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**IDENTIFICATION AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF ESBL
PRODUCING ENTEROBACTERIACEAE AMONG PATIENTS WITH UTI IN
SALMAN BIN ABDUL AZIZ UNIVERSITY HOSPITAL, ALKHARJ, SAUDI ARABIA**

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ABSTRACT

Urinary tract infections (UTI) are not only common nosocomial infections but an important source of morbidity in the community in both hospitalized and out patients Escherichia coli is one of the major cause of UTI, accounting for 70-90% of UTI cases. Their susceptibility pattern against various antibiotics varies area specific studies are required in monitoring the trend and gaining useful knowledge about the type of pathogen causing UTI and their sensitivity pattern. This not only helps in the provision of effective treatment but also guides for establishment of the institutional antibiotic policy.

This cross sectional study was conducted on the urine samples suspected for UTI attending the OPD in a teaching hospital of Kharj Saudi Arabia over a period of six months. All the samples were processed on blood and CLED agar and were incubated at 37°C for 24 hours. Antibiotic susceptibility was tested by Vitek method.

Out of 345 samples received during the study period, bacteria were isolated in 170 samples and enterobacteriaceae were isolated in 137 samples. E. coli was the commonest organism isolated in 44.7% of cases followed by 30% Klebsiella, 4.7% Proteus and 1.31% Citrobacter. Production of ESBL was noted in 37.2% of all the cases with the lion's share of E. coli (38.1%) followed by Klebsiella (35.2%).

It was the first study of its kind conducted in the teaching hospital of Kharj, Saudi Arabia. This study is not only exhibiting the susceptibility pattern of UTI causing organisms, but it would also help in establishing the antibiotic policy of the institute. However, larger population based studies are recommended.

Keywords: ESBL, *E.coli*, ESBL producing *E. coli*, VITEK2

INTRODUCTION

Urinary tract infections (UTI) are not only common nosocomial infections but an important source of morbidity in the community in both hospitalized and out patients [1, 2, 3]. It is the most frequent cause of illness after respiratory tract infections [4]. In young, sexually active women the incidence of UTI exceeds 0.5 episodes per year with about 30% of the women experiencing infections [5].

Escherichia coli is one of the major cause of UTI [6], accounting for 70-90% of UTI cases [7, 8]. Their susceptibility pattern against various antibiotics varies in different geographical locations, eventually leading to empirical therapy, which is based on local susceptibility profiles, geographical location, age and sex of the patient [9]. The changing pattern of pathogen susceptibility is due to extra chromosomal genetic elements, which also carries gene for resistance to number of antibiotics especially in gram negative bacteria [10].

Currently, there is an increasing problem of susceptibility to antibiotics prompting the

need for a good knowledge of antibiotic susceptibility pattern of these pathogens so as to ensure efficient treatment and eradication [11]. Moreover, area specific studies are required in monitoring the trend and gaining useful knowledge about the type of pathogen causing UTI and their sensitivity pattern [12]. This not only helps in the provision of effective treatment but also guides for establishment of the institutional antibiotic policy.

MATERIAL AND METHODS

This observational, prospective 08 months' study was carried out among outpatient departments of Obstetrics and Gynaecology, Urology and Medicine, Department of Microbiology at Salman Bin Abdulaziz University Hospital, Alkharj from 1st January to 31st August 2014. All patients attending the respective outpatient departments clinically suspected for UTI were selected for the purpose of the study during the study period. The total numbers of patients were 345. Patients who had no symptoms suggestive of UTI at the time of observation

were excluded from the study. Suspicion of UTI was made on the basis of urinary symptoms, fever, purulent urine or hematuria. Their urine samples were sent to Diagnostic and Research Laboratory working under supervision of qualified microbiologist. Specimen was collected by standard “clean catch” mid stream method in patients. Before collecting the sample, male subjects were asked to clean the genital part with soap and water while female patients were told to do the genital toilet using soap and water and the vulva was washed and the labia was carefully separated prior to voiding the urine in sterile bottle. Samples were tested for presence of white blood cells and cast.

Samples were examined and processed on the MacConkey agar and cysteine lactose electrolyte deficient medium (CLED) medium by standard loop method and incubated for at least 24 hours at 37⁰C. Plates were observed for bacterial growth. Culture results were interpreted as significant and insignificant according to standard i.e. A growth of $\geq 10^5$ CFU/ml was labeled as significant bacteriuria.

Identification and antimicrobial susceptibility testing using Vitek 2 and E-test

All isolates were identified and tested for susceptibility by the Vitek 2 system (bioMérieux, Marcy l’Etoile, France) using

the card for Gram-negative strains (GN cards) and AST-N0292. The following antimicrobial agents were tested in the study: amikacin, gentamicin, ciprofloxacin, ceftazidime, cefotaxime, piperacillin/tazobactam, and trimethoprim/sulfamethoxazole. The cards were inoculated and incubated in the system according to the manufacturer’s instructions. All results were interpreted using the Advanced Expert System (AES) (software version VT2-R04.03). The isolates were initially screened positive if minimum inhibitory concentration (MICs) of ceftazidime and cefotaxime for these organisms were ≥ 2 mg/L using the Vitek 2 system AST-N0292 card.

