

## Research Article



## Bad Bugs and No Drugs: Activity of Colistin as Waging War against Emerging Metallo- $\beta$ -Lactamases Producing Pathogens

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**Abstract** | Inter-hospital and intra-hospital dissemination of metallo- $\beta$ -lactamase (M $\beta$ L) producing strains possess significant therapeutic challenges.

**Objective:** This study was carried out to evaluate the efficacy of Colistin against M $\beta$ L producers.

**Material and Methods:** This cross-sectional study was conducted in Microbiology Laboratory, Allama Iqbal Medical College, Lahore, Pakistan from 1<sup>st</sup> July 2016 to 25<sup>th</sup> February 2017. A total of 12126 clinical samples were collected from patients presenting to Jinnah Hospital, Lahore. Every sample was processed for bacterial culture. Bacterial identification was performed according to standard guidelines. Every gram-negative isolate was further processed for antimicrobial susceptibility testing by modified Kirby Baur disc diffusion method. Zone sizes were interpreted according to CLSI 2016 guidelines. Next day every carbapenem-resistant isolate were further processed for M $\beta$ L detection by EDTA method, zone size of Carbapenem disc only and Carbapenem disc impregnated with EDTA was compared ( >7 mm increase M $\beta$ L positive, 0-5 mm increase M $\beta$ L-negative).

**Results:** Out of total 12126 samples, 35.9% (n=4361) were culture positive and only 40.5% (n=1770) were Gram negative rods. Of these 9.6% (n=170) were Carbapenem-resistant isolates with 47% (n=80) M $\beta$ L producers. Briefly 51.7% (n=30) *Acinetobacter* species were M $\beta$ L positive, *Pseudomonas* species 38.5% (n=22), *Escherichia coli* 69.5% (n=16), *Klebsiella* species 37.0% (n=10), *Proteus* 66.6% (n=2) and 0% *Citrobacter* spp were M $\beta$ L positive. 32.5% M $\beta$ L positive isolates were from ICU, 21.2% were from OPD, 12.5% were from Surgical Units, 12.5% were from Medical Unit, 17.5% were from Orthopedic Unit, and 3.7% were from Pulmonology ward. Almost 100% resistant was observed in M $\beta$ L positive isolates for Imipenem, Piperacillin+Tazobactam, Ceftriaxone, Co-amoxycylav, Cefoperazone+Sulbactam, Ciprofloxacin, and Amikacin, Doxycycline, and Gentamicin showed 91.2%, 94.0%, and 97.5% resistant rate respectively. No resistance was observed against Colistin.

**Conclusion:** M $\beta$ L producing Gram negative rods are rising in clinical setups. They are becoming a nightmare for clinicians to treat such infections. Colistin remains the only choice of drug for M $\beta$ L positive and Negative isolates with 0% resistant rate except for *Proteus* species, to which it is intrinsically resistant.

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**Keywords** | EDTA, Metallo beta-lactamase(M $\beta$ L), Colistin, *Acinetobacter*

## Introduction

Inter-hospital and intra-hospital dissemination of metallo- $\beta$ -lactamase (M $\beta$ L) producing strains possess significant therapeutic challenges. Early identification of M $\beta$ L-producing strains is a significant step to properly implement infection control measures to stop their spread <sup>(1)</sup>. The first time M $\beta$ L was detected in 1960 in *Bacillus cereus*, <sup>(2)</sup> later on in 1991 first plasmid-mediated M $\beta$ L producing *Pseudomonas aeruginosa* was discovered in Japan <sup>(3)</sup>. Nowadays, it is emerging as a nosocomial threat for critically sick hospitalized patients <sup>(1)</sup>.

Based on the structure and discovery,  $\beta$ -lactam drugs can be classified into four major groups; Penicillin, Cephalosporin, Carbapenems, and Monobactams. The  $\beta$ -lactamase breaks the bonds of beta-lactam ring rendering the antibiotic ineffective <sup>(4)</sup>. The most common mechanism of resistance against  $\beta$ -lactam drugs among bacteria is the production of hydrolytic enzymes, named  $\beta$ -lactamases, which inactivates the  $\beta$ -lactam drugs by disrupting the amide bond of their beta-lactam ring <sup>(5)</sup>. Carbapenems are the most powerful class of beta-lactams and display high activity against Gram positive and Gram negative bacteria <sup>(6)</sup>.

