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EVOLUTION OF AMINOGLYCOSIDE RESISTANCE IN CTX-M-15 PRODUCING *KLEBSIELLA SPP.* IN KURDISTAN PROVINCE, IRAN

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ABSTRACT: Background: the aim of present investigation was evolution of aminoglycoside resistance in CTX-M-15 producing *Klebsiella spp.* Clinical isolates from general hospitals of Kurdistan Province, Iran. **Methods:** Ninety *Klebsiella spp.* isolates were obtained from different clinical specimens. Antibiotic susceptibility pattern and detection of ESBL producing isolates performed by Kirby-Bauer disc diffusion method according to Clinical and Laboratory Standards Institute's (CLSI) guidelines. CTX-M-15 gene screened by PCR amplification. Fisher tests was used to analyses by with STATA software program and p-values below 0.05 were considered as significant. **Results:** Out of 90 clinical isolates the highest and lowest resistance related to cefotaxime and imipenem respectively. The results of detection of ESBL producing isolates showed that 62 isolates (84.4%) were ESBL-positive isolates. Thirty two (42.1%) out of 90 *Klebsiella spp.* isolates caring CTX-M-15 gene. CTX-M-15 was detected in 56.2% of gentamycin resistance, 34.4% of kanamycin resistance, and 56.2% of amikacin resistance. **Conclusion:** the present study showed that there was high frequency of CTX-M-15 gene in ESBL producing clinical isolates in Kurdistan Province, Iran. Moreover, there was no statistically significant relationship between CTX-M-15 production and resistance to aminoglycoside antibiotics in *Klebsiella spp.* isolates.

INTRODUCTION: *Klebsiella spp.* especially *Klebsiella pneumoniae* and *Klebsiella oxytoca* are one of the main causes of hospital infection and a member of Enterobacteriaceae family^{1, 2}. Beta-lactam antimicrobial agents commonly used for the treatment of infections caused by *Klebsiella spp.*, including respiratory, urinary tract infections, and septicemia^{3, 4}.

Unfortunately today there are reports of the prevalence of resistance to beta-lactam antibiotics around the World⁵. The presence of extended spectrum beta-lactamase (ESBLs) enzymes is a major mechanism of resistance to beta-lactam antibiotics in bacteria^{6, 7}. *Klebsiella spp.* and *E. coli* are the major ESBL-producing bacteria in worldwide⁸. Recently, CTX-M remain as the main of ESBL type which hydrolyze third and fourth generation cephalosporins such as cefotaxime and ceftriaxone⁹.

CTX-M category into five subtype including; CTX-M-1 (CTX-M-1, -3, -10, -11, -12, -15, -28 and FEC-1), CTX-M-2 (CTX-M-2, -4, -5, -6, -7, -20 and TOHO-1), CTX-M-8 (CTX-M-8), CTX-M-

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9 (CTX-M-9, -13, -14, -16, -17, -19, -21, -24, -27 and TOHO-2) and CTX-M-25^{10, 11}. In some studies, CTX-M-15 has been reported as the most prevalent CTX-M¹². CTX-M-15 was initially described in New Delhi, India in 1999 and subsequently found in different geographical areas of the World¹³. ESBL-producing isolates often resistance to various antimicrobial agents such as aminoglycosides (e.g. gentamicin, amikacin, and kanamycin) and quinolones (e.g. ciprofloxacin) and which causes failure of treatment in severe infections and a major concern for clinicians¹⁴. Therefore, the aim of present investigation was evolution of aminoglycoside resistance in CTX-M-15 producing *Klebsiella* spp. clinical isolates from Kurdistan province, Iran.

MATERIAL AND METHODS:

Collection of Clinical Isolates: Seventy *K. pneumoniae* and twenty *Klebsiella oxytoca* isolates were recovered from urine, blood, tracheal aspirates, and wound from March 2016 to October 2017 from general hospitals of Kurdistan Province, Iran. Thirty *K. pneumoniae* related to previous study¹⁵. *Klebsiella* strains identified by routine microbiological tests. All strains were kept in -80°C in tryptic soy broth medium¹⁶.

