1   **Metallo-β-lactamases: structure, function, epidemiology, treatment options, and the**

2   **development pipeline**

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20   **Running title:** Treatment options for infections caused by bacteria that produce metallo β-

21   lactamases

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25   Abstract

26   Modern medicine is threatened by the global rise of antibiotic resistance, especially among -

27   negative bacteria. Metallo-β-lactamase (MBL) enzymes are a particular concern and are

28   increasingly disseminated worldwide, though particularly in Asia. Many producers have

29   multiple further drug resistances, leaving few obvious treatment options. Nonetheless, and

30   more encouragingly, MBLs may be less effective agents of carbapenem resistance *in vivo*,

31   under zinc limitation, than *in vitro*. Owing to their unique structure and function, and their

32   diversity, MBLs pose a particular challenge for drug development. They evade all recently

33   licensed β-lactam- β-lactamase inhibitor combinations, although several stable agents and

34   inhibitor combinations are at various stages in the pipeline. These potential therapies, along

35   with the epidemiology of producers and current treatment options, are the focus of this

36   review.

37

Introduction

Antimicrobial therapy is threatened by the global rise of resistance, especially in gram-negative bacteria (1), where resistance to β-lactams is largely mediated by β-lactamases (2). Carbapenems evade most β-lactamases but are hydrolyzed by metallo-β-lactamases (MBLs) as well as by a few active-site serine β-lactamases (SBLs), notably members of the KPC and OXA-48-like groups. MBLs are chromosomal and ubiquitous in some non-fermenters, including *Stenotrophomonas maltophilia*, *Aeromonas* spp*.* and *Chryseobacterium* spp., which are of modest clinical concern. A minority of *Bacteroides fragilis* strains have a chromosomal MBL, CfiA or CcrA, but this is uncommon and only expressed strongly if an upstream insertion sequence provides an efficient promoter (3). More important are the acquired MBLs that are spreading among Enterobacterales and *Pseudomonas aeruginosa* (4); these are associated with extremely-drug-resistant (XDR) phenotypes, with the producers generally also resistant to multiple aminoglycosides, fluoroquinolones, and other agents as well as to β-lactams.

Classification and diversity of metallo-β-lactamases

β-Lactamases are classified by two major systems. The first is based on substrate profiles and vulnerability to inhibitors (5), and places MBLs into its Group 3, whereas Groups 1 and 2 comprise SBLs. The second classifies β-lactamases according to their amino acid sequences, recognising four enzyme classes (6). MBLs form class B whilst SBLs divide among classes A, C and D (7). The MBLs are structurally and mechanistically dissimilar from SBLs, suggesting a separate evolutionary origin.

Class B is further divided into three subclasses, B1, B2 and B3, based on differences in amino acid sequence at the active site, zinc ligands, zinc stoichiometry, loop architecture, and substrate profiles (8). The important acquired MBLs, comprising the IMP, NDM and VIM types

62 fall into subclass B1. They hydrolyze all currently available β-lactam antibiotics except

63 monobactams (e.g. aztreonam) (9), as do most or all other sub-class B1 or B3 enzymes. In

64 contrast, the CphA (subclass B2) MBLs of *Aeromonas* spp. have narrow-spectrum activity

65 directed exclusively against carbapenems. Irrespective of subclass, MBLs are not inhibited by

66 clavulanic acid, sulbactam, tazobactam, avibactam or by developmental penicillanic acid

67 sulfones and diazabicyclooctanes.

68   The important acquired subgroup B1 MBLs (Table 1) are mostly named based on where

69 they were first described; thus, for example, Verona Integron-encoded Metallo β-lactamase

70 (VIM) and New Delhi Metallo β-lactamase (NDM). The first acquired MBL ('imipenemase', IMP-

71 1), was reported from clinical isolates of *P. aeruginosa* and *Serratia marcescens* in Japan in the

72 1990s (10) and its family now includes over 85 sequence variants (11). The first VIM enzyme

73 was found in *P. aeruginosa* in 1997 (12), with over 69 variants since described (11). NDM –

74 now the most prevalent MBL in Enterobacterales and *A. baumannii* – was first identified in

75 2008 in *Klebsiella pneumoniae* and *Escherichia coli* isolates from a patient who had travelled

76 to Sweden from New Delhi, India (13). Twenty-nine NDM variants have since been described,

77 (11).