Phenotypic confirmation of ESBLs was done using E-test (epsilometer assay; bioMérieux, Marcy l’Etoile, France) on Mueller-Hinton agar. A decrease of at least three twofold concentrations in MIC of either ceftazidime or cefotaxime tested in combination with clavulanic acid versus its MIC when tested alone was indicative of phenotypic confirmation of ESBL production [21].

RESULTS

A total of 345 samples were collected during the study period. Of these 135 (39%) were male and 210 (61%) were female patients (**Table 1**). The causative organisms were isolated in 170 (49.27%) cases.

Enterobacteriaceae were isolated in 137/170 (80.5%) cases, while other organisms constituted for 33/170 (19.5%) of cases (**Table 2**).

E coli was found to be the most frequent organism isolated accounting for 76/170 (44.7%) isolates followed by Klebsiella (51/170=30%) and Staphylococcus saprophyticus (09/170=5.2%). Pseudomonas and Proteus accounted for (8/170=4.7%) cases each, while 7/170 (4.11%) cases of both S aureus and S hemolyticus were isolated. Citrobacter and Shingomonas paucimobilis were the least frequent accounting for 02 (1.17%) cases each (**Table 3**).

Production of ESBL amongst enterobacteriaceae was noted in 51/137 (37.22%) cases. Again the E coli were the most frequent ESBL producing organisms with a frequency of 29/76 (38.1%) cases, followed by Klebsiella (18/51 = 35.2% cases), Proteus (02/08 = 25% cases) and Citrobacter (01/02 = 50% cases) as shown in **Table 4**.

Table 5 shows the susceptibility pattern of members of enterobacteriaceae. It is clearly obvious that all the organisms showed 100% susceptibility to meropenem. A higher susceptibility of the organisms was observed for imipenem as well. Piperacillin, amikacin and gentamicin also displayed a higher susceptibility pattern for all the members of

enterobacteriaceae isolates. Nitrofurantoin, cefepime and ciprofloxacin showed an average of 85%, 79% and 72.5% susceptibility respectively for enterobacteriaceae. An average of 76.25% was observed for aoxiclav and 60.5% for norfloxacin. The susceptibility of enterobacteriaceae was the lowest for trimethoprim/sulfam and ampicillin (**Table 5**).

DISCUSSION

It was a cross sectional hospital based study conducted in the Microbiology department of Salman bin Abdulaziz University Hospital. In this study three hundred forty five patient's urine samples received for culture and sensitivity during the period of 1st January to 31st August 2014 were included. Prevalence of UTI was found to be 61% in females and 39% in males (**Table 1**). This finding is in accordance with the finding reported by Alzohairy and Khadri in the same kingdom [13] and other researchers from different regions [14, 15]. The frequency of urinary tract infection is greater in women as compared to men and our results were similar to these reports. This might be owing to anatomic, physiological, physical and sexual factors [16, 17].

Causative Agents and Their Prevalence In UTI

Escherichia coli was the commonest isolate in our study contributing for 44.7% of cases, followed by 30% *Klebsiella pneumoniae* and 4.7% cases of each of *Proteus* and *Pseudomonas* (**Table 3**). Similar findings have been reported by Alzohairy and Khadri, Majumder *et al* and Kausar *et al* [13-15] This study found *Escherichia coli*, *Proteus*, *Pseudomonas* and *Klebsiella* to be amongst the top most five isolates from urine of patients having urinary tract infection. *E coli* and *Klebsiella* infections are more prevalent in adults and *Pseudomonas* in children and elderly [13].

ESBL Production

Enterobacteriaceae are reported to be notorious for ESBL production and subsequently multiple drug resistance. In our study 38.1% of *E coli*, 35.2% of *Klebsiella*, 25% of *Proteus* and 50% of *Citrobacter* were found to ESBL producing isolates. Overall ESBL production was observed in 37.22% of isolates (**Table 4**). It has been stated in the literature that the prevalence of ESBL producing organisms varies from country to country and from center to center within a country [18]. The wide range of ESBL producing isolates, ranging from less than 0.1% in Japan[19], 0-25% in USA [20], 22-75% in India [17] and 61-77% in Pakistan [9, 15].

Susceptibility Pattern

In our study *Escherichia coli* and *Klebsiella* spp isolates were found to be resistant to Ampicillin (100%) our results showed higher resistant rate.in the different part of the world, resistance of *Escherichia coli* and *Klebsiella* spp to Ampicillin have been a higher side and is increasing day by day but there are only few reports which indicate 100% resistance to penicillin [22].

