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The Prevalence of Metallo-*β*-Lactamase-Producing *Pseudomonas aeruginosa* in Egypt: A Systematic Review and Meta-Analysis

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ABSTRACT

Background: Metallo-*beta*-lactamase (MBL)-producing *Pseudomonas aeruginosa* represents a serious hazard to humanity because of its high mortality rate, ability to hydrolyze all beta-lactam antibiotics, including carbapenem, and absence of a clinically approved inhibitor. There are several studies conducted in Egypt that report a heterogeneous incidence of MBL among *Pseudomonas aeruginosa* clinical isolates. **Methods:** We performed a systematic search in MEDLINE [PubMed], Scopus, Google scholar, and Web of Science. Out of 1882 records, 20 studies agreed with the inclusion and exclusion criteria and are included in our review. **Results:** Our investigation revealed a high incidence of MBL-producing *Pseudomonas aeruginosa* of about 33.7% (95% CI: 19.3-48) and MBL-mediated Imipenem resistance among *P. aeruginosa* of about 74.1% (95% CI: 63.5-84.6). Furthermore, based on the included studies and other molecular studies conducted in Egypt, among MBL-encoding genes, bla_{VIM} appeared to be the most prevalent MBL gene in clinically isolated *Pseudomonas aeruginosa* in Egypt. **Conclusion:** This high disseminating rate raises the alarm to support both antimicrobial stewardship activities and infection control programs to prevent further increases.

Keywords: *Pseudomonas aeruginosa; Imipenem-resistant P. aeruginosa MBL; blaVIM, Egypt; Systematic review.*

INTRODUCTION

Pseudomonas aeruginosa (*P. aeruginosa*) is an opportunistic Gram-negative rod that is responsible for 11% of all nosocomial infections and it is recognized as one of the leading causes of nosocomial infections, especially in immunocompromised patients[1].it is listed as Priority 1 (Critical) in the 2017 WHO list of bacteria for which new antibiotics are urgently needed[2]. The CDC has also classified MDR *P. aeruginosa* as a serious threat and therefore listed it in the 2019 Antibiotic Resistance Threat Report[3].

P. aeruginosa can resist the insult effect of antibiotics not only due to its antibiotic-inactivating enzymes (acquired resistance) but also due to its high capability of intrinsic resistance and biofilm formation (adaptive resistance)[4, 5]. Beta-lactamases are enzymes produced by bacteria that confer resistance through hydrolysis of the beta lactam ring, and it is considered the most important mechanism of resistance to *beta*-lactam antibiotics [6].

The most frequently used method for classification of beta lactamases is Ambler classification (a structural classification based on primary amino acids

sequence homology) that categorized beta lactamases into four classes: class A, B, C and D. Classes: A, C, and D are serine-β-lactamases (SBLs), while class B is metallo-β-lactamases (MBLs) that requires zinc at their active sites [7, 8, 9].

MBL-producing *Pseudomonas aeruginosa* represents a real concern due to their robust resistance to all β-lactam antibiotics and no clinically approved inhibitor has been developed[10].

Among the metallo-beta-lactamase enzymes are VIM, IMP, NDM, SPM, and GIM. *Bla*$_{VIM-2}$ is the most widely distributed MBL-encoding gene in *P. aeruginosa* and was detected in over 30 countries[11, 12].

There's a great variability in studies reporting the prevalence of MBL-producing *P. aeruginosa* in Egypt. Therefore, we conducted a systematic review and meta-analysis that combined all of the different incidences of MBL-producing *P. aeruginosa* from different regions of Egypt into a single numerical estimate and also determined the percentage of MBL-mediated Imipenem resistance among *P. aeruginosa* to guide infection control programs in taking preventive measures and thus reduce its growing spread. In addition, we reviewed molecular studies that were not included in the systematic review to identify the most prevalent MBL-encoding gene in Egypt.