78   It is easy to be dismissive of the chromosomal subclass B2 and B3 MBLs, but recent

79 reports highlight *Stenotrophomonas maltophilia* as a multidrug-resistant pathogen in

80 immunocompromised hosts (14). *S. maltophilia* carries a subclass B3 MBL (L1 enzyme), which

81 is unique among MBLs in having four identical subunits (15), in addition to a chromosomally-

82 mediated SBL (L2 enzyme). This combination confers resistance to almost all β-lactams,

83 although minimum inhibitory concentrations (MICs) vary with methodology, because media

84 affect the expression and/or function of these enzymes (16). *Elizabethkingia meningoseptica*

85   has two chromosomal MBLs, a B1 enzyme (BlaB) and a B3 type (GOB) with the former making

86   the dominant contribution to resistance (17).

87

88   **Genetic support of acquired MBLs**

89   Acquired IMP and VIM enzymes generally are encoded by gene cassettes within class 1 or class

90   3 integrons. These may be embedded within transposons, allowing insertion into the bacterial

91   chromosome or plasmids (18). By contrast, the bla_{NDM} gene is not integron-associated and has

92   been observed on narrow-host-range plasmids belonging to incompatibility group IncF, in

93   addition to wide-host-range plasmids belonging to IncA/C, IncL/M, IncH and IncN (19–22). *K.*

94   *pneumoniae* and *E. coli* are the frequent hosts of these plasmids, and there are particular

95   associations with *K. pneumoniae* sequence types (STs) ST11, ST14, ST15 or ST147 and *E. coli*

96   ST167, ST410 or ST617 (23). These should not, however, be seen as global epidemic strains

97   along the lines of *K. pneumoniae* ST258 variants with KPC carbapenemases, for many are

98   common STs without carbapenemases. In *A. baumannii* the bla_{NDM-1} gene is generally located

99   within the composite transposon Tn*125* and embedded between two copies of a strong

100   promoter gene IS*Aba125* (24, 25); it is much less prevalent in this genus than are OXA

101   carbapenemases (Class D).

102   B2 and B3 MBLs are generally chromosomally encoded, ubiquitous in their host species

103   and not transmissible. However, exceptions exist, with horizontal transfer observed. Thus, the

104   AIM-1 MBL (B3) was initially reported, in 2012, to be encoded by a gene inserted in (and

105   atypical of) the chromosome of a *P. aeruginosa* isolate; subsequently, in 2019, it was reported

106   from *K. pneumoniae* (26). The bla_{LMB-1} gene, encoding another subclass B3 enzyme, was

107   reported to be located on a plasmid in *Rheinheimera pacifica* where it was flanked by ISCR

108   mobilization sequences, implying transfer from some other (unknown) source organism. (27).

109   Mobilization of bla_{SMB-1}, encoding a third sub-class B3 enzyme, has occurred similarly (28).

110

111   **Structure and catalytic function of MBLs**

112   Irrespective of subgroup, MBLs contain the αβ/βα fold typical of the metallo-hydrolase /

113   oxidoreductase superfamily (29). The *S. maltophilia* enzyme has four identical subunits (15),

114   whereas other MBLs are monomeric.

115   B1 and B3 MBLs have a shallow active-site groove containing 1 or 2 catalytically

116   functional divalent zinc ions, flanked by flexible loops (29). In contrast, the B2 enzymes have

117   an active site that is less accessible and flanked by a helix (30). Except for these consistencies,

118   MBLs are highly divergent even within subclasses, and have as little as 20% sequence identity

119   between subclasses (7).

120   Mechanistically, the zinc ion(s) activate a water molecule, which acts to open the β-

121   lactam ring (31). There is no covalent intermediate, as with SBL-mediated catalysis. Anionic

122   intermediates have been characterized when MBLs hydrolyze carbapenems (32), but not when

123   NDM-1 enzymes hydrolyze penicillins or cephalosporins (33). In general, imipenem and

124   meropenem are similarly good substrate for MBLs: for example, NDM-1 enzyme displays

125   similar catalytic activity, reflected in values of k_{cat}/K_m ratio, for imipenem (0.09μM^{-1}s^{-1}) and

126   meropenem (0.06μM^{-1}s^{-1}) (34); biapenem is a weaker substrate, owing to high K_m values, but

127   seems unsuitable for high-dose development (35).

128   Figure 1 illustrates the amino acid residues that bind zinc at the active sites of B1, B2,

129   and B3 MBLs (8). Crystal structures of B1 enzymes, including IMP-, VIM-, NDM-, and *B. fragilis*

130   CcrA, (panel A) reveal two zinc-binding sites (Zn1 and Zn2). The Zn1 site contains three histidine

131   residues (His116, His118, and His196), whereas the ligands for the Zn2 site are aspartic acid

6

132   (Asp120), cysteine (Cys221), and histidine (His263) (8). There is only one zinc ion in the active

133   site of the *A. hydrophila* enzyme (subclass B2, panel B), and two in the active site of the *S.*

134   *maltophilia* enzyme (subclass B3, panel C).

