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Resistant Pattern of Extended Spectrum β-lactamase Producing Isolates from Clinical Specimen; An Experience at a Tertiary Care Hospital in Alkharj, Saudi Arabia

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Resistant pattern of extended spectrum β-lactamase producing isolates from clinical specimen; an experience at a tertiary care hospital in Alkharj, Saudi Arabia Improper use of antibiotics has resulted in increasing resistance of microorganisms against traditionally used antimicrobial agents. Production of ESBL by enterobacteriaceae presented the prototype of such increasing resistant pattern. This is not only associated with management problems but also responsible for increase cost of treatment. The resistant pattern of enterobacteriaceae was observed in this study in a tertiary care hospital of central region in Saudi Arabia. A total of 131 non-repetitive isolates of enterobacteriaceae from 200 clinical samples of urine, blood, pus, wound swab, high vaginal swab, sputum were included in the study over a period of eight months. All specimens were inoculated on Blood agar, MacConkey agar and cysteine lactose electrolyte deficient medium (CLED) medium. The resistant pattern was noted and results were compared with the national and international data available. The most frequent sample in our study was urine, followed by swabs, pus and body fluids. Members of enterobacteriaceae were isolated in 131/200 samples of these 84 isolates were positive for ESBL production. E coli was the most frequent organism producing ESBL. The organisms were least resitant to Imipinem, meropenem, amikacin, gentamicin and piperacillin/tazobaztam, while amoxiclav, cefepime, nitrofurantoin and ciprofloxacin showed intermediate susceptibility for ESBL producing enterobacteriaceae. Ceftazidime, trimethoprim/sulfam and norfloxacin were found to be lease effective with poor efficacy in our study.Conclusion and Recommendations: Our study described the resistant pattern of ESBL producing organisms in central region of Saudi Arabia. This study may affect the antibiotic policy to be adopted by the hospital.

Key words: ESBL producing enterobacteriaceae, drug resistance, antibiotic policy.

Indiscriminate use of broad spectrum antibiotics without proper investigation has resulted in dramatic increase in antibiotic resistance over the years[1]. The development of antibiotic resistance limits the choice of antibiotics to be used[2]. Extended spectrum β-lactamase producing enterobacteriaceae member are reported to be notorious for their ability to produce multidrug resistance by hydrolyzing β lactam antibiotics[2,3]. A single organism may harbor multiple ESBLs such as the serine cephalosporinases and Amp C[4]. Escherichia coli is the most frequently

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reported organism in the recent past as a common cause of urinary tract infection in community bases studies and is capable of producing â lactamases, such as CTX-M enzymes[4].

For treatment of gram negative sepsis the extended spectrum â lactams are the most common drugs being used empirically. Threat of treatment failure is mainly due to the emergence of ESBL producing organisms[5]. The bacteria producing ESBL also imparts resistance against some other drugs like quinolones etc[6]. However, the incidence and resistance pattern of antimicrobial resistance of ESBL producing enterobacteriaceae varies from time to time and from place to place[7]. Even from one center to another center the variation has been reported widely in literature. The frequency of ESBL producing organisms in Saudi Arabia is documented to vary from 4.8% to 15.8% in different regions[8-11].

Resistant pattern of ESBL producing organism has a lions' share in constituting the antibiotic policy of an institute. An effective antibiotic policy is effective in combating against drug resistance[7]. Therefore, keeping in view the regional, time-based and intra-institutional variation regarding this subject it is advisable to collect such data with regular and frequent intervals of time.

MATERIALS AND METHODS

Bacterial isolates

A total of 131 non-repetitive isolates of enterobacteriaceae from 200 clinical samples of urine, blood, pus, wound swab, high vaginal swab, sputum were obtained from different clinics of the hospital,(Medicine, Surgery, gynecology and obstetrics, pediatrics) over a period of Eight months (February to September, 2014). The study included patients of all age groups and both sexes. All the specimens were then inoculated on Blood agar, MacConkey agar and cysteine lactose electrolyte deficient medium (CLED) medium. All the media were incubated for 18-24 hours at 37°C. Blood cultures were processed using the BACT/ALERT 3D system (bioMérieux, France).

Plates were observed for bacterial growth. Culture results were interpreted as significant and insignificant according to standard.

Identification and antimicrobial susceptibility testing using Vitek 2

All isolates were identified and tested for susceptibility by the Vitek 2 system using the card for Gram-negative strains (GN cards) and AST-N291. 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-N0291 card[12].

Objective

To see the resistant pattern of ESBL producing enterobacteriaceae clinical isolates in our population

RESULTS

This was a cross sectional retrospective analysis of all the 200 different samples sent for microbiological examination in the diagnostic laboratory of Salman bin Abdulaziz university hospital over a period of eight months i.e. from February 2014 to September 2014.

Overall 200 samples were received during the study period. Most of the samples were urine (47.5%), followed by various swabs (23.5%) and pus (18.5%). Sputum and blood samples accounted for 5.5% and 5.0% respectively. (Table 1)

Out of 200 samples bacterial growth was noted in 168 sample of these members of enterobacteriaceae were isolated in 131 (77.9%) samples, and in the rest 37 (22.1%) samples other organisms like Staphylococcus aureus, Pseudomonas spp., etc. were isolated. (Table 2)

A total of 84 isolates of enterobacteriaceae showed ESBL production. This accounted for 42% (84/200 samples) of all the samples, 50% (84/168 samples) of all the isolates, and 64.1% (84/131 samples) of enterobacteriaceae isolates. Escherichia coli was the most frequent organism producing ESBL with a relative frequency of 42.85% (36/84). Klebsiella with a frequency of

27.38% (23/84) ranked second amongst ESBL producing organisms followed by 14.28% (12/84) isolates of Proteus and 09.52% (08/84) cases of Citrobacter. Sphingomonas were the least frequent accounted for 05.95% (5/84) cases (Table 3).

