

Gram Negative Extended Spectrum Beta-Lactamase Producing Bacteria Prevalence in Jouf Region Tertiary Care Hospital, Saudi Arabia

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Abstract

Extended Spectrum beta lactamase producing pathogens are reported in many clinical samples and pose an emerging threat health. To find the prevalence of ESBL producing Enterobactriacea pathogens isolated from inpatients (Medical and Surgical Wards; Hospital stay > 72 h) admitted to Prince, Mutib, Hospital Sakaka, Jouf. A total of 1043 Enterobactriacea were isolated during April 2015 to October 2016 study period. Vitek -2 compact (biomeriux Leon, France) was used for identification and antimicrobial sensitivity (AST) test. A Total of 115/1043 (11.02%) ESBL producing pathogens were isolated from urine 15.07% (n=242), sputum 13.6 % (n=220), wound 10.8% (n=287) and blood 5.4 % (n=294). *Klebsiella pneumonia* is most dominant followed by *E coli* and *Proteus mirabilis*. Cephamycins and carbapenems were found most effective (100% sensitivity) against the pathogens isolated. The aminoglycosides, beta lactams and Fluroquinolnes class of antibiotics, microorganisms have gained the resistance of 20%, 22.7%, 40-70% respectively. The infection control measures should be taken seriously and making of effective use of antibiotics is need of hour.

Keywords: ESBL, Gram negative bacteria, Antimicrobial sensitivity test, Antibiotics

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INTRODUCTION

The antimicrobial resistance (AMR) is a matter of concern worldwide¹. Genes encoding beta-lactamase and modification of enzymes in bacteria results in drug resistance². The *Enterobacteriaceae* carry the genes for resistance to β lactams and the β -lactamase family is divided into four groups: Extended Spectrum β Lactamases (ESBLs), Amp- C type, carbapenemases and Penicillinases. The antimicrobial resistance results in treatment failure, economic loss and a source of resistant genes in bacteria that leads to risk of human health. Among the members of *Enterobacteriaceae* family (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter. Baumannii* and *Escherichia coli*) are known to produce extended-spectrum β -lactamase (ESBL) and are a serious threat to human health³. *E. coli* the most important pathogen in community acquired infections and have been studied in detail⁴. ESBL producing pathogens delays suitable antibiotic therapy which in turn leads to longer hospital stay and more economic burden⁵. The resistance to antibiotics causes higher mortality rates in patients. In Asia 70% of ESBL infections are due to nosocomial acquired. ESBL producing microorganisms are one of the cause of antimicrobial resistance (AMR)⁶. In Europe year, 1980 first case of ESBL was reported and subsequently in USA. Emergence of ESBL present a significant threat to human health, particularly *klebsiella* and *E. coli* pathogens for which only few antibiotics are available⁷. Countries with low and moderate income are facing problems to overcome or in managing antimicrobial resistance due to lack of surveillance and non-availability of high class microbiology laboratories for diagnosis of pathogens and their antibiograms⁸. The pathogenic microbes belongs to gram positive⁹ and gram negative¹⁰ have been isolated from wound, blood, sputum and urine samples were reported as antibiotic resistant in nature¹¹. The distribution of antimicrobial resistant pathogens changes with time and among the hospitals located in different regions¹². The prevalence of ESBLs in the middle east and Gulf Cooperation countries (GCC) have been reported¹³. The plasmid encoded genes are largely responsible for the ESBL and they belong to class A and are divided into three genotypes CTX-M, SHV and TEM¹⁴. This is the first study

conducted in Jouf region to explore the prevalence of ESBL producing pathogens among the patients attending the Prince, Mutib, Hospital Sakaka, Jouf, Saudi Arabia.

MATERIALS AND METHODS

A retrospective study, was conducted during April, 2015 to October 2016 at secondary care 150 bed hospital in rural area of Northern Province of Saudi Arabia, covering the population of 2.7 million approximately. The total number of 1043 samples (blood, wound, Urine and Sputum) were obtained from patients (Medical and ICUs Wards) attending to the Prince, Muteb, Hospital Sakaka, Jouf, Saudi Arabia..

