PREVALENCE OF \( \beta \)-LACTAMASES ENZYMES AMONG \textit{ENTEROBACTERIACEAE} IN DIFFERENT IRAQI PROVINCES: A REVIEW

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ABSTRACT

Gram negative bacteria are responsible for most infections among people around the world. The level of antibacterial resistance in gram negative bacteria in Iraqi provinces, specifically, Baghdad, Hilla and Karbala, has not been previously studied. This study shows a high resistance rate among the governorates due to gram negative bacteria producing ESBLs and carbapenemase enzymes, these infections are mostly opportunistic. The recognized \( \beta \)-lactamase genes are CTX-M, OXA-10, NDM-1, TEM, SHV,IMP-1 and VIM-2 have found in many isolates. But, less common \( \beta \)-lactamase genes are PER \( \beta \)-lactamase in Hilla city. So as numerous possible factors unique to the Iraqi provinces may have responsible of appearance \( \beta \)-lactamases, comprising the excessive consumption of antimicrobial agents and a large proportion of travelers are mainly from the Iranian, Pakistan and Indian countries.

Keywords: Enterobacteriaceae, SHV, PER, CTX-M, MBLs

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INTRODUCTION

\textit{Enterobacteriaceae} comprising enormous, varied group of gram-negative rods and intestinal tract is a normal habitat in human and animals. \textit{Enterobacteriaceae} including members of aerobes or facultative anaerobes rods have the ability to ferment a wide range of carbohydrates, as they possess a very difficult antigenic structure, and create a diversity of virulence factors and toxins\cite{1}. Morbidity and mortality are worldwide due to infection with gram negative bacteria(GNB), and the resistance to \( \beta \)-lactam agents caused by production of \( \beta \)-lactamase \cite{2}. At the present time, there is global interest about rapid spread of \( \beta \)-lactam resistance among GNB and this is poses a clinical and public health challenge \cite{3}. The production of broad-spectrum \( \beta \)-lactamases among GNB regarded a main cause of multidrug resistance (MDR). Penicillins and expanded-spectrum cephalosporins hydrolyzed by AmpC and extended-spectrum \( \beta \)-lactamases\cite{4}. The
encoded β-lactamases can be either plasmid or chromosomal or both. At least one of chromosomal β-lactamase expressed by most species of Enterobacteriaceae[5]. The name of ESBLs, due to increased activity of spectrum[5]. Bacteria have the ability to produce ESBL are usually resistant to penicillins, third generation oxynimecephalosporins (cefotaxime, ceftazidime, ceftriaxone), first-and second-generation cephalosporins and monobactams (aztreonam) so as susceptible to cephamycins, carbapenems and fourth-generation cephalosporins (cefepime, cefpirome). β-lactamase inhibitors such as tazobactam and clavulanic acid can inhibit the ESBLs production[6]. Enterobacteriaceae species responsible for producing of extended spectrum beta-lactamase, commonly K. pneumoniae which taken from intensive care unit patients[7]. And newly, another GNB such as Acinetobacter baumannii and Pseudomonas aeruginosa[8]. Currently, there are many of ESBLs more than 600 variants have been designated, (CTX-M) have ability to hydrolyzing Cefotaxime, Sulphydryl variable suggested SHV and Temoneria producing of (TEM) (http://www.lahey.org/studies/weibt.htm). SHV and TEM types are most of ESBLs detected in the nosocomial infections caused by GNB[7]. As well as, ESBLs has been found distributed in a worldwide, such as, previous study have showed the producing of extended spectrum beta-lactamase in European countries are greater than United States as well as fewer than Asia and South America[9]. This article focuses on the distribution of broad spectrum β-lactamase producing gram negative bacilli in the Iraq and identification of trials and factors are likely to be dangerous responsible of the diffusion of (ESBLs) and (AmpC) β-lactamase in this country. Moreover, not only ESBLs confer the resistance to β-lactam antibiotics such as expanded-spectrum cephalosporins. Another hydrolyzing β-lactamases, such as AmpC molecular class C β-lactamases can confer antibiotic resistance. Additionally, there are many mechanisms other than β-lactamases may be confer the resistance to β-lactam agents such as; upregulated efflux pumps and decrease of outer membrane porins, especially carbapenem resistance in Pseudomonas aeruginosa. Though, we will cite all the research about resistance of GNB to antimicrobial agents especially B-lactams in this study and determine which of each mechanisms responsible for resistance to most antimicrobial drugs.

