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Full Length Research Paper

Extended spectrum beta lactamases among multi drug resistant *Escherichia coli* and *Klebsiella* species causing urinary tract infections in Khartoum

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This is a descriptive laboratory based case study carried out in Khartoum state hospitals during the period of June, 2007 to April, 2008. The study aimed to evaluate emergence of ESBL among multi drug resistant *Escherichia coli and Klebsiella* species causing nosocomial UTI. Hundred strains of multi drug resistant (MDR) *E. coli and Klebsiella* species causing nosocomial urinary tract infections (UTIs) from two main hospitals from Khartoum (Omdurman teaching hospital and Fedail Hospital) were included in this study. Susceptibility testing was performed against antibiotics commonly used in treatment of urinary tract infections. *E. coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* (49, 38 and 13% respectively) were among the studied isolates. β-Lactamase was produced by all isolates; high resistance level for 3rd generation cephalosporin was noticed. ESBLs were detected in high prevalence among all multi drug resistant *E. coli* and *Klebsiella* species isolates 53%. All isolates were found sensitive to Imipenem and Meropenem. In this study it's recommended that developing guidelines for the early phenotypic detection of ESBL in microbiology laboratories and seeking knowledge of antibiotic susceptibility pattern for empirical antibiotic therapy. Further studies about ESBL occurrence among UTIs are also recommended.

Key words: ESBL in Sudan, multi-drug resistant, MDR *Escherichia coli*, MDR *Klebsiella* spp, urinary tract infection, beta lactamase.

INTRODUCTION

Urinary tract infection (UTI) is a very common infection both in the community and hospital patients and ranks high amongst the most common reasons that compel a patient to seek medical attention (Gastmeier et al., 1998, Magee et al., 1999, Mobley, 2000).

Uropathogens have shown a slow but steady increase of resistance to several agents over the last decade. *Escherichia coli* and other *Enterobacteriaceae* have become less susceptible to commonly used antimicrobials such as trimethoprim/sulfamethoxazole and, in some areas, fluoroquinolones (Winstanley et al., 1997, 1997, Bishara et al., 1997). Study done by (Hassan et al., 2007) in Sudan showed multidrug resistance among uropathogens.

It is well known that the mechanism of antimicrobial resistance could happen by enzymatic inactivation, altered receptors or by altered antibiotic transport (Koneman et al., 1997). Current knowledge on antimicrobial susceptibility pattern of uropathogens is mandatory for appropriate therapy. Extended Spectrum Beta Lactamases (ESBL) hydrolyses expanded spectrum cephalosporins, which are used in the treatment of UTI. They arise by mutations in genes for common plasmid-mediated beta lactamases that alter the configuration of the enzyme near its active site to increase the affinity and hydrolytic ability of the beta lactamases for oxyimino

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compounds while simultaneously weakening the overall enzyme efficiency. Some ESBLs confer high-level resistance to all oxyimino beta lactams, but for other ESBLs resistance is only slightly increased or increased selectively for particular beta lactams. This creates a problem for the clinical laboratory, since organisms producing less active ESBLs can fail to reach current National Committee for Clinical Laboratory Standard's (NCCLS) break points for resistance but can cause significant disease (Katsanis et al., 1994).

The aim of this study is to evaluate emergence of ESBL among multi drug resistant *E. coli and Klebsiella* species causing nosocomial UTI, since the failure of treatment of UTI both complicated and uncomplicated, which are most commonly caused by *Enterobacteriacae*, will increase the rate of morbidity and mortality among UTI patients. Also study sought alternative drugs which can be used to these MDR strains.

MATERIALS AND METHODS

A laboratory based descriptive case study was carried out in order to evaluate emergence of ESBL among multi drug resistant *E. coli and Klebsiella* species causing nosocomial UTI. One hundred bacterial strains were collected from two main (Omdurman Teaching (64) and Fedail (36)) hospital laboratories in Khartoum state during June 2007 to April 2008 as pure isolates in nutrient agar slopes. The study was conducted in the laboratories of Medical Microbiology Laboratory, Faculty of Health Sciences, Omdurman Ahlia University. Personnel data were collected from file records.

Strains of *E. coli and Klebsiella* species with a significant growth ($>10^5$ CFU) that showed resistance to amoxicillin and at least one cephalosporin and collected from urine of patients who fulfill the definition of nosocomial UTI regardless of age were included in this study. No hospital infection outbreaks were reports during study period.

