Systematic surveillance and meta-analysis on the prevalence of Metallo-β-Lactamase producers among carbapenem resistant clinical isolates in Pakistan

Noor ul Ain
University of the Punjab
Samyyia Abrar
University of the Punjab
Rehan Ahmad Khan
University of the Punjab
Abdul Hannan
university of the Punjab
Namrah Imran
University of the Punjab
saba riaz (saba.mmg@pu.edu.pk)
University of the Punjab https://orcid.org/0000-0002-6630-5689

Research

Keywords: Metallo-β-lactamases, Carbapenem resistance, Meta-analysis, New Delhi Metallo-β-lactamase (NDM)

Posted Date: February 11th, 2020

DOI: https://doi.org/10.21203/rs.2.23189/v1

Version of Record: A version of this preprint was published at Journal of Global Antimicrobial Resistance on December 1st, 2020. See the published version at https://doi.org/10.1016/j.jgar.2020.07.024.

Abstract

Background: Rapid emergence of carbapenem resistance (CR) is a health concern of pertinent importance. Epidemiological surveillance of CR at global and indigenous level (Pakistan) can help to improve infection control strategy and establish pharmacovigilance programs. This study evaluate the prevalence of clinically significant CR isolates, and its genetic variant distribution among different geographical regions of Pakistan.

Methods: A meta-analysis was conducted to present the current rate of CR infections and prevalence of Metallo-β-lactamases (MBLs). The proposed subject was researched using robustic electronic databases a) PubMed b) PubMed Central® (PMC), and c) Google Scholar to identify the available literature. Thereafter, relevant data was extracted and statistical analysis was performed using STATA version 14.

Result: A total of 110 relevant studies were identified with 19 meeting the inclusion criteria for the meta-analysis of CR, while 22 for MBLs. Pooled rate for carbapenem resistance was determined to be 0.28 (95% CI: 0.26-0.31) with overall significant heterogeneity (I^2 =99.61%, p<0.001) and significant estimated score ES=0 (Z=22.65, p<0.001). In case of Pakistan, the overall pooled proportion of MBL producers was 0.34 (95% CI: 0.29-0.39) with overall heterogeneity significance (I^2 =99.62%, p<0.001) and respective significant ES=0 (Z=13.17, p<0.001). Conclusively, diverse variants of carbapenemases (VIM, IMP, NDM, KPC, GIM, SIM) along with other co-existing β-lactamase variants (OXA, TEM, SHV, CTX-M) have been reported across the country. However, New Delhi Metallo-β-lactamase (NDM)-variants were reported in predominant literature.

Conclusion: The prevalence of CR isolates in Pakistan is alarming, associated with MBL production primarily evident from the studies. The study emphasizes the need for regular surveillance, pharmacovigilance and antibiotic stewardship programs to ensure the availability of data to the authorities for preemptive measures of infection control.

Introduction

The global spread of antibiotic resistant poses significant therapeutic challenges in clinical and non-clinical settings. Carbapenem drugs are the most potent β-lactam drugs used as an ultimate strategic remedy against pathogens resistant to most broad spectrum antibiotics [1]. Recently, the infection associated with pathogens resistant to carbapenem have been documented for high rates of morbidity and mortality [2, 3]. β-lactamases, the prime factor of carbapenem-resistance (CR) which not only degrade carbapenem but several other beta-lactam drugs [4]. The mobile genetic elements being the disseminators of the gene encoding enzymes variants, produce new β-lactamase variants. [5]. The mobile genetic elements (MGE) facilitate the inter-species spread of the resistance determinant and mutations, that diminish the boundaries between nosocomial bugs and community pathogens [4, 6].

Among the β-lactamases, Carbapenemases are the significant cause of resistance to carbapenem drugs. Moreover, they have been extensively reported in Enterobacteriaceae and non-lactose fermenting Gram negative bacteria (Pseudomonas spp. and Acinetobacter baumannai) [6, 7]. It is pertinent to mention here, that the occurrence of different variants i.e. NDM-type, VIM-type, and IMP-type Metallo-β-lactamases (MBLs) bring them to a concern of prime attention for the region (Pakistan) [4]. Appreciable literature investigates the emergence and epidemics of new variants of MBLs at global level [8, 9, 6], however, the South-Asian countries hold extreme vulnerability [10–12].

Developing nations (South Asia), barely allocate independent funds for health based research with state of art infrastructural growth. Being one of the developing territories, Pakistan has been through the crest & trough of infrastructural development in the recent past. As per national level records, antibiotic resistance imparts 50% to the mortality rate [13]. Likewise, intrinsic and extrinsic issues such as self-medication practices, ignorance, substandard health facilities, unhygienic sanitation/flushing system and indiscriminate use of antibiotics in poultry and livestock are the major factors responsible for reinforcing antimicrobial resistance. Consequently, extreme antimicrobial resistance has left the health sector with limited options of antibiotics that could efficiently treat infections. Afore-mentioned alarming situation grab the attention of researcher in health sector to explore the novel action mechanism of carbapenem drug and accordingly CR. It is very important to mention here that proactive measures should be adopted to suppress the progressive spread of CR across the country.

Conclusively, there is a dire need to assemble the available authentic literature in regards to the current rate of carbapenem resistance across the country. Therefore, a comprehensive study was conducted with the aim to determine the overall pooled prevalence of CR and MBL-production having scope limited to Pakistan. This research also demonstrates a comprehensive explanation of meta-analysis from Pakistan focusing the pathogens resistant to carbapenems and the prevalence of MBLs. Moreover, this review will not only improve infection prevention strategies but will also provide a platform to promote antibiotic stewardship and pharmacovigilance programs. Finally, the current research has also highlighted open ended problems that demand thorough investigation specifically for the regions that lack surveillance data.

Methods - Strategic Approach

Study Design – Analyses schematics

To determine the rate of carbapenem resistance and prevalence of MBLs in Pakistan, a descriptive meta-analysis, based on critical evaluation and scientific investigation was conducted. To achieve the object of study, extensive literature review was carried with subsequent statistical analyses.

