# Phenotypic Detection of Metallo-β - Lactamases Production in Clinical Isolates of Gram Negative Bacilli

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# الكشف المظهري عن انتاج محللات البيتا لاكتام الفلزية في المعزولات الاكلينيكية للعصيات سالبة الجرام

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# Summary and conclusions

Metallo-β lactamases have recently emerged as one of the most worrisome resistance mechanisms owing to their capacity to hydrolyze with the exception of aztreonam, all β-lactams including carbapenems. Their genes are carried on highly mobile elements, allowing easy dissemination and are difficult to detect, posing significant risks due to their ability to participate in horizontal MBL gene transfer. There are no standardized methods for detecting MBLs carrying organisms and despite PCR being highly accurate and reliable, its accessibility is often limited to reference laboratories. Several non molecular techniques have been studied, all taking advantage of the enzyme's zinc dependence by using chelating agents, to inhibit its activity.

The present study aimed to evaluate imipenem- EDTA CDT and imipenem -EDTA DDST (in comparison to MBLs E-test) as simple reliable inexpensive methods for phenotypic detection of MBLs production by Gram negative bacilli.

This study included 115 isolates of Gram negative bacilli obtained from various clinical samples including urine, pus from wounds, LRT samples, burn exudates, blood, vaginal discharge, purulent ascitic fluid, purulent pleural effusion and ear discharge. The most common isolated pathogen was *Ps. aeruginosa* (39%), followed by *A. baumanni* (19%), then *E.coli* (15.7%)*, Klebsiella* (13%), *Proteus* (12%) and *Serratia* (1%).

Out of the 115 Gram negative isolates, 31 (27%) showed resistance to imipenem (16 isolates of *Ps. aeruginosa*, 12 *A. baumannii,* 2 *E.coli spp.* and 1 *Klebsiella spp.)*. The most common samples from which the imipenem resistant isolates were isolated were pus from wounds (29%), followed by urine and LRT samples (22.6%) then burn exuadate (16%), blood

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# *List of Abbreviations*

| Abbreviation | Title |
|---|---|
| *A.baumannii* | *Acinetobacter Baumannii* |
| AHL-SMs | Acylated homoserine lactone signaling molecules |
| API 20 NE | Analytical profile index 20 None Enterobacteriacae |
| ASU | Ain Shams University |
| ASUHs | Ain Shams University Hospitals |
| ATMO | Amino thiazolyl methoxyimino |
| bla | Beta lactamase |
| CDC | Centers for disease control and prevention |
| CDT | Combined disk test |
| CFU | Colony forming unit |
| CLSI | Clinical Laboratory and Standard Institute |
| COPD | Chronic obstructive pulmonary disease |
| DDST | Double disk synergy test |
| *E. coli* | *Escherichia coli* |
| EDTA | Ethylene-diamine-tetraacetic acid |
| ESAC | Extended-spectrum AmpC |
| ESBLs | Extended spectrum beta lactamases |
| FDA | Food and drug administration |
| GIM | German imipenemase |
| ICU | Intensive care unit |
| IMP | Imipenemase |
| KD | Kilo Dalton |
| *K.pneumoniae* | *Klebsiella pneumoniae* |
| KPC | *Klebsiella pneumomiae* carbapenemase |
| LRT | Lower respiratory tract |

| | |
|---|---|
| MBLs | Metallo-beta lactamases |
| MHA | Muller Hinton agar |
| MIC | Minimal inhibitory concentration |
| MPA | Mercaptopropionic acid |
| NAG | N-acetylglucosamine |
| NAM | N-acetylmuramic acid |
| NDM | New Delhi metallo beta lactamase |
| NMC-A | Non metallo-*c*arbapenamase of class A |
| No. | Number |
| OMP | Outer membrane protein |
| OXA | Oxacillinase |
| PBPs | Penicillin binding proteins |
| PCR | Polymerase chain reaction |
| PPV | Positive predictive value |
| *Ps.aeruginosa* | *Pseudomonas aeruginosa* |
| S. *maltophilia* | *Stenotrophomonas maltophilia* |
| SD | Standard deviation |
| SHV | Sulfhydryl Reagent Variable |
| SMA | Sodium mercaptoacetic acid |
| SPM | Sao Paulo metallo beta lactamase |
| *Spp.* | Species |
| TSX | Trimethoprim/ sulfamethoxazole |
| VIM | Veronese imipenemase |

