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Significance of Metallo-β-Lactamase in the Emergence of Carbapenem-Resistant Pseudomonas *aeruginosa*: Future Prospective Ways to Evade Resistance Emergence

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Abstract: The worldwide emergence of Carbapenem-resistant Pseudomonas *aeruginosa* has become a global threat to public health, the clinical industry, the food industry, and the global economy as Carbapenm is the last resort drug for Multi-drug resistant Pseudomonas *aeruginosa* [25, 28]. Pseudomonas *aeruginosa* is a gram-negative bacilli ubiquitously found in nature that causes serious nosocomial infections increasing the severity of the health condition of hospitalized patients and adding to morbidity and mortality. Recent studies have discovered the emergence of Metallo-beta-lactamase genes in CRPA as a critical issue behind the rapid spread of CRPA strains internationally. Along with intrinsic mechanisms, CRPA strains have been found to contain these MBL genes in mobile genetic elements with other antibiotic-resistant genes, exacerbating the situation [3, 5, 29-31, 37, 38, 49]. In this study selected studies have been discussed that have been published in the last ten years in India to understand the epidemiological situation of our country on the significance of MBL genes in CRPA emergence. Furthermore, the impact of it on a global scale can also be delineated. It not only helps to understand the issues in-depth but also, to address the current approaches by which the resistance can be avoided without the constant need for another novel antibiotic. Antibiotic dosage abuse is another crucial factor in the MDRPA emergence [34]. There are multiple novel approaches being studied around the world. Chitosan-encapsulated POMs (Polyoxometalates) are one such solution that shows very promising potential. It is also a cheaper, easily produced, malleable, and bio-sustainable option than other available nanoparticle therapies. This nano-system can work as a suitable drug delivery system for Carbapenem by releasing the desired drug in a dose-dependent manner as well as containing self-antibacterial activity itself, efficient to combat both intrinsic and extrinsic resistance mechanisms in CRPA. Additionally, it can show efficiency in breaking the biofilm barrier created by many CRPA strains as an additional resistance mechanism [27, 41, 43]. The efficiency of the tiny drug carriers can be enhanced many times by immobilizing phage-encoded Tail Spike Proteins on them. This can confirm a targeted delivery of Carbapenem to treat MDRPA and reduce the chances of further emergence of CRPA strains [65, 66, 67]. This review article focuses on the potential of this newer approach to solve the ongoing CRPA issue.

Keywords: *Carbapenem-Resistant Pseudomonas aeruginosa; Metallo-Beta-Lactamase; Phage Therapy; Chitosan Polyoxomolybdate. Phage-Encoded Tail Spike Proteins.*

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I. INTRODUCTION

Carbapenems are one of the antibiotics ubiquitously prescribed to patients worldwide due to their broad spectrum efficiency against Multi-drug resistant Gram-positive and negative bacteria [26, 31]. As a member of the beta-lactam class of antibiotics, carbapenems consist of a beta-lactam ring that binds to the Penicillin Binding Protein (PBP) of bacterial cell walls and hinders bacterial cell wall synthesis [25, 30, 33, 46]. Additionally, they have a significant chemical modification to their ring structure, which involves a C-atom at position 1 [47]. This unique attribute renders high stability to the structure of Carbapenems against most beta-lactamase enzymes produced by antibiotic-resistant bacteria. Due to their less toxic effects than other available last-resort antibiotics such as Polymyxins; Carbapenems are globally preferred by clinicians [28, 31].

They are routinely prescribed as an empiric drug to treat nosocomial infections such as VAP (Ventilator Associated Pneumonia) caused by Pseudomonas *aeruginosa* at healthcare facilities [5, 6, 29, 36]. Pseudomonas *aeruginosa* is

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an opportunistic Gram-negative aerobic bacilli commonly found in nature (water, soil, etc.) [29, 49]. However, they are also cardinal issues for exacerbating clinical complications of hospitalized patients, especially those who are admitted to intensive care units and or with cystic fibrosis and burn wounds [8, 15, 27, 30, 40, 48, 49].

Since the last decade, treating antibiotic-resistant P. *aeruginosa* has been highly distressing due to the emergence of resistance against "last-resort" drug Carbapenem. It is not only a global health threat, but it also adversely impacts food security and creates a substantial socio-economic loss [12, 17, 25, 28, 31, 50]. Unregulated use of antibiotics is a critical factor in the emergence of Carbapenem-resistant P. *aeruginosa* worldwide [1, 2, 7, 12, 20, 23, 27, 30].

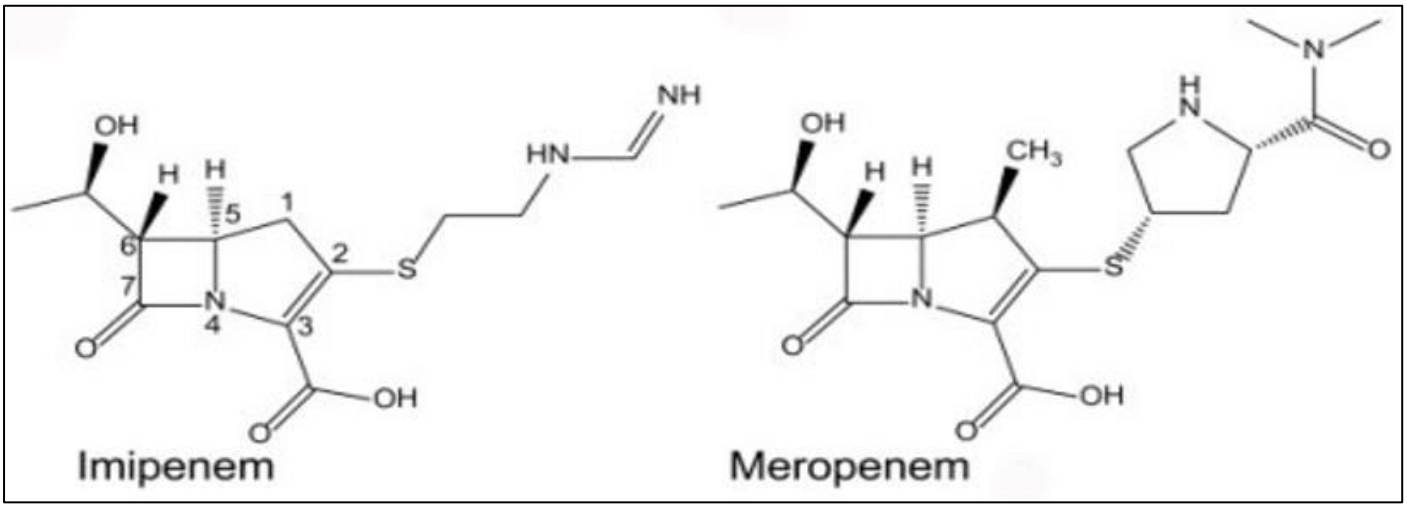

Fig 1 Carbapenem Structures (IMI and MER) [47].

P. *aeruginosa* can achieve antibiotic resistance through two mechanisms: a) the intrinsic mechanism that includes over expression of efflux pumps (*mex* AB, *mex* CD, *mex* EF, *mex* XY), hyperexpression of chromosomal *amp*C gene or porin loss (*opr*D); extrinsic mechanisms are obtained by acquiring antibiotic resistance genes such as ESBLs and Carbapenemases [5, 9, 11, 13, 25, 30, 31, 37, 38].

Throughout the years, intrinsic resistance mechanisms have been extensively studied. However, it has been observed in recent years that the frequency of MBL-producing CRPA (Carbapenem Resistant P. aeruginosa) is rapidly increasing [51].

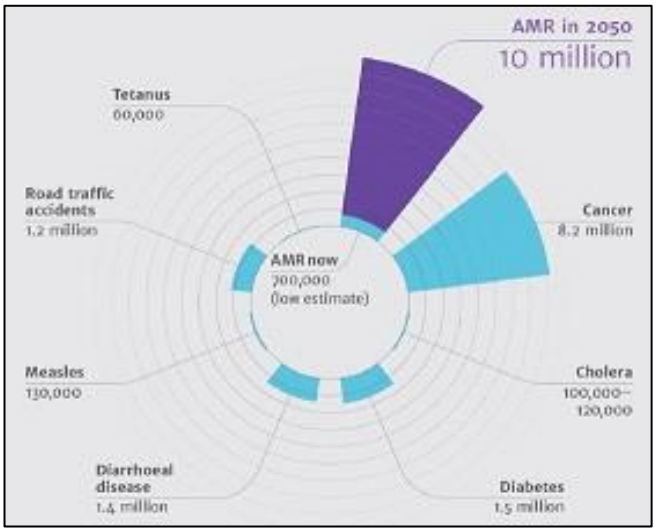

Fig 2 Anticipated Deaths Caused by AMR in 2050 Compared to Other Cardinal Health Issues [43].

Metallo-β-lactamases (MBL) are carbapenemases (class B) present in periplasmic space of gram-negative bacteria that hydrolyze Carbapenem by inactivating the beta-lactam ring using one or two zinc ions at the active site [24-26, 32, 53]. Further investigation corroborates the presence of MBL genes with other antibiotic resistance genes in integrons or mobile genetic elements; which explains the incessant spread of these genes and the prevalence of MDR strains [23, 35]. It is not only confined among clonal spread but it also has become a global issue involving interspecies transmission [3, 32, 33, 38, 51]. Moreover, Pseudomonas *aeruginosa* can also form biofilms on tissues and inanimate objects, which makes the treatment even more difficult. It produces alginate EPS (Extracellular Polymeric Substances) layer and protects the cells in adverse conditions by enhancing antibiotic resistance through eliminating transport of elements [28, 40, 51].

This review article focuses on 10 years of previous research studies on CRPA in India to depict the epidemiological scenario for analyzing the rapid emergence of CRPA across India.

A. Characterization of Metallo-β-Lactamase:

The objective of this epidemiological review is to analyze previous research works on MBL-producing CRPA in the last ten years from 2011 to 2021 to understand the significance of the recently observed rapid increase of MBL genes in the prevalence of MDRPA, leaving the treatment almost impossible. Through the detailed elucidation, we can also recognize possible ways to control morbidity and mortality.

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The rapid clonal dissemination of MBLs (IMP and VIM) can be explained proficiently with the research study by Durgesh G. Deshmukh *et al.* in 2011, which clarifies the high mobility of MBLs as a result of their position in integrons (commonly found in type 1 integrons, type 3 is also observed) that is further embedded in transposons. Thus, MBL genes can also be transferred from P. *aeruginosa* to *Enterobacteriaceae* and vice versa. Other antibiotic-resistant gene cassettes integrated into integrons with MBL genes result in MDRPA strains [3, 34, 35] (Table 1).

Table 1 Susceptibility Shown by MBL and Non-MBL Producing Gram Negative Bacteria in Vitro [3]

Antibiotics	Non-MBL strains (n – 617) %	MBL strains (n – 21)
Ampicillin (10 µg)	2.84	0
Cefazolin (30 µg)	36.36	0
Cephalothin (30 µg)	36.24	0
Cefoperazone (30 µg)	36.36	0
Ceftriaxone (30 µg)	43.56	0
Cefotaxime (30 µg)	44.48	0
Ceftazidime (30 µg)	46.31	0
Ceftazidime/Clavulanic acid (30 µg/10 µg)	52.56	0
Cefepime (30 µg)	60.78	0
Piperacillin (100 µg)	66.74	0
Piperacillin/Tazobactam (100 µg/10 µg)	78.24	0
Imipenem (10 µg)	100	0
Gentamicin (10 µg)	64.88	19.04
Amikacin (30 µg)	77.84	28.6
Ciprofloxacin (5 µg)	65.72	14.3
Levofloxacin (5 µg)	88.68	28.6
Colistin (10 µg)	100	100

MBL: Metallo-β-lactamases

They isolated the majority of MBL-producing bacteria from pus and tracheal secretion samples. Out of a total of 638 gram-negative isolates, eight (1.25%) were IMP-resistant Pseudomonas *aeruginosa*. Seven (87.50%) isolates were MBL-producing strains, and one (12.50%) did not produce either AmpC or any other carbapenemase. It indicates the ability of Pseudomonas *aeruginosa* to show Carbapenem resistance through both intrinsic and extrinsic mechanisms [3, 4].

