

# Prevalence of Metallo- $\beta$ -lactamases Producing *Escherichia coli* Isolated from North of Palestine

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## Introduction

Metallo-beta-lactamase (MBL) producing Gram-negative bacteria are being reported with increasing frequency from several countries and have emerged as a most widespread and clinically significant carbapenem resistance mechanism [1]. Metallo- $\beta$ -lactamase producing pathogens can hydrolyze all types of  $\beta$ -lactams including penicillins, cephalosporins, carbapenems, cephamycins, except monobactams as aztreonam [2]. In addition, their catalytic activities are not affected by available  $\beta$ -lactamase inhibitors such as clavulanic acid, tazobactam and sulbactam [3]. These enzymes belong to Ambler class B  $\beta$ -lactamases based on their amino acid sequence homology and to group 3 according to the Bush classification based on their substrate and inhibitor profiles [2]. Class B  $\beta$ -lactamase are carbapenemases type which depend on zinc ions to hydrolyze the  $\beta$ -lactams and due to this, the presence of divalent cation-chelating agents such as EDTA, MBL catalysis is inhibited [4,5].

Metallo- $\beta$ -lactamases enzymes are either chromosomally encoded (resident MBLs) mainly in environmental bacteria or opportunistic pathogens or clinically relevant metallo- $\beta$ -lactamases are carried on highly mobile genetic elements allowing easy dissemination (acquired or plasmid mediated MBLs) [6,7]. The MBLs which are produced by environmental bacteria, could be precursors for the efficient MBLs present in clinical pathogens that are exposed to increasing doses of antibiotics [8].

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## ABSTRACT

**Objective:** This study aimed to determine the prevalence of Metallo- $\beta$ -lactamases (MBLs) among clinical *E. coli* isolated from North of Palestine.

**Methods:** A total of 79 of *E. coli* isolates were recovered from hospitals in North of Palestine during February-July 2015. A multiplex PCR was used to determine the prevalence of MBLs among these isolates.

**Results:** Results showed that the prevalence of MBLs was 87.4%. The *spm* gene was the most prevalent (86.1%) among these isolates. According to the geographical distribution, results showed that the prevalence of MBLs among *E. coli* isolates recovered from Thabet Hospital-Tulkarm was 75.9%. The *Spm* gene was the most common (72.4%) among these isolates, while the *Imp* gene was detected in 41.4% of isolates. For isolates recovered from Jenin Hospitals, the prevalence was 94% and *spm* gene was detected in all MBL producers, 2 other genes were detected, *Imp* and *Sim*, and the prevalence was 12% and 2%, respectively. A total of 26.1% of MBL *E. coli* producers carried 2 genes.

**Conclusions:** Our results showed high occurrence of MBLs among *E. coli* isolates in Palestine. Based on these results we recommend the continuous monitoring and surveillance of the prevalence, proper control and prevention practices and effective antibiotic use will limit the further spread of MBLs producing isolates within hospitals in this country. Prevalence of MBL producing microorganisms has not been investigated previously.

**KEY WORDS:** Metallo- $\beta$ -lactamases  
*E. coli*  
MBLs  
Palestine

To date, nine different MBL types have been identified in Gram-negative bacilli, namely, IMP-like, VIM-like, GIM-1, SIM-1, SPM-1, KHM-1, AIM-1, NDM and DIM-1[9]. The most widespread MBLs include IMP, VIM, and NDM [5]. The Indian subcontinent, the Balkans regions, and the Middle East are considered to be the main reservoirs of NDM producing pathogens [10].

Metallo- $\beta$ -lactamases were common in *Pseudomonas aeruginosa* and *Acinetobacter* spp., recently they have emerged at an increasing rate among different members of

*Enterobacteriaceae* such as *Escherichia coli* and *Klebsiella pneumoniae* [11,12]. In 2011, outbreaks of NDM-1-producing *E. coli* and *Klebsiella pneumoniae* isolates have been obtained from patients hospitalised in four healthcare facilities in northern Italy [13]. Recently, a study in Nepal showed that the incidence of MBL among *E. coli* recovered from different clinical isolates was 18.98% [12]. Several studies in India showed the prevalence of MBL producing *E. coli* ranged from 1.7%-15% [14-18]. In addition, in India it was shown that 50%-81.8% of *E. coli* resistant/intermediate to carbapenems were MBL producers [19-21]. In Pakistan, it was reported that 71% carbapenem-resistant *E. coli* were MBL producers [22]. In a PCR-Based Nosocomial Surveillance Study in Puerto Rico [23], it was found 5.1% of *E. coli* isolates were MBLs producers. In Nigeria, it was found that the prevalence of MBLs among *E. coli* ranged from 12.5%-41.2% [24,25]. In Iraq, 45.2% of *E. coli* isolated from pregnant and non-pregnant women with genital tract infection were MBL producers [26].

