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# Prevalence of *Pseudomonas aeruginosa* producing Metallo-β- lactamases in wounds and burns infections

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## ABSTRACT:

Metallo β -lactamases (MBLs) producing *Pseudomonas aeruginosa* have been detected from clinical isolates in worldwide with increase in emergence in the last years . The spread of MBLs positive isolates in a localized hospital environment causes not only a therapeutic problem but as well as a serious concern for infection control handling , hence , this study was aimed to determine the prevalence of *P. aeruginosa* producing MBLs isolated from some skin infections (wounds and burns)**.** A total of 57 *P. aeruginosa* were isolated from wounds and burns infections (24 wound swabs and 33 burn swabs) in Al-Hussain Teaching Hospital .Primary screening of carbapenems resistant isolates appeared that 63% (36 isolates) were resistant to imipenem and meropenem .Among the 36 carbapenems resistant isolates that were tested for production MBLs by phenotypic test (CDT)with EDTA inhibitor (as chelating factor ), 34 (94%) were MBLs positive and 59.6% from 57 isolates were positive to MBLs production . MICs values of MBLs producers were higher in imipenem ((≥16-64) µg/ml)) than that in meropenem (( ≥16-32) µg/ml)) . Out of 34 MBLs producer , 24 (72.7%) isolates were founded in specimens of burn swabs , while the wound swabs specimens registered 41.6% . MBLs producing isolates were also tested for antibiotics susceptibility , all isolates (100%)were sensitive to aztreonam and 70.5% to ciprofloxacin . 100% of isolates were resistant to cefoxitin and amoxycillin – clavulanic acid.

**KEYWORDS :** *Pseudomonas* , Metallo-β- lactamases ,wounds, burns ,EDTA.

## INTRODUCTION:

*Pseudomonas* a very important genus, consist of aerobic , gram – negative rods , motile , they are very common in soil and other natural environment , *Pseudomonas aeruginosa* produces a soluble , blue – green pigmentation, in certain condition , especially in weakened hosts , this bacterium can infect the burns , wounds , urinary tract and can cause sepsis , meningitis and abscesses (Tortora *et al*., 2004).The organism is resistant to ,and may multiply in many disinfectants and antiseptics commonly used in hospitals (Greenwood *et al* .,2007). Nosocomial infections with these organisms tend to appear in association with contamination of commercial products such as disinfectants ,blood product, respiratory therapy equipment (Henderson *et al*., 1988) . Burned patients are especially at risk ,the presence of the organism in ward air , dust and in eschar from the burns suggests that infection can be air borne . However ,contact spread is probably more important than the air borne route . transmission may occur directly via the hands of medical staff , or indirectly via contaminated apparatus(Greenwood *et al* .,2007). *P. aeruginosa* produces infection of wounds and burns , giving rise to blue – green pus (Brooks *et al*., 2007).

Drug resistance in these bacteria very common ,these result from inherent resistance (missing high affinity porin some drugs enter through) ;through plasmid – mediated beta lactamases and acetylating enzymes (Louise *et al*., 2004)

Carbapenems are powerful, broad-spectrum antibiotics that have proven to be safe and effective in treating serious infections, it provides better control of Gram-negative bacteria than other beta-lactam antibiotics. It is stable against beta-lactamasese and AmpC beta-lactamase enzymes, making it effective in treating multidrug resistance bacteria (Baughman,2009) . The emergence of carbapenems – resistant organisms is worrisome after microbial treatment options have become very limited (Dahiya *et al*., 2015).

Carbapenemases are β –lactamases belong to numbers of molecular classes : A,B,D. Class A (Bush group 2f) enzymes(KPC,SME, IMI.NMC,GES) are inhibited by clavulanic acid and hydrolyze cephalosporines and penicillins more efficiently than carbapenems (Bush *et al*., 1995; Gladstone *et al*., 2005) . The second class (B enzymes) of carbapenemases (Bush group 3) are metallo - β –lactamases (MBL), and class D carbapenemases , they belong to the OXA family (OXA23 to OXA 27) (Thomson ,2010 ;Gladstone *et al*., 2005). metallo - β -lactamases (MBLs) are able to hydrolyze all β –lactams antibiotics and carbapenems efficiently with the exception of monobactams (aztreonam) and resist to β –lactamase inhibitors but are inhibited by chelating factors such as EDTA (Thomson , 2010). The genes responsible for production of metallo beta lactamases may be chromosomally or plasmid mediated ,the common metallo beta lactamases families include VIM, IMP,GIM,SIM are chromosomally ,the first imipenem resistance located on plasmid was first detected in Japan in 1990 ,in clinical isolate of *Pseudomonas aeruginosa* (Dahiya *et al*., 2015).