**Occurrence of β–lactamases genes among GNB in some Iraqi Provinces:**

Many and varied studies about distribution of GNB producing for AmpC and ESBLs enzymes from various Iraqi provinces. However, in the Iraq provinces many surveys have been showed among AmpC and ESBL–producing gram negative bacteria isolates from the different hospitals. In 2013, study conducted by Al-Niaameet. al.[10] in Baghdad provincerelated to many patients in health centers revealed that 34 clinical sample taken from those patients. The frequency of isolates were as follow (35.29%) P. aeruginosa, (29.41%) E. coli, (20.58%) K. pneumoniae and (14.7%) A. baumannii. Screening test used for testing of Whole isolates for (ESBL) and (AmpC) β-lactamases, the positive ratios for (ESBL) and (AmpC) β-lactamases were 15(44.1%), 7(20.50%) respectively. The isolates were Escherichia coli [7(70%), 1(10%)], Klebsiella pneumonia [5(71.4%), 3(42.8%)] and A. baumannii [3(60%) 3(60%)] respectively. Based on current study, the distribution of ESBLs among E. coli and K. pneumonia are greater in our country as comparison with USA and Canada[11,12]. But the ratio was lower than that have studied from India, approximately 40% E. coli and K. pneumonia isolates taken from urinary tract infections were...
positive for ESBLs. In another study from the Baghdad Governorate, showed that out of the 100 environmental swabs collected from three hospitals, 15 isolate effectively were diagnosed as *P. aeruginosa*, the antimicrobial susceptibility test for these isolates were achieved and exposed all of these isolates (100%) resistant to (Ceftrixone), (Cefepime), (Chloramphenicol) and (Tobramycin), also, this resistance may be due to selective pressure by overused of antibiotics. As well as, via Multiplex (PCR) by using particular primers to OXA-10 and VEB-1 genes. The results discovered 15(100%) environmental isolates were positive for OXA-10 and 6 (40%) for VEB-1 gene in the strains.