METHODS

Search strategy

Four databases, including MEDLINE [PubMed], Scopus, Google Scholar, and Web of Science, were searched for by the following key words: *Pseudomonas aeruginosa* or *P. aeruginosa* or MBL-producing *P. aeruginosa* or metallo-beta-lactamases producing *P. aeruginosa* or multi-drug resistance *P. aeruginosa* and MBL or metallo-beta-lactamases, MBL-encoding gene, and Egypt. The search was restricted to studies published in English only and the filtration of studies was in accordance with the Preferred Reporting Items for Systematic Reviews and Meta Analyses guidelines (PRISMA) [15].

Inclusion criteria were as follows:

1- *P. aeruginosa* clinical samples that were isolated from Egyptian patients.
2- Phenotypic detection of MBL includes Imipenem/EDTA combined disc test (CDT) [16], or Imipenem-EDTA double disc synergy test (DDST)[17], or MBL E-test [18] with or without PCR for detection of MBL-encoding genes.

Exclusion criteria

1- Selection of antibiotic-resistant strains of *P. aeruginosa* (for example, MDR or XDR) for screening of MBL.
2- Unclear materials and methods.
3- Studies that do not report results of MBL screening.
4- Studies that included a small *P. aeruginosa* sample size.
5- Repetitive samples.

Data extraction

From each included study, the following data were extracted by two independent reviewers (HK and MH), publication time, government, methods of screening of MBL, total *P. aeruginosa* isolates, MBL producers, and number of *P. aeruginosa* isolates harboring MBL-encoding genes. The authors' disagreements were settled through discussion. If more than one technique is used the result of MBL E-test is taken because it is the most sensitive [22, 23, 24].

Statistical analysis

Heterogeneity between studies was assessed by I-squared, and the prevalence of MBL-producing *P. aeruginosa* was pooled in a forest plot using OpenMeta [Analyst] by the DerSimonial and Laird random-effects model. Using JASP (version 0.16.1.), the risk of bias within studies was evaluated by visual inspection of the funnel plot and also tested with a non-parametric rank test, Begg's test[19], and the parametric regression test (also known as "Egger's test")[20]. In both cases, low p-values ($P < 0.05$) are indicative of asymmetry and were taken as evidence of publication bias.

RESULTS

Study selection

The study selection process is depicted in **Figure 1** flow chart. Upon initial searching, a total of 1882 studies were identified. All the identified records were first filtered based on title and abstract. Non-relevant, duplicate, and review articles (1822) were excluded. After removal of non-clinical samples, studies were then identified by screening the full text according to the aforementioned criteria of inclusion and exclusion. The extracted data from the selected studies was summarized in **Table 1**.

The prevalence of MBL among *Pseudomonas aeruginosa*

Heterogeneities between the 20 selected studies were as follows:

($I2 = 98.83$ %, $P < 0.001$) as shown in **Table 2**, which indicates high heterogeneity. So, the DerSimonian and Laird random effects model was used[21]. Publication bias was first assessed visually by the funnel plot (**Figure 2**) and slight asymmetry was found, but there was no evidence of publication bias by Egger's regression test (P value = 0.477) and Begg's rank test (P value = 0.351). The pooled prevalence of MBL among