NDM MBL producing *E. coli* was detected in Poland [27]. In Algeria, the first three autochthonous cases of infections caused by NDM-5 MBL-producing *Escherichia coli* strains were recovered from urine and blood specimens, these isolates were coexpressed blaCTX-M-15 with the blaTEM-1 and blaada2 genes [28]. In China, the first description of IncX3 plasmids carrying NDM-1  $\beta$ -lactamases in *E. coli* was reported [29]. In Japan, 90.7% of MBL producing *E. coli* carried IMP-6 gene and 9.3% carried IMP-1 gene, all IMP-6-positive isolates were carried CTX-M, 47% of IMP-6-positive isolates were positive for TEM [30]. VIM-1- $\beta$ -lactamases-producing *E. coli* has been detected in a university hospital in Greece [31]. The first report of IMP-1-producing *E. coli* in Turkey was described by Aktas [32]. In other study in Turkey, 0.0% of multidrug resistant *E. coli* isolated from a tertiary care training and research hospital were producing MBLs [33]. The first report about detection of the blaNDM-1 MBL gene in Australia was described previously [34].

This study was conducted to address part of deficient information in molecular antibiotic resistance characterization and their transmissible potential in Palestine. Therefore, this study aimed to determine the prevalence of MBLs among clinical *E. coli* isolated from North of Pales-

tine using the multiplex PCR technique, as this has not been investigated previously.

## Materials and Methods

A total of 79 isolates of *E. coli* were isolated from urine samples from Hospitals in the North of Palestine during February-July 2012. A total of 50 isolates were recovered from Hospitals in Jenin and 29 from Thabet Hospital in Tulkarm. Antimicrobial susceptibility was determined according to the Clinical and Laboratory Standard Institute (CLSI) using the disk diffusion method [35]. Antibiotic disks (Oxoid) used were Ceftriaxone (CRO) 30 $\mu$ g, Norfloxacin (NOR) 10  $\mu$ g, Nalidixic acid (30 $\mu$ g), Ciprofloxacin (CIP) 5  $\mu$ g, Tetracycline (TE) 30 $\mu$ g, Kanamycin (K) 30 $\mu$ g, Trimethoprim/Sulfamethoxazole (SXT) 1.25/23.75 $\mu$ g, Cefotaxime (CTX) 30  $\mu$ g, Meropenem (MEM) 10  $\mu$ g, Imipenem (IMP) 10  $\mu$ g and Ceftazidime (CAZ) 30 $\mu$ g. The plates were incubated at 37°C for 18-24 hrs. The zone of inhibition were measured in millimetres using a caliper. Strains were classified as resistant or susceptible according to the criteria recommended by CLSI guidelines [35].

*E. coli* DNA was prepared for PCR according to the method described previously [36]. Briefly, cells were scraped off an overnight nutrient agar plate with a sterile loop, washed with 1 ml of 1X Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8]), then the pellet was resuspended in 0.5 ml of sterile distilled H<sub>2</sub>O, and boiled for 10-15 min. The cells then were incubated on ice for 10 min. The debris was pelleted by centrifugation at 11,500 X g for 5 min. DNA concentration was determined using a spectrophotometer and the samples were stored at -20°C until use for further DNA analysis. Polymerase Chain Reaction (PCR) analysis was performed for VIM, IMP, SPM-1, GIM-1 and SIM-1 according to methods described previously [37]. Sequences of primers and size of amplicons are described in Table 1. The PCR reaction mix with a final volume of 25  $\mu$ L, was performed with 12.5  $\mu$ L of PCR premix (ReadyMix™ Taq PCR Reaction Mix with MgCl<sub>2</sub>, Sigma), 0.4  $\mu$ M of each primer and 2  $\mu$ L of DNA template. The cycling conditions were as the following: initial DNA denaturation at 94 °C for 5 min, followed by 36 cycles of 94°C for 30 s, 52°C for 40 s and 72°C for 50 s, followed by a single, final, elongation step at 72 °C

for 5min. The PCR products were detected by electrophoresis through 1.5% agarose gels to determine the size of amplified fragment after ethidium bromide staining.

