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RESEARCH ARTICLE

Prevalence and risk factors of extended-spectrum β-lactamases producing *Enterobacteriaceae* in a general hospital in Saudi Arabia

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ABSTRACT

Objective: To estimate the prevalence and associated risk factors of extended-spectrum β-lactamase producing Enterobacteriaceae (ESBL) in King Khaled General Hospital, Saudi Arabia.

Methods: A twelve -month retrospective study for the presence of ESBL producing Enterobacteriaceae infection was performed by using the Microbiology and Infection Control Departments' database. For all the collected specimens, microbiological identification and antimicrobial sensitivity testing were done using MicroScan WalkAway system and then confirmed by API 20E and E-test respectively.

Results: The prevalence of ESBL producing Enterobacteriaceae infection among studied patients was 22%. The most common types of infections were urinary tract infections representing 59.2%. Previous use of antibiotics, urinary catheter, mechanical ventilation, previous hospitalization, previous intensive care unit admission and nosocomial origin of infection were significant risk factors for acquiring infection. Amikacin had the highest activity against ESBL producing isolates, whereas 20% of isolates were resistant to carbapenems

Conclusions: The study revealed that prevalence of ESBL producing Enterobacteriaceae infection was relatively high. Our findings suggest that invasive devices, the use of antibiotics, prolonged hospitalization especially in the intensive care unit increases the risk of acquiring such infections. A strict antibiotic policy should be addressed especially with observed emergence of carbapenem resistance. Continuous review of need to invasive devices and strict compliance with basic infection control measures are mandatory to limit the spread of ESBL Enterobacteriaceae. *J Microbiol Infect Dis 2014; 4(2): 50-54*

Key words: Enterobacteriaceae, extended-spectrum β-lactamase, prevalence, ESBL, resistant bacteria, risk factors

Suudi Arabistan'da bir genel hastanede genişlemiş spektrumlu β-laktamaz üreten Enterobacteriaceae prevalansı ve risk faktörleri

ÖZET

Amaç: Suudi Arabistan Kral Halid Hastanesi'nde genişlemiş spektrumlu β-laktamaz (GSBL) üreten *Enterobacteriaceae* prevalansı ve enfeksiyon gelişmesinde risk faktörlerinin belirlenmesi

Yöntemler: Mikrobiyoloji ve Enfeksiyon Kontrol Birimleri'nin veri tabanı kullanılarak 12 aylık dönemde geriye yönelik olarak GSBL üreten Enterobacteriaceae ile enfeksiyon görülme oranı belirlendi. Bu sürede içerisinde elde edilen örneklerde mikrobiyolojik tanımlama ve antibiyogram testleri MicroScan WalkAway sistemi kullanılarak yapıldı. Sonuçlar API 20E ve E-test yöntemi ile doğrulandı.

Bulgular: Çalışmaya alınan hastalarda GSBL üreten Enterobacteriaceae prevelansı % 22 olarak bulundu. En sık karşılaşılan enfeksiyon % 59,2 ile üriner sistem enfeksiyonu idi. Antibiyotik kullanımı, üriner kateterizasyon, mekanik ventilasyon, daha önce hastanede yatış, yoğun bakım ünitesinde tedavi görme ve enfeksiyonun hastane kaynaklı olması GSBL üreten Enterobacteriaceae ile enfeksiyon gelişmesi için risk faktörleri olarak bulundu. GSBL üreten bakterlere karşı en etkin antibiyotik amikasinken, izolatların % 20'sinde karbapenemlere karşı direnç saptandı.

Sonuçlar: Bu çalışmanın sonuçları GSBL üreten Enterobacteriaceae oranının yüksek olduğunu göstermektedir. Bulgular; antibiyotik kullanımı, invaziv işlemler ve özellikle yoğun bakım ünitesine olmak üzere uzamış hastane yatışının GSBL üreten bakterilerle enfeksiyon gelişme riskini artırdığını göstermektedir. Gözlenen yüksek karbapenem direnç oranı antibiyotik kullanımıyla ilgili etkin politikalar oluşturulması gerektiğini ortaya koymaktadır. GSBL üreten Enterobacteriaceae yayılmasının engellenmesi için kullanılan invaziv cihazlara ihtiyacın sürekli olarak değerlendirilmesi ve temel enfeksiyon kontrol önlemlerine etkin şekilde uyulması gerekmektedir.