135   Differences in assay methodology between workers make it difficult to compare

136   hydrolytic efficiencies for different MBLs. Variation within e.g. the VIM, IMP, SPM and GIM

137   family appears largely inconsequential (36). Nevertheless, subtle but important evolution

138   may be ongoing, as illustrated in the NDM family. Here, experimental data do not define

139   major differences in the catalytic efficiencies among NDM -1, -3, -4, -5, -6, -7 and -8 enzymes

140   (37) under standard conditions, but differences are seen under zinc deprivation. Thus,

141   studies comparing NDM-1, NDM-4 (Met154Leu) and NDM-12 (Met154Leu, Gly222Asp)

142   demonstrate that the Met154Leu substitution, present in 50% of clinical NDM variants in

143   some locales, enhances the ability to confer resistance at low Zn^{++} concentrations (38, 39).

144   This is potentially important because, as discussed later, zinc is restricted in infection (40) and

145   its scarcity may impede the ability of classical NDM-1 enzyme to confer clinical resistance.

146   NDM variants that have increased affinity for zinc (up to ~10-fold decreased $K_{d, Zn2}$) display

147   selective advantages in experiments that mimic zinc scarcity imposed by the host immune

148   system (41). Perhaps driven by similar pressures, the NDM-15 variant has evolved to function

149   efficiently as a mono- rather than a bi-zinc enzyme (41). In addition, there are suggestions

150   that NDM enzymes are evolving to develop greater thermodynamic stability (37).

151

152   **Epidemiology and distribution of acquired MBLs**

153   Bacteria with IMP, VIM and NDM enzymes have been identified in a range of community,

154   hospital, and environmental settings (42). Their prevalence, and importance relative to serine

155   carbapenemases varies greatly by country.

156

Indian Subcontinent, Asia and Russia. The greatest burden of acquired, plasmid-mediated,

MBLs lies in south and south-east Asia (43), where NDM types are prevalent. As already noted,

bla_{NDM-1} was first identified in bacteria isolated in 2008 from a patient who had travelled to

Sweden from India (44). NDM variants have subsequently been spread worldwide via patient

transfers and travel (45). Epidemiological surveillance has confirmed that NDM-1 and its

variants are widely disseminated throughout India, Pakistan and Bangladesh (46, 47);

moreover, a review of 39 carbapenem-resistant Enterobacterales (CRE) collected in India in

2006-2007 by the SENTRY Antimicrobial Surveillance Program found that 15 harboured bla_{NDM-1}

(48), indicating that it was circulating prior to its 'discovery' in 2008. Enterobacterales with

bla_{NDM} were isolated from public tap water in India (49) and in river systems around pilgrimage

sites (42) demonstrating the gene has become established beyond healthcare environments.

In India there is frequent co-carriage with other carbapenemases in Enterobacterales

(50); thus, in 2012, among 113 non-clonal CRE isolates at a Mumbai hospital, 106 produced

NDM enzymes and 21 of these also have a second carbapenemase, most often an OXA-48-like

(n=17) or VIM-type (n=4). Surprisingly, given that most international reports of NDM enzymes

relate to Enterobacterales, *P. aeruginosa* was the most common MBL host (24%) among 3414

carbapenem-resistant gram-negative bacteria collected from community and hospital settings

in North India (51), with bla_{NDM-1} (36%) the most prevalent carbapenemase gene followed by

bla_{VIM} (18.4%).

Although KPC is the principal carbapenemase among Enterobacterales (CPE) in China,

a survey across 25 provinces showed that 32% of phenotypic carbapenem resistance in

Enterobacterales was linked to bla_{NDM-1} (52) whilst a study (2012-16) of clinical *Enterobacter*

cloacae across three tertiary hospitals found bla_{NDM-1} to be the most common carbapenemase

180 gene (80%), followed by bla_{IMP-26} (8%) and bla_{IMP-4} (6%) (53). The importance of IMP MBLs,

181 particularly IMP-4, in China has been underscored by others; thus, multiple Enterobacterales

182 species carrying a plasmid encoding IMP-4 enzyme were identified from patients with

183 epidemiological links to China (54), and surveillance at a Beijing hospital highlighted both IMP-

184 4 and NDM-1 in *K. pneumoniae* (55). Co-localisation of bla_{NDM-9} and the plasmid-mediated

185 colistin resistance gene *mcr-1* was seen in an *E. coli* strain recovered from retail chicken meat

186 in Guangzhou, China (56). Having been recognized 30 years ago in Japan, IMP-type enzymes

187 are now endemic there, though not highly prevalent (57).