**Table 1.** Target genes for PCR amplification, amplicon size, primer sequences and annealing temperature.

Target gene	Primer sequence 5'→3'	Annealing temperature	Amplicon size
<i>Imp</i>	Imp-F GGA ATA GAG TGG CTT AAY TCT C Imp-R CCA AAC YAC TAS GTT ATC T	52°C	188bp
<i>Vim</i>	Vim-F GAT GGT GTT TGG TCG CAT A Vim-R 5-CGA ATG CGC AGC ACC AG-3	52°C	390bp
<i>Gim</i>	Gim-F TCG ACA CAC CTT GGT CTG AA Gim-R AAC TTC CAA CTT TGC CAT GC	52°C	477bp
<i>Spm</i>	Spm-F AAA ATC TGG GTA CGC AAA CG Spm-R ACA TTA TCC GCT GGA ACA GG	52°C	271bp
<i>Sim</i>	Sim-F TAC AAG GGA TTC GGC ATC G Sim-R TAA TGG CCT GTT CCC ATG TG	52°C	570bp

ERIC (Enterobacterial repetitive intergenic consensus) PCR was performed using Primer ERIC1: 5'-ATG TAA GCT CCT GGG GAT TCA C-3' and Primer ERIC2: 5'-AAG TAA GTG ACT GGG GTG AGC G-3'. Each PCR reaction mix was performed in a final volume of 25 µL containing 12.5 µL of PCR premix with MgCl<sub>2</sub> (Ready-Mix<sup>TM</sup> Taq PCR Reaction Mix with MgCl<sub>2</sub>, Sigma), 1 µM of each primer, 3 µL DNA template. In addition, the master mix was modified by increasing the concentration of dNTPs to 0.4 mM, 3 mM MgCl<sub>2</sub> and 1.5 U of Taq DNA polymerase. DNA amplification was carried out using the thermal cycler (Mastercycler personal, Eppendorf, Germany) according to the following thermal conditions: initial denaturation for 2 min at 94°C was followed by 30 cycles of initial denaturation 94°C for 50 s, 50°C for 40 s and 72°C for 1 min, with a final extension step at 72°C for 5 min. The PCR products were analyzed by electrophoresis on 1.5 % agarose gel stained with ethidium bromide. The gel images were scored using a binary scoring system that recorded the presence and absence of bands as 1 and 0, respectively. A binary matrix was analyzed by the unweighted pair group method for arithmetic averages (UPGMA), using SPSS statistics software version 20 (IBM).

## Results

The antimicrobial susceptibility pattern revealed that these clinical *E. coli* strains were less resistant (24.1%) to Kanamycine and Ceftriaxone, while showed high resistant to Trimethoprim/Sulfamethoxazole (67.1%) and Tetracycline (60.8%). Data are presented in Table 2.

**Table 2.** Antibiotic resistance of 79 *E. coli* isolates recovered from different clinical samples-Palestine.

Antibiotic	Resistant strains %
Ciprofloxacin	38.0 %
Trimethoprim/Sulfamethoxazole	67.1%
Ceftriaxone	24.1%
Tetracycline	60.8%
Nalidixic acid	36.7%
Norfloxacin	29.1%
Kanamycin	24.1 %
Cefotaxime	41.8%
Ceftazidime	41.8%
Meropenem	38%
Imipenem	44.3%

Our results showed that the prevalence of MBLs among *E. coli* isolated from North of Palestine was 87.4%. The *spm* gene was the most prevalent (86.1) among these isolates. According to the places, results showed that the prevalence of MBLs genes in *E. coli* isolates recovered from Thabet Hospital Tulkarm using multiplex PCR technique was 75.9%. The *Spm* gene was the most common (72.4%) among these isolates, while the *Imp* gene was detected in 41.4% of isolates. Isolates recovered from Jenin Hospitals, the prevalence was 94% and *spm* gene was detected in all MBL producers. Other 2 genes were detected, *Imp* and *Sim*, and the prevalence was 12% and 2%, respectively. A total of 26.1% of MPL producers carried 2 genes. Data are presented in Table 3 and Figure 1.