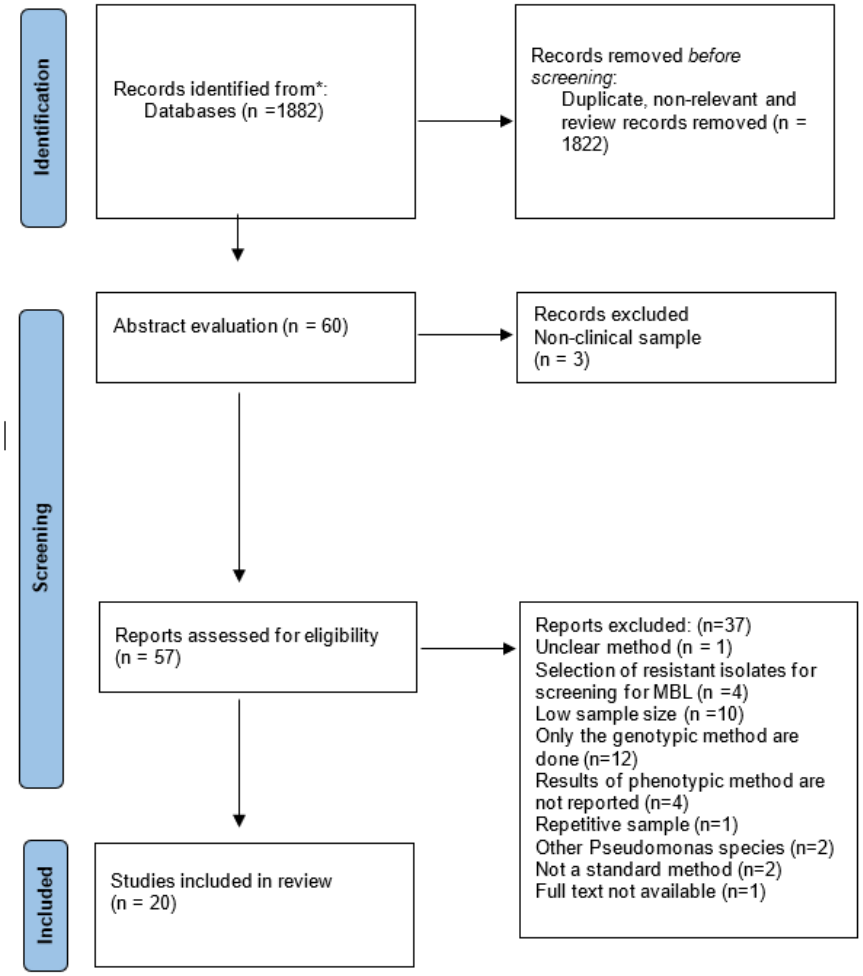

Figure 1. Flowchart of the Literature Selection Process using PRISMA flow diagram

1802 *Pseudomonas aeruginosa* isolates was 33.7% (95% CI: 19.3-48) as depicted in **Figure 3**.

Prevalence of MBL among Imipenem resistant *Pseudomonas aeruginosa*

Out of the 20selected studies, 13 studies only report MBL prevalence among Imipenem resistant *P. aeruginosa*. Diab et al[25]reported a higher number of MBL producers relative to Imipenem resistant isolates. This is due to screening of MBL was not restricted to Imipenem resistant isolates. So, out of 36 Imipenem resistant isolates, there are 32 and 31 MBL producers by the MBL E-test and CDT respectively.

Out of 1200 *P. aeruginosa* isolates, 347 were Imipenem resistant and of those, 254 were MBL producers. The pooled prevalence, heterogeneity, and publication bias tests are summarized in table 2. (The funnel plot and forest plot are depicted in **Figures 2** and **3** respectively).

DISCUSSION

P. aeruginosa is one of the leading causes of many nosocomial infections. Unfortunately, these bacteria have a high ability of resistance to many antibiotics due to their intrinsic and extrinsic resistance. Several studies have found that MBL producing *P. aeruginosa* has a high mortality rate[57-61]. Carbapenems are the drugs of choice for treating severe infections caused by AmpC or ESBL-producing *P. aeruginosa.*[62] But, they have poor stability to metallo-beta lactamase enzymes[63]. This highlights the significance of epidemiological studies that reveal the prevalence of MBL producers among *P. aeruginosa*.

To our knowledge, this is the first systematic review and meta-analysis conducted in Egypt that summarized the heterogeneous incidence rate of MBL among pseudomonas in one numerical estimate. Our study revealed that the overall pooled prevalence is 33.7% (95% CI: 19.3-48) which is relatively similar to