Since early 1990, new genes have been encoded for metallo –beta- lactamase enzymes they are registered in over the world in important medical bacteria such as *Pseudomonas aeruginosa* (Zavascki *et al*., 2005 ; Aghamiri *et al*., 2014) .Resistance to imipenem by *P.aeruginsa* resulting from MBL recorded to be an important cause of hospital infections and is a critical therapeutic problem all over the world (Franco *et al.*,2010). In addition, the increased mortality rates have been documented for patients with bacteria of *P. aeruginsa* producing metallo- beta-lactamase especially because of insufficient empirical treatment (Zavascki *et al.*, 2006). Because these bacteria are the main causes of hospital infections in our hospitals, especially burns patients and the emergence of isolates, including resistance to carbapenems (Varaiya *et al.*, 2008), this study was aimed to isolating and diagnosis of *Pseudomonas aeruginosa* infections of burns and wound and the study of production of metallo –beta- lactamases.

## MATERIALS AND METHOS:

### Bacterial isolates:

A total of 57 isolates of *Pseudomonas aeruginosa* were collected from two types of skin infected specimens ( wound swabs and burn swabs ) in the Al-Hussain Teaching Hospital in Al-Nasiriyah city ,Thi –Qar province ,Iraq. Bacterial isolates were identified by using API 20 E ((Bio Merieux, France)) and biochemical tests according to (Holt *et al*., 1994; Collee, *et. al*., 1996).

### Antibiotic susceptibility tests:

Susceptibility testing of isolates were determined by standard disk diffusion method on Mueller Hinton agar(BD&BBL , USA) according to CLSI recommendations (CLSI,2009), antibiotics disks tested (symbol /in µg) were imipenem * (IMI /10) , meropenem * (MEM/10), ceftazidime * (CAZ /30) ,cefoxitin * (FOX /30), Amoxicillin-clavulanic acid*** (AMC/20-10), gentamycin** (CN)(10), amikacin** (AK /30) , cephalothin *** (CF /30) , tobramycin** (TB /10 µg) , ciprofloxacin** (CIP /5 µg) , oxacillin** (OX /1) , piperacillin ** (PI /100) , aztreonam** * (ATM / 30).

* Mast group Ltd. Merseyside.U.K. , **:Bioanalyse, Turkey
***:BD&BBL, USA

**MBLs production tests:**

Resistant isolates to imipenem and meropenem were suspected to MBLs production .MBLs were detected by Combined Disk (CD) Test using EDTA (Varaiya *et al*., 2008 ; Qu *et al*., 2009; Upadhyay & Bhattacharjee , 2010 ) as described below :

***Combined Disk (CD) Test***

EDTA (0.5 M)solution was prepared by dissolving 186.1 g of EDTA in 1000 ml of distilled water.The PH was adjusted to 8.0 using NaOH and sterilized by autoclaving (Sambrook & Russell , 2001).Two of each 10 µg –imipenem and meropenem disks were placed on the plates of MHA which were inoculated by test organism, and appropriate amount (10 µL) of an EDTA solution were added to one of imipenem and one of meropenem disk . The inhibition zones of the imipenem and meropenem alone and with EDTA were measured after 24 hr of incubation in air at 35 °C. An increased in the zone of inhibition around the imipenem –EDTA disk and meropenem –EDTA disk ≥ 7 mm were registered as a positive result.

**Minimal inhibitory concentration (MIC):** The MICs were determined according to the method of (Stock & Ridgway, 1987 ; Piddock , 1990) .The values were compared with the break points recommended by CLSI (2006).