188   NDM MBLs are the second-most-prevalent carbapenemases after OXA-48 in the

189 Middle East, excepting Israel (58, 59). This probably reflects extensive interactions with the

190 Indian subcontinent. As in India, there is significant penetration of bla_{NDM} into *P. aeruginosa*,

191 where a much greater proportion of carbapenem resistance appears to be carbapenemase-

192 mediated than in Europe or the USA. Thus, in the Gulf Cooperation Council countries, bla_{VIM}

193 was found in 39% of carbapenem-resistant *P. aeruginosa* isolates (60), with most hosts

194 belonging to internationally-disseminated high risk clones, including ST235, ST111, ST233,

195 ST654 and ST357 (60). These lineages seem unusually adept at acquiring extrinsic resistance

196 genes. In Dubai, 32% of resistant *P. aeruginosa* isolates produced VIM-type MBLs (61), though

197 a larger proportion had outer membrane impermeability.

198   The proportion of carbapenem-resistant *P. aeruginosa* harboring MBLs in Russia rose

199 from 4.5% between 2002-04 to 28.7% between 2008-10 (62), largely reflecting the spread of

200 an XDR bla_{VIM-2}-positive ST235 high-risk clone, also present in Belarus and Kazakhstan (62).

201 NDM is reported as the predominant carbapenemase among Enterobacterales in St Petersburg

202 (63, 64), whereas OXA-48 is predominant in Moscow (65).

203

204   **Europe**. Although Italy had earlier reported both IMP and VIM enzymes (66), Greece was the

205   first European country to report extensive dissemination of Enterobacterales with MBLs.

206   Specifically , *K. pneumoniae* with VIM carbapenemases were reported from multiple hospitals

207   in 2003–7, and multi-locus sequence typing identified three major clonal complexes (CCs);

208   CC147, CC18 and CC14 among the producers (67). By 2006, 20% of *K. pneumoniae* isolates

209   collected from hospital wards and 50% of those from ICUs monitored by the Greek System for

210   the Surveillance of Antimicrobial Resistance were carbapenem-resistant, largely owing to the

211   spread of the bla_{VIM-1} cassette (68). By 2010, KPC had displaced VIM to become the dominant

212   carbapenemase in Greece, largely through the spread of a *K. pneumoniae* ST258 variant (69).

213   Nonetheless, VIM-types remained scattered, and may now be re-emerging due to suppression

214   of the KPC carbapenemases via the use of ceftazidime-avibactam (70).

215   Elsewhere in Europe concern about carbapenemases grew following a flurry of press

216   interest in NDM enzymes from 2008-10, and with the spread of *K. pneumoniae* ST258/512

217   lineages with KPC carbapenemases in Italy from 2010. The UK, taken as an exemplar, recorded

218   a few *P. aeruginosa* and Enterobacterales with IMP and VIM MBLs before 2008. Thereafter,

219   Enterobacterales with NDM enzymes increased (46). Most early cases were imports via

220   patients who had travelled to (and often been hospitalized in) the Indian sub-continent.

221   Multiple NDM variants have subsequently been reported in the UK, with NDM-1 the most

222   frequent among Enterobacterales, followed by NDM-5 and NDM-7 (71). In contrast, VIM

223   variants account for 91% of the (uncommon) MBLs in *P. aeruginosa*, again associated with

224   international high-risk clones ST235, ST111, ST233 and ST357 (72).

225   While referral of CPE isolates to the national reference laboratory has increased 100-

226   fold since 2008, many producers are from screening rather than clinical samples. OXA-48 is

227   now the fastest-spreading carbapenemase but isolates with NDM enzymes account for 20-25%

228   of CPE submitted. A growing minority of these, particularly *E. coli*, have both NDM- and OXA-

229   48-like enzymes (71, 73).

230   In 2012, the European Centre for Disease Prevention and Control launched its

231   'European Survey of Carbapenemase-Producing Enterobacteriaceae (EuSCAPE)' project. The

232   geographic distribution of enzyme types were estimated by national experts across 38

233   European countries in 2015 (74). A random sample of carbapenem-susceptible and -non-

234   susceptible *K. pneumoniae* and *E. coli* subsequently were collected prospectively to determine

235   the occurrence of carbapenemases (75). The results, published in 2017, revealed SBLs (KPC or

236   OXA-48 enzymes) were more prevalent than MBLs in most countries but that MBLs were

237   widely scattered and were the most prevalent carbapenemases among Enterobacterales in a

238   few countries. Thus, VIM enzymes were the dominant carbapenemases in Hungary and NDM

239   in Serbia and Montenegro. The prevalence of NDM enzymes in the latter countries tallies with

240   early descriptions of producers linked to these Balkan states. It is unclear whether these

241   originated as imports from India or as independent local gene escapes from the unknown

242   source organism (76).