Non-probability sampling technique was used and selected samples were taken as isolated multidrug resistant *E. coli and Klebsiella* species.

Antimicrobial susceptibility testing was performed, to confirm the multidrug resistance, by using Kirby-Bauer susceptibility testing technique with commercially available disks (Oxoid-UK).

Amoxycillin (30 µg) nalidixic acid (30 µg), nitrofurantoin (50 µg), ciprofloxacin (1 µg), gentamicin (10 µg), amikacin (10 µg), cefuroxime (30 µg) and trimethoprim / sulphamethoxazole (1.2 / 23.8 µg) were disks used (National Committee for Clinical Laboratory Standards, 2001).

Beta lactamase test was performed to confirm the production of beta lactamase enzymes by using Nitrocefin disk which is recommended by National committee for Clinical Laboratory Standards (NCCLS) and the World Health Organization (WHO) (O'Callaghan et al., 1972).

Etest was used to determine the MIC of ceftazidime and cefotaxime (Cormican et al., 1996).

ESBL production was detected by double disk diffusion method. Discs containing ceftazidime (30 µg), cefotaxime (30 µg) respectively, were placed 25 mm (centre to centre of the discs) from the co-amoxiclav disc. Inoculated plates were incubated aerobically at 37°C for 18 - 24 h. After overnight incubation at 37°C, a clear extension of the edges of the inhibition zone of any of the antibiotics towards the disc containing clavulanic acid was regarded as a phenotypic confirmation of the presence of ESBL (Therrien et al., 2000).

Antimicrobial susceptibility test for alternative drugs were performed by Kirby-Bauer susceptibility testing technique. These include Imipenem (30 µg), Meropenem (50 µg), Aztreonam (30 µg), Pipercillin / tazobactum (1 µg). The data was analyzed by using Statistical Package for Social Studies (SPSS)

Ethical consideration

This study didn't involve human subject. No personnel identifying data were used so, informed consent was not sought.

RESULTS

One hundred bacterial strains identified as *E. coli* (49%), *K. pneumoniae* (38%) or *Klebsiella oxytoca* (13%) and resistant to amoxicillin and at least one cephalosporin were studied. Strains were identified from urine sample collected from patients with defined nosocomial UTI in ages between four days to ninety-one years. Pre susceptibility patterns of 100 selected isolates were done (Table 1).

Isolates that were found to be resistant to amoxicillin were suspected of harbouring one or more β-lactamases. Nitrocefin tests were performed and they all gave positive results.

All of the isolates were resistant to amoxicillin, naldixic acid, gentamycin, trimethoprim/sulphamethoxazole. Nitrofurantoin, ciprofloxacin and cefuroxime resistance was seen among most of isolates. Amikacin showed a better susceptibility pattern, especially in *Klebsiella* species (Table 1).

MIC for cefotaxime and ceftazidime by using Etest was obtained in this study. Cefotaxime was susceptible 13.6% and ceftazidime 7.8% of all multidrug resistant *E. coli and Klebsiella* species.

Fifty three isolates (53%) indicated the production of ESBL with double disc diffusion, using cefotaxime and ceftazidime disks (Photo 1).

All isolates were 100% susceptible for imipenem and meropenem. Pipracillin/tazobactum showed a good activity (74.05%) against tested isolates, while azterionam showed a poor activity as there were only 19.23% susceptible isolates. The study revealed that there were a high resistance pattern for cefoxitin and cefipime, 100 and 97.09% respectively.

DISCUSSION

Antimicrobial resistance is now recognized as an increasingly global problem, especially Gram-negative bacteria (Slama, 2008).

Increasing resistance to broad spectrum cephalosporins amongst *E. coli* and *Klebsiella* species predominantly due to the production of ESBLs were reported from different countries (Bouchillon et al., 2002, Khanfar et al.,

Table 1. Antimicrobial resistance profiles of isolates by disk diffusion method from urine samples (n = 100).

Isolate	*E. coli* (%)	*K. pneumoniae* (%)	*K. oxytoca* (%)
AMX	100	100	100
NA	100	100	100
F	100	97.37	100
CIP	97.96	100	92.31
CN	100	100	100
AK	69.39	39.47	30.77
CXM	95.92	100	100
SXT	100	100	100

Key: AMX, Amoxicillin; NA, Naldixic Acid; F, Nitrofurantoin; CIP, Ciprofloxacin; CN, Gentamicin; AK, Amikacin, CXM, Cefuroxime; SXT, Trimethoprim / sulphamethoxazole.