A research paper published in 2012 conducted in Tamil Nadu, India on the influence of blaVIM and blaIMP (two prevalently found MBL strains in India) for the emergence of CRPA (Carbapenem-resistant P. *aeruginosa).* They reported that out of 61 isolated samples of CRPA, 36 isolates were identified for $bla_{VIM/IMP}$ production by both the PCR and the MBL screening test with IMP discs dipped in EDTA. 34 isolates were also positive in the phenotypic test Modified Hodge Test in Muller-Hinton agar for detection of Carbapenemase, but 2 samples showed negative results. These two samples were retested using Muller-Hinton agar with 70 mg/L zinc sulphate concentration. Now these two samples were detected as MBL producers emphasizing the impact of zinc ions in MBL activity [24]. On the other hand, 6 samples were MHT positive but were negative for the MBL screening test. This confirms again intrinsic approaches such as loss of porins (*opr*D) and overexpression of efflux pump taken by CRPA. Some MBL screening test positive samples including CRPA were not recognised in PCR detection of $bla_{VIM/IMP}$. It infers the presence of other variants of MBL genes (SPM1, GIM-1, SIM-1 and NDM-1) [3, 23, 35]. They further elaborate on the possibility of engendering false positive results for the MBL screen test. It has been reported in the case of P. *aeruginosa* that EDTA increases the cell membrane permeability. Hence, the MBL screening test is not considered a standardized test by CLSI guidelines even though it shows high accuracy [1].

This is further validated by another study published in the same year from a tertiary care hospital in Chennai. K. Arunagiri *et al.* found that 70.1% of their 67 MDRPA samples were positive in CDT, an MBL-screening test specific in this study for $bla_{VIM/IMP}$. Among these samples, 87.2% strains predominantly contained bla_{VIM} gene and only 4.3% had bla_{IMP}. However, they also found, similar to the observation of the study by M. Shanthi Amudhan and colleagues, that 4 MDRP CDT-positive isolates did not produce $bla_{IMP/VIM}$ type of MBLs. Thus, they remained negative in genotypic PCR amplification. Furthermore, three of twenty CDT-negative isolates were genotypically positive for bla_{VIM} gene. The non-identification can happen due to several reasons. The presence of bla_{VIM} gene in these isolates can be cryptic and might show increased expression after prolonged exposure to the Carbapenem drug. They also discussed that exertion of 10 µg of IMP/MER was unsuitable for the MBL-screening method which can contradict the result considering the different MIC values of various MBL producers (optimum range being 8-9 µg). Therefore, antibiotic concentration is evidentially a crucial factor for the upregulation of Carbapenem-resistant PA strains [34]. Moreover, it can also result from overexpression of ESBL/ Amp C enzymes and alteration in bacterial membrane permeability because of EDTA [5, 37]. 17 (25.37%) MDRP isolates were positive for phenotypic CDT but negative for genotypic PCR. It indicates the significance of intrinsic mechanisms such as the alteration of oprD2 in the emergence of MDRPA [2].

Srujana Mohanty *et al*. reported in 2013 that 7/38 (18.4%) CARPA samples in their study were also resistant to Colistin, an antibiotic under the Polymyxin type. The highly toxic effects limit its use in the treatment of CARPA. It highlights on incessant cyclic nature of the emergence of MDRPA eliminating therapeutic options. More CARPA samples were MDRPA than Carbapenem susceptible isolates. However, any notable deviation in MIC50 and MIC90 of Colistin was not observed for CA susceptible, CA-resistant MBL-producing, and non-producing strains except MIC90 being the highest for MBL-producing CRPA. MIC50 for MER and IMP were two to four times higher for MBL-producing CARPA samples compared to the CARPA MBL non-producers [15].

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Antibiotic	COLISTIN MIC µg/ml					
	Carbapenem Sensitive		Carbapenem – Resistant			
			MBL- Positive		*MBL- Negative*	
	MIC 50	MIC 90	MIC 50	MIC 90	MIC 50	MIC 90
PSEUDOMONAS	1.25	2	0.87	4	1.5	3

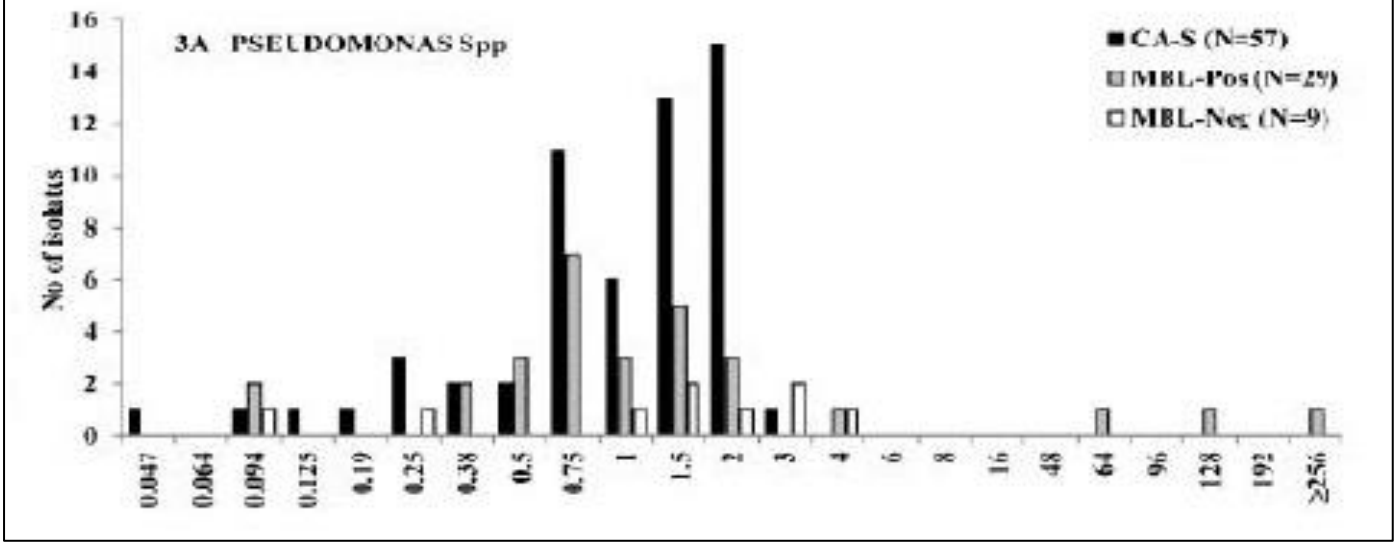

Fig 3 MIC50 and MIC90 of MBL and Non-MBL Producing Pseudomonas spp. [15]

Kolhal Veerappa et al. worked on the interlink between IMP-resistant MBL-positive Pseudomonas *aeruginosa* and a high mortality and morbidity rate in 2014. Their research data ratified it as 42.86% (6/14) of MBL-positive IRPA samples caused VAP (Ventilator-associated pneumonia) in patients that led to death within 4 days**.** 57.14% (8/14) of IRPA samples isolated from patients in ICU severed the complications and caused a lengthy recovery of 21 ± 4.95 days. Whereas, 80% (8/10) patients in ICU carrying IR MBLNP strains exhibited faster recovery within 6.125±2.1 days. A striking observation was made in this study as they reported the isolation of IRPA samples from patients who had IMP therapy three weeks ago [54]. It implies towards the correlation between prior introduction of Crabapenem and the duration of hospitalization with the emergence of Carbapenem resistance which is similar to other studies [37, 54].

The research paper published by Shikha Ranjan and colleagues in 2015 reported MHT (Modified Hodge Test) as the most efficient phenotypic method to detect beta-lactamase-producing PA. They considered the Epsilometer test (E-test) as standard as it is a more convenient and easily achievable test than genotypic detection test. They compared MHT with other commonly used phenotypic tests such as CDT (Combined Disk Test), DDST (Double-Disk Synergy Test) using EDTA+IMP/CAZ, EDTA disk Penetration Test (PT) CAZ, ceftizoxime, cefepime, and cefotaxime. MBL E-test detected 24 samples (15%) as MBL-producing P. *aeruginosa*. MHT showed positive results for 26 samples, which includes additional 2 samples that probably imply the production of other kinds of beta-lactamases (A, C, or D). Other phenotypic positive results were as follows: CDT (23), DDST (17), PT (15). CDT and PT tests also showed false positive results. However, DDST was the most (100%) accurate method for MBL detection. EDTA is a metal ion chelator that can inactivate any MBL as it requires one or two zinc ions at its tunable active site [24, 26, 32]. EDTA also shows excellent membrane permeability and deleterious effects on bacteria. Thus, the presence of EDTA in phenotypic methods to identify MBL-producing PA creates contradictions by producing false-positive results [10].

Noteworthy findings were observed by Deepjyoti Paul *et al*. in their work published in 2016. Two clinical samples of P. *aeruginosa* harboring *the* bla_{VIM2} gene were isolated from patients admitted to the Silchar Medical College and Hospitals, India. bla_{VIM2} gene was successfully horizontally transferred through a conjugative approach using Inc F plasmid of 30 kb from E. *coli* JM107 donor strain to E. coli recipient strain B. However, the transfer remained unsuccessful in this study from P. *aeruginosa* to E. *coli*. This failure is inconclusive in determining the possibility of the gene being horizontally transferred among various bacterial species in nature, health care setups, and *in vivo* [23]. Both of the isolated bla_{VIM2} genes found in gene cassettes co-existing with bla_{NDM-1} and bla_{VEB-1} (ESBL) genes. This perpetuates the significance of the presence of bla_{VIM2} in gene cassettes along with other antimicrobial resistance genes in the prevalence of MDRPA. Interestingly, bla_{NDM-1} gene could not be hybridized with bla_{VIM2} in a 30kb

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plasmid but in a 24kb plasmid. They also found *the* bla_{NDM-1} gene in a plasmid that is aberrated from other studies that found bla_{NDM-1} to be a chromosomal gene. This insinuates a possible genetic location shift of MBL genes [14].

Lavanya Mohanam *et al.* published their study on the co-existence of MBL genes in CRPA in 2017. 10.3% (22/213) of their P. *aeruginosa* isolates were IMP resistant and the MBL gene was encoded in Integron 1. They discussed the superiority of genotypic methods (91%; 20/22) for detecting MBL presumptively, over phenotypic methods (82%; 18/22) that involve the application of EDTA. EDTA being a metal ion chelator, inactivates MBLs by chelating zinc ions essential for the activity of these enzymes [24]. Thus, the sensitivity of the phenotypic methods is not as reliable as genotypic ones. The co-existence pattern of the MBLs was as follows: three (14%) isolates with bla_{VIM} and bla_{NDM}; two (9%) isolates showed bla_{IMP} and bla_{NDM} and one (5%) isolate had both bla_{VIM} and bla_{IMP}. One sample co-exhibited (4.5%) three MBLs (bla_{IMP}, bla_{VIM} and bla_{NDM}) together. 10 (45%) isolates containing class I Integron did not show positive results in the amplification of variable regions Int I based on 5'CS and 3'CS primers. A recombination at 5' CS of Int I with the *tns* module of Int 2, which is similar to Tn402 - the progenitor of class I Int, can result in these negative results.