## RESULTS:

By disk diffusion susceptibility testing ,all of the 57 *pseudomonas aeruginosa* isolates (100%) were resistant to oxacillin ,amoxicillin + clavulanic acid , cephalotin and pipracillin , it was found that 82% of the isolates were resistant to cefoxitin ,63% were resistant to imipenem and meropenem , while sensitivity of isolates to ciprofloxacin were more than other antibiotics , it was registered 72% (Figure 1).

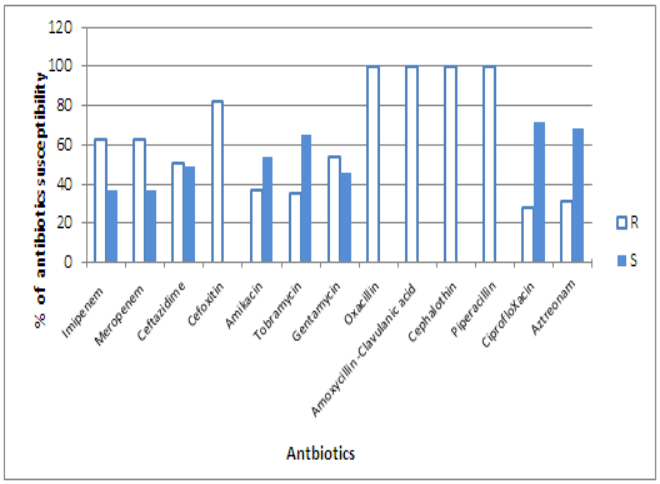


Figure (1): Antibiotics susceptibility of *Pseudomonas aeruginosa* isolates (no=57). R : Resistant , S: Sensitive.

Thirty six (63%) isolates which resistant to carbapenems (imipenem and meropenem) were used to detection MBLs by combined – disk test (CDT), 34 (94%) of the tested isolates were MBLs positive to each imipenem and meropenem resistant isolates (Figure2). Two isolates (6%)of carbapenem resistant were negative to production of MBLs by this test . In compared to total number (57)of isolates that use in this study , the results were illustrated 59.6% of all isolates of *Pseudomonas aeruginosa* were positive to MBLs production , and 40.4 % were negative to these enzymes ( Table 1).

Table 1: Prevalence of MBLs producing isolates by combined –disk test (CDT).

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| Carbapenems | No. of carbapenem resistant isolates | No. &(%) of MBLs producing isolates (no=36) | No. &(%) of MBLs Non producing isolates (no=36) | No. &(%) of MBLs producers from total isolates (no=57) | No. &(%) of MBLs Non Producers from total isolates (no=57) |
|---|---|---|---|---|---|
| Imipenem | 36 | 34(94) | 2(6) | 34(59.6) | 23(40.4) |
| Meropenem | 36 | 34(94) | 2(6) | 34(59.6) | 23(40.4) |

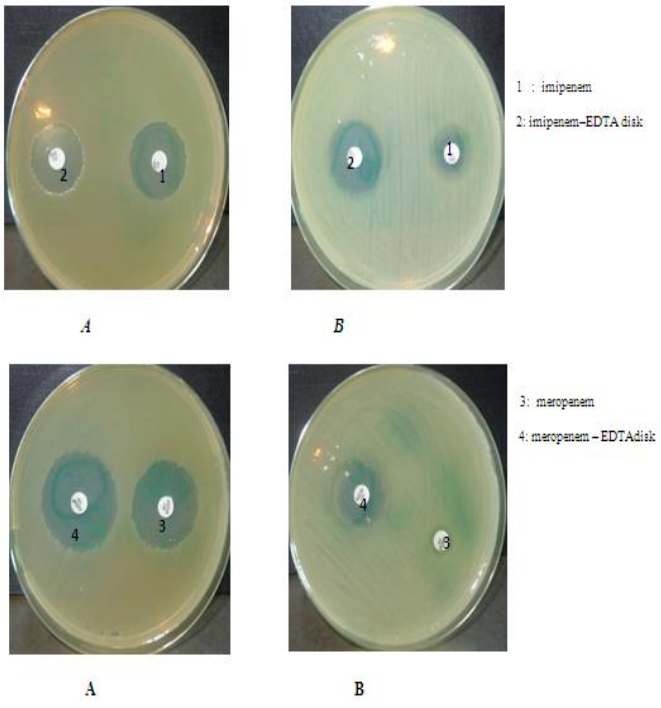


Figure ( 2) : Combined –Disk Test (CDT) for detection MBLs producing *P.aeruginosa* .A: negative result , B : positive result, showing enhancement of zone inhibition around imipenem-EDTA (1) and meropenem – EDTA(4).