Ambler molecular classification scheme based on amino acid sequence criteria, classified  $\beta$ -lactamase producing strains into four diverse molecular classes A, B, C, and D <sup>(7)</sup>. Class A includes extended-spectrum beta-lactamases (ESBLs) blamable for resistance to broad-spectrum Cephalosporin <sup>(7)</sup>. Class B includes metallo-beta-lactamase (M $\beta$ Ls) <sup>(7)</sup>. Class C beta-lactamases AmpC enzymes, are widespread among gram-negative bacteria and are naturally occurring with a serine at its active site. Class D was also known as oxacillinases similarly to class A and C beta-lactamase, it also contains a serine at its active site <sup>(7)</sup>. The Bush-Jacoby classification scheme of beta-lactamases is based on substrate/inhibitor specificity mentioned that M $\beta$ L belonging to Group 3 <sup>(8)</sup>.

M $\beta$ Ls usually possess a broad hydrolysis profile that includes all  $\beta$ -lactam antibiotics including Carbapenems. Two recognized types of Carbapenemases are (i) serine  $\beta$ -lactamases (ii) M $\beta$ L <sup>(9)</sup>. Structurally and functionally, M $\beta$ L is a unique group; they differ structurally from the other beta-lactamases by their requirement for a zinc ion at the active site. Functionally, they were once distinguished primarily by their

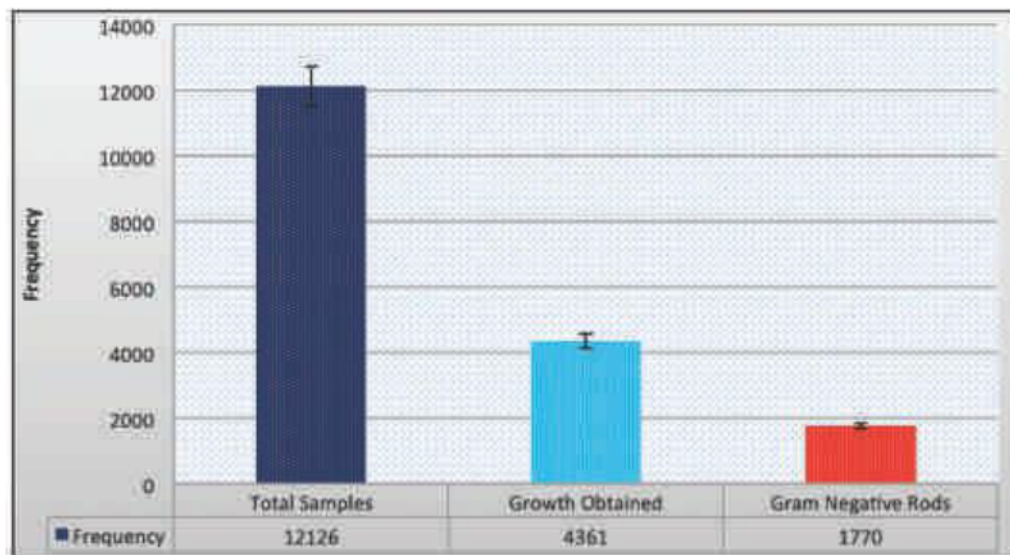
ability to hydrolyze Carbapenems, but some serine beta-lactamases now have also acquired that ability. <sup>(10)</sup>

The detection of  $\beta$ -lactamases can be divided into two groups phenotypic and genotypic detection methods. Phenotypic methods include (i) Minimum inhibitory concentration (MIC) by agar dilution (ii) MIC by E-Test (iii) Modified Hodge's Test (iv) EDTA disc diffusion method (v) Vitek MIC detection (vi) Nitrocefin, chromogenic cephalosporin substrate which changes color from yellow to red upon beta-lactamase mediated hydrolysis Double-disc synergy test (DDST). The genotypic method of detection includes (I) PCR for the specific genes, (ii) DNA probes (iii) Cloning and sequencing <sup>(11)</sup>. This study was carried out to evaluate the efficacy of Colistin against M $\beta$ L producers and to potential assess the unitization of Colistin for infections control.

## Material and Methods

This cross-sectional study was conducted at Microbiology Section, Pathology Department, Allama Iqbal Medical College (AIMC), Lahore, Pakistan, during the period of eight months (1<sup>st</sup> July 2016 to 25<sup>th</sup> February 2017). A total of 12126 clinical samples (urine, blood, pus, pus swabs, tips, respiratory samples, body fluids) were collected for all ages and both gender patients presenting to Jinnah Hospital, Lahore. Every sample was cultured on blood and MacConkey agar. Selectively CLED (cysteine lactose electrolyte deficient) for urine samples, blood, MacConkey and chocolate agar were used for respiratory samples and other body fluids. After 24 hours of incubation at 37 °C bacterial identification was done by colonial morphology, Gram stain, and biochemical profile. After identification, all those isolates that were gram-negative rods were further processed for antimicrobial susceptibility testing by modified Kirby Baur disc diffusion method and zone sizes were interpreted according to CLSI 2016 guidelines. Next day all those isolates that were resistant to Meropenem (MEM) or Imipenem (IPM) were further processed for M $\beta$ L detection.