Anahtar kelimeler: *Enterobacteriaceae*, genişlemiş spektrumlu β-laktamaz, prevalans. GSBL, dirençli bakteri, risk faktörleri

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INTRODUCTION

In the last twenty years, antimicrobial resistance especially extended-spectrum β-lactamases (ESBLs) have spread among *Enterobacteriaceae*, particullarly *Escherichia coli* and *Klebsiella pneumoniae*.[1] B-lactamases are enzymes that hydrolysis β-lactam ring thus destroying the activity of β lactam antibiotics. ESBLs were discovered in 1983 and they can hydrolyze oxyimino-cephalosporins, and monobactams, but not cephamycins or carbapenems.[2] ESBLs together with resistance to other antibiotics such as chloramphenicol, trimethoprim, tetracyclines, sulphonamides and aminoglycosides are encoded by genes present on large plasmids.[3] Majority of ESBL associated infections are resistant to various antibiotics, leaving only limited compounds as a therapy.[4] Presently, carbapenems became the first line antimicrobials for the treatment of such infections.[1] The spread of ESBLs has certain consequences such as hindering effective treatment, poor outcomes, prolonged hospitalization and increase treatment costs.[5] Risk factors for ESBLs associated infections include patients' prior comorbidities (such as diabetes mellitus, renal failure, immunosuppression, neoplastic diseases, etc.), long hospital stay, advanced age, use of mechanical interventions (urinary catheters, venous catheters, endotracheal tubes) and previous therapy with broad spectrum antimicrobials.[6] The prevalence of ESBLs differs between countries and hospitals. In Saudi Arabia the prevalence of ESBLs varies greatly in different regions. It was shown to be 11% in eastern province whereas, in Abha (southern region) and Riyadh (central region) it was reported to be 27.5% and 36% respectively.[7-9] This study was conducted to describe the prevalence of ESBLs among *Enterobacteriaceae* in our hospital over a period of one year and to identify risk factors for infections.

METHODS

The current study was conducted in King Khaled General Hospital (KKGH), Saudi Arabia between January and December 2012. KKGH is a 300-bed tertiary care facility with approximately 23,000 admissions per year. Microbiological results were reviewed retrospectively. Only positive cultures for *Enterobacteriaceae* in different specimens from inpatients were included. If two similar cultures from the same patient were encountered, only one was included. For analysis, we defined cases as those patients with isolates classified as ESBL producers, and controls as patients with isolates negative for ESBL production. For each patient, age, sex, previous hospitalization (last six months) or intensive care unit admission, nosocomial infection (any infection presented within 48 hours of admission and was not diagnosed at that time),[10] use of invasive devices (more than 48 hours) during current hospital stay and previous antibiotic use (more than seven days) were recorded from infection control surveillance data. Type of specimen, species of bacteria and antibiotic sensitivity pattern were also recorded.

Microbiological methods

The identification of Enterobacteracae species, antibiotic sensitivity testing and ESBL production were done by MicroScan WalkAway 96 (Siemens, Sacramento, USA) with its panels (negative breakpoint combo42). Results were interpreted by Microscan software program, following Clinical and laboratory Standards Institute (CLSI) guidelines.[11] Confirmtion of species identification and ESBL production were done by API 20E (bioMerieux, France) and two ESBL E test strips for ceftazidime and cefotaxime with and without clavulanate (AB Biodisk, Sweden) respectively. ESBL diagnosis was considered if MIC was reduced by ≥3 twofold dilutions with clavulanic acid.

Statistical analysis

The analysis was done using the statistical software Open Epi (Open Source Epidemiologic Statistics for Public Health) Version 3.01. The qualitative data were presented in the form of number and percentage. Two by two tables are used to evaluate the association between a possible risk factor (Exposure) and an outcome (Disease). The risk was estimated using odds ratio, 95% confidence interval and Chi-Square. Statistical value of $p < 0.05$ was considered to be significant.

RESULTS

A total of 1870 patients with *Enterobacteriaceae* isolates were included in the study. Out of these, 412 (22 %) were ESBL producers. The mean age of the case group (± standard deviation) was 53.7 ± 17.5years, and (54.6%) of them were male. The mean age of the control group (± standard deviation) was 55.3 ± 16.9 years, and (53.4%) of them were male. E coli had the highest prevalence (61.2%) followed by *Klebsiella pneumonia* (22.8%) as demonstrated in Table 1.