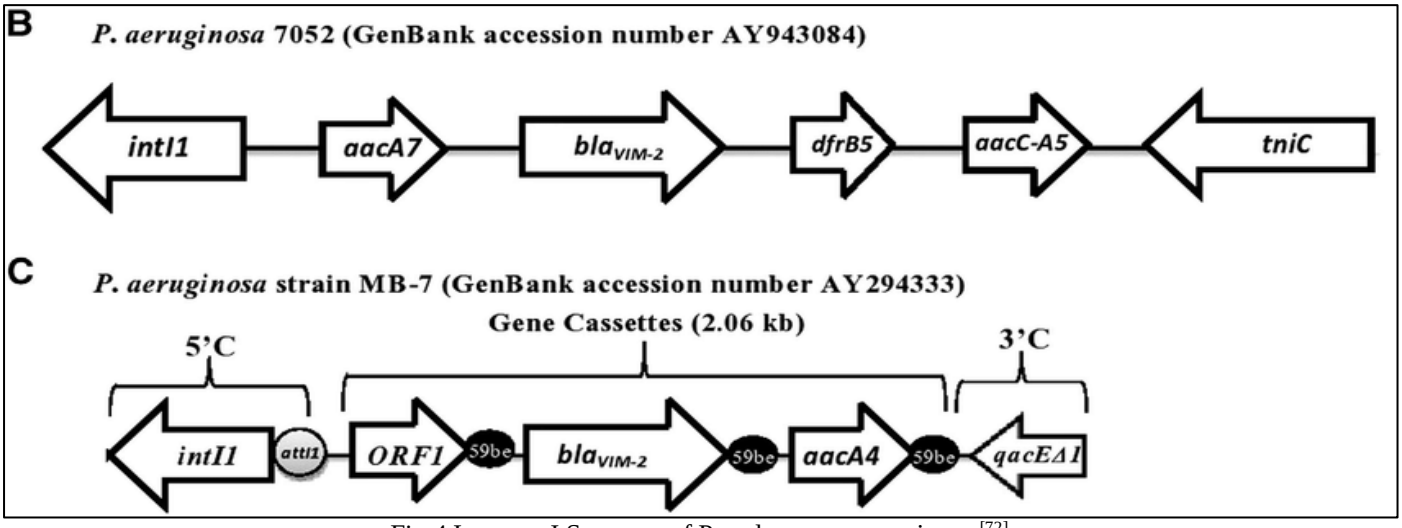

Fig 4 Integron I Structure of Pseudomonas *aeruginosa* [72]

This indicates the genetic relatedness of class I and II integrons [16, 35].

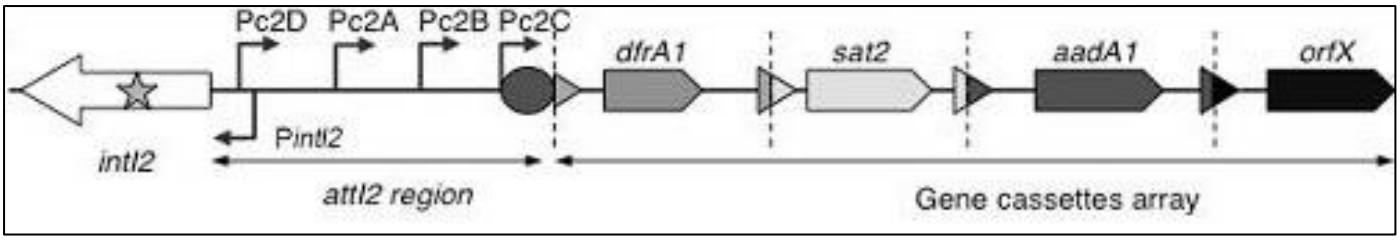

Fig 5 General Structure of Class II Integrons [73]

Agila Kumari Pragasam and her team focused on the genetic relatedness of CRPA circulating across hospital facilities in India in 2018 and found alarming data exhibiting novel CRPA clones derived from prevalent high-risk international clones (ST235, ST357). 40% and 20% of their 507 CRPA isolates, were carbapenemase and ESBL+carbapenemase-producing CRPA. 33% of the carbapenemase-producing strains had a single carbapenemase [bla_{VIM} (21%), bla_{NDM} (8%), bla_{GES} (3%) and bla_{IMP} (1%)]. 6.4% co-exhibited double carbapenemases such as $bla_{NDM + VIM}$ in 3%, $bla_{NDM + GES}$ in 2%, $bla_{VIM + GES}$ in 1%, $bla_{IMP + VIM}$ in 0.2%, $bla_{IMP + NDM}$ in 0.2%. Rest 1% carried triple carbapenemases, $bla_{IMP + VIM + GES}$ (0.6%) and $bla_{IMP + VIM + NDM}$ (0.2%). 13% of ESBLs and carbapenemase co-producing isolates showed one gene each of ESBL and carbapenemase. Multiple ESBL with carbapenemase-producing isolates were 5.6%. 156 carbapenemase-producing isolates were further tested for epidemiological study. They found 32 (21%) and 15 (10%) of these 156 isolates belonged to high-risk international clones ST357 and ST235. 38 isolates (24%) showed novel emerging sequence types (ST) among which >80% of single-loci variants were derived from high-risk international clones. This strikingly alarming observation that evinces regional dissemination of ST variants disputes the inception of Pseudomonas *aeruginosa* to be non-clonal [30].

In 2018, K. Ellapan *et al.* published their study on the different multi-drug resistance mechanisms involving both intrinsic (porin loss and resistance–nodulation–cell division (RND) for efflux pump systems) and extrinsic (carbapenemase production); and the accumulation of

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virulence genes ([*opr*D]; [*mex*AB and *mex*CD]) in CRPA. In accordance with their study, MBL genes are commonly encoded in integron gene cassettes in bacterial chromosomes as a part of a large gene island co-existing with other virulence genes. MBLs can also be transmitted through plasmids as well. All these factors significantly impart to the production of MDRPA. Among 156 CRPA isolates, 48.7% were MBL-producing out of which, 1 isolate had both bla_{VIM} + bla_{NDM} (7.1%). Nine isolates had porin loss (5.8%) and two of those also harbored bla_{VIM} gene. *mexA* gene was predominantly expressed in efflux pump-positive isolates. *algD* expression, which is important for biofilm production was detected in 93% of CARPA isolates. Other virulence genes were also significantly detected such as *alg*U (89%), *rhl*R (84%), *las*R (81%), and *exo*S (76%) which are mainly involved in virulence factors like alginate production, motility regulation, biofilm production, quorum sensing, toxin production, etc. The *las*B and *plc*H genes that are involved in the disruption of the human immune system and physiological barriers were observed in 94% and 92% of CRPA [55].

A similar work by Nishu Verma *et al*. in 2019 confirms the co-harboring of more than one MBL gene in CRPA isolates [15]. They detected bla_{VIM} in 30 isolates (30/102, 29.1%) and bla_{NDM-1} in 29 (29/102, 28.4%) isolates. These are the two most prevalent MBL gene variants found in most Asiatic countries and VIM-2 is the most prevalently found MBL worldwide [32, 38]. Six (6/102, 5.88%) CRPA isolates had a combination of both bla_{VIM} and bla_{NDM-1} genes. Two (2/102, 0.02%) more isolates co-harbored bla_{VIM} and bla_{NDM-1} along with bla_{SPM} and bla_{GIM}, respectively. All the samples were detected as MBL producers using both genotypic and phenotypic (only in CDST) methods. However, DDST could not recognize one isolate of bla_{VIM} and bla_{NDM-1} producer and the isolate that had bla_{VIM} and bla_{NDM-1} with bla_{SPM} [18].

Another research paper on plasmid-encoding MBLs in P. *aeruginosa* by Deepjyoti Paul and his team published in the same year describes MBL genes (bla_{VIM2} and bla_{NDM1}) self-conjugative using an IncF plasmid of 40 kb (bla_{NDM-1}; IncFIA) and 55 kb (bla_{VIM}, IncFIB). The stability of these transformant (E. coli DH5α) and transconjugant (azide-resistant E. *coli* J53) plasmids was weaker than parental plasmids found in PA samples carrying bla_{VIM2} and bla_{NDM1}. SDS was the most effective eliminating agent in their study for plasmids carrying MBL genes with complete elimination in a single treatment, followed by EtBr and Acridine orange with complete elimination in the second and third treatments. They also report that the elimination of MBL gene-carrying plasmids could successfully reverse a CR PA strain to a susceptible one. The significant decrease in the bla_{VIM-2}-encoding plasmid copy number under antibiotic pressure (Ertapenem and Aztreonam) corroborates the importance of controlled and application of appropriate doses of Carbapenems [27, 34]. Even though in this particular study, increasing the concentration of IMP and MER could successfully reduce plasmid copy numbers this should be further analyzed using a larger sample size of MBL-producing CRPA. Additionally, SDS can cause false negative results for the plasmid elimination test as similar to EDTA, it acts on bacterial membranes and increases the membrane permeability. Hence, these isolates become susceptible to Carbapenems [19].

S. Sarkar *et al*. studied the beta-lactamase profile and biofilm production ability in MDR P. *aeruginosa* in 2020 in Kolkata. Biofilm-producing CRPA shows increased resistance against antibiotics and detergents than planktonic cells making the infections more difficult to treat. They reported that 53.34% of 394 PA isolates were carbapenemase-producing detected using the multiplex PCR genotypic method. bla_{NDM-1} (68.75%) and bla_{VIM} (18.75%) were the most prevalent carbapenemase genes and co-production of bla_{NDM-1}+ bla_{VIM} was observed in 12.5% isolates. In this study, ESBL (36.80%) was the most commonly found beta-lactamase enzyme using the DST phenotypic method. AmpC and carbapenemase (MBL) producers were 51 (12.94%) and 49 (12.43%) using the DST method. Biofilm was produced by 158 (40.10%) isolates. Further analysis represented that out of 204 MDRPA (204/394; 51.77%), 8 were strong, 17 were moderate, and 52 were weak biofilm-producers. This data infers that biofilm formation is an additional resistance mechanism for less resistant strains of CRPA. However, this additional mechanism may have significance in the protection and transmission of MBL genes among CRPA strains [39].

The research paper published by K. Mahesh Kumar *et al*. in 2021 reported MHT (18, 75%) as the most efficient phenotypic method followed by CDT (17, 70.83%) and E-test (14, 58.33%) method. Among 24 CRPA, 11 (46%) showed *the* bla_{VIM2} gene as the prevalent MBL variant, similar to other previously discussed studies substantiating it as the predominant MBL gene worldwide [4, 38, 56]. Overall, the resistance mechanisms are induced by the lack of drug penetration and inactivation of Carbapenem and other antibiotics involving beta-lactam hydrolyzing enzymes. Metallo-beta lactamase enzymes require bivalent zinc cations as co-factors for their hydrolyzing activity, which can be inhibited with a metal ion chelator [24]. These MBL-producing isolates cause bacteremia, Ventilator associated pneumonia (VAP) in patients admitted especially in ICU, and are often associated with outbreaks of nosocomial infection in hospital settings. MBLs are found both in bacterial chromosomes and plasmids. Hence, early detection and appropriate diagnosis are critical to combat the emergence of CRPA and treat the infections effectively [56].

B. Future Perspective for CRPA Treatment:

- *Phage Therapy:*

Ongoing research brings a beacon of hope to mitigate current critical issues caused by Carbapenem-resistant Pseudomonas *aeruginosa*. Recent studies on phage therapy show potential as a promising tool to treat infections caused by CRPA [61]. Phages having bacteria naturally as hosts, can target pathogenic bacteria more spontaneously and accurately. Databases like phage-banks are available to find bacteriophages particularly active against Pseudomonas *aeruginosa* [61].