**Table 3.** Distribution of MBL genes among *E. coli* isolated from Jenin and Tulkarm hospitals.

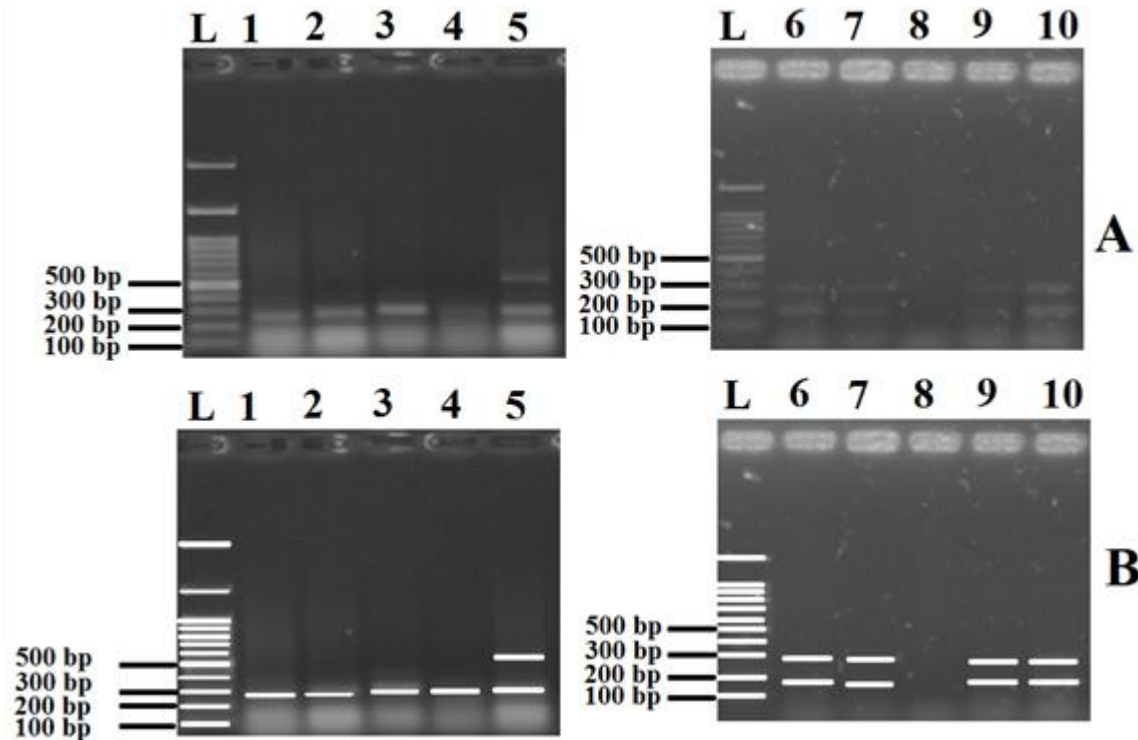
Location (N)	MBL genes n (%)				Total
	<i>spm</i>	<i>Spm+Imp</i>	<i>Spm+Sim</i>	<i>Imp</i>	
Jenin (50 isolates)	40 (80)	6 (12)	1 (2)	0 (0.0)	47 (94)
Tulkarm (29 isolates)	10 (34.5)	11 (37.9)	0 (0.0)	1 (3.4)	22 (75.9)
Total (79)	50 (63.3)	17 (21.5)	1 (1.3)	1 (1.3)	69 (87.4)

Multiple β-lactamase producing isolates have been detected (data not shown).

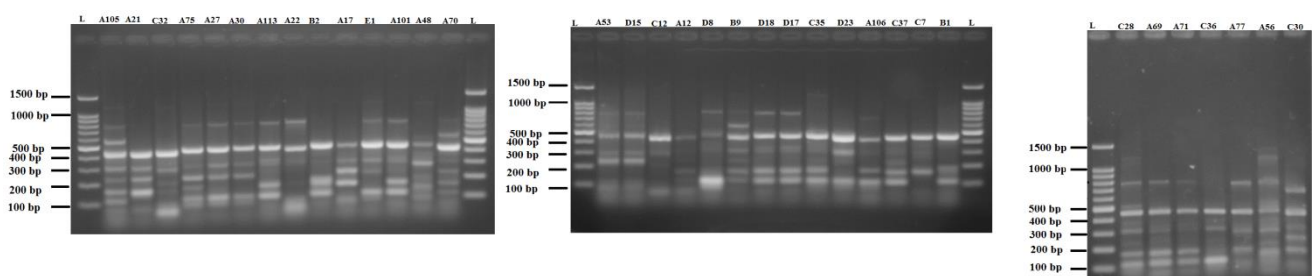
ERIC-PCR analysis of 35 isolates from Jenin Hospitals which carried genes for MBLs were genetically diverse and comprised a heterogeneous population with a total 16

ERICPCR clusters at a (50%) similarity level. Data are presented in Figures 2 and 3.

**Figure 1.** A. Multiplex PCR profile specific for MBLs. L: Lanes ladder; Lanes 1-4 for *Spm* gene (271 bp), lane 5 for *Spm* gene (271 bp) and *Sim* gene (570 bp), lane 6,7 and 9 for *Imp* gene (188 bp) and *Spm* gene (271 bp) and lane 9 for negative control. B. It is the same as A but bands are demarcated to be obvious.



