacin, ceftriaxone/sulbactam, amikacin, gentamycin were mostly used. Most of the cases in this study were resistant to commonly used drugs such as levofloxacin, ceftriaxone, ciprofloxacin and gentamicin. This might be due to the abuse of these drugs, leading to resistance

of these organisms. One of the reasons of abuse is availability of drugs over the counter without prescription. Furthermore, the use of fake and substandard drugs may also be the contributory factors of drug resistance. So, it is essential to take medication until the full-prescribed dose is finished. Stopping the medication too early may allow bacteria to grow, which may result in resistance to the antibiotics.

In the present study, most of the pathogens of Enterobacteriaceae, were sensitivity rates of amikacin 28 (77.8%), meropenem 22 (61.1%), imipenem 21 (58.38%), ertapenem 22 (61.1%), nitrofurantoin 18 (50%). Similarly, the antimicrobial sensitivity pattern of ESBL producing Enterobacteriaceae and *Escherichia coli* showed 100% sensitivity to amikacin, imipenem, ertapenem, and nitrofurantoin. The main reason of amikacin sensitive may be due to the fact that these drugs have not been over-used. Nitrofurantoin showing moderate sensitivity may be due to its unique structure, mechanism of action and being more effective in urinary tract than other tissue.

CONCLUSION

This study demonstrated that the majority of hospital acquired urinary tract infection in Urosurgery and Renal Medicine unit, Yangon Speciality Hospital (2017-2018) was due to Enterobactericeae. The most common pathogen of ESBL producers were *Escherichia coli* and *Klebsiella pneumonia*e and they were multidrug resistant. These organisms were sensitive to amikacin, imipenem, ertapenem, and nitrofurantoin and were resistance to commonly used antibiotics, levofloxacin, ceftriaxone, ciprofloxacin and gentamicin. As the drug resistance among these microorganisms becomes an evolving process, routine surveillance and monitoring studies should be conducted to help physician to start the most reasonable empirical treatment, to develop evidence based guidelines for antibiotics use.

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ETHICAL APPROVAL

This study was Ethical approved by Research and Ethics Committee, University of Medicine 1.

CONFLICT OF INTEREST

There are no conflicts of interest.

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