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Novel phages like DRL P1 are ideal candidates for such therapy due to their high specificity towards Pseudomonas sp.; which curtails non-specific targeting to commonly found beneficial bacteria in human body like E. *coli*, Streptococcus sp, Bacillus sp., etc. [61]. The phage body of DRL P1 consists of a head (diameter 197.47 nm), a neck, a contractile tail that assures high specificity (93 nm), a base plate, and tail fiber geometry. Having a large capsid diameter contributes to its better survival chances. Additionally, DRL P1 shows clear plaque in the double agar assay method, which confirms their lytic ability against Pseudomonas sp. The phage is also capable of affecting biofilms of mono or mixed culture. S. Sharma *et al*. also demonstrated the compatibility of DRL P1 in diverse storage procedures for long-term use including lyophilization. These advantageous features of DRL P1 make it a potential phage choice for therapy to cure CRPA infections . The large capsid of DRL P1 can also be used as a carrier for Carbapenem drug for targeted antibiotic therapy. This can be achieved by an infusion method as demonstrated in figure 7 [61] for cytotoxic drugs or by achieving capsid modification of engineered VLPs [59, 63].

AM.P2, a phage specific to Pseudomonas *aeruginosa* - found in wastewater and the human gut, shows clear plaque with a 5mm diameter and turbid edge in the double agar assay method as described in the research paper by Menon *et al*. This is critical because it implies the efficiency of AM.P2 phage to diffuse through agar and attack planktonic bacterial cells due to their small head size. Its noteworthy infection efficiency (0.1 phage/bacterium) and high lytic nature make it a prospective phage therapy choice. Moreover, the AM.P2 phage being a member of *podoviridae*, contains a noncontractile tail that requires a simpler method to infect host cells than phages like DLR P1 with contractile tail. This is why; AM.P2 can also be used for therapeutic purposes for bacterial cell lysis or as a drug carrier for Carbapenem [64].

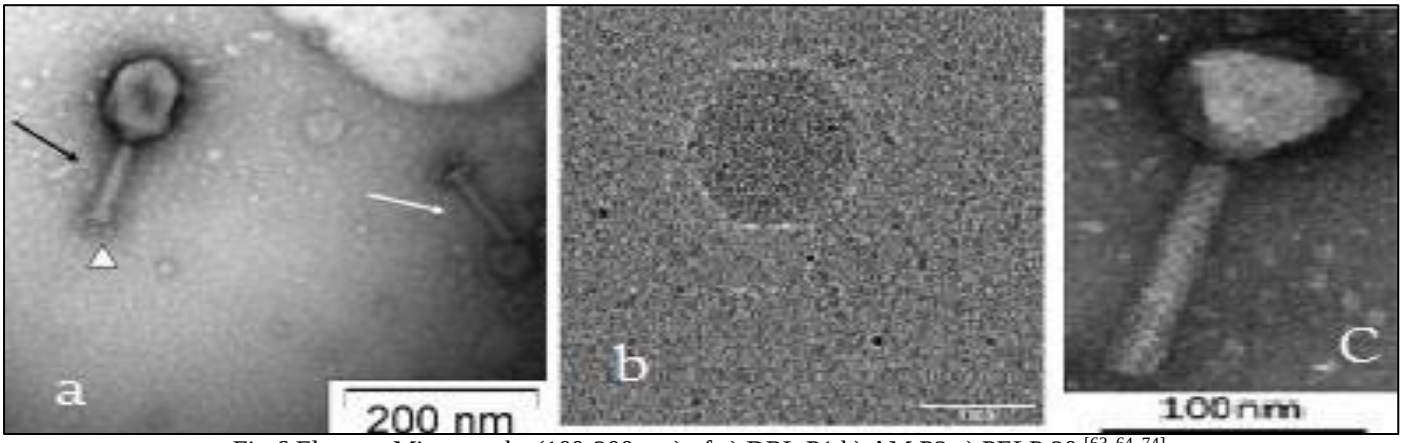

Fig 6 Electron Micrographs (100-200 nm) of a) DRL P1 b) AM P2 c) PELP 20 [63, 64, 74]

Zara Chegini *et al*. mentioned in their paper that the PELP 20 phage proved to be a highly effective phage against biofilm-forming P. *aeruginosa* strains when tested *in vivo* in the mice model with chronic lung infections caused by P. *aeruginosa* as 3-log phage could reduce the biofilm [40]. Furthermore, phages also exhibit a broad spectrum host range against PA and inhibit bacteria from spreading into the blood. These unique qualities are highly desirable in candidates for selecting a phage therapy with appropriate phage types [40, 65, 66, 67].

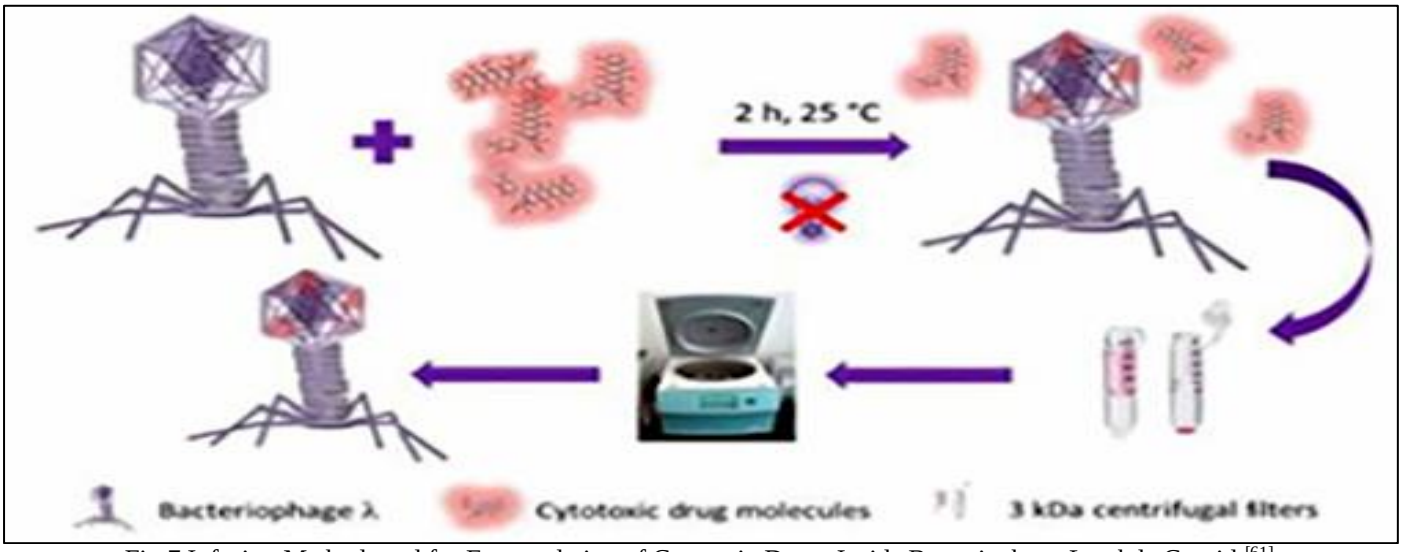

Fig 7 Infusion Method used for Encapsulation of Cytotoxic Drugs Inside Bacteriophage Lambda Capsid [61]

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P. Serwer *et al*. explained phage capsids are gated that can be manipulated with highly specific heat and chemical treatment [41]. However, manipulating the phage gate is attainable *in vitro* but it enhances complications for drug delivery *in vivo*. This challenge may be overcome by using genetic engineering tools appropriate for introducing a short nucleotide sequence only essential for opening the phage gate to escort Carbapenem drug into the CRPA cell [44, 57, 58].

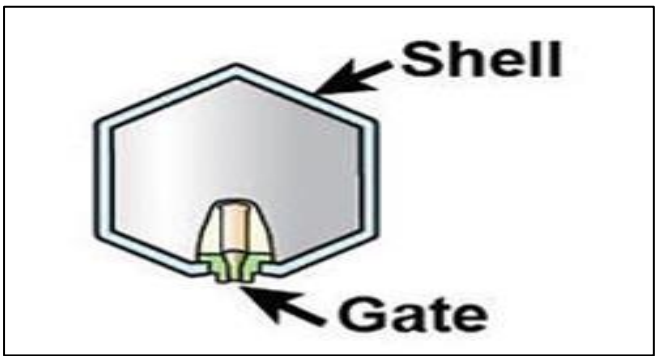

Fig 8 Schematic Illustration of Gated Structure of Phage Capsid [41]

Even though further extensive studies are an exigency to confirm the efficacy of these phages in patients, it can be deduced from this discussion that phage therapy can resolve the enigma of dearth of suitable clinical treatment for MDR P. *aeruginosa*. The efficiency of the above-mentioned phage types can be increased by using a cocktail phage therapy and/or combination phage therapy where the phages work in synergy with a chosen antibiotic, in this case, Carbapenem. Phage cocktails are not only useful for inhibiting bacterial infection but also for increasing affectivity via eliminating phage-resistant CRPA cells [40].

➢ *Other Approaches:*

The limitations of phage therapy in curing patients infected with biofilm-producing Carbapenem-resistant PA, phage resistance and the difficulties faced with high specificity of bacterio-phages towards particular PA strains while treating multiple species cultures *in vivo* and complications involved in drug loading and releasing led the researchers towards other novel approaches that can resolve this emerging issue effectively if suitably exerted [40].

Polyoxometalate (POM) is an inorganic chemical compound that has been being studied since 1826 as a potential multi-dimensional answer for Carbapenem-resistant bacterial strains. Species of Polyoxotungstates such as $[P_2W_{18}O_{62}]$ and $[PT_{12}W_{10}O_{40}]$ reduce β-lactamase production in bacterial cells [43]. Thus, it reverses beta-lactam (Carbapenem) antibiotic-resistant bacteria to susceptible ones and reinforces the beta-lactam-containing drug in treatment. More than one research data can be found that corroborates the excellence of Polyoxotungstates e.g. -lacunary keggin species of $[PW_{11}O_{39}]^{7-}$ as an antibacterial agent that works in synergy with beta-lactam-based antibiotics. Keggin-type $[SiMo_{12}O_{40}]^{4-}$ also shows a synergistic effect with beta-lactam antibiotics (Piperacillin, Oxacillin, and cefazolin) against VRSA. Polyoxomolybdates have been observed to be less active compared to polyoxotungstates and polyoxovandates when tested against gram-negative bacteria H. *pylori* [42]. Choosing the right POM that may work in synergy with Carbapenem is extremely challenging, yet it demands immediate consideration to find an approachable and time-sensitive therapeutic solution for Carbapenem-resistant Pseudomonas *aeruginosa*. Additionally, POMs induce cytokine and chemokine production, main chemicals involved in human inflammation signaling pathways further confirming immunological response (IL-17, TNF, NF-kappa B) [43].

Nadia I. Gumerova *et al*. exhibited the impact of the charge; size; chemical composition and their combination for the efficiency of POMs as antibacterial therapy. They chose 29 different POMs with various structured species for this study. They observed inactivity or less active inorganic POMos can be alleviated via adding an anionic pyrophosphate gr; Arsenic group (highly potent to inhibit bacterial activity); or a more suitable clinical choice - organic moieties [42, 45].