243

244   **North America**. Infections due to Enterobacterales carrying bla_{VIM-2}, bla_{VIM-7}, bla_{IMP-4} and bla_{IMP-18} genes were recorded in the USA prior to 2005 but, in general, MBLs remained extremely rare

246   (1, 77). In 2010, Enterobacterales harboring NDM-1 were isolated from three patients in

247   different states (78) and, as with many contemporaneous cases in the UK and elsewhere, the

248   source patients had all recently been in India or Pakistan (21). Subsequent expansion of NDM

249   enzymes in the US has been less marked than the UK, with KPC carbapenemases becoming

250   considerably more prevalent. Nevertheless, up to December 2017, 379 CPE with NDM

251 carbapenemases were reported to the CDC from 34 States, with just under a third (109) from

252 Illinois (79), where an outbreak was associated with contaminated endoscopes.

253   Enterobacterales with NDM enzymes have been increasing in Canada since 2008 and

254 these MBLs are now the second-most-common carbapenemases in the country, with a higher

255 prevalence in the Western Provinces (80). Surveillance conducted between 2007-2015 in

256 Toronto revealed that, among 291 clinical CPE, 51% had NDM enzymes, and 24% of these

257 patients had never received healthcare abroad nor travelled to high-risk areas (81), suggesting

258 the enzymes are established locally. In 2019 a novel MBL, bla_{CAM-1}, was identified from isolates

259 that were collected in 2007 (82). No subsequent isolates harboring this gene have been

260 reported.

261

262 **Africa**. Paucity of data means the prevalence of CPE carrying MBLs in Africa is difficult to

263 estimate. Apparent infrequency may reflect true rarity, limited sampling, or a lack of

264 infrastructure for accurate detection. CPE with VIM MBLs nonetheless have been identified in

265 Nigeria, Morocco, Algeria, Tunisia, Tanzania, and South Africa; and those with NDM enzymes

266 in Kenya, Nigeria, Morocco, Algeria, Tunisia, Tanzania, and South Africa (83, 84). Infections

267 caused by Enterobacterales producing MBLs are reported from both imported and local cases,

268 raising concerns regarding emerging endemicity (85). Those with IMP-type enzymes have been

269 identified in small numbers in Morocco, Tunisia, and Tanzania, and appear genuinely

270 uncommon (84). An outbreak caused by *Klebsiella* spp. carrying bla_{NDM-5} was reported from a

271 neonatal unit in Nigeria (86). A concern is that African patients are strongly represented in

272 medical tourism to India, which is a risk factor for colonisation with Enterobacterales producing

273 MBLs (87).

274

275 **Rest of the world**. KPC enzymes dominate among carbapenemases from Enterobacterales in

276 Latin America, with (unusually) some penetration also into *P. aeruginosa*. Nonetheless,

277 Enterobacterales with NDM enzymes are endemic in Brazil, with several outbreaks reported

278 (88). Early case reports of MBL-producing Enterobacterales in Latin America often concerned

279 Proteeae, including *Providencia* spp. and *Morganella* (89, 90), which are infrequent hosts of

280 bla_{NDM} elsewhere. This creates a treatment issue since these genera are inherently resistant to

281 polymyxins and newer-generation tetracyclines, which remain options against other MBL-

282 producing Enterobacterales (below).

283 Unique to South America is the wide distribution in Brazil of *P. aeruginosa* with SPM-1

284 MBL (91), principally associated with an ST277 clone. Outcomes of severe infections with this

285 clone are often poor, reflecting a lack of good treatment options (92).

286 Carbapenemases are rare in Australasia, but there is spread of bla_{IMP-4} among

287 Enterobacterales (93), as in parts of China. *E. cloacae* is a major host, with dissemination

288 mediated by an IncHI2 plasmid (94). Production of IMP-4 enzyme has also been recorded in

289 *Salmonella* spp. from domestic pets (95) and seagulls (96), but the significance of this is

290 uncertain.