Table 2 gives MICs of selected carbapenem antibiotics to MBLs positive isolates by phenotypic test (CDT). It was appeared from results , all 34 isolates were resistant to imipenem and meropenem , and MICs values of imipenem were higher ((≥16-64) µg/ml) than that in meropenem ((≥16-32) µg/ml).

Table (2): $MIC_S$ of selected carbapenem antibiotics to MBLs positive isolates(no= 34)

| Carbapenems | Ranges of MICs (µg/ml) | No. &(%) of resistant isolates | No. &(%)of sensitive isolates |
|---|---|---|---|
| Imipenem | ≥16-64 | 34(100) | 0 |
| Meropenem | ≥16-32 | 34(100) | 0) |

*P.aeruginosa* strains isolated from two types of skin infected specimens were 24 (42.1%) wound swabs and 33(57.8%) in burn swabs. Out of 34 MBLs producing isolates, 24(72.7%) isolates were founded in specimens of burn swabs, while the wound swabs specimens were registered the lower percentage(41.6%) of MBLs producing isolates (Table 3).

Table 3: Distribution of MBLs producing isolates according to types of skin infected specimens

| Types of skin infected specimens | No. & (%)of *Ps.aeruginosa* Isolates | No. & (%)of MBLs producing isolates |
|---|---|---|
| Wound swabs | 24(42.1) | 10(41.6) |
| Burn swabs | 33(57.8) | 24(72.7) |
| Total | 57 | 34(59.6) |

The results in figure 2 showed antibiotic susceptibility of metallo - β –lactamases producing isolates , these isolates were 100% sensitive to Aztreonam and 70.5% to ciprofloxacin . The highest (100%) of resistance were observed in cfoxitin and amoxicillin –clavulanic acid , and isolates were appeared high rates of resistance to other antibiotics were included cephalothin (97%) , gentamycin (88.2%)and ceftazidime , piperacillin in percentage 85.2%.

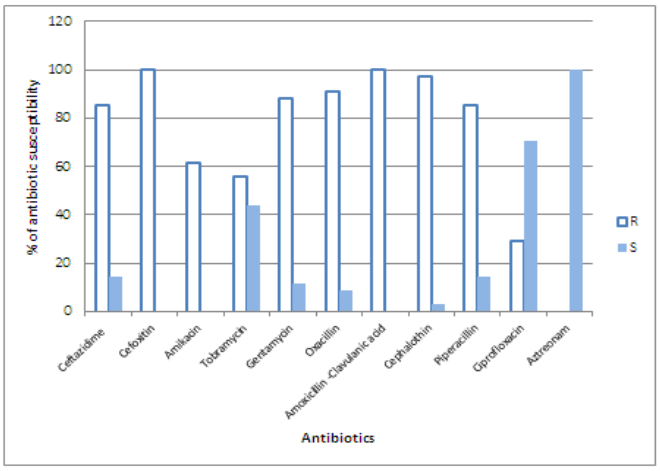


Figure2 : Antibiotics susceptibility of MBLs - producing isolates (no=34). R : Resistant, S: Sensitive.