Bacterial isolation and identification

Samples (blood, wound, sputum and urine) were streaked on blood agar , MacConkey agar , and chocolate agar and were incubated in aerobic condition at 35°C growth was observed after 24 hrs and isolates were identified based on morphological and biochemical characteristics using standard procedures of Clinical and Laboratory Standard Institute (CLSI)¹⁵.

Antimicrobial Susceptibility Test

The automated Vitek 2 Compac system was used to perform antimicrobial susceptibility tests as per clinical laboratory standards institute (CLSI) protocols¹⁵. The ESBL isolates were confirmed by the method described by bilal et al., 2018²⁹ and the following American type culture collection strains *Klebsiella pneumoniae* ATCC 700603, *Proteus mirabilis* ATCC BAA-856 and *E. coli* ATCC 25922 were used as controls.

The vitek 2 antimicrobial susceptibility test (AST) card is composed of Amikacin (30 μ g), Gentamicin (10 μ g), Ertapenem (10 μ g), Imipenem (10 μ g), Meropenem (10 μ g), Cephalothin (30 μ g), Cefuroxime (30 μ g), Cefoxitin (30 μ g), Ceftazidime (30 μ g), Ceftriaxone (30 μ g), Cefepime (30 μ g), Aztreonam (30 μ g), Ampicillin (10 μ g), Amoxicillin-Clavulanate (20+10 μ g), Piperacillin-Tazobactam (100/10 μ g), Colistin (10 μ g), Trimethoprim-Sulfamethoxazole (1.25+23.75 μ g) Nitrofurantoin (300 μ g), Ciprofloxacin (5 μ g), Levofloxacin (5 μ g), Tigecycline (15 μ g).

Statistical analysis

The study data was analyzed with the aid of SPSS V.17 (statistical package for the social sciences) software.

RESULTS

Demographic and Distribution of Isolates

The 1.5 year retrospective study revealed that 1043 patients were admitted in Prince Muteb hospital during April 2015- October 2016. The microorganisms were isolated from various clinical samples urine 15.07% (n=242), sputum 13.6 % (n=220), wound 10.8% (n=287) and blood 5.4% (n=294)(Fig. 1). A total of 115/1043 (11.02%) pathogens were isolated that are ESBL producing *Enterobacteriaceae* and were obtained from 73 (63.4%) males and 42 (36.5%) females and amongst the most predominant ESBL pathogen was *Klebsiella pneumoniae* 36.5% (n=42) followed by *E. coli* 34.7 % (n=40) and *Proteus* 28.6% (n=33). It is worth to mention that 73 patients (63.4%) were non Saudis and 94 (81.7%) were remained

in hospital for more than 3 days. The demographic profile of population is Shown in Table 1.

Antimicrobial resistance of ESBL producing *Enterobacteriaceae*

A high rate of resistance was observed in *Klebsiella pneumoniae* isolated from blood, urine, wound and pus against the antimicrobials. Among the aminoglycosides the resistance rate was found as gentamycin (60-89%) and Amakacin (20-28%). Cephalosporins: ceftazidime (100%), cefotaxime (100%), cefepime (98%) and Fluroquinolones: levofloxacin (40-67.7%), ciprofloxacin (40-83%) and Sulpha drug: trimethoprim/sulfamethoxazole (50%)(Fig. 2) *Proteus mirabilis* showed the resistance pattern towards aminoglycosides as gentamycin (79-89%) and amakacin (20-43%), Cephalosporins: ceftazidime (100%), cefotaxime