Reviewing Literature And Study Selection

The object of the study was achieved via developing a systematic plan, starting with reviewing vast literature using tailored computational efforts i.e. PubMed, PMC, Web of Science, and Google Scholar. The keywords used for the comprehensive search were: Carbapenem resistance, Metallo beta lactamases (MBLs),

MBLs in Pakistan, MBL genes, Detection of MBLs and MBLs in different geographical areas of Pakistan. Prominent and relevant literature was listed by taking into account the theme, executive summary and findings of the studies. Additionally, the inclusion criteria were also extended to the relevant cited references of the same idea.

Inclusion Vs Exclusion Criteria

Author names, publication year, sample size, number of carbapenem resistant isolates and reported rate of MBLs were the key parameters for the extraction of data in the current research. Thereafter, the summarized data was classified into 4 groups on the basis of geographical locations in the country i.e. a) Punjab, b) Islamabad Capital Territory (ICT) c) Sindh d) Khyber Pakhtunkhwa (KP).

Brief revision of all the selected articles (n = 110) was conducted and irrelevant papers were omitted (Fig. 1). To proceed with the analyses step, two groups were organized, Group 1& 2. Group 1 presented the rate of CR while Group 2 depicts the rate of MBLs. It is pertinent to mention that all the selected studies (group 1 & 2) were based on clinical isolates only. Moreover the respective inclusion criteria for Group 1 were studies reporting the rate of CR from all regions of Pakistan while for Group 2 the selected criteria were literature representing MBL rate through any of the phenotypic or molecular detection of gene variants across the country.

A collective exclusion criteria were formulated for both the groups, based on the successive arguments. Since the scope of this research was limited to clinical relevance, therefore, studies related to environment and animal specimens were excluded. Likewise, case reports, posters or abstracts, and duplicates were also removed. Similarly individual exclusion criteria were also designed for Group 1 & 2. Studies not clearly mentioning the rate CR were excluded in case of Group 1, while for Group 2 exclusion was adopted for the studies without experimenting the rate of MBL and taking into account β-lactamases alone other than MBLs.

Data Extraction

The extracted data for prevalence of CR and rate of MBL-producers detected by phenotypic tests including combination disc test (CDST), modified Hodge Test (MHT), and epsilometer test (E-Test), and molecular detection tool for gene variants (i.e., PCR) was organized in tables and reviewed twice before analyses to remove any error/ omission (Table 1).

Table 1

Year-wise distribution of articles reporting CR rate and MBL-producing isolates from different regions of Pakistan.

Sr. no.	Region	Publication year	Study duration	Authors	Sample size	Isolate specific study	Carbapenem resistant isolates.	MBL Positive	Phenotypic detection tests			
									DDST	CDST	MHT	E-te
Punjab												
1	Lahore	2019	2016–2017	Awan et al. [35]	12126	Acinetobacter baumannii	170	-	no	no	yes	no
2	Lahore	2018	2015	Akhtar et al [36]	100	All gram negative pathogens	-	89	no	yes	yes	no
3	Lahore	2018	2016–2017	Naz et al. [37]	12126	All gram negative pathogens	170	80	no	yes	no	no
4	Lahore	2018	2015–2017	Ain et al. [26]	942	All gram negative pathogens	142	90	no	yes	yes	ye
5	Faisalabad	2018	2016–2017	Rasool et al [9]	152	All gram negative pathogens	103	58	no	yes	yes	no
6	Lahore	2016	2013–2014	Javed et al. [25]	1,168	Escherichia coli and Klebsiella pneumoniae	134	131	yes	yes	yes	no
7	Lahore	2016	2014	Anwar et al [38]	112	Acinetobacter baumannii	66	63	yes	yes	yes	no
8	Lahore	2015	not mentioned	Shan et al. [39]	200	Pseudomonas Aeruginosa	56	49	no	yes	no	no
Sindh												
9	Karachi	2017	2014	Fakhuruddin Indha [10]	100	Acinetobacter species	95	2	yes	yes	yes	
10	Karachi	2016	2009–2012	Khan et al. [28]	114	All gram negative pathogens	114	104	no	no	no	no
11	Karachi	2014	2001	Guhar et al [12]	142	Gram negatives (non fermenting spp.)	114	108	no	yes	no	no
12	Karachi	2013	2009–2010	Sultan et al. [40]	114	Enterobacteriaceae spp	-	107	no	no	yes	ye
13	Karachi	2008	2001	Irfan et al. [11]	142	Acinetobacter and Pseudomonas aeruginosa	115	108	yes	no	no	no
ICT												
14	Islamabad	2018	2015	Humayun et al [41]	277	Klebsiella pneumoniae	14	8	no	yes	no	no
15	Rawalpindi	2018	2016	Braun et al. [42]	7857	all gram negative pathogens	82	-	no	no	no	no
16	Islamabad	2014	2011	Hasan et al. [34]	90	Acinetobacter baumannii	59	59	no	yes	yes	no
17	Islamabad	2014	2009	Qamar et al [43]	71	Enterobacter cloacae	-	10	no	no	yes	no
18	Islamabad	2013	not mentioned	Nahid et al. [44]	356	All gram negative pathogens	160	131	yes	yes	yes	no

Sr. no.	Region	Publication year	Study duration	Authors	Sample size	Isolate specific study	Carbapenem resistant isolates.	MBL Positive	Phenotypic detection tests			
									DDST	CDST	MHT	E-te
19	Islamabad	2013	2011	Day et al. [45]	175	Enterobacteriaceae	32	32	no	no	no	no
20	Rawalpindi	2011	2010	Perry et al. [46]	200	Enterobacteriaceae	-	37	no	yes	yes	no
21	Rawalpindi	2010	2009	Kaleem et al.[47]	2347	All gram negative pathogens	50	39	no	no	no	ye
KPK												
22	Peshawar	2015	2013–2014	Ilyas et al [48]	550	Pseudomonas aeruginosa	70	18	no	yes	no	no
23	Peshawar	2017	not mentioned	Ullah et al [49]	102	Pseudomonas aeruginosa	-	29	yes	no	no	no
24	Swabi	2018	2016–2017.	Jamil et al [27]	240	Escherichia coli	25	12	no	yes	no	no

Statistical Analysis

The pre-published data of carbapenem resistance and MBL prevalence from different areas of Pakistan was interpreted into two groups for future analysis. Afterwards, the screened data was evaluated for the statistical analysis. The statistical analysis was conducted using STATA Version 14.1 (College Station, Texas, USA) according to a previous study [14]. The non-uniformity in statistical data (Statistical heterogeneity) was investigated using I^2 statistics (Measure of inconsistency) at a signified level of 5%. The significant P-value, suggested by literature as 0.05, was used for conversion of meta-analysis results to definite tests of statistics. Using the Random Effects Model (REM), a pooled prevalence of 95% Confidence interval (CI) was obtained. A funnel plot and Begg tests were carried out to investigate the publication partiality using data statistically and graphically.