291

292 **MBL function in resistance, *in vitro* and *in vivo***

293 For many years MBLs were perceived as clinically unimportant chromosomally-encoded

294 enzymes from non-pathogenic organisms, notably *Bacillus cereus* (97, 98). This perception

295 changed with recognition that MBLs confer much of the resistance seen in *Chryseobacterium*

296 spp. and *E. meningoseptica* (99) and with heightened awareness of the morbidity and mortality

297 associated with *S. maltophilia* bacteraemia (100, 101). Interest then escalated with the

298   discovery and proliferation of acquired MBLs, especially NDM-1, which drew extensive press

299   coverage in 2010.

300   Many MBL producers are broadly resistant *in vitro* and, on this basis, real concern exists

301   about lack of treatments. On the other hand, there is evidence that *in vivo* resistance to

302   carbapenems may be less than it appears *in vitro*, because susceptibility tests are

303   conventionally done in media (e.g. cation-adjusted Mueller-Hinton broth) with high zinc

304   concentrations (102), whereas the host immune system imposes a state of zinc deprivation in

305   infection (40, 103). This lack of zinc may not only impede the catalytic function of MBLs but

306   may also interfere with their protein folding (102) and may promote degradation of the

307   enzyme in the periplasm (104).

308   Several preclinical studies suggest a disconnect between high-level *in-vitro* resistance

309   to carbapenems associated with NDM-1 enzymes, but a weak ability to protect against

310   carbapenems in standard murine infection models (105). Moreover, NDM enzymes appear less

311   effective than other carbapenemases in causing resistance to carbapenems in patients (106,

312   107). Thus, mortality in severe infections due to Enterobacterales with bla_{NDM} appears

313   relatively low, ranging from 13% (108) - 55% (109), when compared to that seen with bacteria

314   expressing other MBLs (18% to 67%) (13), or KPC carbapenemases (41% to 65%) (110, 111).

315   Good clinical outcomes have been reported despite treatment with agents to which NDM

316   enzymes confer resistance *in vitro* (106, 107, 112). As yet, there are no studies that confirm or

317   refute whether the higher numbered NDM alleles, encoding variants with their greater affinity

318   for zinc (above), are better able to cause clinical resistance than NDM-1 (39, 41).

319   Finally, it should be underscored that whilst these indications that NDM MBLs are less

320   potent *in vivo* are intriguing, they should be approached with caution. Double-blinded

321   randomized-controlled trials have not been conducted, and existing outcome data are subject

322 to various biases (113, 114). For VIM MBLs, clinical outcomes correlate with carbapenem MICs,

323 implying little or no such *in vitro/in vivo* discordance (115).

324

325 **Current treatment options**

326 Limited data exist to inform clinicians on the optimal treatment for infections caused by MBL-

327 producing gram-negative bacteria (106). Co-trimoxazole remains the standard of care for

328 infections due to *S. maltophilia,* but most Enterobacterales with acquired MBLs also have *sul*

329 and *dfr* genes, conferring resistance. Resistances to fluoroquinolones and aminoglycosides are

330 often present alongside genes encoding acquired MBLs. In particular, bla_{NDM} genes are often

331 linked to the genes encoding ArmA or RmtB methyltransferases, which modify ribosomes to

332 block binding of aminoglycosides, including plazomicin; bla_{IMP} and bla_{VIM} generally occur within

333 integrons that often also carry *aac(6')*, encoding an acetyltransferase that compromises

334 amikacin and tobramycin, though not gentamicin or plazomicin (116). A thorough review of

335 treatment options for MDR and XDR Enterobacterales is available (117). This highlights

336 observational studies comparing monotherapy to combination therapy for bloodstream

337 infection (BSI) involving CRE, although few of these were specifically identified as having MBLs

338 (118, 119).

339

340 **Colistin**. Colistin is the current mainstay of treatment for infections due to MBL-producers. A

341 multinational survey of MBL-producing Enterobacterales and *P. aeruginosa* conducted from 2012-

342 2014 found >97% susceptibility among MBL-producing *P. aeruginosa* (variously with IMP-, VIM-

343 and NDM- enzymes), and >85% for MBL-producing Enterobacterales (>86.1% NDM-type,

344 >88.9% IMP-type >88.9% IMP-type) (83). Exceptions are *Proteeae* and *Serratia* spp., which

345 have intrinsic polymyxin resistance.

346   For bacteria harboring KPC and OXA-48 carbapenemases, colistin has recently been

347   shown less effective than microbiologically-active β-lactamases inhibitor combinations (120),

348   making it plausible that an active β-lactam likewise would be more efficacious than colistin

349   against MBL producers. Of note, the emergence of colistin resistance during treatment, with

350   secondary transmission of resistant variants is a concern (121, 122).