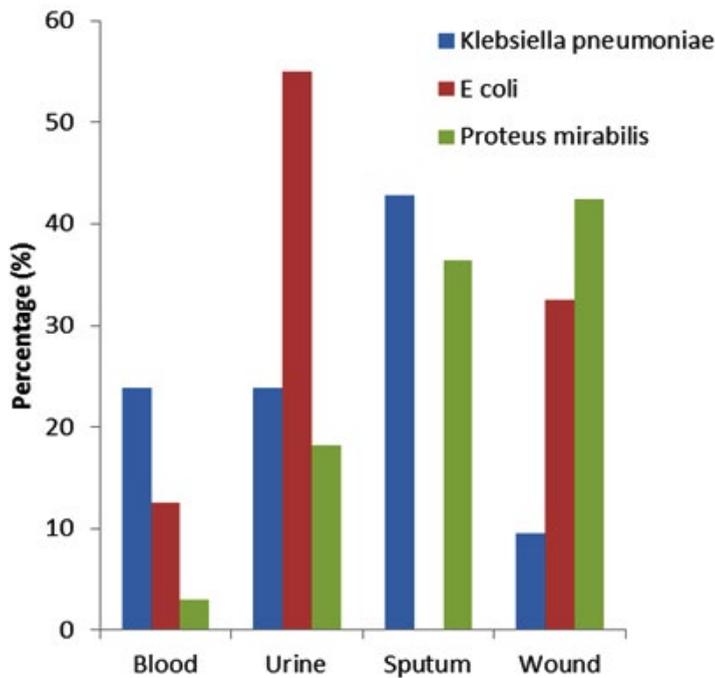


Fig. 1. Percentage of ESBL producing *Enterobacteriaceae*.

Table 1. The prevalence of ESBL producing *Enterobacteriaceae* in the study subjects

Gender	Total No. (%)	Nationality		Hospital stay	
		Saudi	Non- Saudi	< 3 days	> 3 days
Male	73(63.4)	26	47	09	64
Female	42(36.5)	16	26	12	30
Total	115	42 (36.5%)	73(63.4%)	21(18.2%)	94(81.7%)

(100%), cefepime (98%) and Fluroquinolones: levofloxacin (67.7-71%), ciprofloxacin (40-83%) and Sulpha drug: trimethoprim/sulfamethoxazole (100%) and for tigecycline resistance (67-71%)(Fig. 3). The ESBL producing *E Coli* the rate of resistance pattern towards antibiotics was lower as compared to *Klebsiella pneumonia* and *Proteus mirabilis*. The aminoglycosides were found resistant (18-27%).

Cephaloprorins: ceftazidime (100%), cefotaxime (100%), cefepime (68%) and Fluroquinolones: levofloxacin (18%), ciprofloxacin (60%) and Sulpha drug: trimethoprim/sulfamethoxazole (22.7%) and for tigecycline resistance (13.6%).(Fig. 4)

The cephamycins (Cefoxitin), Amoxicillin-Clavulanate, Piperacillin-Tazobactam and carbapenems (Meropenem, Ertapenem and