Results

Distribution of articles describing the rate of CR and prevalence of MBLs

To demonstrate the overall rate of CR and prevalence of MBLs an initial search was launched that shortlisted 110 published papers. Among the total, 24 studies were proceeded for further analysis. As per the inclusion criteria, 19 papers on rates of CR and 22 describing the frequencies for MBL-producers were selected. The maximum number of studies 41.67% (n = 10) were observed from ICT, followed by Punjab 33.34% (n = 8) on the second, Sindh 25% (n = 6) third, and KP 12.5% (n = 3) last. However, no study on the subject was found from the peripheral regions of the country including Baluchistan region and Gilgit Baltistan. In the year 2018, the subject was highly researched followed by 2016 with a decreasing trend in the descendent past years (Table 4).

Table 4
Year-wise distribution of published article from different geographical areas of Pakistan.

Year	Punjab	KPK	Sindh	ICT	Annual Total Publications
Upto 2009	0 (0%)	0 (0%)	1 (20.00%)	0 (0%)	1 (4.16%)
2010	0 (0%)	0 (0%)	0 (0%)	1 (12.50%)	1 (4.16%)
2011	0 (0%)	0 (0%)	0 (0%)	1 (12.50%)	1 (12.50%)
2012	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
2013	0 (0%)	0 (0%)	1 (20.00%)	2 (25.00%)	3 (12.50%)
2014	0 (0%)	0 (0%)	1 (20.00%)	2 (25.00%)	3 (12.50%)
2015	1 (12.50%)	1 (33.33%)	0 (0%)	0 (0%)	2 (8.34%)
2016	2 (25.00%)	0 (0%)	1 (20.00%)	0 (0%)	3 (12.50%)
2017	0 (0%)	1 (33.33%)	1 (20.00%)	0 (0%)	2 (8.34%)
2018	4 (50.00%)	1 (33.33%)	0 (0%)	2 (25.00%)	7 (29.17%)
2019	1 (12.50%)	0 (0%)	0 (0%)	0 (0%)	1 (4.16%)

In detail, 9 studies researched all types of Gram negative pathogens whereas a few studies were found to be isolate specific, for example, Acinetobacter spp. (n = 4), Pseudomonas aeruginosa (n = 3), Enterobacteriaceae (n = 3), Klebsiella pneumoniae (n = 1), Escherichia coli (n = 1), Enterobacter cloacae (n = 1), Acinetobacter & Pseudomonas (n = 1), and K. pneumoniae & E. coli (n = 1). In this research, among the 24 investigations, 92% (n = 22) papers were associated with hospitalized patients, while 8% (n = 2) have presented the cases from in-patients as well as out-patients departments (OPD). None of the studies demonstrate any gender associated with the rate of CR or MBL infections. Moreover, blood cultures and pus specimens were dominant in all studies followed by tracheal secretion. To understand the gastro-intestinal tract CR infections, stool samples were taken into account in two different studies. Only one study was based on neonates specifically while there was no age limit in remaining studies (Table 1).

Laboratory Methods For Determining The MBLs Prevalence

Among the variable phenotypic methods used for MBLs detection in 22 studies, the most common method was combination disc test (CDST) (68.18%, n = 15), followed by modified Hodge test (MHT) (n = 12, 54.54%) and double disc synergism test (DDST) (n = 6, 27.27%). E-test for MBLs detection was performed by only three studies whereas Minimum inhibitory concentrations (MIC) of carbapenem drugs (Imipenem, Meropenem) was determined in six articles.

Molecular Methods Used To Determine The Frequency Of MBLs

Among the 18 studies included in MBL detection, PCR based gene detection method was used by 12 (66.67%) of the studies NDM gene was reported (n = 5, 27.78%) to be the most prevalent among MBL producing isolates. Existence of other β-lactam genes (OXA, TEM, SHV, SIM, and SPM) in combination with bla_{MBL} is also reported. Other MBL gene variants included IMP (n = 8) and VIM (n = 7).

Rate Of CR In Different Geographical Regions Of Pakistan

To explain the rate of CR across the country, 18 articles were reviewed. Among them, 7 (38.89%) were from Punjab, 6 (33.34%) from Islamabad/Rawalpindi, 2 (11.12%) from KP, and the remaining 3 (16.67%) from Sindh. Based on the extracted data from the reviewed literature (Figure. 2; Table 2), the overall pooled proportion of CR was 0.28 (95% CI: 0.26–0.31). The overall heterogeneity was significant (I^2 = 99.61%, P < 0.001) and significant ES = 0 (Z = 22.65, P < 0.001). The pooled proportion of CR-producer for Punjab was observed to be 0.17 (95% CI: 0.14–0.19) with significant heterogeneity (I^2 = 99.23%, P < 0.001) and significant ES = 0 (Z = 12.55, P < 0.001). In case of ICT, the pooled proportion of CR was determined to be 0.18 (95% CI: 0.14–0.22) with respective significant heterogeneity (I^2 = 98.99%, P < 0.001) and corresponding ES = 0 (Z = 8.19, P < 0.001). Moreover, for KP and Sindh, the pooled proportion of CR was estimated to be 0.12 (95% CI: 0.10–0.14) and 0.86 (95% CI: 0.75–0.96) respectively, while their significant ES was calculated to be ES = 0 (Z = 10.35, P < 0.001) and ES = 0 (Z = 16.07, P < 0.001) respectively.