The M $\beta$ L detection was performed by EDTA method, A 0.5M EDTA solution was prepared (186.1gm disodium EDTA + 2H<sub>2</sub>O (Sigma) per 1000 ml distilled water). Sodium hydroxide (NaOH) was used to adjust PH 8.0. The solution was sterilized by autoclaving. Every carbapenem resistance isolate was



**Figure 1:** Breakup of total specimens

inoculated on Mueller-Hinton agar, two disks of Imipenem (10 µg) or Meropenem (10 µg) (Oxoid) were placed on center with the disc to disc difference 25 mm, 04 µl (750 µg EDTA) of 0.5 M EDTA solution was added on one of them. Plates were incubated at 35°C for 18-24 hours.

Next day zone size of simple and EDTA impregnated disc was compared, if there was >7 mm increase in the zone size of EDTA impregnated disc as compared to simple disc, this indicated MβL production by the isolates, while 0-5 mm increase in the zone size indicates MβL-negative isolates. For quality control purpose *Pseudomonas aeruginosa* (MβL Producer) strain was used as a positive control and *Pseudomonas aeruginosa* (MβL Non-producer) was used as a negative control.

Data was analysed and frequency percentages were calculated using SPSS 21.0.

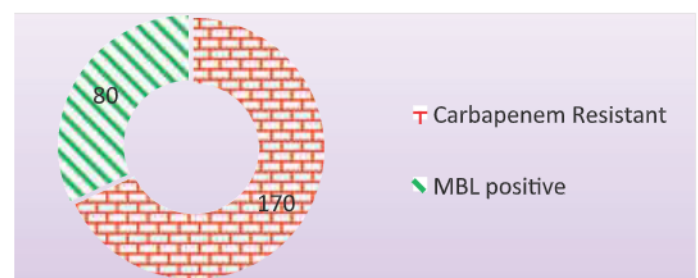
## Results

During the study period, out of total specimens n=12126, bacterial growth was obtained in 35.9% (n=4361) samples, of which 40.5% (n=1770) were Gram-negative rods (GNR) (Figure 1).

Out of these 1770 GNRs, 9.6% (n=170) isolates were carbapenem-resistant isolates; of them 47% (n=80) isolates were MβL producer (Figure 2).

Out of total n=170 carbapenem-resistant isolates, Out of 58 *Acinetobacter* species isolates, 51.7%<sup>(30)</sup> were MβL positive. Out of 57 *Pseudomonas* species

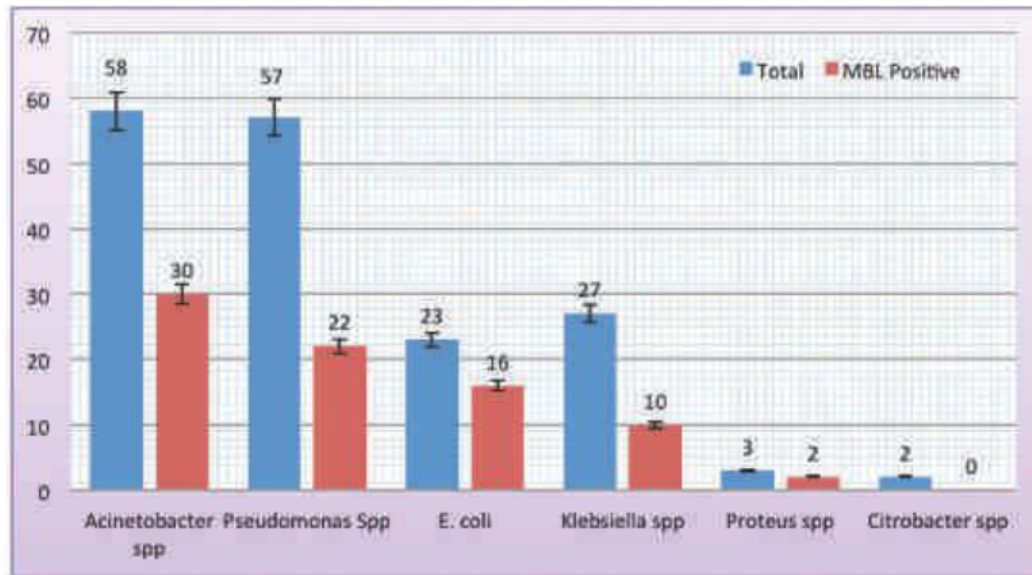
isolates 38.5%<sup>(22)</sup> were MβL positive, Out of 23 *Escherichia coli* isolates 69.5%<sup>(16)</sup> were MβL positive, Out of 27 *Klebsiella* species isolates 37.0%<sup>(10)</sup> were MβL positive. Out of 3 *Proteus species* isolates 66.6%<sup>(2)</sup> were MβL positive and all *Citrobacter* spp isolates were MβL negative (Figure 3).