When compare to other studies in Saudi Arabia [13] our result slightly higher side.

E.coli (85%), *Klebsiella* spp, (80%), *Proteus* (86%0, *Citrobacter* (89%) are more susceptible to nitrofurantoin; smiller findings were reported in other country [14].

In this present study overall Imipenem resistance were 2.7% to *E.coli* and 1.2% to *Klebsiella* spp, whereas, all isolates of uropathgens were 100% to Meropenem so our results are comparable with other reports [13]. In this study, the combination of piperacillin and beta lactamases inhibitors tazobactam showed best result against all urinary isolates (91 to 94%) suggesting that this antibiotics can still be used for the treatment of UTI [23]. Quinolones, especially, Ciprofloxacin have been used for UTI in recent past, in the present study however, *E.coli* and *Klebsiella* spp show slightly higher resistance to ciprofloxacin (29% and 35% respectively)

which is consistent with the previous reports [13]. Other studies show that Quinolones are still active against UTI [23].

Most powerful antibiotics in our study are Imipenem, Meropenem, Amikacin, Nitrofurantoin, and Gentamicin, which shows 91-100% against urinary isolates, are comparable to other report [14].

In more than 60 % cases shows their resistance against Cefotaxime, Amoxiclavate, Trimethoprim Norfloxacin. similar reports were published in different parts of the world [14], which raises the question regarding rationality to empirically use of these antibiotics in UTI without culture and sensitivity reports.

In this study the most active antibiotics against all Enterobacteriaceae were Imipenem, Amikacin, Meropenem, Nitrofurantoin, Gentamicin. Higher resistance

were found against Ampicillin, norflaxacin and cotrimoxazole. Antimicrobial resistance patterns reported from different regions are different and antimicrobial resistance increase.

CONCLUSION

It was the first study of its kind conducted in the teaching hospital of Kharj, Saudi Arabia. This study is not only exhibiting the susceptibility pattern of UTI causing organisms, but it would also help in establishing the antibiotic policy of the institute. However, larger population based studies are recommended.

Note: our study was approved by the Institutional Review Board of Salman Bin Abdul Aziz University.

Competing Intrests

Author disclose no potential conflicts of interest

Table 1: Distribution of Samples According to Gender (n=345)

Gender	Number	Percent
Male	135	39
Female	210	61
Total	345	100

Table 2: Distribution of Total Isolated Micro-Organisms (n=170)

Organism Isolated	Number	Percent
Enterobacteriaceae	137	80.5
Other organisms	33	19.5

Table 3: Distribution of Different Groups of Micro-Organisms (n=170)

ORGANISM	NUMBER	PERCENT
<i>E. Coli</i>	76	44.7
<i>Pseudomonas</i>	8	4.7
<i>Klebsiella</i>	51	30
<i>Proteus</i>	8	4.7

<i>Citrobacter</i>	02	1.31
<i>Staphylococcus aureus</i>	7	4.11
<i>S. saprophyticus</i>	9	5.2
<i>S. heamolyticus</i>	7	4.11
<i>Sphingomonas paucimobilis</i>	2	1.17
Total	170	100

Table 4: Distribution Of Esbl Producing Enterobacteriaceae By Vitek2 Method (n=51)

Organism	Number	Percent
<i>E. coli</i> (76)	29	38.1
<i>Klebsiella</i> (51)	18	35.2
<i>Proteus</i> (8)	02	25
<i>Citrobacter</i> (02)	01	50

Table 5: Susceptibility Pattern of Enterobacteriaceae

Antibiotics	<i>E. coli</i> (76)		<i>Klebsiella</i> (51)		<i>Proteus</i> (08)		<i>Citrobacter</i> (2)	
	S	R	S	R	S	R	S	R
Ampicillin (10µg)	0	100	0	100	1.9	98.1	2.4	97.6
Amoxiclav (100/20µg)	72.3	27.7	74.6	25.4	79	21	81.2	18.8
Piperacillin/tazobactam (100/10 µg)	91.4	8.6	86.7	13.3	93.2	6.8	94.1	5.9
Amikacin (30µg)	91	9	83	17	88.5	11.5	86.4	13.6
Gentamicin (10µg)	89	11	86	14	92	08	90	10
Cefepime	83	17	81	19	73	27	79	21
Ceftazidime (30µg)	48	52	37.4	52.3	41.6	58.4	51	49
Nitrofurantoin	85	15	80	20	86	14	89	11
Ciprofloxacin (5µg)	71	29	65	35	76	24	78	22
Imipenem (10µg)	97.3	2.7	98.8	1.2	99	1	98.9	1.1
Meropenem	100	0	100	0	100	0	100	0
Trimethom/sulfam	53	47	60	40	71	29	77	23
Norfloxacin	51	49	58	42	63	37	70	30

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