**Figure 2.** DNA fingerprints generated by ERIC-PCR analysis of 35 clinical *E. coli* isolates carried genes for MBLs recovered on 1.5% garose gel. L: Lanes contained ladder, while other lanes for ERIC-PCR products.



## Discussion

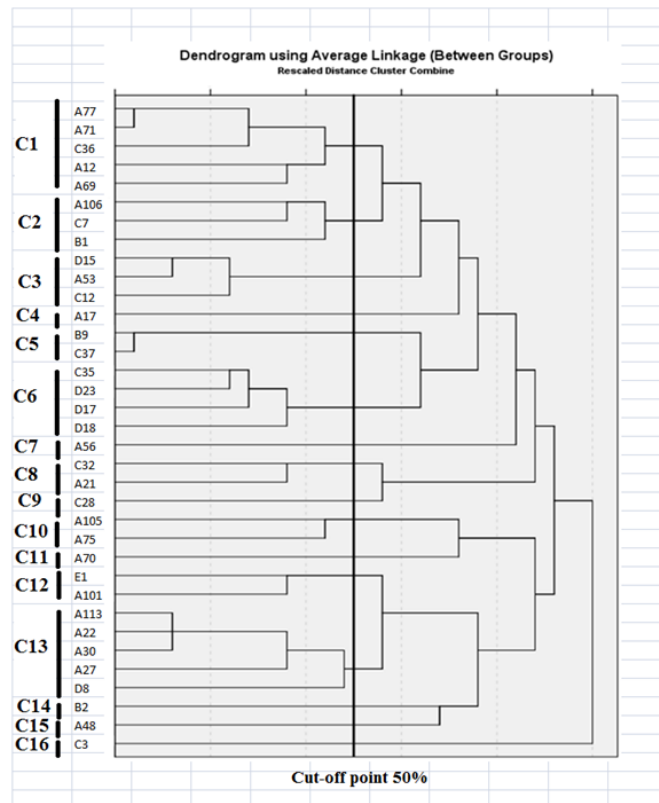
The increase in the rates of antibiotic resistance is a major cause for concern in isolates of the *Enterobacteriaceae* family. Beta-lactamses antimicrobial agents are considered one of the main treatment for serious infections. Carbapenems are considered the most active of these antibacterials, which can be used for the treatment of infections caused by ES $\beta$ L-producing microorganisms, particularly *Escherichia coli* [7]. The early detection of  $\beta$ -lactamase producing microorganisms would be important to avoid

the intrahospital dissemination of such strains and to reduce death rates among patients [16].

Studies on carbapenemase producing *E. coli* were very limited when compared to those on non-fermenters. In Palestine there are no studies about MBL producing microorganisms till now. Results of this study showed that the prevalence of MBLs among *E. coli* in North of Palestine using multiplex PCR technique was 87.4%. In other countries, the prevalence of MBL producers *E. coli* ranged from 1.7%-45.2% [12,14,18,24,26]. Finding of this re-

search showed that *Spm*-type MBL was the most common in *E. coli* isolates and this result is inconsistent with other studies in Japan [30], which showed that 100% of MBL producing *E. coli* carried IMP gene. The most widespread MBLs in other bacterial species include IMP, VIM, and NDM [5].

**Figure 3.** Dendrogram of 35 *E. coli* isolates carried genes for MBLs based on the UPGMA method derived from analysis of the ERIC-PCR profiles at a 50% similarity level. C: Cluster



The high prevalence of MBLs producers among *E. coli* isolates in Palestine may be due to several risk factors such as long term exposure to antibiotics in hospitals, prolonged hospitalization, incorrect therapy, nursing home residency, severe illness, catheterization and movement of health staff in the hospital leading to dissemination of these pathogens throughout the hospital [38,39]. Results of the study showed a differences between the prevalence of MBLs between the 2 governorates, Tulkarm and Jenin, which are both located in North of Palestine. Geographical variation in the occurrence rate of  $\beta$ -lactamases production have been detected from different countries and even from hospital-to-hospital within the same country [40-42]. Multiple  $\beta$ -lactamase has been detected in many of these isolates (data not shown) and a total of 26.1% of MBL producers carried 2 genes, this was an alarming finding.