351

352   **Tigecycline, omadacycline and eravacycline**. These tetracyclines have strong *in vitro* activity

353   against many MBL-producing Enterobacterales, except *Proteeae*, although not against *P.*

354   *aeruginosa*. During November 2018, 275 unique Enterobacterales isolates carrying bla_{NDM}

355   collected by the US Centers for Disease Control were tested with tigecycline (86.5%

356   susceptible, based on a ≤2 μg/ml FDA breakpoint), eravacycline (66.2% susceptible, based on

357   a ≤0.5 μg/ml FDA breakpoint) and omadacycline (59.6% susceptible, based on a ≤4 μg/ml

358   breakpoint) (123). The higher susceptibility rate for tigecycline than eravacycline reflects the

359   higher FDA breakpoint for Enterobacterales; in Europe both agents have an identical 0.5 μg/ml

360   breakpoint and eravacycline is the more active on a simple gravimetric basis, though it is

361   unclear whether this confers clinical advantage (124). Merits of omadacycline are its minimal

362   known drug interactions and that it can be administered orally (125), however, it has the least

363   relevant license (for community-acquired bacterial pneumonia and acute bacterial skin and

364   skin structure infections) in relation to the clinical burden of MBL producers.

365   Whilst the *in vitro* activity of these tetracyclines is encouraging, there are multiple

366   caveats. First, tigecycline carries an FDA 'black box' warning of increased mortality when the

367   drug was used as monotherapy (126); second, both tigecycline and eravacycline have failed to

368   achieve non-inferiority to comparators in one or more clinical trials (VAP and diabetic foot

369   infection for tigecycline, cUTI for eravacycline); third, there is little provenance for tetracyclines

370   as monotherapy in the severely-ill patients who commonly develop infections due to MBL-

371   producing opportunists; fourth, particularly for tigecycline, the disparity between EUCAST (S

372    $\leq$0.5 μg/ml) and FDA (S $\leq$2 μg/ml) breakpoints creates categorization uncertainty; last, the lack

373   of anti-*Proteeae* activity is important in Latin America, where *Providencia* spp. are frequent

374   hosts of *bla*$_{NDM}$ (127). Given these uncertainties, the best advice is to consider these

375   tetracyclines in combination against MBL producers, not as monotherapy.

376

377   **Aztreonam**. Aztreonam is stable to MBLs, though activity is lost against organisms that co-

378   produce ESBLs or AmpC enzymes (128), which are common in MBL-producing

379   Enterobacterales. Clinical experience as monotherapy is lacking for MBL producers, although

380   some success has been recorded when aztreonam was used in combination with ceftazidime-

381   avibactam (129, 130), with avibactam serving to inhibit ESBLs. Six out of ten patients survived

382   following treatment with this combination during an outbreak of *K. pneumoniae* with NDM-1,

383   OXA-48, and CTX-15 β-lactamases in Barcelona (129). Although no adverse events were

384   reported, the safety is unclear, and it is difficult to match the 1.5g +0.5g q6h regimen of

385   aztreonam-avibactam that is presently being developed (below).

386

387   **Fosfomycin**. Fosfomycin commonly retains full *in vitro* activity against MBL-producing

388   Enterobacterales, and has been successful trialed, very recently, as an IV agent in cUTI (131).

389   It may be an option against MBL producers - particularly *E. coli*, which is more susceptible than

390   other Enterobacterales - but it is mainly advocated for use in combination due to concerns

391   about emergence of resistance, particularly in *Klebsiella* spp. (132). Fosfomycin has little direct

392   antipseudomonal activity, with typical MICs above breakpoints. However, *in vitro* synergy is

393 seen when fosfomycin is combined with meropenem against MBL-producing *P. aeruginosa*

394 strains (133), suggesting a need for *in-vivo* exploration.

395

396 ## Development Pipeline

397 The development pipeline represents four main strategies against MBL producers: (i)

398 protection of MBL-stable-monobactams from other co-produced β-lactamases, as e.g. with

399 aztreonam-avibactam; (ii) development of β-lactams stable to MBLs as well as SBLs, as with

400 e.g. cefiderocol and BOS-228, (iii) combinations of cephalosporins and carbapenems with

401 triple-action diazabicyclooctanes (DBOs), and (iv) direct inhibition of MBLs with cyclic

402 boronates, thiols, chelators, dicarboxylic acids, and other agents.

403

404 ## Aztreonam-avibactam

405 Aztreonam-avibactam is the first antibiotic to be developed under a public-private partnership

406 agreement (134, 135), with partial finance from the European Union's Innovative Medicine's

407 Initiative and, latterly, also the US Biomedical Advanced Research and Developmental

408 Authority (BARDA). A prospective randomized phase 3 study (NCT03580044) begins in 2020

409 to determine efficacy, safety, and tolerability versus best available therapy (BAT) for

410 hospitalized adults with complicated intra-abdominal infections (cIAI), nosocomial pneumonia

411 (NP), complicated UTI, or BSI due to MBL-producing gram-negative bacteria (135).