Table 1. Characteristics of studies included in the meta-analysis

Authors	Published time	Government or city	Total sample size	Imipenem resistant isolates	MBL producers	Method of detection of MBL
Abaza et al.[14]	2017	Alexandria	30	NA	30	MBL E-test-PCR
Salah Eldin et al.[26]	2012	Cairo	180	36	35	MBL E-test- Imipenem/EDTA CDT -DDST
Amer et al.[27]	2016	Tanta	46	NA	30	Imipenem/EDTA CDT
Ali and Abdel-Razik .[28]	2009	Cairo	56	16	15	MBL E-test -PCR- Imipenem/EDTA CDT -DDST
El-Mahdy et al.[29]	2019	Mansoura	80	34	18	Imipenem/EDTA CDT-PCR
Mousa et al[30]	2021	Assuit	74	NA	26	Imipenem/EDTA CDT-DDST
Hashem et al.[31]	2017	Ismailia	147	NA	25	Imipenem/EDTA CDT-DDST-PCR
Zafer et al.[32]	2014	Cairo	122	48	33	Imipenem/EDTA CDT -PCR
Raouf et al.[33]	2018	Minia	70	20	17	MBL E-test-PCR
Diab et al.[25]	2013	Giza	50	36	41*	MBL E test- Imipenem/EDTA CDT -PCR
Abbas et al.[13]	2018	Zagazig	50	5	2	Imipenem/EDTA CDT-PCR
El-Mosallamy et al.[34]	2015	Benha	100	25	15	Imipenem/EDTA CDT-PCR
Makharita et al.[35]	2020	Cairo	36	10	6	Imipenem/EDTA CDT
Abd El-Baky et al.[36]	2013	Minia	58	NA	31	Imipenem/EDTA CDT
El-Maraghy et al.[37]	2015	Ismailia	65	25	24	Imipenem/EDTA CDT
El-Naggar et al.[38]	2011	Mansoura	200	NA	41	Imipenem/EDTA CDT-DDST$^{\beta}$
Bahey et al.[39]	2019	Tanta	40	NA	17	Imipenem/EDTA CDT
Shaheen et al.[40]	2010	Zagazig	45	21	15	Imipenem/EDTA CDT-PCR
Gerges and Amin[41]	2014	Zagazig	85	40	32	Imipenem/EDTA CDT-PCR
Amer. et al[42]	2007	Zagazig	261	31	10	MBL E-test-PCR

Abbreviation: ***NA:****Not available,* ***Imipenem/EDTA CDT:*** *imipenem-EDTA combined disk method,* ***DDST:*** *double disk synergy test,* ***PCR:*** *Polymerase chain reaction*:The number of MBL producers is higher than the number of Imipenem resistant isolates as the screening is not restricted to carbapenem resistant isolates.*

Table 2. Statistics of the Meta-analysis

Study/subgroup	Included studies number	Pooled Prevalence	Heterogeneity		Egger's test	Begg's test
			I-squared	P-value		
MBL among *P. aeruginosa*	20	33.7%	98.83 %	<0.001	0.477	0.351
MBL among Imipenem resistant *P. aeruginosa*	13	74.1%	88.59 %	<0.001	0.612	0.435

a meta-analysis study of MBL prevalence among *P. aeruginosa* in Iran that reported that the prevalence of MBL among *P. aeruginosa* was 32.4 %[64] and lower than a study conducted in China that reported 149 MBL producers among 329 *P. aeruginosa* (45.28%) [65], but higher than a study conducted in India that reported 15 MBL producers among 127 isolates of *P. aeruginosa* (11.8%)[66]. The overall MBL prevalence in *P. aeruginosa* in Egypt is substantially higher than in Europe. For instance, reports from Spain and Italy revealed a 0.1% and 1.3% MBL prevalence among P. aeruginosa, respectively[67, 68]. In Mexico, the MBL prevalence in *P. aeruginosa* was estimated at 0.7%, which is very low compared to our study[69].