The coexistence of different classes of  $\beta$ -lactamases in a single bacterial isolate may pose therapeutic challenges, this will seriously limited treatment options. In addition, may pose diagnostic challenge that high-level expression of certain  $\beta$ -lactamases such as the AmpC  $\beta$ -lactamases may mask the recognition of the ES $\beta$ BLs and it may result in a fatal and an inappropriate antimicrobial therapy [43]. The presence of AmpC  $\beta$ -lactamases and ES $\beta$ BLs in a single isolate decreases the effectiveness of the  $\beta$ -lactam- $\beta$ -lactamase inhibitor combinations, while MBLs and AmpC  $\beta$ -lactamases confer resistance to carbapenems [44]. In addition, coexistence of different classes of  $\beta$ -lactamases in a single bacterial isolate poses a serious concern for infection control management, associated with increases in length and cost of hospital stays [23]. Coexistence more than one type of  $\beta$ -lactamases was reported from different species of *Enterobacteriaceae* including *E. coli* [28,30,44,45,46].

The ERIC-PCR typing of MBLs-producing isolates showed various DNA banding profiles. This clonal diversity suggests that most of the strains have been unable to be maintained or spread in different settings of hospital. This observation challenges many conventional thoughts about the nosocomial epidemiology of antibiotic resistance including  $\beta$ -lactamase. These isolates recovered mostly from urine of patients treated mainly in hospitals, sharing significant patient demographics (all isolates used in ERIC-PCR typing are from patients from Jenin Governorate) and isolate characteristics including antibiotic resistance profiles differed. It is clearly indicates that multiple clones of these  $\beta$ -lactamases producing isolates were widespread in these hospitals but not sporadic. This supporting the suggestion that the high rate and extensive inappropriate use of antibiotic especially cephalosporins in the country could be the only major cause [46].

MBLs producing Gram-negative bacteria are an increasing public health problem worldwide because of their resistance to all  $\beta$ -lactams except Aztreonam. In conclusion, our results showed high occurrence of MBLs among *E. coli* isolates in Palestine. Based on these results we recommend the continuous monitoring and surveillance of the prevalence, proper control and prevention practices and effective antibiotic use will limit the further spread of MBLs producing isolates within hospitals in this country.

## Conflict of Interest

We declare that we have no conflict of interest.