MBLs prevalence and distribution in different geographical regions of Pakistan

To present prevalence rate and distribution of MBLs, 22 articles were peer-reviewed: 7 (31.8%) from Punjab and ICT each, 3 (13.6%) from KP and 5 (22.7%) from Sindh. Based on the available literature (Fig. 3; Table 3), the overall pooled proportion of MBL producers was 0.34 (95% CI: 0.29–0.39) with overall heterogeneity significance (I^2 = 99.62%, P < 0.001) and significant ES = 0 (Z = 13.17, P < 0.001). The pooled proportion of MBL-producers for Punjab and ICT was 0.32 (95% CI: 0.20–0.44) and 0.22 (95% CI: 0.12–0.31) respectively, and corresponding significant heterogeneity was found to be (I^2 = 99.54%, P < 0.001) and (I^2 = 98.57%, P < 0.001) with significant ES = 0 (Z = 5.28, P < 0.001) and ES = 0 (Z = 4.57, P < 0.001) respectively. Furthermore, in case of KP the pooled

proportion of MBL producers was estimated to be 0.10 (95% CI: 0.03–0.17) with significant ES = 0 (Z = 2.78, P < 0.01) whereas for Sindh, it was found to be 0.68 (95% CI: 0.22–1.14) with significant heterogeneity (I^2 = 99.79%, P < 0.001) and ES = 0 (Z = 2.88, P < 0.001).

Discussion

The past few decades, infections associated with extensive drug resistance have been highly reported by the literature, as a cause of high mortality and morbidity rates. However, researchers tailored the usage of Carbapenem, for treatment of life threatening infections. The issue of increasing drug resistance specifically CR needs to be highlighted by collecting the data, enlightening the major findings, experimenting with the techniques and tools for easy availability to health care practitioners and the concerned authorities. This research compiles a brief description for the first time in Pakistan that demonstrates the rate of CR and incidence prevalence of MBLs from different geographical regions of the country, with alarmingly high rates of CR and incidence of MBLs [15–18].

Multiple analyses techniques were adopted by various researchers in past, however meta-analysis was the most recommended numerical technique [19, 20]. The findings of meta-analysis are paragraphed as follows.

As per statistics, the overall pooled proportion of CR was 28%. Interesting, high rates of CR have been reported from Iran, wherein 2016 the resistance to imipenem was 55% [95% confidence interval (CI), 53.0– 56.5] [20]. However, the steady increase of CR in Iran is evident from the studies published in advancing years with a pooled prevalence of resistance to imipenem and meropenem to be 81.1% (95% CI 76.6–84.9) and 83.6% (95% CI 78.7–87.5), respectively [19]. More recently, a similar study from Iran, based on the prevalence and mechanisms of carbapenem resistance in A. baumannai has reported the pooled frequency of carbapenem resistance as 85.1% (95% confidence interval [CI]: 82.2–88.1) [21]. However, the rate of CR in Enterobacteriaceae reported by a meta-analysis study from the US was 0.3–2.93 infections per 100,000 person-years [22].

The Pakistan's overall pooled proportion for MBL production was 34%. However, in the Asian continent, only one meta-analysis report from Iran, specifically on P. aeruginosa has reported the pooled prevalence of VIM-1 associated MBL production to be 13% (95% confidence interval isolate = 10.5–16.5%] [23]. It is worth noticing that the maximum number of studies from Pakistan are based on the Acinetobacter spp. which emphasizes the highest incidence of MBL-producers in Acinetobacter spp. This can be related to the several other studies from the continent of Asia, where a high rate of CR is found to be associated with Acinetobacter spp. [24–29] Differences in the proportion of MBLs have been documented from all over the world based on surveys of community-acquired and nosocomial infections. However, we have found no meta-analysis study specifically on Metallo-β-lactamases.

Among 24 studies included, 22 focused on the in-door patients in hospital settings, which indicates that the resistance to carbapenem is more often associated with hospital acquired infections. A meta-analysis study describing the risk factors of CR in Enterobacteriaceae presented that the use of medical devices generated the highest pooled estimate (odds ratio [OR] = 5.09; 95% confidence interval [CI] = 3.38 to 7.67), followed by carbapenem use (OR = 4.71; 95% CI = 3.54 to 6.26) [30]. The highest incidence rate of MBL producers has been recorded from Sindh. Moreover, the rate of MBL producers has found to be varying among different studies. This variation may be associated with different factors like availability and use of antibiotics, difference of nosocomial and community-based studies, and methods to determine the MBL-producers [15, 16, 31]. The studies that have employed molecular methods to determine the gene variants have also reported the co-existence of other beta-lactamase genes with MBL related genes [12, 32, 33]. This aspect of the study is in concordance with reports from different regions of the world where the simultaneous presence of other beta-lactam genes with metallo β-lactamase genes have been described [34–36].

The NDM gene variant was found to be the most prevalent genetic determinant of MBL-production. The region being the epicenter for this gene variant in the community pathogens, bring out the need for detailed studies to prevent further dissemination. Many studies from the continent have reported a high prevalence of the NDM type MBL variant [37, 38]. Some of the studies included here have also reported the serine beta-lactamases, OXA variants, and KPCs to be the causative agents of carbapenem resistance. [12, 38, 39]. Limited literature and research on the subject suggest to consider the matter earnestly. Currently, there is no data published regarding the overall proportion of carbapenem resistance and MBL-production from Pakistan.

Conclusion And Recommendations

This investigation develop a comprehensive article on CR and MBLs by applying meta-analysis numerical approach for the available data across Pakistan. Based on the analysis results, the following conclusion were drawn.

The application of meta-analysis showed an increasing trend in the rate of CR and MBL incidence in the health care settings. It has been observed that no literature is available from the area of Baluchistan and Gilgit Baltistan, hence, the research communities are appreciated to work on the proposed subject in terms of making the data available.

In Pakistan self-medication is a common practice and number of healthcare facilities are low, resistance to antibiotics is increasing which mitigate their efficacy. Standard operating procedures and laws are the needed to standardize the use of antibiotics to prevent critical consequences in the future. In the era where antibiotic resistance is at its verge, the differences in CR and MBL molecular epidemiology for every country is very important for the counter actions and proactive action plans. Moreover, the study suggests effective intervention, such as implementation of national action plan, infection control programs, targeted therapeutics, antibiotic stewardship programs and proper disinfection methods in hospital settings that may play a beneficial role in combating the problem.