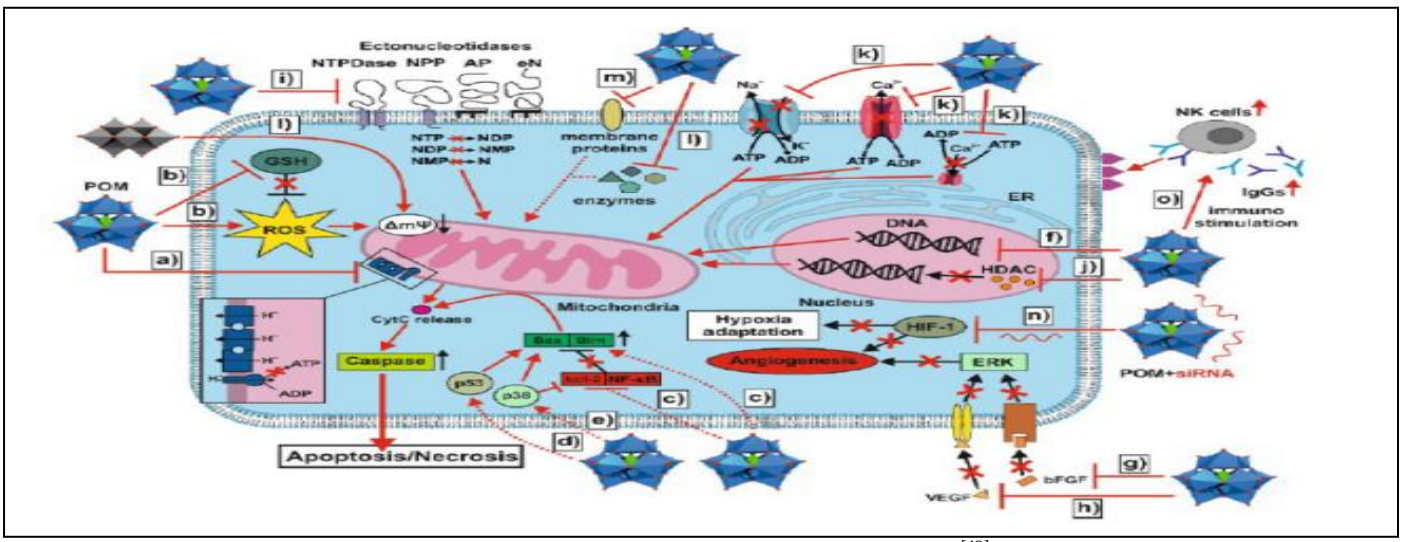

Fig 9 Mode of Action of POMs as an *antibacterial* [43]

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Polyoxovanadate, $[V_{10}O_{28}]$ has shown the potency to transform ATPase pumps into ion channels that can cause cell death by disrupting the bacterial cell ion gradient, which is a remarkable finding considering the intrinsic mechanisms (porin loss, overexpression of efflux pumps) involved in the emergence of CRPA. However, effective Polyoxovanadates also may not show antibacterial activity against gram-negative bacteria such as Pseudomonas *aeruginosa* [43].

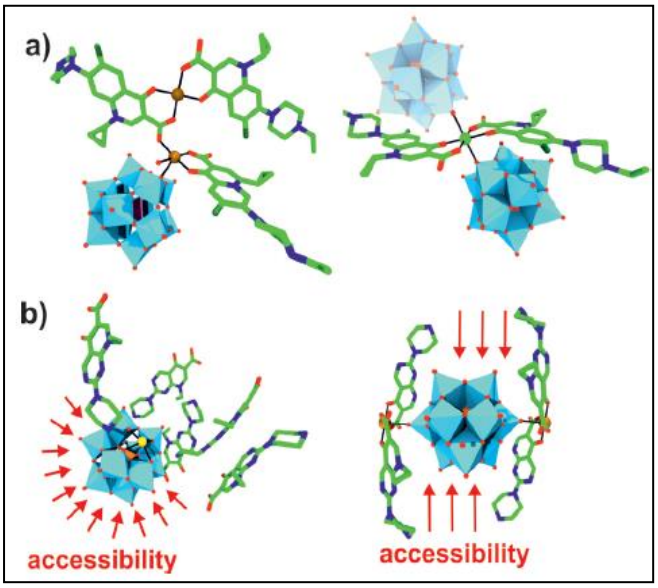

Fig 10 Illustration of Different Types of Assemble of POM-Drug (Quinolone) Hybrid

"a)[Cu2 (Enro)3H2O] [SiW12O40] (left) and H2[Ni(Enro)2][SiW12O40] (right). The adjacent POM found in the crystal structure of H2[Ni(Enro)2][SiW12O40] is indicated by a transparent molecule; however, in solution this site is most probably occupied by a solvent molecule. b) [HPPA]5[PW11CdO39] (left) and [Cu(PPA)2]2[PW12O40] (right). Red arrows indicate the accessible interaction sites. Light blue polyhedra are {WO6}, orange polyhedra {PO4},green sticks carbon, dark blue sticks nitrogen, dark green sticks fluorine, brown spheres copper, green sphere nickel, yellow sphere cadmium, red sticks and spheres oxygen." [75]

A. Bijelic *et al*. discussed POM-drug hybrids were linked using Transition Metals (TM) such as (Cu, Zn, Ni, Co) for linkage [27, 75]. Copper and Zinc are essential trace elements required in human bodies [21]. Accessibility is a crucial factor for attaining high efficiency from the desired POM-drug complex. Carbapenems can also be conjugated to desired POM structure in such an arrangement with adequate accessibility to both POM and drug [75]. The transition metal groups in the aforementioned illustration can be substituted using organic linkers with alleviating medicinal properties such as *para*-amino benzoic acid [76, 77].

POMs are negatively charged chemical compounds. It has been observed that the bioavalability of POMs rapidly increase with introduction of positively charged bio-organic compound. It is also essential to reduce the toxic side effects of POM. Chitosan is a ubiquitously found cationic biopolymer that can be used to encapsulate the chosen POM structure [28, 43]. It is a sustainable option for biomedical purposes with low toxicity that can be easily derived from chitin found in the shells of shrimps and lobsters. As discussed in dissertation paper of Greta R. Patzke *et al*., the positive charge of Chitosan has been observed to elevate the antibacterial activity of POM and especially increase the cell uptake and bioavailability. Its presence also releases POM-drug hybrid in the system in a controlled manner. Chitosan itself has some antibacterial properties. Its antimicrobial activity depends majorly upon the degree of deacetylation of chitosan and its solubility at the pH. When active, chitosan can easily adhere itself to the negatively charged ions pertaining to lipids, proteins, external DNAs, polysaccharides on bacterial cell walls, or Exopolysaccharide substances of biofilms of mono or mixed cultures. Low MW CT can permeate through bacterial cells and inhibit the transcription process by directly interfering with DNA molecules [42]. It also can form a gel-like structure that can block pores and channels on bacterial cells and shut nutrient transmission. It is also able to chelate metal ions and retain water molecules that can hinder bacterial pathways and cause cell lysis [22, 24, 28, 42, 43].

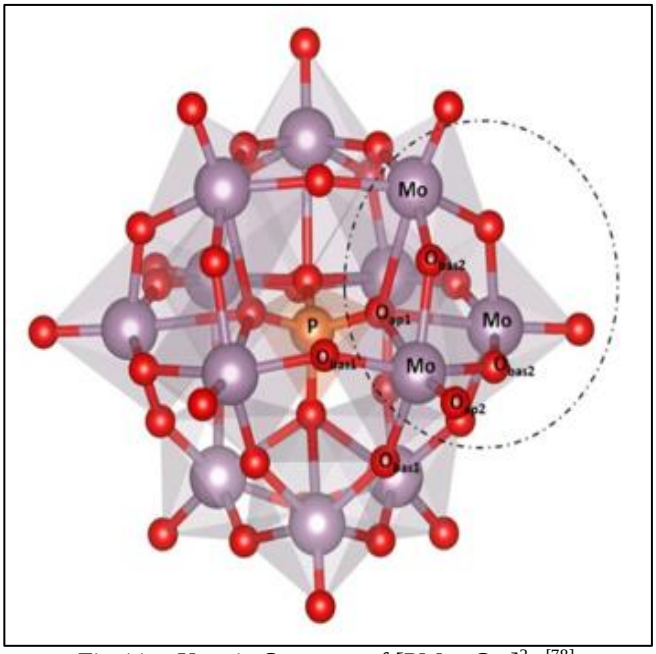

Fig 11 α-Keggin Structure of $[PMo_{12}O_{40}]^{3-}$ [78]

Fiorani *et al*. tested low MW CT nanocomposites with POMs (decavanadate $[V_{10}O_{28}]^{6-}$, Keggin species phosphovanadomolybdic $[PMo_{10}V_2O_{40}]^{5-}$ and decatungstate $[W_{10}O_{32}]^{4-}$) for their antibacterial activity and found out the more positively charged hybrids such as CT-$[PMo_{10}V_2O_{40}]^{5-}$ and CT-$[W_{10}O_{32}]^{4-}$ to be most active. Another study elaborates on the surprising fact that upon organic-inorganic hybridization of $[PMo_{12}O_{40}]^{3-}$ with chitosan exhibited significant antibacterial activity while another tested polyoxotungstate-chitosan hybrid showed no activity against E. *coli* [42, 43].

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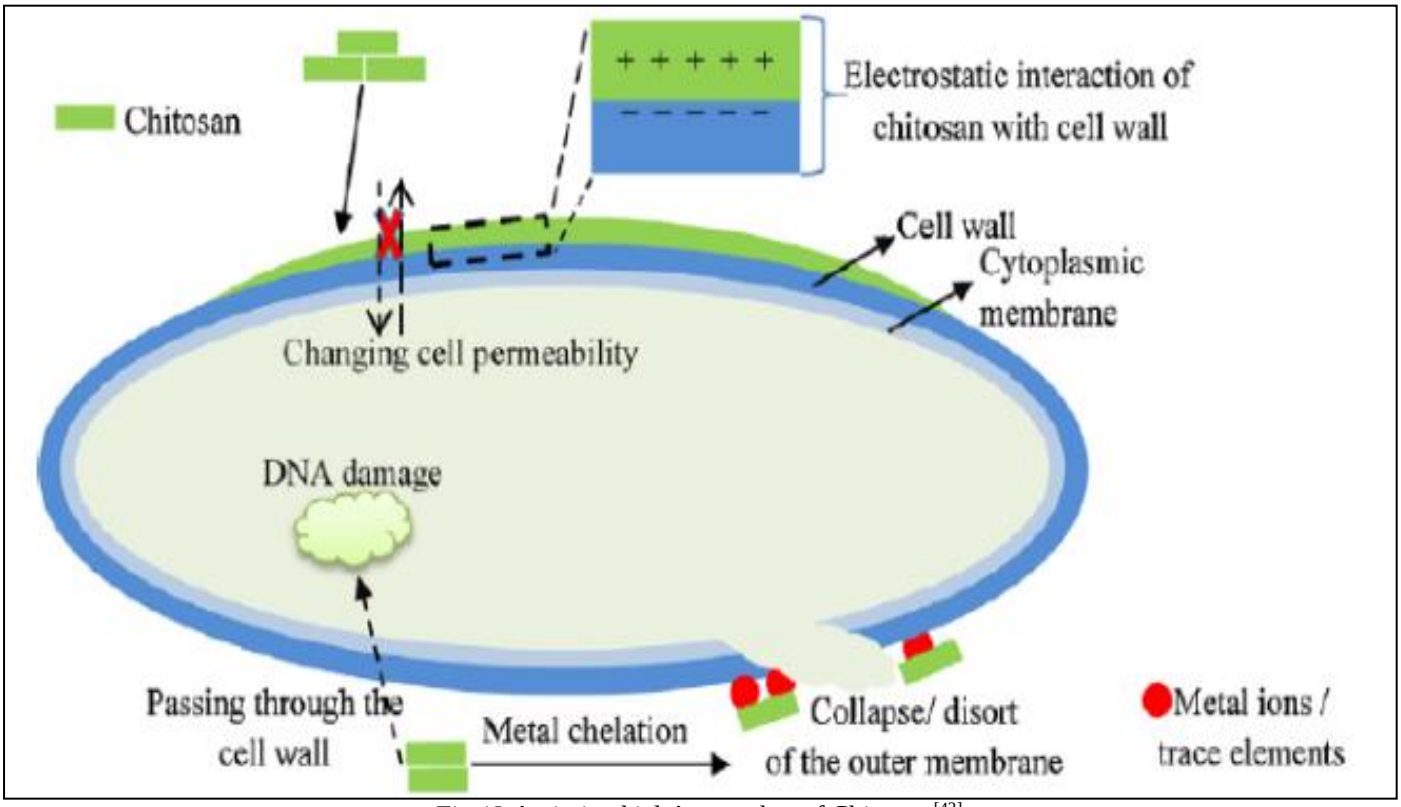

Fig 12 Antimicrobial Approaches of Chitosan [43]

Even though polyoxotunstates showed high efficiency in more than one study, the less toxicity and better biocompatibility of Molybdenum should be considered. Moreover, unlike Molybdenum, Vanadate and Tungsten have no significant effect on the human body. Molybdenum is sometimes required in the human body as a co-factor in various metabolic pathways and biochemical reactions and shows additional benefits [79, 80, 81, 82]. Vanadium doped Polyoxomolybdate also shows higher structural stability and enhanced H-atom adsorption ability [78]. The plasticity of POMs in their size, shape, structure, and incorporation of desired metal ions or organic groups to enhance their activity according to the requirement makes POMs in medical science a demanding field. [45].