## References

- Rasheed JK, Kitchel B, Zhu W, Anderson KF, Clark NC, Ferraro MJ, et al. New Delhi metallo- $\beta$ -lactamase producing Enterobacteriaceae, United States. *Emerg Infect Dis*. 2013; 19:870-8.
- Bush K. Bench-to bedside review: The role of  $\beta$ -lactamases in antibiotic-resistant Gram-negative infections. *Crit Care*. 2010;14 (3): 224.
- Bebrone C. Metallo-beta-lactamases (classification, activity, genetic organization, structure, zinc coordination) and their superfamily. *Biochem Pharmacol*. 2007;74:1686-701.
- Kong KF, Schneper L, Mathee K. Beta-lactam antibiotics: from antibiosis to resistance and bacteriology. *APMIS*. 2010;118 (1):1-36.
- Patel G, Bonomo RA: "Stormy waters ahead": global emergence of carbapenemases. *Front Microbiol*. 2013,4:48.
- Cornaglia G, Giamarellou H, Rossolini GM. Metallo- $\beta$ -lactamases: a last frontier for  $\beta$ -lactams. *Lancet Infect Dis*. 2011;11:381-93.
- Deshmukh DG, Damle AS, Bajaj JK, Bhakre JB, Patwardhan NS. Metallo- $\beta$ -lactamase-producing clinical isolates from patients of a tertiary care hospital. *J Lab Physicians*. 2011;3(2):93-7.
- Meini MR, Llarrull LI, Vila AJ. Evolution of Metallo- $\beta$ -lactamases: Trends revealed by natural diversity and in vitro evolution. *Antibiotics (Basel)*. 2014;3(3):285-316.
- Hammami S, Boutiba-Ben Boubaker I, Ghozzi R, Saidani M, Amine S, Ben Redjeb S. Nosocomial outbreak of imipenem-resistant *Pseudomonas aeruginosa* producing VIM-2 metallo- $\beta$ -lactamase in a kidney transplantation unit. *Diagn Pathol*. 2011; 6:106.
- Dortet L, Poirel L, Nordmann P. Worldwide dissemination of the NDM-type carbapenemases in Gram-negative bacteria. *Biomed Res Int*. 2014;2014:249856.
- Walsh TR. Emerging carbapenemases: a global perspective. *Int J Antimicrob Agents* 2010; 36(Suppl 3):S8-S14.
- Bora A, Sanjana R, Jha BK, Mahaseth SN, Pokharel K. Incidence of metallo-beta-lactamase producing clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* in central Nepal. *BMC Res Notes*. 2014;7:557.
- Gaibani P, Ambretti S, Berlingeri A, Cordovana M, Farruggia P, Panico M, et al. Outbreak of NDM-1-producing Enterobacteriaceae in northern Italy, July to August 2011. *Euro Surveill*. 2011;16(47):20027.
- Datta S, Wattal C, Goel N, Oberoi JK, Raveendran R, Prasad KJ. A ten year analysis of multi-drug resistant blood stream infections caused by *Escherichia coli* & *Klebsiella pneumoniae* in a tertiary care hospital. *Indian J Med Res*. 2012;135(6):907-12.
- Usha K, Kumar E, Gopal DS. Occurrence of various beta-lactamase producing gram negative bacilli in the hospital effluent. *Asian J Pharm Clin Res*. 2013;6(Suppl 3):42-6.
- Wadekar MD, Anuradha K, Venkatesha D. Phenotypic detection of ESBL and MBL in clinical isolates of Enterobacteriaceae. *Int J Curr ResAca Rev*. 2013;1(3):89-95.
- Chakraborty A, Adhikari P, Shenoy S, Shrikala B, Rao S, Biranthabail D, et al. Expression of ESBL, MBL and AmpC  $\beta$ -lactamases by extra intestinal *Escherichia coli* isolates: correlation with treatment and clinical outcome. *J Microbiol Infect Dis*. 2013;3:150-6.
- Chakraborty A, Adhikari P, Shenoy S, Saralaya V. Characterization of plasmid mediated AmpC producing *Escherichia coli* clinical isolates from a tertiary care hospital in South India. *Indian J Pathol Microbiol*. 2014;57(2):255-8.
- Bhaskar MM, Anand R, Harish BN. Prevalence of blaNDM-1 producing blood isolates of *Escherichia coli*, *Klebsiella* species and *Enterobacter* species in a tertiary care centre in South India. *J Microbiol Res Rev*. 2013;1(6): 61-8.
- Sangeetha KT, Golia S, Vasudha CL. Phenotypic detection of metallo beta-lactamase in Gram-negative bacterial isolates. *CIBTech J Microbio*. 2014;3(1):5-10.
- Basireddy S, Singh M, Reddy H, Ali S, Kabra V. Metallo- $\beta$ -Lactamase producing clinical isolates in a tertiary care hospital. *Int J Sci Res*. 2014;3(10):1324-6.
- Kaleem F, Usman J, Hassan A, Khan A. Frequency and susceptibility pattern of metallo-beta-lactamase producers in a hospital in Pakistan. *J Infect Dev Ctries*. 2010;4(12):810-3.
- Robledo IE, Aquino EE, Vázquez GJ. Detection of the KPC gene in *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* during a PCR-based nosocomial surveillance study in Puerto Rico. *Antimicrob Agents Chemother*. 2011;55(6):2968-70.
- Enwuru NV, Enwuru CA, Ogonnia SO, Adepoju-Bello AA. Metallo- $\beta$ -lactamase production by *Escherichia coli* and *Klebsiella* Species isolated from hospital and community subjects in Lagos, Nigeria. *Nat Sci*. 2011;9(11):1-5.