**Figure 2:** Frequency of MβL positive among Carbapenem-resistant isolates

**Table 1:** Department wise frequency distribution of MβL positive isolates

Wards	Carbapenem-resistant		MβL positive	
	Frequency	%	Frequency	%
ICU	66	38.8%	26	32.5%
OPD	32	18.8%	17	21.2%
Surgical Unit	29	17.0%	10	12.5%
Medical Unit	20	11.7%	10	12.5%
Orthopedic Ward	19	11.1%	14	17.5%
Pulmonology ward	4	2.3%	3	3.7%
Total	170	100%	80	100%



**Figure 3:** Frequency of carbapenem-resistant MBL positive isolates

Department wise distribution showed MBL positive isolates were 32.5% from ICU, 21.2% OPD, 12.5% Surgical Unit, 12.5% Medical Unit, 17.5% Orthopedic Unit, and 3.7% Pulmonology ward (Table 1).

Of total, n=80 MBL positive isolates male and female were 65% (n=52) 35% (n=28) respectively (Table 2).

**Table 2:** Gender wise distributions of MBL positive isolates

Genders	Carbapenem-resistant		MBL positive	
	Frequency	%	Frequency	%
Male	98	57.6%	52	65%
Female	72	42.3%	28	35%
Total	170	100%	80	100%

**Table 3:** Antimicrobial resistant patterns of MBL positive and MBL negative isolates

Drugs	MBL positive	MBL negative
Imipenem	100%	100%
Piperacillin + Tazobactam	100%	100%
Ceftriaxone	100%	100%
Co-amoxycylav	100%	100%
Cefoperazone + Sulbactam	100%	100%
Amikacin	91.2%	80.2%
Gentamicin	97.5%	90.0%
Doxycycline	94.0%	90%
Ciprofloxacin	100%	93.4%
Colistin	0%	0%

Table 3 Showed alarmingly high rate of drug resistance among MBL positive isolates as compare to MBL negative isolates. Colistin remains the only choice of drug for MBL positive and negative isolates with 0% resistant rate.

## Discussion

The emergence of MBL producing strains is the significant cause of morbidity and mortality in health-care settings. The pervasiveness of multidrug-resistant MBL producing Enterobacteriaceae strains have been expanding, persistently <sup>(12)</sup>. Bacterial resistance to beta-lactam antibiotics agents is accomplished by any of the following systems. Initially, by the creation of beta-lactam hydrolyzing beta-lactamase compounds, second, by the usage of beta-lactam insensitive cell wall trans-peptidases, third, by the dynamic removal of beta-lactam particles from Gram-negative cells by method of efflux pumps. <sup>(13)</sup>

The present study reported that out of total 1770 gram-negative rods (GNRs), 9.6% (170) were Carbapenem-resistant isolates, of them 47% (80) isolates were MBL producers. Similarly, Parviz, et al. have reported 39.0% of all isolates were MBL positive <sup>(14)</sup>, Atta, R. O., et al. <sup>(15)</sup> have reported that out of 140 *P. aeruginosa* strains, 12.9% were carbapenem-resistant, of which 88.8% found to be MBL producers by the DDST and the disk potentiation tests.

Most frequent MBL positive isolates in present study were 51.7% *Acinetobacter* species, followed by *Pseudomonas* species 38.5% <sup>(22)</sup>, *Escherichia coli* 69.5% <sup>(16)</sup>,

*Klebsiella* species 37.0%<sup>(10)</sup>, *Proteus* 66.6%<sup>(2)</sup>, and 0% *Citrobacter* spp (Figure 3). Our results are supported by previous study conducted by *Fatima et al.*<sup>(16)</sup> from Army Medical College, National University of Sciences and Technology, Rawalpindi, which reported that out of 50 carbapenem-resistant isolates, 78% were MβL producers, most frequent MβL producers were *Acinetobacter baumannii* 84% followed by *Pseudomonas aeruginosa* 78%, *Escherichia coli* 71%. Slight variation in MβL positivity rate may be attributed due to the difference in sample size, MβL detection technique, and study population.