## DISCUSSION:

In recent years, metallo – beta – lactamases (MBLs) have emerged among some G⁻ pathogens, such as *Pseudomonas aeruginosa* , members of Enterobacteriaceae, *Acinetobacter baumanii* ,and *Proteus mirabilis* (Walsh *et al*.,2005**).** *Pseudomonas aeruginosa* originally resistant to number of antibiotics such as newer β –lactam antibiotics or can develop resistance during therapy causing to high mortality and morbidity (Basak *et al*., 2009). The results of this study indicate that the isolates of *Pseudomonas aeruginosa* showed a high percentage of resistance to bata – lactam antibiotics such as oxacillin, amoxycillin – clavulanic acid, cephalothin, piperacillin and cefoxitin,these may be due to production β – lactamases enzymes . β –lactamases is the most common mechanism of β –lactaman tibiotics resistance in G ⁻ bacteria . Newer β –lactamases that hydrolyze cephamycins (e.g. cefoxitin ), oxyimino and zwitterionic cephalosporins are of increasing concern because they restrict therapeutic options , result treatment failures and resistance by production β –lactamases are increasing in occurrence (Bush ,2001; Bradford ,2001), the isolates were also had high resistant to carbapenems (imipenem and meropenem), this finding was supported by other study carried out in Iran, it was founded high frequency of resistance by *P. aeruginosa* against imipenem and meropenem (100%)(Vaez *et al*., 2015), and not agreement with (Basak *et al*., 2009) who founded 92.2% of *Pseudomonas aeruginosa* were sensitive to imipenem and meropenem. All these carbapenems resistant isolates were resistant to amoxycillin – clavulanic acid . Clavulanic acid is β –lactamases inhibitors that using in inhibition extended –spectrum β –lactamases (ESBL) (Mentec *et al*., 1992), this property used in differentiation of ESBLs from AmpC - β – lactamases produced by bacteria which have 3rd generation of cephalosporins as their substrates, but not inhibited by clavulanic acid (Chow *et al*., 1991). AmpC β –lactamases are types of β – lactamases (cephalosporinases) , they confer resistance to narrow – expanded, and broad spectrum cephalosporins, aztreonam ,beta – lactam – beta – lactamase inhibitor combinations and poorly inhibited by clavulanic acid , they are inhibited by cloxacliilin (Bush *et al*., 1995). Gram negative bacteria expressing AmpC β –lactamases are usually resistant to all β –lactam antibiotics except carbapenems (Ratna *et al*., 2003) , Due to these reasons and because these enzymes are resistant to clavulanic acid, but sensitive to carbapenems, these properities do not apply on our isolates that resistant to clavulanic acid and carbapenems (imipenem and meropenem) . Therefore, resistance against carbapenems is not attributed to the AmpC β –lactamases.

One of carbapenem resistant mechanisms mediated by production carbapenem – hydrolyzing β –lactamases (Nordmann & Poirel ,2002). Carbapenemases are divers enzymes that different in their ability to hydrolyze carbapenems and other beta – lactam antiobiotics (Thomson , 2010) , two types of carbapenemases based on molecular study: serine enzymes having a serine moiety in the active site, and metallo – beta – lactamases (MBLs) , need divalent cations ,as metal cofactors for enzyme activity, usually zinc (Bush *et al*., 1995). In the past two decades there have several reports record the levels of MBLs in the bacterial community (Bush ,1998; Livermore ,2002). The specific confirmatory tests of MBLs that involve chelating agents as inhibitions (e.g. EDTA) , chelators are specific inhibitor of MBLs but not inhibited other beta – lactamase (Kim *et al*., 2007 ; Thomson , 2010) .In this study , 94 percent of imipenems and meropenem resistant isolates were positive for MBLs production ,and 59.6 % of total isolates of *Pseudomonas aeruginosa* were positive to these enzyme , our results are concordance with (Varaiya *et al*., 2008) who founded 60 (25%) of *Pseudomonas aeruginosa* were resistant to carbapenems and 50(20.8%) were MBLs producers confirmed by phenotypic test. A study in Pakistan was founded 100% of imipenem resistant *P. aeruginosa* were MBLs producers ( Irfan *et al*., 2008). In this study all isolates of *P. aeruginosa* obtained from two types of skin infected specimens (wounds and burns) ,all wounds are contaminated by bacteria from two sources, endogenous such as the gastrointestinal tract , or exogenous from environment (Kirketerp – Moller *et al*., 2008) ,it is primarily causes inflammation of skin and subcutaneous tissues if the safety of the skin and mucous membrane is damaged . it causes wound infection after surgical procedures , different types of injuries ,burns and dermatitis (Smith *et al*., 2012),it is considered one of the most apportunistic pathogen in chronic wound (Morsi *et al*., 2016) .one of the more informative human disease result from *Pseudomonas aeruginosa* is bacteremia in severe burn victims ( Japoni *et al*., 2009) . High percentage of *P. aeruginosa* was isolated from burns , this may be due to a highly adaptable bacteria that could rapidly develop resistance *to various types* of extended – spectrum antibiotics . It can live in hospital medium characterized by intensive antimicrobial use , and thus it can be transmitted readily among hospitalized burn patients (Lambert , 2002) .