25. Chika E, Malachy U, Ifeanyichukwu I, Peter E, Thaddeus G, Charles E. Phenotypic detection of Metallo- $\beta$ -Lactamase (MBL) enzyme in Enugu, Southeast Nigeria. *Am J Biol chem pharm Sci*. 2014;2(2):1-6.
26. Ahmad SS, Ali FA. Detection of ESBL, AmpC and Metallo Beta-Lactamase mediated resistance in Gram-negative bacteria isolated from women with genital tract infection. *Eur Sci J*. 2014;10(9):193-209.
27. Baraniak A, Izdebski R, Fielt J, Gawryszewska I, Bojarska K, Herda M, et al. NDM-producing Enterobacteriaceae in Poland, 2012-14: inter-regional outbreak of *Klebsiella pneumoniae* ST11 and sporadic cases. *J Antimicrob Chemother*. 2015; pii: dkv282.
28. Sassi A, Loucif L, Gupta SK, Dekhil M, Chettibi H, Rolain JM. NDM-5 carbapenemase-encoding gene in multidrug-resistant clinical isolates of *Escherichia coli* from Algeria. *Antimicrob Agents Chemother*. 2014;58(9):5606-8.
29. Ho PL, Li Z, Lo WU, Cheung YY, Lin CH, Sham PC, et al. Identification and characterization of a novel incompatibility group X3 plasmid carrying bla NDM-1 in Enterobacteriaceae isolates with epidemiological links to multiple geographical areas in China. *Emerg Microbes Infect*. 2012;1(11):e39.
30. Yano H, Ogawa M, Endo S, Kakuta R, Kanamori H, Inomata S, et al. High frequency of IMP-6 among clinical isolates of metallo- $\beta$ -lactamase-producing *Escherichia coli* in Japan. *Antimicrob Agents Chemother*. 2012;56(8):4554-5.
31. Scoulica EV, Neonakis IK, Gikas AI, Tselentis YJ. Spread of bla(VIM-1)-producing *E. coli* in a university hospital in Greece. Genetic analysis of the integron carrying the bla(VIM-1) metallo-beta-lactamase gene. *Diagn Microbiol Infect Dis*. 2004;48(3):167-72.
32. Aktas Z, Satana D, Kayacan C, Ozbek B, Gurler N, Somer A, et al. Carbapenem resistance in Turkey: Repeat report on OXA-48 in *Klebsiella pneumoniae* and first report on IMP-1 beta-lactamase in *Escherichia coli*. *Afr J Microbiol Res*. 2012;6(17):3874-8.
33. Altun Ş, Tufan ZK, Yağcı S, Önde U, Bulut C, Kınıklı S, et al. Extended Spectrum Beta-lactamases, AmpC and metallo beta-lactamases in emerging multi-drug resistant Gram-negative bacteria in intensive care units. 2013;2: 707.
34. Poirel L, Lagrutta E, Taylor P, Pham J, Nordmann P. Emergence of metallo- $\beta$ -lactamase NDM-1-producing multidrug-resistant *Escherichia coli* in Australia. *Antimicrob Agents Chemother*. 2010;54(11): 4914-6.
35. Clinical Laboratory Standards Institute (CLSI): Performance standards for antimicrobial susceptibility testing. Twenty second informational supplement. Wayne, PA, USA: CLSI: M100-S22. 2012.
36. Adwan G, Adwan K, Jarrar N, Salama Y, Barakat A. Prevalence of seg, seh and sei genes among clinical and nasal *Staphylococcus aureus* isolates. *Br Microbiol Res J*. 2013;3:139-49.
37. Ellington MJ, Kistler J, Livermore DM, Woodford N. Multiplex PCR for rapid detection of genes encoding acquired metallo- $\beta$ -lactamases. *J Antimicrob Chemother*. 2007;59(2):321-2.
38. Waiwarawooth J, Jutiworakul K, Joraka W. The prevalence and susceptibility patterns of ESBL-producing *Klebsiella pneumoniae* and *Escherichia coli* in Chonburi hospital. *J Infect Dis Antimicrob Agents* 2006; 23(2):57-65.
39. Kateregga JN, Kantume R, Atuhaire C, Lubowa MN, Ndukupi JG. Phenotypic expression and prevalence of ESBL-producing Enterobacteriaceae in samples collected from patients in various wards of Mulago Hospital, Uganda. *BMC Pharmacol Toxicol* 2015;16:14.
40. Jain A, Roy I, Gupta MK, Kumar M, Agarwal SK. Prevalence of ESBL producing gram negative bacteria in septicemic neonates in a tertiary care hospital. *Indian J Med Microbiol*. 2003;52:421-5.
41. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. *Clin Microbiol Rev*. 2005;18(4):657-86.
42. Livermore DM. Current epidemiology and growing resistance of Gram-negative pathogens. *Korean J Intern Med*. 2012;27(2):128-42.
43. Oberoi L, Singh N, Sharma P, Aggarwal A. ESBL, MBL and Ampc  $\beta$ -lactamases producing superbugs-havoc in the intensive care units of Punjab India. *J Clin Diagn Res*. 2013;7:70-3.
44. Chatterjee SS, karmacharya R, Madhup SK, Gautam V, Das A, Ray P. High prevalence of co-expression of newer  $\beta$ -lactamases (ESBLs, Amp-C- $\beta$ -lactamases, and metallo- $\beta$ -lactamases) in Gram-negative bacilli. *Indian J Med Microbiol*. 2010;28(3), 267-8.
45. Adwan K, Jarrar N, Abu-Hijleh A, Adwan G, Awwad E. Molecular characterization of *Escherichia coli* isolates from patients with urinary tract infections in Palestine. *J Med Microbiol*. 2014;63:229-34.
46. Adwan G, Abu Jaber A. Frequency and molecular characterization of  $\beta$ -lactamases producing *Escherichia coli* isolated from North of Palestine. *Br Microbiol Res J*. 2016;11(5).