The loss or alteration of the outer membrane porin protein OprD is the most widespread mechanism of resistance to imipenem in *P. aeruginosa* [70, 71], but our study revealed that MBL-mediated imipenem resistance to *P. aeruginosa* is one of the major mechanisms. Out of 347 Imipenem resistant isolates, 254 are MBL producers with a pooled prevalence of 74.1% (95% CI: 63.5-84.6). This slightly similar to studies conducted in Pakistan and Brazil. In Pakistan Ameen *et al.* found that MBL production was confirmed in 74 of 114 Imipenem-

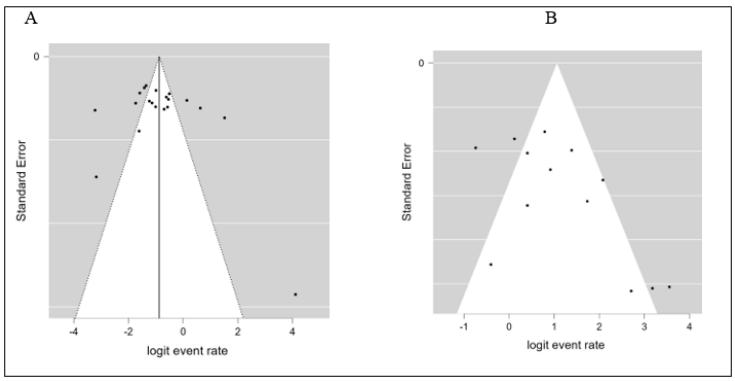

Figure 2. Funnel plot for publication bias. The effect sizes calculated from each study (Logit event rate) against their respective standard errors are displayed in a funnel plot with a pseudo 95% confidence interval (SEs). (A) The prevalence of MBL among *Pseudomonas aeruginosa*. (B) Prevalence of MBL among Imipenem resistant *Pseudomonas aeruginosa.*