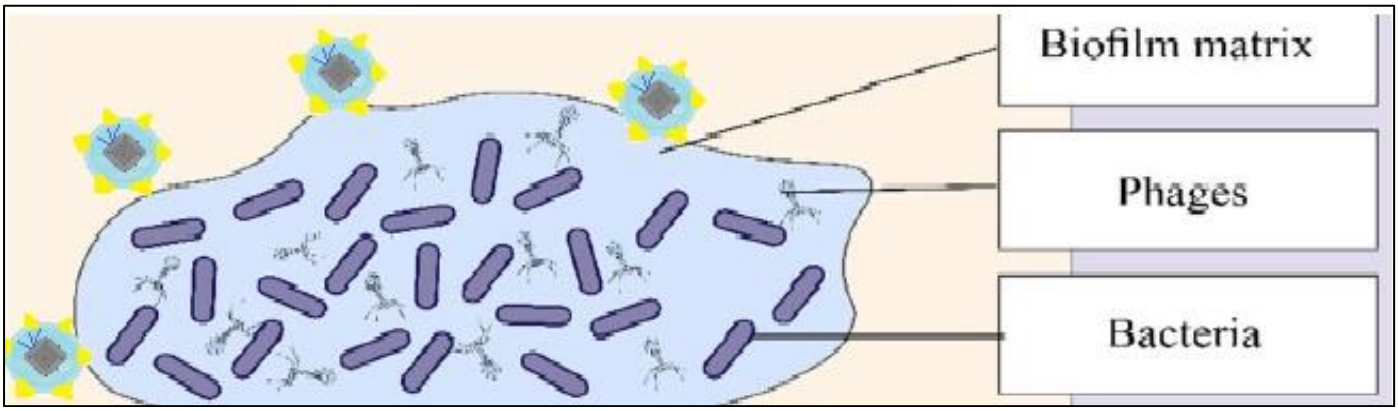

Fig 13 Schematic Illustration of Conjectured Chitosan (Sky Spheres) Encapsulated POM-Carbapenem (IMP/MER) Hybrid (Grey Beads with Blue Drug Tail Like Tag) with Immobilized Phage Coded Depolymerase (Yellow Triangle) for Specific Targeting of Biofilm Producing MDR Pseudomonas Aeruginosa. (Image of Phage Infected Bacterial Biofilm Matrix Source: [40])

Albeit, Chitosan encapsulated POMs seem to be highly promising against Carbapenem-resistant Pseudomonas *aeruginosa* and especially against biofilm-forming strains, still the major drawback of using this nano-system is the non-specificity. However, this can be deciphered by equipping the chitosan-POM nanosystem with Phage-encoded EPS degrading enzymes, which are referred to as TSPs. These enzymes take up a spike shape after protrusion from virion particles. Most of the known lyases are derived from *podovirus*. P. *aeruginosa* produces alginate EPS (D-Mannuronate-L-Guluronate) for biofilm formation unattached to bacterial cell walls. Glonti *et al*. found phage PT 6 (Podoviridae) able to lyse alginate EPS of P. *aeruginosa*. PT 6 phages have been observed to be highly specific toward particular strains of P. *aeruginosa*, which secures the targeting [65].

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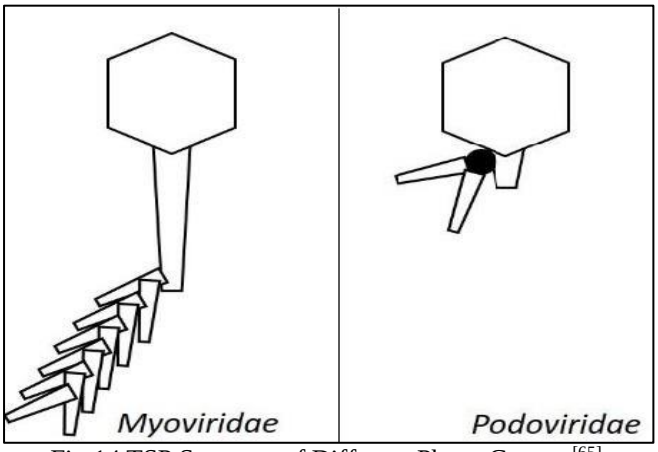

Fig 14 TSP Structure of Different Phage Genera [65]

TSPs can be derived separately from whole phage virions via cloning particular phage genome in bacterial or eukaryotic systems (e.g. E. *coli*) [66, 69, 84]. Immobilization of these enzymes on the chitosan-encapsulated POM carrying Carbapenem can successfully lead to achieving the highly specific targeting of Carbapenem towards the infection site caused by MDR PA in the patient body. PT 6 encoded alginate lyases can be immobilized on chitosan capsules via adsorption, co-polymer linkages or covalent binding [67, 68, 70, 83].

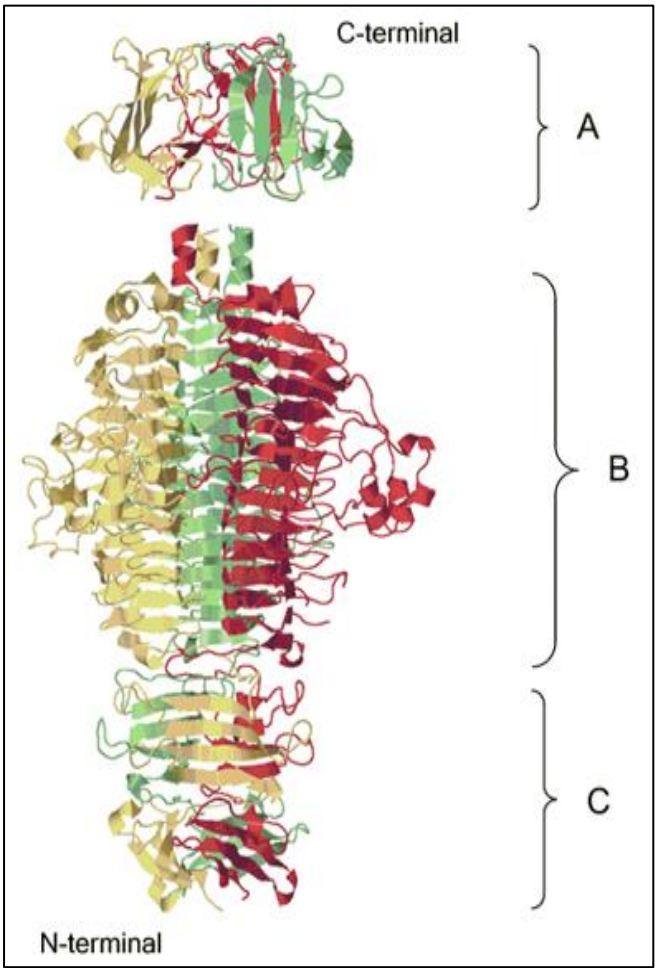

Fig 15 Domains of Phage Encoded Tailspike Protein/Depolymerase [71]

A. Latka *et al*. described **(Fig. 13)** three domains of TSPs that consists of a flexible host receptor binding N-terminal domain, a large central part responsible for host recognition and specificity in enzymatic activity that also imparts in maintaining the trimeric structure of TSPs, and a C-terminal domain that acts as a intra-molecular chaperone also responsible for host receptor recognition and to maintain the trimerization. The N-terminal and C-terminal have been observed to contain conserved sequences, while the central domain is variable and can be mutated to adapt to broader range of similar host specificity [71].

This nano-system possibly can attain effective concentration of Carbapenem at infection site without applying of high dose of Carbapenem [20, 27].

II. CONCLUSION

Emergence of CRPA is an incessant global threat. Understanding it and recognizing the issues including bacterial mechanisms, violation of clinical rules and regulations is an exigency to address it properly. Beta-lactam antibiotics like Carbapenems are undeniably one of the greatest discoveries in human history. Finding a better novel solution without any chances of resistance development is not only highly ambitious also seems mythical at present clinical situation. However, suitably designed approach to deliver Carbapenem might resolve the issue. Chitosan encapsulated POMs have been extensively studied for their great potential in medical field. Most of the resistance mechanisms taken by CRPA cells can be undermined by this nano-system eventually leading to the safe delivery of Carbapenem and CRPA cell death. This nanosystem almost works like human immune system, where the innate approaches are served by the CS-POM nanocomposite. The adaptive immunity like specificity can be brought in by introducing phage depolymerase enzymes that would also help breaking the alginate biofilm barrier created by CRPA strains. Needles to mention that more research works are required to make it a reality.

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➢ *Conflict of Interest*

There is no conflict of interest involved in this study and it is solely conducted for a thorough review purpose only.

ABBREVIATION

- CRPA/CARPA: Carbapenem-Resistant Pseudomonas *aeruginosa*
- MDR: Multi Drug Resistant

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- MDRPA: Multi Drug Resistant Pseudomonas *aeruginosa*
- PA: Pseudomonas *aeruginosa*
- CR: Carbapenem Resistant
- IRPA: Imipenem Resistant Pseudomonas *aeruginosa*
- MBL: Metallo-β-lactamase
- ESBL: Extended Spectrum- β-lactamase
- EPS: Extracellular Polymeric Substances
- MHT: Modified Hodge Test
- CDT: Combined Disc Test
- DDST: Double Disc Synergy Test
- PT: Phenotypic Test
- PCR: Polymerase Chain Reaction
- EDTA: Ethylene Diamine Tetraacetic Acid
- SDS: Sodium Dodecyl Sulfate
- IMP: Imipenemase
- VIM: Verona Integron-encoded MBL
- IMP: Imipenem
- MER: Meropenem
- CAZ: Ceftazidime
- EtBr: Ethidium Bromide
- ICU: Intensive Care Unit
- VAP: Ventilator-associated pneumonia
- POM: Poly Oxo Metalate
- POMo: Poly Oxo Molybdate
- CS: Chitosan
- TSP: Tail Spike Protein
- VRSA: Vancomycin-Resistant Staphylococcus aureus
- ST: Sequence Typing
- MIC: Minimum Inhibitory Concentration

REFERENCES

[1]. Amudhan MS, Sekar U, Kamalanathan A, Balaraman S. bla(IMP) and bla(VIM) mediated carbapenem resistance in Pseudomonas and Acinetobacter species in India. J Infect Dev Ctries. 2012 Nov 26;6(11):757-62. doi: 10.3855/jidc.2268. PMID: 23277500.

[2]. Arunagiri K, Sekar B, Sangeetha G, John J. Detection and characterization of metallo-beta-lactamases in Pseudomonas aeruginosa by phenotypic and molecular methods from clinical samples in a tertiary care hospital. West Indian Med J. 2012 Nov;61(8):778-83. PMID: 23757897.