# Introduction

The introduction of carbapenems into clinical practice represented a great advance for the treatment of serious bacterial infections caused by β-lactam-resistant bacteria. Due to their broad spectrum of activity and stability to hydrolysis by most β-lactamases including the extended spectrum ß -lactamases (ESBLs) and AmpC, the carbapenems have been the drugs of choice for treatment of infections caused by penicillin- or cephalosporin-resistant Gram-negative bacteria ***(Zhanel et al.,2007)***. However there has been an increase in the prevalence of carbapenem resistance reported mainly for *Pseudomonas aeruginosa (Ps.aeruginosa)* and *Acinetobacter spp.* in several countries ***(Gladstone et al., 2005)***

Carbapenem resistance is through lack of drug penetration (i.e., porin mutations and efflux pumps) and/or carbapenem-hydrolyzing β-lactamases. Based on molecular studies, two classes of carbapenem-hydrolyzing enzymes have been described: serine enzymes, possessing a serine moiety at the active site, and metallo-β-lactamases (MBLs) which require divalent cations as cofactors for enzyme activity ***(Bush, 2001; Gladstone et al., 2005).***

MBLs are group of zinc based β-lactamases. They are naturally occurring in several Gram positive and Gram negative organisms. The genes responsible for MBL production may be chromosomally or plasmid-mediated and hence pose a threat of spread of resistance by gene transfer among the Gram-negative bacteria ***(Gladstone et al., 2005)***.

The MBLs encoded on transferable genes include IMP, VIM, SPM, and GIM. Most, if not all, genes encoding IMP ,VIM ,SPM as well as GIM are found as gene cassettes in class 1 integrons carried by transferable large plasmids ***( Castanheira et al.,2004)*** although IMP-1 allele, $bla_{\text{IMP-1}}$ was also found on class 3 integrons ***(Collis et al.,2002)***.

Acquired MBLs is one of the most worrisome resistance mechanisms because they can hydrolyze with the exception of aztreonam, all β-lactams including carbapenems. In addition, their genes are carried on highly mobile elements, allowing easy dissemination. They are also not susceptible to therapeutic serine β- lacatamase inhibitors (such as clavulanate and sulfones) and there's no therapeutic inhibitor for them ***(Walsh et al., 2005).***

MBLs genes have spread from *Ps. aeruginosa* to members of *Enterobacteriaceae* ***(Peleg et al.,2005)*** and MBLs producing isolates were associated with higher morbidity &mortality ***(Walsh et al., 2005).***

With the global increase in the occurrence and types of MBLs, early and accurate detection is crucial for implementation of strict infection control practices and treatment with alternative antimicrobials ***(Nordmann and Poirel, 2002).***

Despite polymerase chain reaction (PCR) being highly accurate and reliable in detection of MBLs, its accessibility is often limited to reference laboratories and it's of limited practical use for daily application in clinical laboratories because of the cost ***(Franklin et al., 2006).*** Thus, the development of a simple and inexpensive testing method for detecting MBLs production is necessary.

Several non molecular techniques have been studied, all taking advantage of the enzyme's zinc dependence by using chelating agents, such as EDTA or 2- mercaptopropionic acid (MPA), to inhibit its activity ***(Franklin et al., 2006).***

E-test (MBL E-test) gradient formats were developed for detection of MBLs based on the reduction of imipenem or ceftazidime minimal inhibitory concentrations (MICs) in the presence of EDTA or 2-MPA. The Etest MBL results were in 100% agreement with the results from the genotypic and biochemical methods ***(Timothy et al., 2002).***

# Aim of the work

Evaluation of imipenem- EDTA combined disk test and imipenem -EDTA double disk synergy test (in comparison to metallo-β-lactamase E-test) as simple reliable inexpensive methods for phenotypic detection of metallo-β-lactamases production by Gram negative bacilli isolated from different clinical specimens.

# Antibiotic Resistance in Gram Negative Bacilli

The emergence of antibiotic-resistant Gram negative pathogens including *Acinetobacter spp., Pseudomonas aeruginosa (Ps. aeruginosa)*, and *Enterobacteriaceae* is a major public health concern, particularly in hospitals and other health care settings ***(Bhavnani et al., 2006).*** This is because:

1- Antibiotic-resistant organisms appear to be biologically fit and are capable of causing serious, life-threatening infections.

2- The presence of multi-resistant strains of these organisms has been associated with prolonged hospital stay, higher health care costs, and increased mortality, particularly when initial antibiotic therapy does not provide coverage of the causative pathogen.

3- Antibiotic-resistant organisms are difficult to manage, because treatment options are limited.

4- The increase in the prevalence of drug-resistant pathogens is occurring at a time when the discovery of new antibiotic agents is slowing down dramatically.

Consequently, there is concern that in the not-too-distant future, we may be faced with a growing number of potentially untreatable infections ***(Slama, 2008).***