Antibiotic Susceptibility Pattern and Detection of ESBL Producing Isolates: Antibiotic susceptibility pattern of nine antibiotic disk including; ceftazidime (30µ), cefotaxime (30µ), ciprofloxacin (5µg), amikacin (30µg), gentamicin (10µg), kanamycin (10µg), colistin (10µg), imipenem (10µ), and co-trimoxazole (1.25+23.75µg) (Roscoe, Denmark) performed by Kirby-Bauer disc diffusion method according to Clinical and Laboratory Standards Institute's (CLSI) guidelines. Detection of ESBLs was tested by ceftazidime, cefotaxime by and without clavulanic acid on Muller-Hinton agar plates. *Escherichia coli* ATCC 25922 and *K. pneumoniae* ATCC 7881 were used as negative and positive controls, respectively¹⁷.

Screening for CTX-M-15 Gene by Polymerase Chain Reaction (PCR): After DNA extraction by Kit (Sina Clon Co. Iran), all ESBL positive isolates screened of CTX-M-15 gene by PCR amplification using specific primers as previously described by Mendonca et al.¹⁸ The thermocycler program was

adjusted as: initial denaturation at 95 °C for 3 min followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 59 °C for 30 s, elongation at 72°C for 45 s, and final elongation at 72 °C for 5 min¹⁸. Then, PCR products analyzed on 2% agarose gels stained with DNA safe stain. *E. coli* ATCC 25922 containing CTX-M-15 gene was used as positive control strain.

Statistical Analysis: Data analyzed by Fisher tests with stata software program v12 and p-values ≥ 0.05 were considered as significant.

RESULTS:

Antibiotic Susceptibility Pattern and Detection of ESBL Producing Isolates: Out of 90 clinical isolates 54 isolates (60%) to ceftazidime, 58 isolates (64.4%) to cefotaxime, 27 isolates (30%) to ciprofloxacin, 31 isolates (34.4%) to amikacin, 31 isolates (34.4%) to gentamicin, 24 isolates (26.7%) to kanamycin, 9 isolates (10%) to colistin, 6 isolates (6.7%) to imipenem, and 52 isolates (57.8%) to co-trimoxazole were resistance. The results of detection of ESBL producing isolates showed that 76 isolates (84.4%) were ESBL-positive isolates.

Screening for CTX-M-15 Gene: Thirty two (42.1%) of the ESBL producing *Klebsiella* spp. isolates carrying CTX-M-15 gene **Fig. 1**. CTX-M-15 was detected in 18(56.2%) of gentamicin resistance, 11(34.4%) of kanamycin resistance, and 16(50%) of amikacin resistance **Table 1**. No significant association was found between aminoglycoside resistance and presence of CTX-M-15 gene.

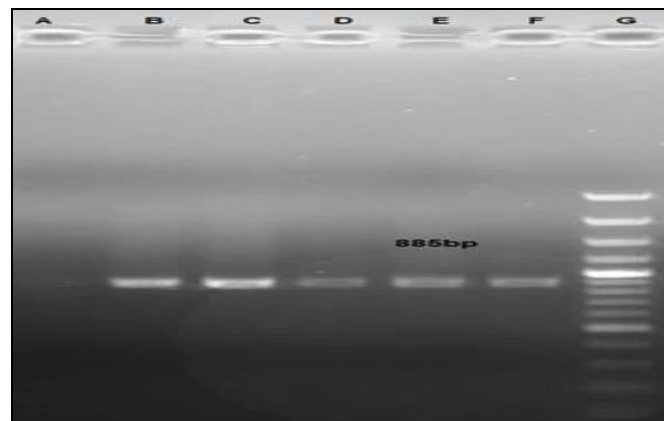


FIG. 1: GEL ELECTROPHORESIS OF PCR PRODUCTS IN KLEBSIALLE SPP. ISOLATES A: Negative Control, B: Positive control for CTX-M-15, C, D, E, and F: Positive isolates for CTX-M-15 885 bp, G: Marker-100 bp