[3]. Deshmukh DG, Damle AS, Bajaj JK, Bhakre JB, Patwardhan NS. Metallo-β-lactamase-producing clinical isolates from patients of a tertiary care hospital. Journal of laboratory physicians. 2011 Jul;3(02):093-7.

[4]. Kim MJ, Bae IK, Jeong SH, Kim SH, Song JH, Choi JY, Yoon SS, Thamlikitkul V, Hsueh PR, Yasin RM, Lalitha MK. Dissemination of metallo-β-lactamase-producing Pseudomonas aeruginosa of sequence type 235 in Asian countries. Journal of Antimicrobial Chemotherapy. 2013 Dec 1;68(12):2820-4.

[5]. Kaur J, Chopra S, Mahajan G. Modified double disc synergy test to detect ESBL production in urinary isolates of Escherichia coli and Klebsiella pneumoniae. Journal of clinical and diagnostic research: JCDR. 2013 Feb 1;7(2):229.

[6]. Kumari M, Verma S, Venkatesh V, Gupta P, Tripathi P, Agarwal A, Siddiqui SS, Arshad Z, Prakash V. Emergence of blaNDM-1 and blaVIM producing Gram-negative bacilli in ventilator-associated pneumonia at AMR Surveillance Regional Reference Laboratory in India. PLoS One. 2021 Sep 8;16(9):e0256308.

[7]. Hirabayashi A, Kato D, Tomita Y, Iguchi M, Yamada K, Kouyama Y, Morioka H, Tetsuka N, Yagi T. Risk factors for and role of OprD protein in increasing minimal inhibitory concentrations of carbapenems in clinical isolates of Pseudomonas aeruginosa. Journal of medical microbiology. 2017 Nov;66(11):1562-72.

[8]. Kumar R, Srivastva P, Rishi S, Dahiya SS, Hemwani K, P.S Nirwan. Detection and antimicrobial susceptibility pattern of Pseudomonas aeruginosa isolates in various clinical samples with special reference to metallo beta lactamase from a tertiary care hospital in Jaipur, India. Natl J Med Res. 2014 Jun. 30; 4(02):128-31.

[9]. Kaur J, Chopra S, Mahajan G. Modified double disc synergy test to detect ESBL production in urinary isolates of Escherichia coli and Klebsiella pneumoniae. Journal of clinical and diagnostic research: JCDR. 2013 Feb 1;7(2):229.

[10]. Ranjan, Shikha; Banashankari, Gunjiganur Shankarappa; Sreenivasa Babu, Poolakunta Ramaiah. Evaluation of phenotypic tests and screening markers for detection of metallo-β-lactamases in clinical isolates of Pseudomonas aeruginosa: A prospective study. Medical Journal of Dr. D.Y. Patil University 8(5):p 599-605, Sep–Oct 2015. | DOI: 10.4103/0975-2870.164977.

[11]. Kotwal A, Biswas D, Kakati B, Singh M. ESBL and MBL in cefepime resistant Pseudomonas aeruginosa: an update from a rural area in Northern India. Journal of clinical and diagnostic research: JCDR. 2016 Apr 1;10(4):DC09.

[12]. Kar B, Sharma M, Peter A, Chetia P, Neog B, Borah A, Pati S, Bhattacharya D. Prevalence and molecular characterization of β-lactamase producers and fluoroquinolone resistant clinical isolates from North East India. Journal of Infection and Public Health. 2021 May 1;14(5):628-37.

[13]. Pragasam AK, Vijayakumar S, Bakthavatchalam YD, Kapil A, Das BK, Ray P, Gautam V, Sistla S, Parija SC, Walia K, Ohri VC. Molecular characterisation of antimicrobial resistance in Pseudomonas aeruginosa and Acinetobacter baumannii during 2014 and 2015 collected across India. Indian journal of medical microbiology. 2016 Oct 1;34(4):433-41.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25oct750

[14]. Paul D, Dhar D, Maurya AP, Mishra S, Sharma GD, Chakravarty A, Bhattacharjee A. Occurrence of co-existing bla VIM-2 and bla NDM-1 in clinical isolates of Pseudomonas aeruginosa from India. Ann Clin Microbiol Antimicrob. 2016 May 6;15:31. doi: 10.1186/s12941-016-0146-0. PMID: 27154587; PMCID: PMC4859973.

[15]. Mohanty S, Maurya V, Gaind R, Deb M. Phenotypic characterization and colistin susceptibilities of carbapenem-resistant of Pseudomonas aeruginosa and Acinetobacter spp. J Infect Dev Ctries. 2013 Nov 15;7(11):880-7. doi: 10.3855/jidc.2924. PMID: 24240048.

[16]. Mohanam L, Menon T. Coexistence of metallo-beta-lactamase-encoding genes in *Pseudomonas aeruginosa*. Indian J Med Res. 2017 Jul;146(Supplement):S46-S52. doi: 10.4103/ijmr.IJMR_29_16. PMID: 29205195; PMCID: PMC5735570.

[17]. Nathwani D, Raman G, Sulham K, Gavaghan M, Menon V. Clinical and economic consequences of hospital-acquired resistant and multidrug-resistant Pseudomonas aeruginosa infections: a systematic review and meta-analysis. Antimicrobial resistance and infection control. 2014 Dec;3:1

[18]. Verma N, Prahraj AK, Mishra B, Behera B, Gupta K. Detection of carbapenemase-producing *Pseudomonas aeruginosa* by phenotypic and genotypic methods in a tertiary care hospital of East India. J Lab Physicians. 2019 Oct-Dec;11(4):287-291. doi: 10.4103/JLP.JLP_136_19. PMID: 31929692; PMCID: PMC6943860.

[19]. Paul D, Chanda DD, Chakravarty A, Bhattacharjee A. An insight into analysis and elimination of plasmids encoding metallo-β-lactamases in Pseudomonas aeruginosa. Journal of Global Antimicrobial Resistance. 2020 Jun;21:3-7. DOI: 10.1016/j.jgar.2019.09.002. PMID: 31518725.

[20]. Hirabayashi A, Kato D, Tomita Y, Iguchi M, Yamada K, Kouyama Y, Morioka H, Tetsuka N, Yagi T. Risk factors for and role of OprD protein in increasing minimal inhibitory concentrations of carbapenems in clinical isolates of Pseudomonas aeruginosa. Journal of medical microbiology. 2017 Nov;66(11):1562-72.

[21]. Plum LM, Rink L, Haase H. The essential toxin: impact of zinc on human health. International journal of environmental research and public health. 2010 Apr;7(4):1342-65..

[22]. Wade N, Tehrani KH, Brüchle NC, van Haren MJ, Mashayekhi V, Martin NI. Mechanistic Investigations of Metallo-β-lactamase Inhibitors: Strong Zinc Binding Is Not Required for Potent Enzyme Inhibition. ChemMedChem. 2021 May 18;16(10):1651-9.

[23]. Kashyap A, Gupta R, Sharma R, Verma VV, Gupta S, Goyal P. New Delhi metallo beta lactamase: menace and its challenges. J Mol Genet Med. 2017 Oct 30;11(4):299.

[24]. Saini S, Bharati K, Shaha C, Mukhopadhyay CK. Zinc depletion promotes apoptosis-like death in drug-sensitive and antimony-resistance Leishmania donovani. Scientific Reports. 2017 Sep 5;7(1):10488.

[25]. Kim SW, Lee JS, Park SB, Lee AR, Jung JW, Chun JH, Lazarte JM, Kim J, Seo JS, Kim JH, Song JW. The importance of porins and β-lactamase in outer membrane vesicles on the hydrolysis of β-lactam antibiotics. International journal of molecular sciences. 2020 Apr 17;21(8):2822.

[26]. González JM, Meini MR, Tomatis PE, Martín FJ, Cricco JA, Vila AJ. Metallo-β-lactamases withstand low Zn (II) conditions by tuning metal-ligand interactions. Nature chemical biology. 2012 Aug;8(8):698-700.

[27]. Rai M, Kon K, Gade A, Ingle A, Nagaonkar D, Paralikar P, da Silva SS. Antibiotic resistance: can nanoparticles tackle the problem. Antibiot. Resist. 2016.

[28]. Kumar M, Curtis A, Hoskins C. Application of nanoparticle technologies in the combat against anti-microbial resistance. Pharmaceutics. 2018 Jan 14;10(1):11.

[29]. Rhodes NJ, Wunderink RG. Empiric Carbapenems for Nosocomial Pneumonia: Is Hindsight Clearer in 2020?. Chest. 2021 Mar 1;159(3):897-9.

[30]. D Pragasam AK, Veeraraghavan B, Anandan S, Narasiman V, Sistla S, Kapil A, Mathur P, Ray P, Wattal C, Bhattacharya S, Deotale V. Dominance of international high-risk clones in carbapenemase-producing Pseudomonas aeruginosa: Multicentric molecular epidemiology report from India. Indian Journal of Medical Microbiology. 2018 Jul 1;36(3):344-51.

[31]. Meletis G. Carbapenem resistance: overview of the problem and future perspectives. Therapeutic advances in infectious disease. 2016 Feb;3(1):15-21.

[32]. Queenan AM, Bush K. Carbapenemases: the versatile β-lactamases. Clinical microbiology reviews. 2007 Jul;20(3):440-58.

[33]. PN SR. Extended Spectrum Beta-Lactamases-A Comprehensive Review. 2015.

[34]. Shaikh S, Fatima J, Shakil S, Rizvi SM, Kamal MA. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. Saudi journal of biological sciences. 2015 Jan 1;22(1):90-101.

[35]. Diene SM, Rolain JM. Carbapenemase genes and genetic platforms in Gram-negative bacilli: Enterobacteriaceae, Pseudomonas and Acinetobacter species. Clinical Microbiology and Infection. 2014 Sep 1;20(9):831-8.

[36]. Tsao LH, Hsin CY, Liu HY, Chuang HC, Chen LY, Lee YJ. Risk factors for healthcare-associated infection caused by carbapenem-resistant Pseudomonas aeruginosa. Journal of microbiology, immunology and infection. 2018 Jun 1;51(3):359-66.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25oct750

[37]. Upadhyay S, Mishra S, Sen MR, Banerjee T, Bhattacharjee A. Co-existence of Pseudomonas-derived cephalosporinase among plasmid encoded CMY-2 harbouring isolates of Pseudomonas aeruginosa in north India. Indian Journal of Medical Microbiology. 2013 Jul 1;31(3):257-60.

[38]. S Alsaadi LA, Al-Dulaimi AA, Rasheed Al-Taai HR. Prevalence of bla VIM, bla IMP and bla NDM Genes in Carbapenem Resistant Pseudomonas Aeruginosa Isolated from Different Clinical Infections in Diyala, Iraq. Indian Journal of Public Health Research & Development. 2020 Feb 1;11(2).

[39]. SARKAR S, DUTTA S, NAMHATA A, BANERJEE C, SENGUPTA M, SENGUPTA M. Beta-lactamase Profile and Biofilm Production of Pseudomonas aeruginosa Isolated from a Tertiary Care Hospital in Kolkata, India. Journal of Clinical & Diagnostic Research. 2020 Oct 1;14(10).

[40]. Chegini Z, Khoshbayan A, Taati Moghadam M, Farahani I, Jazireian P, Shariati A. Bacteriophage therapy against Pseudomonas aeruginosa biofilms: a review. Ann Clin Microbiol Antimicrob. 2020 Sep 30;19(1):45. doi: 10.1186/s12941-020-00389-5. PMID: 32998720; PMCID: PMC7528332

[41]. Serwer P, Wright ET, Gonzales CB, Serwer P, Wright ET, Gonzales CB. Phage capsids as gated, long-persistence, uniform drug delivery vehicles. Current and Future Aspects of Nanomedicine. 2020 Apr 20.

[42]. Bijelic A, Aureliano M, Rompel A. The antibacterial activity of polyoxometalates: structures, antibiotic effects and future perspectives. Chemical. Communications. 2018;54(10):1153-69.