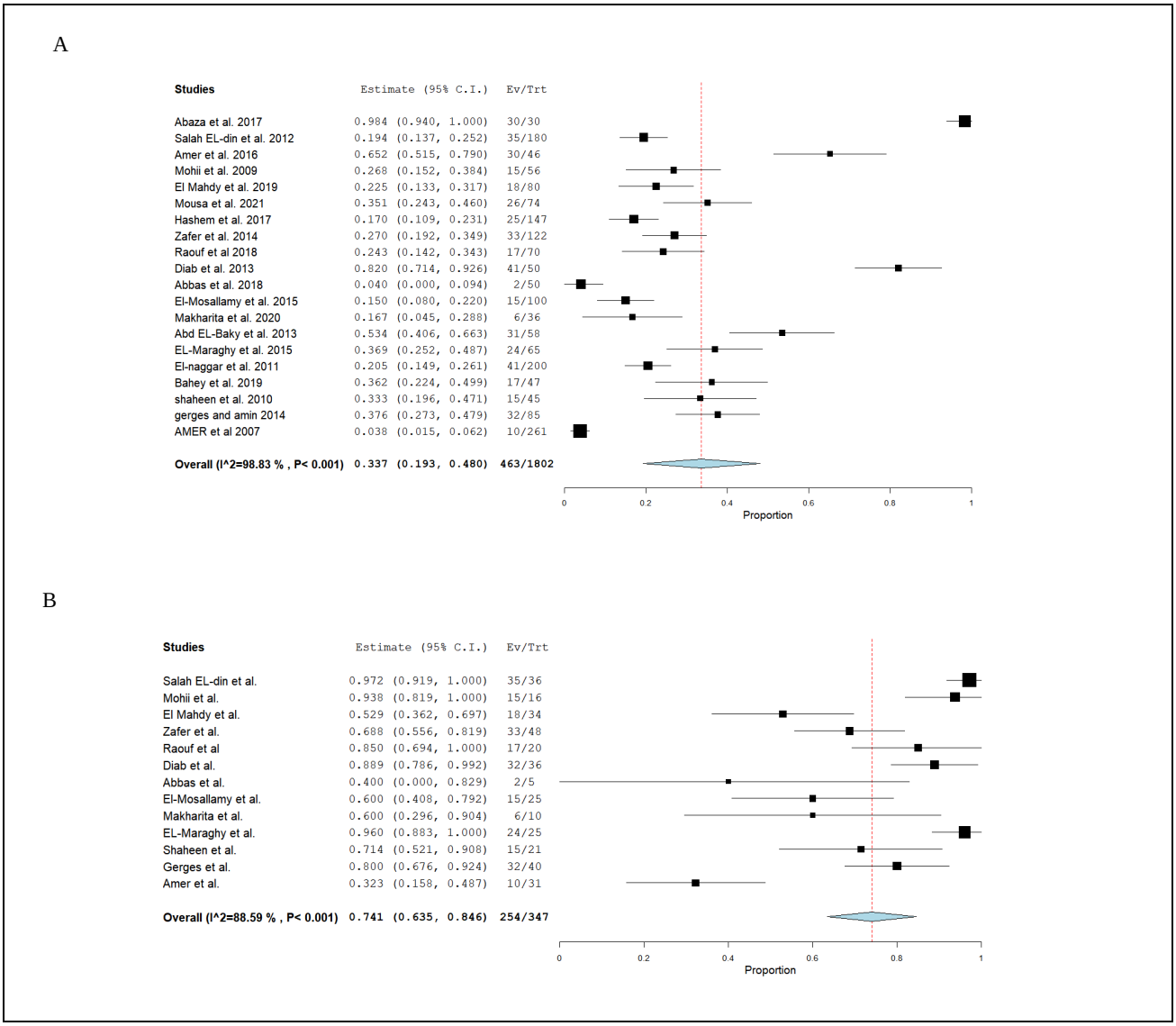

Figure3: Forest plot of the studies included in this meta-analysis. (A) The prevalence of MBL among *P. aeruginosa*. (B) The Prevalence of MBL among Imipenem resistant *P. aeruginosa.*

Table 3. Distribution of MBL-genes in the included studies

Author	Tested isolates	MBL-encoding genes					
		bla$_{GIM}$	*bla*$_{SIM}$	*bla*$_{SPM}$	*bla*$_{VIM}$	*bla*$_{IMP}$	*bla*$_{NDM}$
Abaza et al.[14]	total (30)	NA	NA	SPM-1=19	0	IMP-1=11	NA
Ali and Abdel-Razik.[28]	Imipenem resistant *P. aeruginosa* (n=16)	NA	NA	NA	1	9	NA
El-Mahdy et al.[29]	carbapenem resistant *P. aeruginosa* (n=34)	NA	NA	NA	18	NA	1
Hashem et al.[31]	MBL producer isolates (n= 25)	GIM-1=12	SIM-1=12	SPM-1=6	5 (VIM-1=1 VIM-2=1 VIM-4=3)	IMP-1= 1	0
Zafer et al.[32]	Imipenem resistant isolates (n=48)	0	0	0	28 (VIM-2=28, VIM-1=0)	IMP-1=1 IMP-2=0	2
Raouf et al.[33]	Imipenem resistant isolates (n=20)	NA	NA	NA	9 (VIM-1=8, VIM-2=1)	0	NA
Diab et al.[25]	total isolates (n=50)	NA	NA	NA	VIM-2=35	0	NA
Abbas et al.[13]	Not clear	NA	NA	NA	0	NA	NA
EL-Mosallamy et al.[34]	Imipenem resistant isolates(n=25)	0	0	2	13	0	NA
Gerges and Amin[41]	Imipenem resistant (n=40)	NA	NA	NA	40	25 both (VIM +IMP)	NA
Shaheen et al.[40]	total isolates (n=45)	NA	NA	NA	15	0	NA
Amer et al.[42]	Imipenem resistant isolates=10	NA	NA	NA	NA	8	NA

NA: not available

Table 4 Characteristics of molecular studies that are not involved in meta-analysis