The distribution of ESBL-producing *Enterobacteriaceae* in different type of specimens is shown in Table 2. The clinical characteristics and possible

risk factors for ESBLs related infections are demonstrated in Table 3 and the sensitivity rates of ESBL producing *Enterobacteriaceae* to antibiotics are demonstrated in Figure 1.

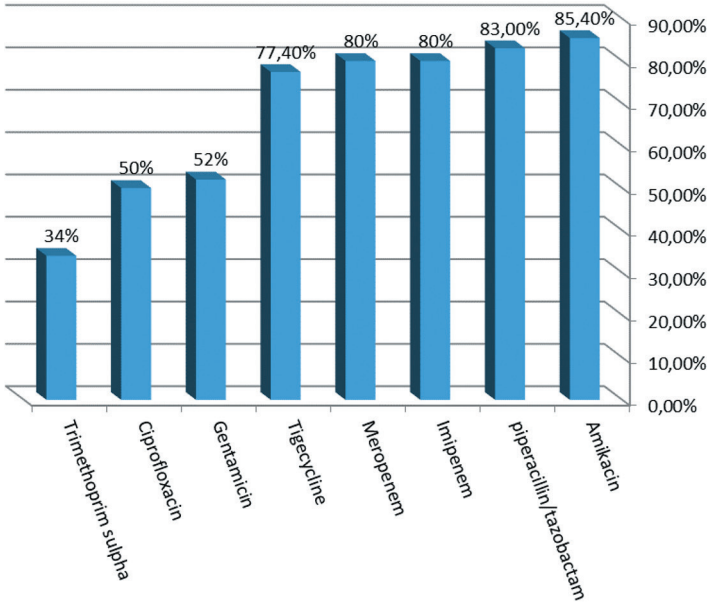

Figure 1. Antimicrobial Susceptibility rates of ESBL Producing Enterobacteriaceae.

Table 1. Distribution of ESBLs isolates according to species

ESBL producing species	n (%)
Esherichia coli	252 (61.2)
Klebsiella pneumoniae	94 (22.8)
Enterobacter aerogenes	25 (6.1)
Enterobacter cloacae	21 (5.1)
Klebsiella oxytoca	9 (2.2)
Proteus mirabilis	9 (2.2)
Citrobacter spp.	2 (0.4)
Total	412 (100)

Table 2. Distribution of ESBL producing isolates according to type of the specimen

Clinical Specimen	ESBL producers (%)
Urine	244 (59.2)
Blood culture	28 (6.8)
Others	52 (12.6)
Wound swab	72 (17.5)
Sputum	16 (3.9)
Total	412 (100)

Table 3. Clinical features and potential risk factors for ESBLs producing *Enterobacteriaceae* infections

Risk factors	No. of ESBL (%) (n=412)	Non-ESBL (n=1458)	OR (95% CI)	p value
Male gender	225 (54.6)	778 (53.4)	1.05 (0.84-1.3)	0.35
Old age (≥65 years)	208 (50.4)	760(52)	0.94 (0.75-1.17)	0.3
Nosocomial origin of infection	277 (67)	900(62)	1.27 (1.0-1.60)	0.024
Indwelling urinary catheter	298 (72)	976(67)	1.29 (1.01-1.64)	0.022
Central venous catheter	242 (58.7)	798(54.7)	1.18 (0.94-1.47)	0.083
Mechanical ventilation	139 (33.7)	354(24.3)	1.59 (1.25- 2.01)	0.0001
Previous antibiotic use	378 (92)	1278(88)	1.6 (1.07-2.30)	0.01
Cephalosporins	324 (79)	1069(73)	1.34 (1.03-1.74)	0.02
Fluoroquinolones	182 (44)	546(37)	1.32 (1.06-1.65)	0.01
Recent operation	185 (44.9)	683(46.8)	0.92 (0.74-1.15)	0.26
Previous hospitalization (B6 months)	282 (68.4)	878(60.2)	1.43 (1.14-1.81)	0.001
Previous ICU admission	217 (52.7)	694(47.6)	1.22 (0.99-1.53)	0.04