Another study was accomplished by researchers Al-Marjaniet. al., recorded that a whole of isolates 92 taken from numerous infections such as *A. baumannii* and *P. aeruginosa* examined for (ESBLs) and metallo β-lactamase production by two tests included that : double disc synergy(DDS) and disc potentiating tests. The ratios of extended spectrum B-lactamases were (35.2%) and (25%) of *A.baumannii* and *P.aeruginosa* respectively, also (41.1%) *A. baumannii* and (20%) *P.aeruginosa* isolates were positive for (MBLs). Moreover, PCR method achieved for revealing of β-lactamase (TEM), (SHV),(CTX-M), (OXA-1),(IMP-1) and (VIM-2) genes. The resistance of these isolates were very highly for beta-lactam drugs and (MDR). The results of ESBLs genes detection explain, that all *A.baumannii* and *P.aeruginosa* isolates were positive for ESBLs and MBLs production contained of (CTX-M gene), also *A. baumannii* isolates producing of ESBLs and MBLs were (16.6 %) and (42.8%) respectively carried of (TEM gene), on the other hand, all the ESBLs and MBLs *P.aeruginosa* producers were negative for blaTEM gene. 42.8 % of *A. baumannii* isolates were carried for blaIMP-1 type. While (VIM-2) gene didn’t found in the isolates producing for(ESBLs or MBLs),Alternate study in Baghdad city achieved by Mohammed, revealed out of 50 *K. Pneumonia* isolates,13 (26%) were ESBL producer by CDT, and *Klebsiella pneumoniae* isolates which positive for ESBLs were resistant to several antibiotics. Thus (CTX-M) gene was widespread among *K. pneumoniae*. In addition to, other study carried out by (Al-Marjani and Khadam, 2016) exhibited the prevalence of blaCTX-M gene in *A. baumannii* isolates in Iraq and reason of international development of (CTX-M)gene in *A. baumannii* due to spread of CTX-M group in Iraq.In 2016, Study published by Al-Charrakhet. al. in Baghdad province was aimed at detection of Metallo-β-Lactamase in *P. aeruginosa* isolates as demonstrated 16 (21.3%) out of 75 *P. aeruginosa* isolates grown on MacConkey agar enhanced with Meropenem 4mg/L. So as 6 (37.5 %) isolates were very resistant to Carbapenem by MIC of various antibiotics, MIC ≥16 μg/ml whereas Imipenem-EDTA combined disc test (CDST) revealed 4 (25%) isolates were positive for MBL production. The molecular method (PCR) exposed that (blaIMP), (blaSPM-1 genes) hidden in 3 (50%), 1(16.6%) of the isolates were resistant to carbapenem, respectively. While (blaVIM) gene was not found. The occurrence of MDR *P. aeruginosa* isolates particularly the bacteria resistant to Carbapenem were elevated in Baghdad governorate. The (blaIMP) gene was predominant in the (MBLs) genes in *P. aeruginosubacteria*.Recent study achieved by Al-Khafajiet. al., in Baghdad showed that 57Enterobacteriaceae species out of 45 clinical specimens gained from infected wounds and burns in three Iraqi hospitals. The PCR analysis revealed that 5 *K. pneumonia* isolates and 3 *E. coli* isolates are containing blaIMP encoding formetallo-β-lactamase.A previous study in Hilla province showed 12/13(92%) K.pneumonia isolates were recognized as ESBL-producers but the results of PCR exhibited that both TEM and SHV enzyme were highly distribution among species of Enterobacteriaceae. While Al-Hilli, 2010 showed 7/18(38.8%) Enterobacteriaceae spp. were positive.
for blaCTX-M but negative for blaOXA gene. As well as, 7 Enterobacteriaceae spp. were able to produce AmpC β-lactamase enzyme. Al-Charrakhet et al.,[22] in Hilla region, they found 65/88 (73.8%) K. pneumoniae strains were resistant to β-lactam antibiotics and the percentage of β-lactamase-producer strains was 58.4%, as well as the determination of isolates producing ESBLs β-lactamase was in only 8% of these isolates. ESBL-producers of Klebsiella strains tested for Plasmid profile and the resistance against penicillin, ampicillin, amoxicillin, tetracycline, rifampin, and erythromycin so as production of ESBLs were due to genes located on conjugative plasmids although there is genes express from resistance to cephalothin, cefazolin, cephalaxin and gentamycin which have the location on chromosome. Al-Charrakhi,[23] found that 87.5% of gram-negative rods exhibited high beta-lactam resistance and 75% of these isolates were highly resistant to erythromycin. So as only one isolate of P. aeruginosa among all gram-negative rods was most resistant. Another study in Hilla city conducted by Al-Dahmoshiet et al.,[24] which found that ESBL determination by two method such as double disc synergy test and ESBL Chromatic medium very important and accurate where the result showed that 19(48.7%) for double disc synergy test and 22(56.4%) for ESBL Chromatic medium. So as he found carbapenemase resistant isolates were (10.3%) and AmpC production among diarrheagenic E. coli were (7.7%) whereas (10.3%) of isolates were produce of MBL. Previous study in Hilla city-Iraq showed that examination of (ESBL) and (KPC) conducted by double disc synergy test and ESBL Chromatic medium for (ESBLs), while test strip for (MIC) and chromCRE medium for (KPC). The DDS test result was 10 (43.5%) isolates of K. pneumonia were positive and 12 (52.2%) isolates were positive by Chromatic ESBL medium while using MIC test for detection of KPC revealed 3(13%) isolates were positive and 4(17.4%) isolates were positive by Chrom CRE medium.