2008).

Many other reports from different countries and regions showed different prevalence rates of ESBLs producing *Entrobacteriaceae* causing urinary tract infections. *K. pneumoniae* and *E. coli* are the most common ESBL-positive species, but all *Enterobacteriaceae* can harbor plasmid-mediated ESBL genes (Bouchillon et al., 2002, Lautenbach et al., 2001). In this study the ESBLs producing MDR uropathogen *E. coli and Klebsiella* species were 53%. This finding is a little bit higher than those obtained from the studies done by (Bouchillon et al., 2002) from Egypt and (Kadar et al., 2005) in Saudi Arabia, where ESBL were produced by 40.9 and 40.3% respectively. Another study carried out in India (Mohammed et al., 2005) showed that ESBL was positive in 42% and Supriya et al. (2003) detected ESBL production in 48.3%, as determined by the double disc synergy test, which was much higher than that obtained by Ibukun et al. (2001) from Nigeria in which ESBL production was only 20.8%. The result of this study is much less than Mohanty et al. in India 2003, where they observed ESBL production in 71.5% of the Gram-negative bacilli.

Resistance to additional classes of antibiotics rather than beta lactams has been noted among ESBLs producing *E. coli* and *Klebsiella* species. With resistance to each additional class of antibiotics, infections related with ESBL producing bacteria become a greater therapeutic challenge (Emily et al., 2005).

This study showed that all isolates were sensitive to the carbapenems which are the most common alternative drugs used for treatment of ESBL producing bacteria. Similar results were observed in the study done by Kadar et al. (2005) who revealed that, more than 89% of the ESBL producers were susceptible to imipenem and meropenem. However, use of alternative drug which is very broad spectrum and expensive drug as first line for treatment of ESBL-positive bacteria will significantly increases cost of treatment and will contribute to carbapenem resistance in other organism (Nordman et al., 2002; Wright et al., 2008).

High prevalence rate of ESBL-producing bacteria among MDR *E. coli* and *Klebsiella* species were shown in this study. This rate is alarming and need special consideration. This study investigated only the frequency of ESBL producing among MDR *E. coli and Klebsiella* species causing nosocomial UTI and this is limitation of the study. The prevalence rate of ESBL producing non-MDR *E. coli and Klebsiella* species in the country were not studied and it was out of this study aim. Further studies about this issue are needed.

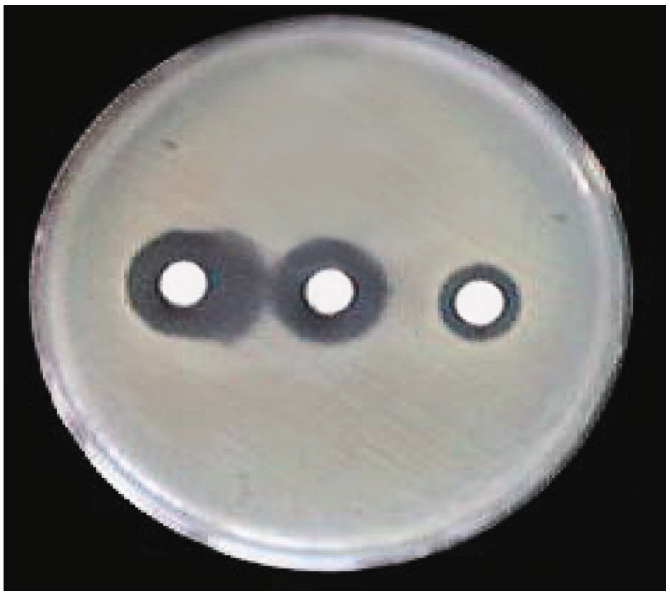

Photo 1. *Klebsiella pneumoniae* showing ESBL production by double disk diffusion method. Ceftazidime (left disc) a clear extension of the edges of the inhibition zone of the antibiotic towards the disc containing clavulanic acid (middle disc) while cefotaxime (right disc) showed negative test result.

ESBL producers are associated with increased morbidity and mortality, especially amongst patients on intensive care and high-dependency units (John et al., 2002). Updated knowledge of the common antibiotic-sensitivity patterns must be sought when starting empirical antibiotic therapy in Sudanese patients with urinary tract infection. Guidelines for the early phenotypic detection of ESBL in microbiology laboratories are needed. Further studies about ESBL occurrence among UTIs are also recommended.

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