Author(publication year)	Tested isolates	MBL-encoding gene					
		bla$_{GIM}$	bla$_{SIM}$	*bla*$_{SPM}$	*bla*$_{VIM}$	*bla*$_{IMP}$	*bla*$_{NDM}$
Zafer et al.(2015)[43]	MBL producer isolates (n=33)	0	0	0	28(VIM-2)	1	2
Farhan et al.(2019)[44]	carbapenem-resistant isolates (n=21)	11	NA	8	11	9	NA
Gaballah et al.(2019)[45]	carbapenem-resistant isolates (n=32)	0	NA	0	27	0	0
Essa and Afifi.(2007)[46]	Imipenem resistant isolates (n=40)	NA	NA	NA	16	0	NA
Hassuna et al.(2020)[47]	carbapenem-resistant isolates (n=32)	NA	NA	0	31 (VIM-1=31 ,VIM-2=0)	0	4
El-Domany et al(2017).[48]	total isolates (n=114)	NA	NA	NA	8	5	NA
Ramadan et al.(2018) [49]	carbapenem-resistant isolates (n=22)	NA	NA	NA	11	4	6
Soliman et al.(2020)[50]	carbapenem-resistant isolates (n=7)	NA	NA	NA	7 (VIM-2=3, VIM-24=4)	NA	NA
Basha et al.(2020)[51]	11 selected XDR P. aeruginosa	NA	NA	NA	VIM-1=2	0	NDM-1=10
El-Domany et al. (2016)[52]	Imipenem resistant isolates (n= 14)	11	1	4	8	5	NA
Emara et al. (2020)[53]	total isolates (n=114)	NA	SIM-2=1	SPM-1 =4	NA	NA	NA
Kishk et al. (2016)[54]	total isolates (n=26)	NA	NA	NA	7	6	NA
Elhabibi et al. (2016)[55]	total isolates (n=200)	NA	NA	NA	VIM-2=85	NA	NA
Abo-Alella et al. (2021)[56]	carbapenem-resistant isolates (n=6)	NA	NA	NA	6	4	1

Abbreviation: *NA: not available*

resistant isolates (64.9%)[72] and in Brazil, Franco *et al.* reported that 53 out of 69 (76.8%) Imipenem-resistant *Pseudomonas aeruginosa* isolates produced MBL[73].

The high incidence of MBL producer and MBL-mediated Imipenem resistance could be attributed to the misuse of carbapenem and lack of antimicrobial stewardship in Egypt [74, 75].

In the studies included in our review, *bla*$_{VIM}$ seemed to be the most prevalent among MBL-encoding genes, and this is consistent with other molecular studies conducted in Egypt as shown in table 3.For instance Zafer *et al.* demonstrated a 85% of *P. aeruginosa* MBL producers harbored *bla*$_{VIM}$[43].Furthermore, Farhan et al. and Gaballah et al. revealed that out of 21 and 32 carbapenem resistant *P. aeruginosa* isolates, *bla*$_{VIM}$ was identified in 11 (52.3%) [44] and 27 (84.4%) [45]respectively. Similar findings were recorded in neighboring nations. In Sudan, for example, the, *bla*$_{VIM}$ was found in 28 (38.9%) of the 72 positive MBL genes [76]. Moreover, In Saudi Arabia the *bla*$_{VIM}$ was identified in all MBL producers [77, 78]. In Libya, all *P. aeruginosa* MBL-positive strains produced *bla*$_{VIM-2}$[79] and in Iran a systematic review and meta-analysis showed that, the *bla*$_{VIM}$ is the prevalent in Iranian burn centers (21.4%)[80].

There are some limitations to our study. For example, our results do not fully reflect the prevalence of MBL-producing *P. aeruginosa* in Egypt, as not all regions in Egypt report the prevalence of MBL producers among *P. aeruginosa* clinical isolates. Also, there are some variations among the aforementioned phenotypic methods for screening of MBL.

Giving the fact that selective pressure of antibiotics is the driving force of evolution and diversity of beta lactamases and MBL could be evolved from carbapenem use [81, 82]antimicrobial stewardship should be implemented strictly, especially for carbapenem overuse as empirical therapy in Egypt and infection control programs must be strengthened considering this high disseminating rate.

CONCLUSION

In conclusion, our results revealed a high incidence of MBL producers among *P. aeruginosa* clinical isolates and that MBL is a major mechanism of resistance in imipenem-*resistant P. aeruginosa*. Further investigations are needed to identify other possible mechanisms of imipenem resistance in *P. aeruginosa.*

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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