[25] Abbas and Jarallah explained 17 out from 117 K. pneumoniae isolates were positive for ESBL production so as these isolates tested for the existence of blaTEM gene by PCR and observed in only 13 isolates, the presence of blaTEM gene in Hilla province was very high in compared with previous study recorded that 57.1% of E.coli and K.pneumonia isolates were contained of (TEM) gene.[27] Another study in Hilla city, Abbas and Jarallah demonstrated 91 out of 117 K. pneumoniae isolates were ESBL producers. All isolates were exposed to three phenotypic tests for carbapenemase production, the result was (65%), (82%) and (100%) for imipenem-EDTA disk, modified Hodge test and KPC CHROM agar, respectively while PCR assay reveals presence of blaNDM-1, blaVIM and blaOXA-23 in ratio of 17.6%, 82.3%, and 88.2%, respectively. Additionally, the PCR method indicated that 13 (76.5%) of carbapenemase producers hidden blaTEM, blaSHV, blaCTX-M, blaOXA-1 and only 10 (58.8%) isolates hidden blaPER genes. As well as in the same study all the isolates were positive for carbapenemase additional examined for AmpC production by two methods (modified three dimensional MTDT and AmpC disk test) and the result was as follows: 3 (17.6%), 2(11.8%) were AmpC producers, respectively. Also by PCR assay blaAmpC was noticed in 13 (76.5%) of the isolates. In a recent study, conducted by Jarallah and Abbas revealed 10/17 (58.8%) carbapenem-resistant K.pneumonia hidden a blaPER gene by PCR. However, this regarded first report about discovery of PER β-lactamase in Hilla city. However, Abbas had been reported that 9 out of 23 isolates of Klebsiella spp. were contained of ESBL enzymes by disk method but when he used the PCR technique for detection of blaCTX-M gene found only 2 isolates were positive for this gene. Bunyan et al.,[29] stated that 16/21 (76.1%) isolates of P. aeruginosa were positive for (IMP), (VIM), (SPM), (SIM) and (GIM) by polymerase chain reaction.
methods. A study conducted in the city of Karbala showed that 60 out of 158 \textit{P. aeruginosa} isolates were very resistant to antipseudomonal agents such as carbapenems because of increasing treatment of this group particularly Meropenem\cite{31}. Another study in the same province revealed that all \textit{K.pneumoniae} isolates were tested for ampicillin, amoxicillin-clavulanic acid and cefotaxime, then the result was very resistant for these agents, while the resistance proportions were noticed to those agents among \textit{E. coli} (96.9\%, 92.3\% and 87.7\%, respectively) and \textit{P. mirabilis} (100\%, 78.3\% and 52.2\%, respectively). Conversely, carbapenems agents (imipenem and meropenem) were very effective against all isolates\cite{32}. A recent study also in Karbala- Iraq explained 23/38(60.53\%) \textit{P.mirabilis} isolates were positive for \textit{bla TEM} and 13(34.21\%) for \textit{blaSHV} so as 9(23.6\%) for both \textit{bla TEM} and \textit{blaSHV}\cite{33}. Furthermore, the occurrence of ESBL in the Karbala Province was very little in compared with other provinces in Iraq.

**CONCLUSION**

We found the worldwide distribution of antimicrobial resistance among pathogenic gram negative bacteria and is also a serious problem in the Iraqi hospitals. Though, the size of the problem is not completely described due to the absence of studies about detecting resistance strategies. Traveling to the states where definite classes of extended spectrum B-lactamases or metallo B-lactamases are widespread is a risk factor that is probable to remain, if not escalate. Diverse controlling plans to decrease antibiotic resistance among gram negative bacteria in the Iraqi hospitals exclude (1) implementation of antibiotic control programs in health institutions, (2) Do not use antibiotics without consulting doctor (3) Initiate awareness campaigns on the use of antibiotics among the community, (4) Educating about basic precautions to reduce infections such as: hand hygiene.

**ETHICAL CLEARANCE**

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

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