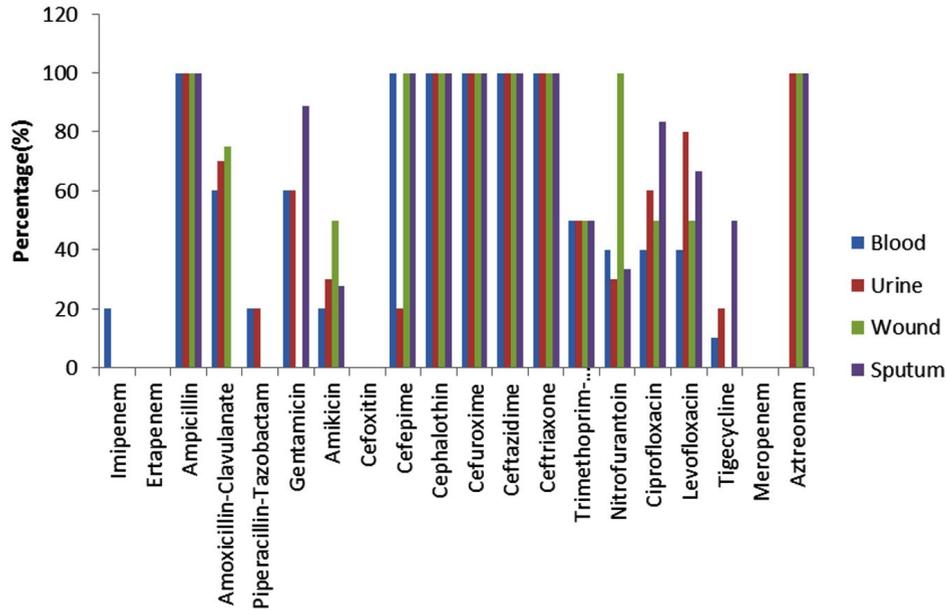


Fig. 2. Antibiotic resistance pattern of ESBL producing *Klebsiella pneumoniae*

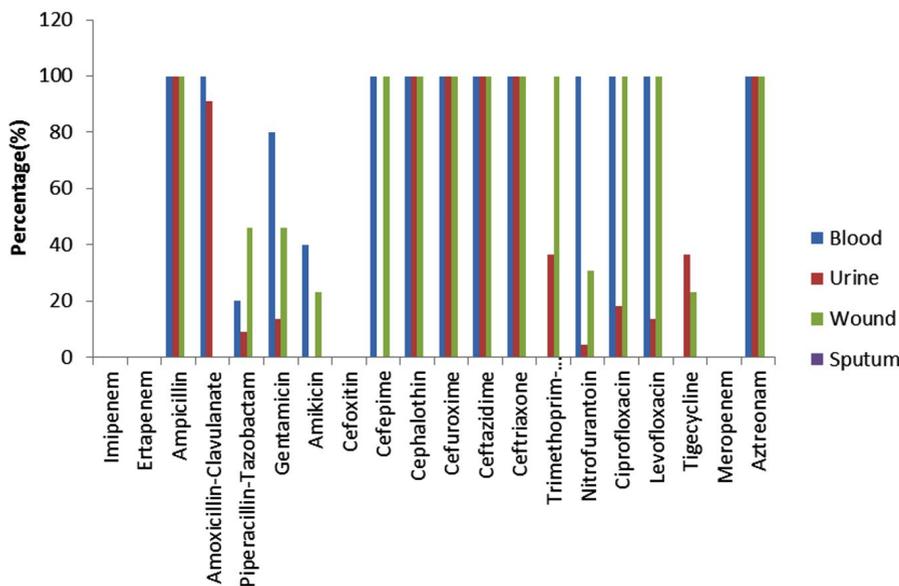


Fig. 3. Antibiotic resistance pattern of ESBL producing *E. Coli*

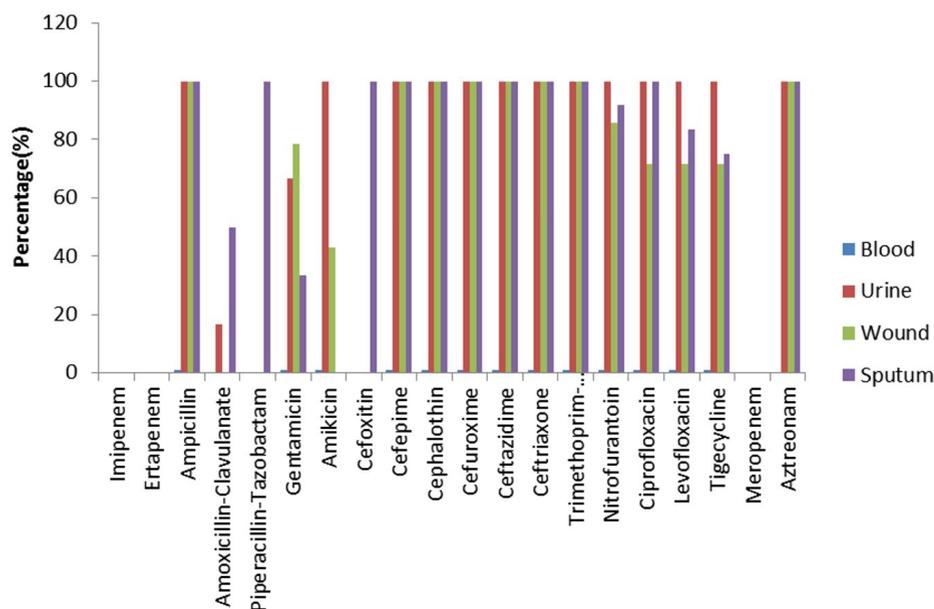


Fig. 4. Antibiotic resistance pattern of ESBL producing *proteus mirabilis*

imepenem) were effectively sensitive against all the isolated ESBL producers. ESBL producing *Klebsiella pneumonia* strains from sputum showed an intermediate susceptibility of 11% towards Cefoxitin. We did not find any strain resistant to meropenem and Ertapenem. However, *Klebsiella pneumonia* isolated from blood samples showed 20% resistance to imipenem.