A study was conducted by Pandya *et al* in 2011 from India have reported that out of 450 gram-negative clinical isolate 6%<sup>(23)</sup> were carbapenem-resistant, of which 96.3% were MβL positive, MβL positivity was high in *Pseudomonas* 10% followed by *Klebsiella* sp 7.2% *Acinetobacter* species 7.1% and *E.coli* 3%<sup>(17)</sup>. Another study by Irfan *et al* from Karachi, Pakistan, in 2008 reported that MβL was detected in 96.6 % carbapenem-resistant *Acinetobacter* isolates, whereas 100% carbapenem-resistant *Pseudomonas aeruginosa* isolates were MβL producers.<sup>(18)</sup> The major difference in this study was that all isolates were taken from intensive care unit of a tertiary hospital.

In the present study, distribution of MβL positive isolate was 32.5% from ICU, 21.2% from OPD, 12.5% from Surgical Unit, 12.5% from Medical Unit, 17.5% from Orthopedic Unit, and 3.7% from Pulmonology ward (Table 1). Our study is in the favor of the previous study from College of Medical Sciences, Bharatpur, Nepal conducted by Bora *et al.*<sup>(19)</sup> reported that *E. coli*, 53.56% MβL positive isolates were recovered from the patient admitted to intensive care units (ICU) and 46.34% from the patient admitted to different hospital wards. Likewise, among the MβL positive *K. pneumonia*, isolates 58.97% were recovered from the patients admitted to ICU and 46.15% were recovered from the patients admitted to different hospital wards.

The present study reported 100% resistant of MβL positive isolates for Imipenem, Piperacillin-Tazobactam, Ceftriaxone, Co-amoxiclav, Cefoperazone-sulbactam, Ciprofloxacin, while Amikacin, Doxycycline, and Gentamicin showed 91.2%, 94.0%, and 97.5% resistant rate was observed respectively. The high rate of drug resistance among MβL positive isolates as compared to MβL negative isolates. Colistin remains

the only choice of drug for MβL positive and Negative isolates with 0% resistant rate (Table 3). Another study by Ranjan *et al* in 2014 reported that out of 350 isolates of *P. aeruginosa*, MβL was detected in 16.5% isolates by the E-test method; Resistance trend was significantly higher in the MβL-positive strains with 100% resistance to Ciprofloxacin, Tobramycin, and Meropenem, followed by Imipenem 93.1% and Gentamicin 89.6%. The frequency of multidrug-resistant (MDR) and pan drug-resistant (PDR) strains was significantly higher among the MβL positive group as compared to that in the non-MβL group 55.1% vs. 7.8% ( $P < 0.0001$ ) and 8.6% vs. 0.6% ( $P = 0.0006$ ), respectively<sup>(20)</sup>.

Similarly, Asghar *et al* reported that *P. aeruginosa* was most susceptible to Imipenem 65.9%, Amikacin 62.7%, Meropenem 58.7%, and Piperacillin/Tazobactam 57.2 %. MβL-producing *P. aeruginosa* were identified in 15.9% isolates.<sup>(21)</sup> Another study reported that among 89 different bacterial isolates as follows *Escherichia coli* ( $n=43$ ), *Klebsiella pneumonia* ( $n=18$ ), *Pseudomonas aeruginosa* ( $n=10$ ), *Enterobacter cloacae* ( $n=6$ ), *Acinetobacter baumannii* ( $n=5$ ), *Klebsiella oxytoca* ( $n=4$ ), *Proteus mirabilis* ( $n=2$ ) and *Salmonella paratyphi* ( $n=1$ ). MIC testing showed that 58/89 (65%) and 29/89 (32%) isolates were resistant to meropenem and colistin, respectively, whereas 27/89 (30%) isolates were resistant to both antibiotics<sup>(22)</sup>. Combination therapy of Colistin with Meropenem is recommended even against that organism which is resistant to carbapenem alone. It has been proven that combination therapy of Colistin and Meropenem has very good synergistic effects and results in significantly lowering of MICs. So combination therapy of Colistin and meropenem is recommended against MBL producing Gram-negative rods<sup>(23)</sup>.

## Conclusion

MβL producing Gram negative rods are rising in clinical setup and Colistin is the most effective antibiotics against MβL producing carbapenemases resistant isolates.

## Author's Contribution

**SN:** Lab work and data collection

**FR:** Study design and supervised the lab work

**MS:** Manuscript writing and reference management

**SI:** Manuscript editing and study plan

**AIA:** Supervised the study and proof reading

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