OR: odds ratio, CI: confidence interval

DISCUSSION

The rate of ESBLs in bacterial species differs greatly all over the world, and rapidly changing from time to time.[12] The prevalence of ESBLs was reported to be over 10% in east Europe, 3.5% in a Canadian study and 20-48.8% in Asia.[13-15] Within the Arabian Gulf region, ESBL prevalence ranged from a low of 7.5% in Kuwait to as high as 41% in United Arab Emirates.[16,17] In Saudi Arabia ESBL detection was reported to be 27.5% in *K. pneumoniae* and 36% in *Enterobacteriaceae*. In other studies, It was 15.8% and 8.9% in blood cultures and urinary isolates, respectively.[7-9,18] In our study the prevalence of ESBL was (22%)in *Enterobacteriaceae*. When compared to regional and international data, the ESBL prevalence in our institution tends to be towards the higher limit. This can be attributed to availability of broad spectrum antibiotics, the haphazard use of many of them with lack of strict antibiotic policy to control their use. Their spread cannot be prevented due to improper isolation of patients with ESBL producing strains.

ESBL-producing E. coli is of concern as an important community-acquired pathogen. Community acquired ESBL associated infections are mostly urinary tract infections (UTIs), however some patients suffer from intra-abdominal infections and bacteremia.[1] In Our study (33%) of ESBL associated infections were acquired within two days of admission. Similarly, Ben Ami et al. showed that the prevalence of community acquired ESBL associated infections was 34.6%.19 On the other hand, in a study conducted in the eastern region of Saudi Arabia, only 37.9% of the bacteremia due to ESBL producing isolates were hospital acquired.[20] Also, in a nationwide study conducted in Spain, 51% of ESBL-producing E. coli strains were isolated from outpatients.[21]

In our study E coli had the highest prevalence (61.2%) among ESBL producers followed by *K. pneumoniae* (22.8%) and most ESBL isolates were detected in urine samples (59.2%).The same findings were observed in other studies and explained by the fact that hospitalized patients suffer frequently from UTIs, and *Enterobacteriaceae* (mainly *E. coli*) are the most common isolated organisms in these infections.[6,19,22,23] In our study, amikacin was quite active against ESBL isolates, which was reported also by Rubio-Perez et al. and attributed it to the reduction of aminoglycosides usage to avoid renal toxicity.[6]

In the current study high level of carbapenem (20%) resistance has been observed among ESBL isolates. Carbapenems are the first line of treatment of these organisms and, until recently, carbapenems resistance was rare among Enterobacteriaceae.[24] Carbapenem resistance can be due to production of carbapenemases, the poor binding of carbapenems to penicillin-binding proteins present in the bacteria, the over-expression of multidrug efflux pumps by the bacteria or lack of porins in the bacterial cell membrane.A combination of resistance mechanisms can result with a significant rate of resistance.[25] In our facility the high rate of carbapenem resistance could have been predisposed by the wide use of carbapenems as empirical treatment because its broad spectrum, activity against ESBL isolates and relatively less side effects compared to amikacin.

Based on our results, antibiotics useage particularly cephalosporins and fluoroquinolones were significantly associated with ESBL associated infections. Antimicrobial exposure is considered to be an important risk factor for ESBL related infections as it allows resistant mutants to become the dominant strains.[26-28] Cephalosporin and fluoroquinolone exposure was reported as risk factor for multidrug resistant bacteria infections, previously.[28-30]

In our study although central venous catheter was not considered as risk factor for ESBL producing *Enterobacteriaceae* infections, mechanical ventilation and urinary catheter were both considered as risk factors. Similar findings were reported by other studies.[15,19,29]

Previous hospitalization, and nosocomial origin of infection were significantly associated with ESBL acquisition which was described by other authors.[19,26] On the other hand, old age, male gender, and recent operation were not significantly associated with ESBL producing *Enterobacteriaceae* infections in our study.

Our study has limitations. As noted, high rate of carbapenem resistance has been observed but since it was a retrospective study, the exact rate and mechanism of this resistance could not be confirmed.

In conclusion, this study suggests there is a high prevalence of ESBL associated infections in our setting, which can be attributed to prolonged un-reviewed invasive interventions, unrestricted use of antibiotics, prolonged and probably unnecessary hospital stay. These isolates pose a special therapeutic challenge especially with the growing resistance to carbapenems. Therefore, strict antibiotic policy, continuous review of the need to in-

vasive devices, minimizing duration of hospital stay together with strict compliance to infection control precautions would serve as the most efficient way of preventing the spread of these organisms. We also recommend that carbapenem resistance should be thoroughly investigated by further research to know the magnitude and mechanism of such resistance.

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