TABLE 1: N (%) FREQUENCY OF ANTIMICROBIAL RESISTANCE BASED ON RESISTANT GROUPS OF *KLEBSIELLA SPP.* ISOLATES

| Antibiotics | ESBL n=76 | CTX-M-15 n=32 | Total isolates n=90 |
|----------------|--------------|------------------|------------------------|
| Ceftazidime | 42(55.3%) | 26(81.2%) | 54(60%) |
| Cefotaxime | 51(67.1%) | 26(81.2%) | 58(64.4%) |
| Ciprofloxacin | 23(30.3%) | 18(56.2%) | 27(30%) |
| Amikacin | 23(30.3%) | 16(50%) | 31(34.4%) |
| Gentamycin | 26(34.2%) | 18(56.2%) | 31(34.4%) |
| Kanamycin | 18(23.7%) | 11(34.4%) | 24(26.7%) |
| Colistin | 7(9.2%) | 5(15.6%) | 9(10%) |
| Imipenem | 5(6.6%) | 4(12.5%) | 6(6.7%) |
| Co-Trimoxazole | 43(56.6%) | 31(96.9%) | 52(57.8%) |

DISCUSSION: Members of the genus *Klebsiella* are gram-negative bacteria of human intestinal flora and opportunistic pathogens¹⁹. In recent decades, a significant increase in drug resistant *Klebsiella spp.* isolates has been recorded Worldwide, which has led to health problems and increased mortality rates^{20, 21}. In our findings the highest resistance related to ceftazidime and cefotaxime. Also; the lowest resistance related to imipenem and colistin.

This results similar to study conducted by Azadpour *et al.*, in Khorramabad, Iran²². The prevalence of cephalosporinases, carbapenemases and ESBLs are one of the most important causes of drug resistance²³. In our study, 88.6% of isolates were ESBL-producing *Klebsiella spp.* In investigations by Sharif in Iran, Mokaddas in Kuwait and Al-Zarouni in the United Arab Emirates the prevalence of ESBL-producing isolates recorded 41.4%, 31.7% and 41%²⁴⁻²⁶, which were lesser than our findings. The prevalence of ESBL producing isolates in different regions is different due to different causes, including differences in the pattern of antibiotic used^{27, 28}.

In some study CTX-M-15 considered as one of the main of ESBLs⁹. Almost CTX-M-15 producing isolates hydrolysis the ceftazidime¹⁰. The present results indicated that isolates harboring CTX-M-15 identified in 84.4% of ESBL producing isolates and 26 isolates of 32 CTX-M-15 producing isolate resistance to ceftazidime. similarity, in the study Peerayeh *et al.*, in Iran 63.5% of ESBL-producing strains was ESBL producers and all of them resistance to ceftazidime²⁹. Aktas *et al.*, in Istanbul reported 14.8% ESBL producing *K. pneumoniae* and 35% of them carrying CTX-M-15 ESBLs³⁰. Ensor *et al.*, in Kuwait found 91% of the ESBL-producing *K. pneumoniae* carrying CTX-M-15

gene and all of isolates were sensitive to meropenem and imipenem³¹.

Therefore, in this study, we decided to investigate the resistance to aminoglycosides (gentamycin, kanamycin and amikacin) in CTX-M-15 carrying isolates. Right now, the resistance to aminoglycosides is slower than β -lactams. Lee *et al.*, in Korea reported nearly 30% resistance to gentamycin and kanamycin in *K. pneumoniae* clinical isolates³². Tumaini *et al.*, in Tanzania recorded 77% and 1.45% respectively, resistance to gentamycin and kanamycin in *K. pneumoniae* isolates³³. This rate of aminoglycosides resistance was consistent with the results of present study. On the other hands, in our results, the rate of resistance to amikacin, gentamycin, and kanamycin in CTX-M-15 producing isolates were 50%, 56.2% and 34.4% that similar to the result of Peerayeh *et al.*²⁹ Ma *et al.*, in Taiwan described 87.2% and 43.4% resistant to gentamicin and amikacin in ESBL-producing *K. pneumoniae* strains³⁴. Based on the results of our findings determination of drug resistance pattern in CTX-M-15 carrying isolates, imipenem and colistin is the effective antimicrobial agent.

CONCLUSION: In conclusion, the present study revealed a high prevalence of CTX-M-15 gene in ESBL producing clinical isolates in Kurdistan Province, Iran. Moreover, there was no statistically significant relationship between CTX-M-15 production and resistance to aminoglycoside antibiotics in *Klebsiella spp.* isolates.

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CONFLICT OF INTEREST: Nil

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