In recent study , MBLs isolates were observed in higher rate (72.7%) with burn specimens , these may be due to empirical treatment by different beta lactams antibiotics which including carbapenems with burn patients that infected by *Pseudomonas aeruginosa* lead to production of MBLs in the case of burn patients are non response to carbapenems , these results are similar with study conducted by Fallah *et al*., (2013) in Tehran , showed 57.9% of *Pseudomonas aeruginosa* isolated from burn wards were MBLs producers. Study in Belgrade was indicated to higher production of MBLs (36.5%) (Jovcic *et al*., 2011). Occurrence MBLs producing *Pseudomonas aeruginosa* is a serious problem in hospital departments especially in burn units . These isolates can causes a big problem in treatment and spread of resistance between other bacteria (Khosravi & Mihani , 2008) .

Results of this study were showed that, MBLs producers of *Pseudomonas aeruginosa* were resistant to other antibiotics in high rates especially beta – lactam – antibiotics which included cefoxitin , amoxycillin –clavulanic acid , cephalothin , oxacillin , ceftazidime and piperacillin , in construct , isolates showed high sensitivity to monobactam (aztreonam).MBLs can strongly hydrolyze all bata lactam except monobactam (atreonam)(Saderi *et al*., 2008), this exception is due to MBLs bind to aztreonam with low affinity and location of the antibiotic within the active site of the enzyme in appropriate hydrolysis, MBLS are resistant to β –lactamase inhibitors commercially available , but they are sensitive to metal ion chelators , this due to metallo β –lactamases dependent on zinc ions in active site of these enzymes , thus binding of metal chelators

like EDTA by zinc leads to inhibition of enzyme (Halat *et al*., 2016) .

MBLs producing isolates were also resistant to other β –lactam antibiotics which used in this study as aminoglycosides (amikacin,tobramycin and gentamycin) , in *Pseudomonas aeruginosa* , the coexistence genes responsible for resistance to carbapenems and aminoglycosides has been reported , particularly as part of class 1 integrons (Odumosu *et al*., 2013).Multi drug resistant (MDR) it is mean resistance to antimicrobial agents included three or more anti – pseudomonal classes (aminoglycosides , carbapenems , pencillins / cephalosporins and fluroquinolones) (Magiorakos, 2011), in current study , MBLs producers isolates were appeared MDR (pencillins , cephalosporins , carbapenems and aminoglycodides ), this finding is similar with study of Irfan *et al*., (2008) who founded a high percentage of MBLs producing isolates among MDR *Pseudomonas aeruginosa* .MDR *Pseudomonas aeruginosa* is causes mortality and morbidity in burn patients which result 4-60% nosocomial infections in various parts of world (Carmeli *et al*., 1999). Ciprofloxacin is antibiotic of fluoroquinolones group was active against our isolates of MBLs producers , similar results were obtained by (Yakupogullari *et al*., 2007) . The emergence of metallo – beta – lactamases producing multidrug resistant *Pseudomonas aeruginosa* is a big health problem because it leaves the doctor with almost no therapeutic options for treatment(Viedma *et al*., 2012) .We conclude that resistant to carbapenems( imipenem and meropenem) due to MBLs among *Pseudomonas aeruginosa* isolates has occurred in high rates in patients with skin infected (wounds and burns) , and the MBLs producing isolates affects mostly burn patients . MDR seem in the MBLs producing isolates .We recommend that reasonable use of antibiotic therapy , misuse of antibiotics may cause the spread and prevalence of resistance against effective drugs such as carbapenems . Highlight need for detection MBLs in correct reliable phenotypic test in laboratory ,This helps to choose the right treatment .

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