DISCUSSION

In this study 115 ESBL producing *Enterobacteriaceae* were found among inpatients (Medical and ICUs wards) in Prince Muteb Hospital, Jof, Saudi Arabia. Our results revealed ESBL producing *E. coli* and *klebsiella pneumoniae* is significantly higher than previous studies reported from Abha, Riyadh, Dammam and Al Khobar, Saudi Arabia¹⁶⁻¹⁸. The ESBL producing *proteus mirabilis* in our study subjects were found 36.8% which is higher as compared to other studies¹⁸.

In Europe, Turkey, Russia and Poland the prevalence of ESBLs was found 2-47%¹⁹, from the southern America (Brazil) 60%^{20,21}. In Asia, ESBL producing *Enterobacteriaceae* (*E. coli*) 5% in Japan, 57% in China and Thailand were reported. In Pakistan the prevalence was reported as

highest (73%)²². In Middle East, Kuwait, and UAE the ESBLs were reported 25% and 36% respectively^{23,24}. In our study 115/1043 were ESBL producing *Enterobacteriaceae* (*K. pneumoniae*, *E. coli* and *P. mirabilis*). To our knowledge, this is the first study conducted in the northern region of Saudi Arabia to explore the ESBLs prevalence and its susceptibility pattern. The frequency of ESBLs in *E. coli*, *K. pneumoniae* and *P. mirabilis* 36.5%, 34.7% and 28.6% respectively. From Al Khobar, the *E. coli* and *K. pneumoniae* was reported 10.3% and 12.3%²⁵. This variation may be due to differences in the number of isolates (either Tertiary care hospital or community based).

Further, in our study we did not find any strains resistance to cephamycins and carbapenems. The efficiency of these drug classes has been attributed to the stability of beta lactamases. Hence, cephamycins and carbapenems are the choice of drugs for treating the patients with infections caused by ESBL producing *Enterobacteriaceae*. The ESBL producing *P. mirabilis*, *E. coli* and *K. pneumoniae*, isolates in our study are resistance to all the generations (I- IV) of cephalosporins in hospitalized patients. Whereas, Menyfah et al.,(2018) reported that,

ampicillin, Trimethoprim-Sulfamethoxazole showed highest resistance to *E. coli* isolates in Riyadh Saudi Arabia²⁵.

Aminoglycosides, Nitrofurantoin and sulpha drugs were 70% resistance to clinical isolates in our study. Whereas, in Norway, *E. coli* and *K. pneumoniae* showed high resistance to aminoglycoside due to the presence of modified enzymes AAC(6)-1b²⁶. Higher rate of resistance against ciprofloxacin, levofloxacin, ceftriaxone, ceftazidime, Tiglycyline, Azetranonam and Ampicillin in community is probably due to over use, misuse or self-mediations.

Previous studies has shown that, the antibiotic resistance against the Ceftriaxone, Cefuroxime, Ciprofloxacin, Ampicillin for ESBL producing pathogens^{17,27,28}. However, our study also revealed that, the antibiotic resistance against Levofloxacin, Cefepime and Aztreonam. This ineffectivity against the Gram negative bacteria occur due to the mutations and spread of ESBLs with in the *Enterobacteriaceae* family and extensive use of these antibiotics for prophylaxis.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

BA, SR & MAH designed the retrospective study plan. JI, Z, FNS collected the data of the study and later double checked it. M and A analyzed the data, drafted the manuscript, compiled information from the literature, and designed the Figures and tables. M, A, BA, SR & MAH wrote the manuscript. All authors read and approved the manuscript.

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None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript

ETHICS STATEMENT

Not applicable.

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