Abbreviations

CR: Carbapenem resistance, CDST: Combination disc test , DDST: Double disc synergism test, E-Test: Epsilometric test, MBLs: Metallo β-lactamases, MHT: Modified Hodge Test, MIC: Minimum inhibitory concentration, NDM: New Delhi Metallo-β-lactamase

Declarations

This is part of Ph. D thesis of Ms. Noor Ul Ain.

Ethics approval and consent to participate

This study was approved by local ethical committee (Citilab and Research Center Ref # 27th – 17CLRC/27th).

Consent for publication

Not applicable

Availability of Data

Please contact author for data requests

Funding

No funding was provided for this study

Authors' Contributions

Study concept and design of the study: (Saba Riaz); data collection: (Noor Ul Ain, Abdul Hannan and Namrah Imran); Statistical analysis: (Rehan Ahmad Khan), Preparation of draft, editing and reviewing: (Noor Ul Ain)

Acknowledgements

We would like to pay our gratitude towards College of Statistical and Actuarial Sciences, University of the Punjab and Citilab and Research center, Lahore Pakistan for supporting the study.

Competing interests

The authors declare that they have no competing interests

Authors' details

[1]Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan, [2]College of Statistical and Actuarial Sciences, [3]Citi Lab and Research Center, Faisal Town Lahore, Pakistan.

References

1. Watkins RR, Bonomo RA (2013) Increasing prevalence of carbapenem-resistant Enterobacteriaceae and strategies to avert a looming crisis. Expert review of anti-infective therapy 11 (6):543-545
2. Cai B, Echols R, Magee G, Arjona Ferreira JC, Morgan G, Ariyasu M, Sawada T, Nagata TD (2017) Prevalence of Carbapenem-Resistant Gram-Negative Infections in the United States Predominated by Acinetobacter baumannii and Pseudomonas aeruginosa. Open Forum Infectious Diseases 4 (3). doi:10.1093/ofid/ofx176
3. Nordmann P, Poirel L (2019) Epidemiology and diagnostics of carbapenem resistance in gram-negative bacteria. Clinical Infectious Diseases 69 (Supplement_7):S521-S528
4. Codjoe F, Donkor E (2018) Carbapenem resistance: a review. Medical Sciences 6 (1):1
5. Meletis G (2016) Carbapenem resistance: overview of the problem and future perspectives. Therapeutic advances in infectious disease 3 (1):15-21
6. Logan LK, Weinstein RA (2017) The epidemiology of carbapenem-resistant Enterobacteriaceae: the impact and evolution of a global menace. The Journal of infectious diseases 215 (suppl_1):S28-S36
7. Gniadek TJ, Carroll KC, Simner PJ (2016) Carbapenem-resistant non-glucose-fermenting Gram-negative bacilli: the missing piece to the puzzle. Journal of clinical microbiology 54 (7):1700-1710
8. Shin S, Jeong SH, Lee H, Hong JS, Park M-J, Song W (2018) Emergence of multidrug-resistant Providencia rettgeri isolates co-producing NDM-1 carbapenemase and PER-1 extended-spectrum β-lactamase causing a first outbreak in Korea. Annals of clinical microbiology and antimicrobials 17 (1):20
9. Al-Zahrani IA, Alasiri BA (2018) The emergence of carbapenem-resistant Klebsiella pneumoniae isolates producing OXA-48 and NDM in the Southern (Asir) province, Saudi Arabia. Saudi medical journal 39 (1):23
10. Bhatia R, Narain JP (2010) The growing challenge of antimicrobial resistance in the South-East Asia Region-Are we losing the battle? The Indian journal of medical research 132 (5):482