[43]. Conti, Simona. Biomedical Studies on Chitosan Materials and Polyoxometalate Nanocomposites as Versatile Anticancer and Antimicrobial Drug Prototypes. PhD diss., University of Zurich, 2019.

[44]. Gutiérrez D, Fernández L, Rodríguez A, García P. Practical method for isolation of phage deletion mutants. Methods and protocols. 2018 Jan 17;1(1):6.

[45]. Gumerova NI, Al-Sayed E, Krivosudský L, Čipčić-Paljetak H, Verbanac D, Rompel A. Antibacterial activity of polyoxometalates against Moraxella catarrhalis. Frontiers in chemistry. 2018 Aug 14;6:336.

[46]. Armstrong T, Fenn SJ, Hardie KR. JMM Profile: Carbapenems: a broad-spectrum antibiotic. Journal of medical microbiology. 2021 Dec 10;70(12):001462.

[47]. Jeon JH, Lee JH, Lee JJ, Park KS, Karim AM, Lee CR, Jeong BC, Lee SH. Structural basis for carbapenem-hydrolyzing mechanisms of carbapenemases conferring antibiotic resistance. Int J Mol Sci. 2015 Apr 29;16(5):9654-92. doi: 10.3390/ijms16059654. PMID: 25938965; PMCID: PMC4463611.

[48]. Chaudhari V, Gunjal S, Mehta M. Antibiotic resistance patterns of Pseudomonas aeruginosa in a tertiary care hospital in Central India. Int J Med Sci Public Health. 2013 Apr 1;2(2):386-9.

[49]. Lee YL, Ko WC, Hsueh PR. Geographic patterns of carbapenem-resistant Pseudomonas aeruginosa in the Asia-Pacific Region: results from the Antimicrobial Testing Leadership and Surveillance (ATLAS) program, 2015–2019. Antimicrobial agents and chemotherapy. 2022 Feb 15;66(2):e02000-21.

[50]. Wong MH, chi Chan EW, Chen S. Isolation of carbapenem-resistant Pseudomonas spp. from food. Journal of Global Antimicrobial Resistance. 2015 Jun 1;3(2):109-14.

[51]. Acharya M, Joshi PR, Thapa K, Aryal R, Kakshapati T, Sharma S. Detection of metallo-β-lactamases-encoding genes among clinical isolates of Pseudomonas aeruginosa in a tertiary care hospital, Kathmandu, Nepal. BMC Res Notes. 2017 Dec 8;10(1):718. doi: 10.1186/s13104-017-3068-9. PMID: 29216906; PMCID: PMC5721655.

[52]. Li F, Chen D, Li L, Liang D, Wang F, Zhang B. Analysis of metallo-β-lactamases, oprD mutation, and multidrug resistance of β-lactam antibiotic-resistant strains of Pseudomonas aeruginosa isolated from Southern China. Current Microbiology. 2020 Nov;77:3264-9.

[53]. Poirel L, Naas T, Nicolas D, Collet L, Bellais S, Cavallo JD, Nordmann P. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-beta-lactamase and its plasmid- and integron-borne gene from a Pseudomonas aeruginosa clinical isolate in France. Antimicrob Agents Chemother. 2000 Apr;44(4):891-7. doi: 10.1128/AAC.44.4.891-897.2000. PMID: 10722487; PMCID: PMC89788.

[54]. Babu KV, Visweswaraiah DS, Kumar A. The influence of Imipenem resistant metallo-beta-lactamase positive and negative Pseudomonas aeruginosa nosocomial infections on mortality and morbidity. J Nat Sci Biol Med. 2014 Jul;5(2):345-51. doi: 10.4103/0976-9668.136181. PMID: 25097412; PMCID: PMC4121912.

[55]. Ellappan K, Narasimha HB, Kumar S. Co-existence 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. 2018 Mar 1;12:37-43.

[56]. Kumar KM, Saikumar C. To Study The Antimicrobial Susceptibility Pattern Of Pseudomonas Aeruginosa And Detection Of Metallo Beta Lactamase Producing Strain With Special Reference To blaNDM-1 And blaVIM. Annals Of The Romanian Society For Cell Biology. 2021;25(1):2998-3013.

[57]. Xu H, Bao X, Wang Y, Xu Y, Deng B, Lu Y, Hou J. Engineering T7 bacteriophage as a potential DNA vaccine targeting delivery vector. Virology Journal. 2018 Dec;15:1-8.

[58]. Brown WL, Mastico RA, Wu M, Heal KG, Adams CJ, Murray JB, Simpson JC, Lord JM, Taylor-Robinson AW, Stockley PG. RNA bacteriophage capsid-mediated drug delivery and epitope presentation. Intervirology. 2003 Jan 30;45(4-6):371-80.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25oct750

[59]. Carmody CM, Goddard JM, Nugen SR. Bacteriophage capsid modification by genetic and chemical methods. Bioconjugate chemistry. 2021 Mar 4;32(3):466-81.
[60]. Duyvesteyn HM, Santos-Pérez I, Peccati F, Martinez-Castillo A, Walter TS, Reguera D, Goñi FM, Jiménez-Osés G, Oksanen HM, Stuart DI, Abrescia NG. Bacteriophage PRD1 as a nanoscaffold for drug loading. Nanoscale. 2021;13(47):19875-83.
[61]. Anand T, Virmani N, Bera BC, Vaid RK, Kumar A, Tripathi BN. Applications of personalised phage therapy highlighting the importance of bacteriophage banks against emerging antimicrobial resistance. Def Life Sci J. 2020;5(4):305-14.
[62]. Dostalova S, Jimenez AM, Vaculovicova M, Adam V, Kizek R. Phage Capsid for Efficient Delivery of Cytotoxic Drugs. Int. J. Pharmacol. Pharm. Sci.:601-4.
[63]. Sharma S, Datta S, Chatterjee S, Dutta M, Samanta J, Vairale MG, Gupta R, Veer V, Dwivedi SK. Isolation and characterization of a lytic bacteriophage against Pseudomonas aeruginosa. Scientific Reports. 2021 Sep 29;11(1):19393.
[64]. Menon ND, Kumar MS, Satheesh Babu TG, Bose S, Vijayakumar G, Baswe M, Chatterjee M, D'Silva JR, Shetty K, Haripriyan J, Kumar A. A Novel N4-Like Bacteriophage isolated from a wastewater source in South India with activity against several multidrug-resistant clinical Pseudomonas aeruginosa isolates. Msphere. 2021 Feb 24;6(1):10-128.
[65]. Knecht LE, Veljkovic M, Fieseler L. Diversity and Function of Phage Encoded Depolymerases. Front Microbiol. 2020 Jan 10;10:2949. doi: 10.3389/fmicb.2019.02949. PMID: 31998258; PMCID: PMC6966330.
[66]. Witte S, Zinsli LV, Gonzalez-Serrano R, Matter CI, Loessner MJ, van Mierlo JT, Dunne M. Structural and functional characterization of the receptor binding proteins of Escherichia coli O157 phages EP75 and EP335. Computational and Structural Biotechnology Journal. 2021 Jan 1;19:3416-26.
[67]. Kaushal J, Singh G, Arya SK. Immobilization of catalase onto chitosan and chitosan–bentonite complex: a comparative study. Biotechnology Reports. 2018 Jun 1;18:e00258.
[68]. Budriene S, Gorochovceva N, Romaskevic T, Yugova LV, Miezeliene A, Dienys G, Zubriene A. β-Galactosidase from Penicillium canescens. Properties and immobilization. Central European journal of chemistry. 2005 Mar;3:95-105.
[69]. Bartell PF, Lam GK, Orr TE. Purification and properties of polysaccharide depolymerase associated with phage-infected Pseudomonas *aeruginosa*. Journal of Biological Chemistry. 1968 May 10;243(9):2077-80.
[70]. Hassan ME, Yang Q, Xiao Z, Liu L, Wang N, Cui X, Yang L. Impact of immobilization technology in industrial and pharmaceutical applications. 3 Biotech. 2019 Dec;9(12):440. doi: 10.1007/s13205-019-1969-0. Epub 2019 Nov 8. PMID: 31750038; PMCID: PMC6841786.
[71]. Latka A, Maciejewska B, Majkowska-Skrobek G, Briers Y, Drulis-Kawa Z. Bacteriophage-encoded virion-associated enzymes to overcome the carbohydrate barriers during the infection process. Applied microbiology and biotechnology. 2017 Apr;101:3103-19.
[72]. Bashar S, Sanyal SK, Sultana M, Hossain MA. Emergence of IntI1 associated bla VIM-2 gene cassette-mediated carbapenem resistance in opportunistic pathogen Pseudomonas stutzeri. Emerging Microbes & Infections. 2017 Jan 1;6(1):1-3.
[73]. Jové T, Da Re S, Tabesse A, Gassama-Sow A, Ploy MC. Gene expression in class 2 integrons is SOS-independent and involves two Pc promoters. Frontiers in microbiology. 2017 Aug 15;8:1499.
[74]. Sahota, J.S., Smith, C.M., Radhakrishnan, P., Winstanley, C., Goderdzishvili, M., Chanishvili, N., Kadioglu, A., O'Callaghan, C. and Clokie, M.R.J., 2015. Bacteriophage delivery by nebulization and efficacy against phenotypically diverse Pseudomonas aeruginosa from cystic fibrosis patients. *Journal of aerosol medicine and pulmonary drug delivery*, *28*(5), pp.353-360.
[75]. Bijelic A, Aureliano M, Rompel A. Polyoxometalates as potential next-generation metallodrugs in the combat against cancer. Angewandte Chemie International Edition. 2019 Mar 4;58(10):2980-99.
[76]. Nisa ZU, Akhtar T. para-aminobenzoic acid-A substrate of immense significance. Mini-Reviews in Organic Chemistry. 2020 Sep 1;17(6):686-700.
[77]. Rosbottom I, Toroz D, Hammond RB, Roberts KJ. Conformational and structural stability of the single molecule and hydrogen bonded clusters of para aminobenzoic acid in the gas and solution phases. CrystEngComm. 2018;20(46):7543-55.
[78]. Aspera SM, Arevalo RL, Nakanishi H, Kasai H, Sekine S, Kawai H. Vanadium doped polyoxometalate: induced active sites and increased hydrogen adsorption. Journal of Physics: Condensed Matter. 2020 Feb 13;32(19):195001.
[79]. Ni D, Jiang D, Kutyreff CJ, Lai J, Yan Y, Barnhart TE, Yu B, Im HJ, Kang L, Cho SY, Liu Z. Molybdenum-based nanoclusters act as antioxidants and ameliorate acute kidney injury in mice. Nature communications. 2018 Dec 21;9(1):5421.
[80]. Schwarz G, Belaidi AA. Molybdenum in human health and disease. Interrelations between essential metal ions and human diseases. 2013:415-50.
[81]. Novotny JA. Molybdenum nutriture in humans. Journal of Evidence-Based Complementary & Alternative Medicine. 2011 Oct;16(3):164-8.
[82]. Domingo JL. Vanadium and tungsten derivatives as antidiabetic agents: a review of their toxic effects. Biological trace element research. 2002 Aug;88:97-112.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25oct750

[83]. Verma ML, Kumar S, Das A, Randhawa JS, Chamundeeswari M. Enzyme immobilization on chitin and chitosan-based supports for biotechnological applications. Sustainable Agriculture Reviews 35: Chitin and Chitosan: History, Fundamentals and Innovations. 2019:147-73.

[84]. Topka-Bielecka G, Dydecka A, Necel A, Bloch S, Nejman-Faleńczyk B, Węgrzyn G, Węgrzyn A. Bacteriophage-derived depolymerases against bacterial biofilm. Antibiotics. 2021 Feb 10;10(2):175.