Table 1. Different samples collected for enterobacteriace (n = 200)

Samples	Number	Percent
Urine	95	47.5
Swab	47	23.5
Pus	37	18.5
Sputum	11	5.5
Blood	10	5.0

Table 2. Distribution of total isolated micro-organsims (n = 168)

Samples	Number	Percent
Enterobacteriaceae	131	77.9
Other organisms	37	22.1

Table 3. Distribution of esbl producing enterobacteriaceae by vitek2 method (n = 84)

Samples	Number	Percent
Urine	95	47.5
Swab	47	23.5
Pus	37	18.5
Sputum	11	5.5
Blood	10	5.0

Susceptibility pattern of ESBL producing organisms is shown in table 4. It is apparent that meropenem, imipenem, piperacillin/tazobactam, gentamicin and amikacin are the most effective drugs against ESBL producing organisms in our study. The susceptibility to above said drugs ranged from 83% to 100% with a mean of 93.3% in our study. Amoxiclav, cefepime, nitrofurantoin and ciprofloxacin showed intermediate susceptibility for ESBL producing enterobacteriaceae with a mean of 76.87%, 79%, 85% and 72.5% respectively. We found that ceftazidime, trimethoprim/sulfam and norfloxacin were found to be lease effective with poor efficacy in the said study (Table 4).

DISCUSSION

Changing pattern of antibiotic resistance has created many problems in the management of serious infections worldwide. Members of enterobacteriaceae are notorious for producing resistance against commonly and widely used antibiotics. Production of ESBL adds gravity to the situation. Since the first ever report of ESBL producing enterobacteriaceae in the mid 1980s[13], quite a number of studies have been published reporting such organisms in various clinical isolates. This also has affected greatly the antibiotic policy determination of any institute. This would be the first study of its kind in Salman bin abdulaziz university hospital, Kharj Saudi Arabia.

Table 4. Susceptibility pattern of ESBL producing enterobacteriaceae

Antibiotics	*E. coli* (36)		*Klebsiella* (23)		*Proteus* (12)		*Citrobacter* (8)	
	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/tazobactum (100/10µg)	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

In present study, majority samples were urine (47.5%) followed by swabs (23%) and sputum (18.5%). Sample from body fluids accounted for 21.5%. Roshan et al described ESBL production in 45.1% of urine samples, 30.5% of pus and 7.5% in body fluids[2]. The selection of sample may be different in an inpatient setting versus OPD settings. Majority of our samples were referred from OPD. This may explain the slight disparity amongst the distribution of various clinical samples in our study from that of Roshan et al.

Enterobacteriaceae were found to be the most frequent (77.9%) organisms isolated in our study. This finding is in complete accordance with the findings of Qamar et al[14] and Seetha et al[15], who had described the frequencies of enterobacteriaceae to be 78.5% and 67.92%. However the frequency of samples collected and examined may vary from center to center.

Escherichia coli was found to be the most frequent ESBL producing organisms in our study (42.85%) followed by Klebsiella (27.38%). The similar finding has been reported by Somily et al from the eastern region of the kingdom, however the percentage of E coli (6.6%) described by them is much lower than our study[16]. Researchers from other countries have reported 61.7%[2] and 58%[17] of E coli in their studies. Our results are in accordance with the findings of international data available, however the percentage is much higher than reported from the central region of the kingdom. This is an established fact that the percentage of ESBL producing organisms reported from central region is much lesser than other regions of the kingdom, a fact that has been accepted by Somily et al[16]. Relative frequency of ESBL producing species of Klebsiella was found to 27.38% in present study. Roshan et al described the relative frequency of Klebsiella spp to be 21.1%[2]. Another study from central region i.e. Riyadh has described 55% of ESBL Klebsiella spp in their study. This is quite apparent that the relative frequencies of Klebsiella species is very much variable not only from one region to another in the kingdom, and amongst various countries worldwide.

Amikacin, imipenem, piperacillin/ tazobactam, meropenem and gentamicin were associated with the least resistance against ESBL producing enterobacteriaceae in our study. Similar findings have been reported by Hassan et al from within the kingdom[12]. Cefepime, ciprofloxacin and amoxiclav exhibited intermediate resistance against ESBL producing enterobacteriaceae in our study. Once considered drug of choice in the infections with enterobacteriaceae, ciprofloxacin is now becoming less effective with intermediate and highly variable resistant pattern. Although, lower resistant pattern has been described by Roshan et al and Hassan et al[2, 12] for ciprofloxacin, the drug efficacy in treating infections with ESBL producing enterobacteriaceae is now getting controversial. Some authors have considered ciprofloxacin may serve as an alternative choice for infections caused by ESBL producing enterobacteriaceae[19] as poor efficacy has been shown by this drug[2]. Nitrofurantoin is not commonly used drug in UTI in the kingdom, therefore the resistance is not that high for this drug. However, variable results have been described for nitrofurantoin resistant pattern in ESBL producing enterobacteriaceae[2]. The least effective drugs in our study with highest resistant in ESBL producing enterobacteriaceae were cotrimoxazole and norfloxacin. Similar observations have been described by Roshan et al [2], Hassan et al[12] and Al-Zahran and Akhtar[20].

CONCLUSION

The most difficult infections to be treated in a tertiary care hospital are the ones caused by ESBL producing enterobacteriaceae, as a continuously changing resistant pattern is being observed over a period of time. The institutes are recommended to carry out long term broad based studies before establishment of the antibiotic policy of the hospital so as to ensure quality treatment provided to patients.

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