11. Ellappan K, Narasimha HB, Kumar S (2018) Coexistence of multidrug resistance mechanisms and virulence genes in carbapenem-resistant Pseudomonas aeruginosa strains from a tertiary care hospital in South India. Journal of global antimicrobial resistance 12:37-43
12. Ain NU, Iftikhar A, Bukhari SS, Abrar S, Hussain S, Haider MH, Rasheed F, Riaz S (2018) High frequency and molecular epidemiology of metallo-β-lactamase-producing gram-negative bacilli in a tertiary care hospital in Lahore, Pakistan. Antimicrobial Resistance & Infection Control 7 (1):128
13. Situation Analysis Report on Antimicrobial Resistance in Pakistan, Findings and Recomendations for Antibiotic Use and Resistance. (2018). Global Antibiotic Resistance Paternship and the center for Disease Dynamics, Economics & Policy
14. Nyaga VN, Arbyn M, Aerts M (2014) Metaprop: a Stata command to perform meta-analysis of binomial data. Archives of Public Health 72 (1):39
15. Rasool MH, Zaheer M, Hassan MF, Shafique M, Qamar MU (2019) Isolation and Antimicrobial Susceptibility Paradigm of Carbapenem Resistant Metallo-Beta-Lactamase Producing Gram Negative Rods. Pakistan Journal of Zoology 51 (3)
16. Indhar F, Durrani MA, Bux A, Sohail M (2017) Carbapenemases among *Acinetobacter* species isolated from NICU of a tertairy care hospital in Karachi. JPMA 67 (1547)
17. Irfan S, Zafar A, Guhar D, Ahsan T, Hasan R (2008) Metallo-β-lactamase-producing clinical isolates of *Acinetobacter* species and *Pseudomonas aeruginosa* from intensive care unit patients of a tertiary care hospital. Indian journal of medical microbiology 26 (3):243
18. Guhar D, Irfan S, Zafar A, Ahsan T, Hasan R Detection of metallo-β-lactamase producing clinical gram-negative non-fermenting isolates. In: Proceedings of 2014 11th International Bhurban Conference on Applied Sciences & Technology (IBCAST) Islamabad, Pakistan, 14th-18th January, 2014, 2014. IEEE, pp 73-74
19. Beigverdi R, Sattari-Maraji A, Emaneini M, Jabalameli F (2019) Status of carbapenem-resistant Acinetobacter baumannii harboring carbapenemase: First systematic review and meta-analysis from Iran. Infection, Genetics and Evolution
20. Pourhajibagher M, Hashemi FB, Pourakbari B, Aziemzadeh M, Bahador A (2016) Antimicrobial resistance of Acinetobacter baumannii to imipenem in Iran: a systematic review and meta-analysis. The open microbiology journal 10:32
21. Nasiri MJ, Zamani S, Fardsanei F, Arshadi M, Bigverdi R, Hajikhani B, Goudarzi H, Tabarsi P, Dabiri H, Feizabadi MM (2019) Prevalence and Mechanisms of Carbapenem Resistance in Acinetobacter baumannii: A Comprehensive Systematic Review of Cross-Sectional Studies from Iran. Microbial Drug Resistance
22. Livorsi DJ, Chorazy ML, Schweizer ML, Balkenende EC, Blevins AE, Nair R, Samore MH, Nelson RE, Khader K, Perencevich EN (2018) A systematic review of the epidemiology of carbapenem-resistant Enterobacteriaceae in the United States. Antimicrobial Resistance & Infection Control 7 (1):55
23. Sedighi M, Salehi-Abargouei A, Oryan G, Faghri J (2014) Epidemiology of VIM-1-imipenem resistant Pseudomonas aeruginosa in Iran: A systematic review and meta-analysis. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences 19 (9):899
24. Biglari S, Hanafiah A, Mohd Puzi S, Ramli R, Rahman M, Lopes BS (2017) Antimicrobial resistance mechanisms and genetic diversity of multidrug-resistant Acinetobacter baumannii isolated from a teaching hospital in Malaysia. Microbial Drug Resistance 23 (5):545-555
25. Salehi B, Goudarzi H, Nikmanesh B, Houri H, Alavi-Moghaddam M, Ghalavand Z (2018) Emergence and characterization of nosocomial multidrug-resistant and extensively drug-resistant Acinetobacter baumannii isolates in Tehran, Iran. Journal of infection and chemotherapy 24 (7):515-523
26. Lean S-S, Suhaili Z, Ismail S, Rahman NIA, Othman N, Abdullah FH, Jusoh Z, Yeo CC, Thong K-L (2014) Prevalence and genetic characterization of carbapenem-and polymyxin-resistant Acinetobacter baumannii isolated from a tertiary hospital in Terengganu, Malaysia. ISRN microbiology 2014
27. Chen Z, Liu W, Zhang Y, Li Y, Jian Z, Deng H, Zou M, Liu Y (2013) Molecular epidemiology of carbapenem-resistant Acinetobacter spp. from XiangYa Hospital, in Hunan Province, China. Journal of basic microbiology 53 (2):121-127
28. Sinha N, Agarwal J, Srivastava S, Singh M (2013) Analysis of carbapenem-resistant Acinetobacter from a tertiary care setting in North India. Indian journal of medical microbiology 31 (1):60
29. Aksoy MD, Çavuşlu Ş, Tuğrul HM (2015) Investigation of metallo beta lactamases and oxacilinases in carbapenem resistant Acinetobacter baumannii strains isolated from inpatients. Balkan medical journal 32 (1):79
30. van Loon K, Voor AF, Vos MC (2018) A systematic review and meta-analyses of the clinical epidemiology of carbapenem-resistant Enterobacteriaceae. Antimicrobial agents and chemotherapy 62 (1):e01730-01717
31. Javed H, Ejaz H, Zafar A, Rathore AW, ul Haq I (2016) Metallo-beta-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae*: A rising threat for hospitalized children. JPMA 66 (1068)
32. Jamil J, Haroon M, Sultan A, Khan MA, Gul N, Kalsoom (2018) Prevalence, antibiotic sensitivity and phenotypic screening of ESBL/MBL producer *E. coli* strains isolated from urine; District Swabi, KP, Pakistan. JPMA The Journal of the Pakistan Medical Association 68 (11):1704-1707
33. Khan E, Irfan S, Sultan BA, Nasir A, Hasan R (2016) Dissemination and spread of New Delhi Metallo-beta-lactamase-1 Superbugs in hospital settings. J Pak Med Assoc 66:999-1004
34. Balm M, La MV, Krishnan P, Jureen R, Lin R, Teo J (2013) Emergence of K lebsiella pneumoniae co-producing NDM-type and OXA-181 carbapenemases. Clinical Microbiology and Infection 19 (9):E421-E423
35. Iraz M, Özad Düzgün A, Sandallı C, Doymaz MZ, Akkoyunlu Y, Saral A, Peleg AY, Özgümüş OB, Beriş FŞ, Karaoğlu H (2015) Distribution of β-lactamase genes among carbapenem-resistant Klebsiella pneumoniae strains isolated from patients in Turkey. Annals of laboratory medicine 35 (6):595-601
36. Polotto M, Casella T, de Lucca Oliveira MG, Rúbio FG, Nogueira ML, de Almeida MT, Nogueira MC (2012) Detection of P. aeruginosa harboring bla CTX-M-2, bla GES-1 and bla GES-5, bla IMP-1 and bla SPM-1 causing infections in Brazilian tertiary-care hospital. BMC infectious diseases 12 (1):176
37. Rahman M, Prasad KN, Gupta S, Singh S, Singh A, Pathak A, Gupta KK, Ahmad S, Gonzalez-Zorn B (2018) Prevalence and molecular characterization of new Delhi metallo-beta-lactamases in multidrug-resistant Pseudomonas aeruginosa and Acinetobacter baumannii from India. Microbial Drug Resistance 24 (6):792-798

38. Baran I, Aksu N (2016) Phenotypic and genotypic characteristics of carbapenem-resistant Enterobacteriaceae in a tertiary-level reference hospital in Turkey. Annals of clinical microbiology and antimicrobials 15 (1):20
39. Hasan B, Perveen K, Olsen B, Zahra R (2014) Emergence of carbapenem-resistant *Acinetobacter baumannii* in hospitals in Pakistan. Journal of medical microbiology 63 (1):50-55

Tables 2-3

Due to technical limitations, Tables 2-3 are provided in the Supplementary Files section.

Figures

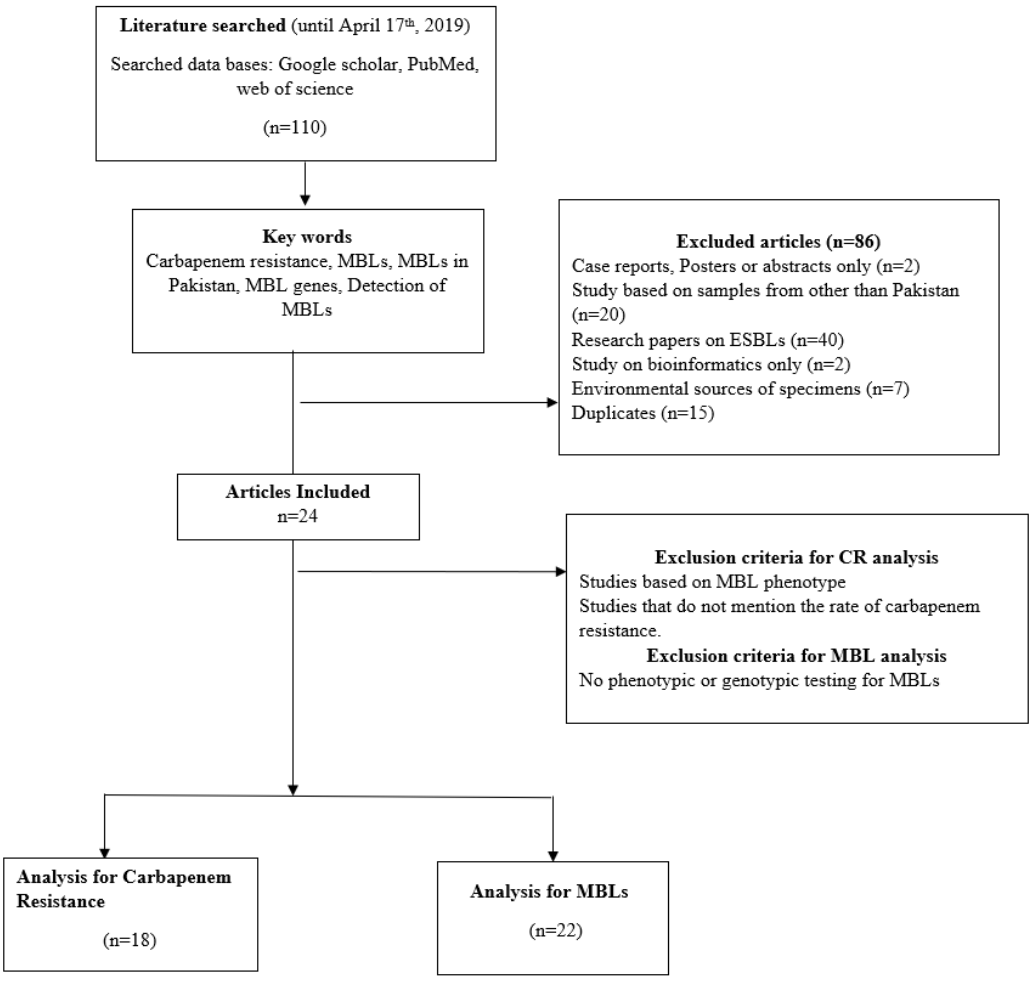

Figure 1

Flow diagram of the study selection, literature search, and article selection

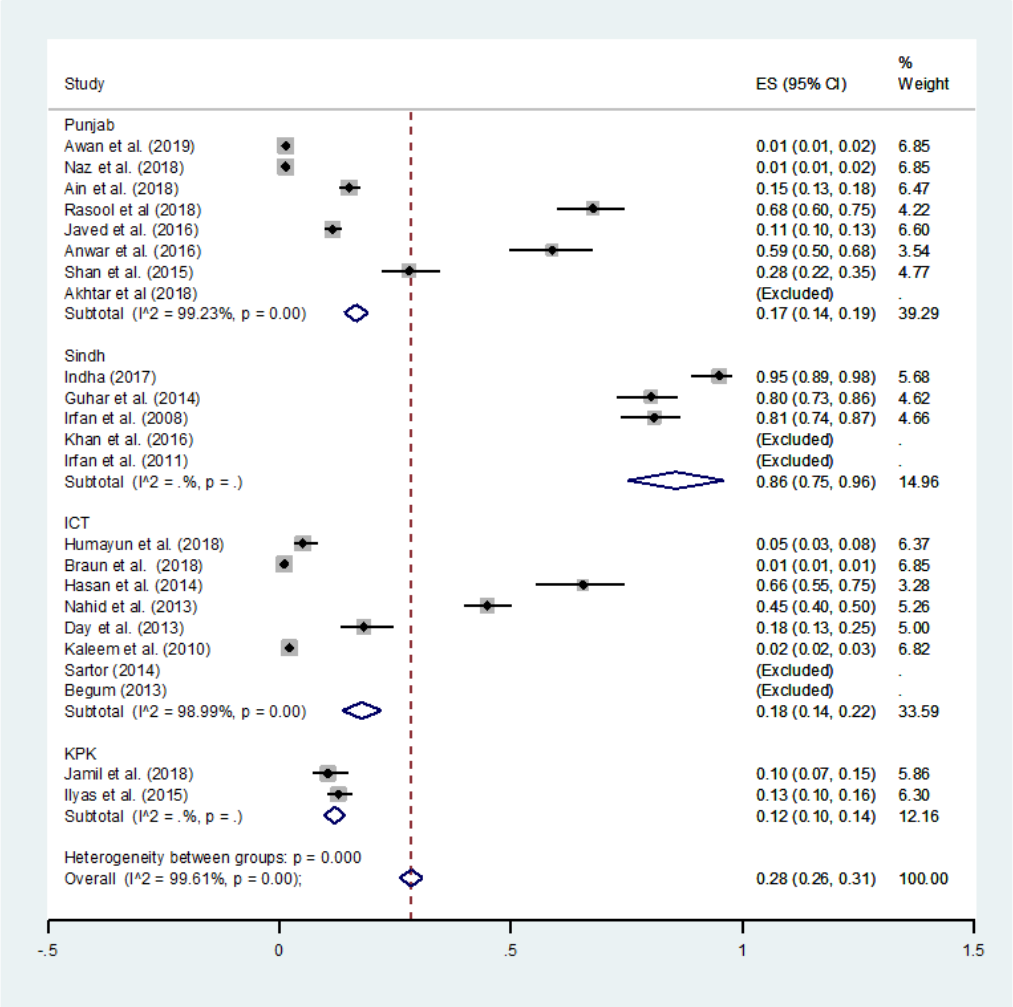

Figure 2

Proportion estimates of Carbapenem resistant clinical isolates in Pakistan. Midpoint of each horizontal line segment shows the proportion estimate in each study. Rhombic mark shows the pooled proportion from all studies

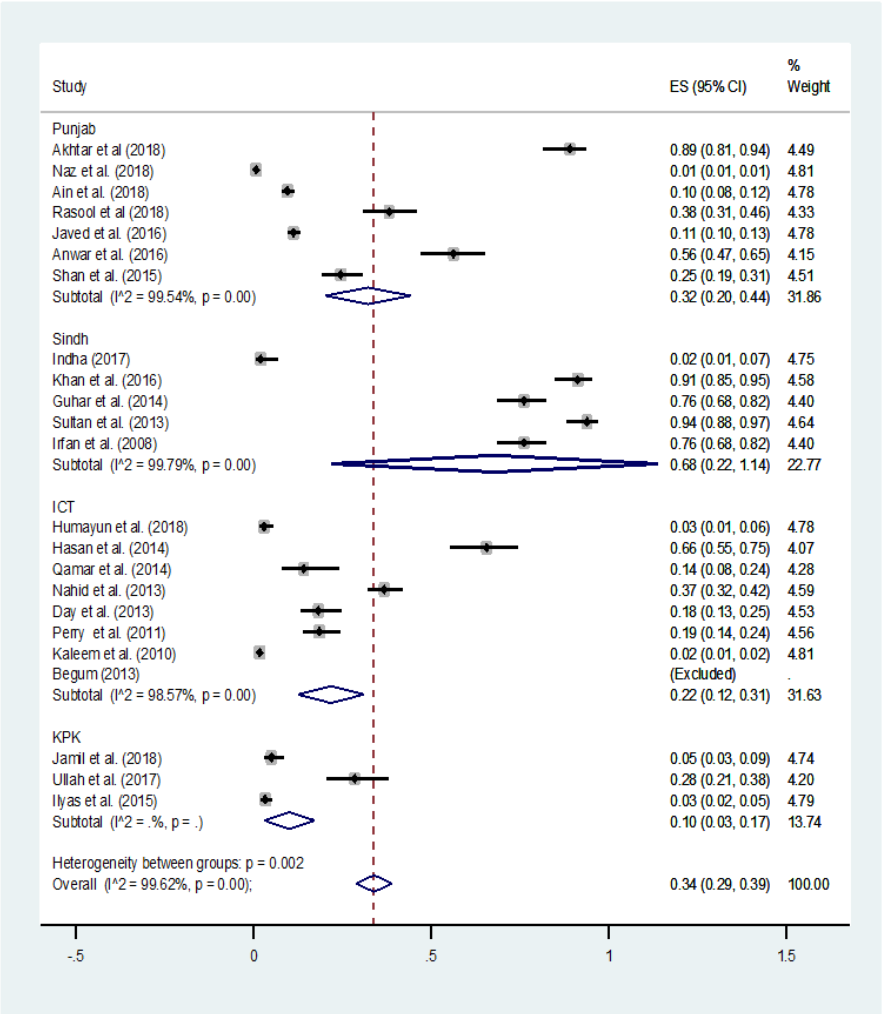

Figure 3

Proportion estimates of MBL-producing clinical isolates in Pakistan. Midpoint of each horizontal line segment shows the proportion estimate of MBL in each study. Rhombic mark shows the pooled proportion from all studies

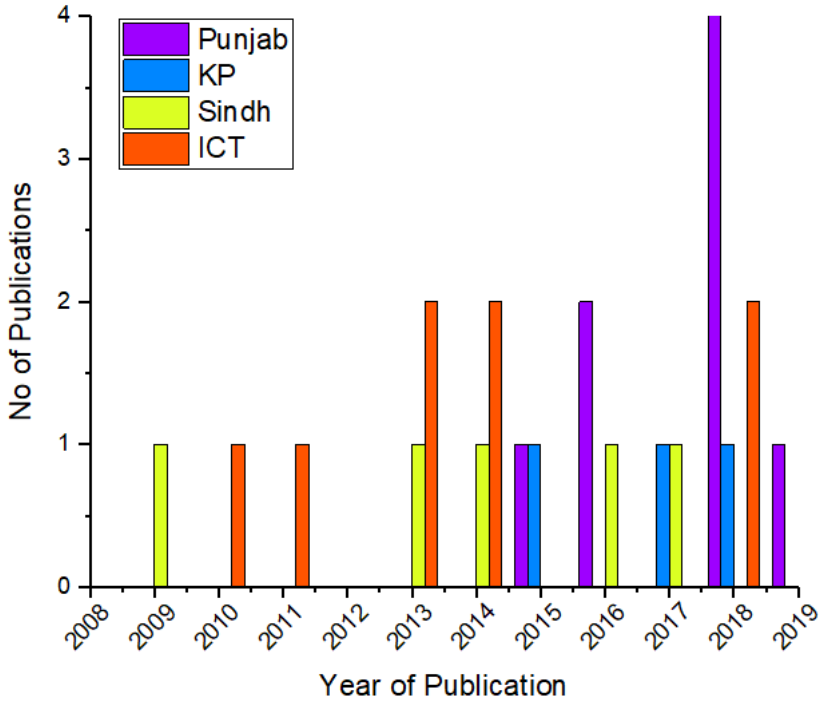

Figure 4

Published research on CR & MBLs across Pakistan

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Tables23.docx