412 Aztreonam evades hydrolysis by MBLs (128) but is compromised by the ESBL and AmpC

413 enzymes that are co-produced by many MBL-positive CPE. These SBLs are inhibited by

414 avibactam, a diazabicyclooctane (DBO) (136, 137) and, consequently, MBL-producing

415 Enterobacterales that also carry ESBLs or AmpC are susceptible to aztreonam-avibactam *in*

416 *vitro* (138) and *in vivo* (139). The combination is less reliably active against MBL-producing *P.*

417 *aeruginosa* (140), because aztreonam has weak anti-pseudomonal activity.

418 Considerable interest exists, because the safety and efficacy of aztreonam are well

419 established, and because avibactam was established to be effective at inactivating ESBLs and

420 AmpC enzymes during trials with ceftazidime. Moreover, case reports suggest success against

421 infections caused by MBL producers when aztreonam was co-administered with ceftazidime-

422 avibactam (see aztreonam section above) (129, 130).

423

424 **MBL-stable β-lactams**

425 **Cefiderocol (S-649266).**   Cefiderocol (S-649266) is a novel parenteral siderophore

426 cephalosporin designed by Shionogi & Co. Ltd., with a catechol linked to its 3-position side

427 chain. It is licensed in the USA for cUTI and in the EU and UK for 'treatment of infections due

428 to aerobic gram-negative organisms in adults with limited treatment options' (141). It is

429 retained among developmental agents here, rather than being included in the established

430 treatments, because there is little published experience with MBL producers to date (142,

431 143).

432 Critically, the catechol moiety forms a chelation complex with ferric iron and this

433 complex is actively accumulated by gram-negative bacteria, which are forced to scavenge this

434 essential element (144). Cefiderocol has good activity *in vitro* under iron starvation, against

435 gram-negative bacteria, including CPE, *P. aeruginosa* and *A. baumannii* (145)*.* It is relatively

436 stable to both SBLs and MBLs (144), however, the MICs for Enterobacterales and non-

437 fermenters with NDM carbapenemases tend to be slightly higher than those for isolates of the

438 same species with other carbapenemase types (146). Cefiderocol proved effective against

439 carbapenem-resistant *P. aeruginosa* (expressing IMP-1 enzymes), *A. baumannii* (expressing

440 OXA-51-like enzymes) and *K. pneumoniae* (expressing NDM-1 enzymes) in immunocompetent

441 rat respiratory tract infection models, achieving a ≥3-log reduction in the number of viable

442 bacteria in the lungs when dosed over 4 days so as to recreate the human exposures of a 2g

443 q8h 3h-IV infusion regimen (147). Efficacy reduced when the infusion time was reduced to 1h,

444 owing to a lower percentage of the dosing interval during which free-drug concentrations were

445 above the MIC (% T_f >MIC) (147). Interestingly, the mean % T_f >MIC required for a 1-$\log_{10}$

446 reduction was 18-24% greater for *A. baumannii* isolates (expressing OXA-23 or OXA-24) in the

447 murine lung infection model than for Enterobacterales expressing NDM-1, NDM-4 or KPC-2

448 enzymes and for *P. aeruginosa* isolates expressing IMP-1 or VIM-10 MBLs (148).

449 In humans, the 2g IV q8h 3h-infusion regimen provided >90% probability of target

450 attainment (PTA) with 75% T_f>MIC for MICs of ≤4µg/ml for patients with normal renal function

451 (149). A phase 3 trial (NCT03032380) has shown non-inferiority to meropenem in nosocomial

452 pneumonia (150). Less encouragingly, another trial (NCT02714595), found excess deaths in the

453 cefiderocol arm, compared with 'best available therapy,' for patients with severe infections

454 caused by carbapenem-resistant gram-negative pathogens (151). Full analysis is awaited but,

455 notably, deaths were mostly associated with *Acinetobacter* infections (152), not

456 Enterobacterales.

457

458 **BOS-228 (formerly LYS228).** BOS-228 is a monobactam and, like aztreonam, is stable to MBLs

459 (153). Unlike aztreonam, it is also stable to many potent SBLs, including carbapenemases,

460 ESBLs, and AmpC types (154); moreover it binds strongly to PBPs1a and 1b of Enterobacterales

461 as well as to PBP3, which is the sole target of aztreonam (155). BOS-228 had an MIC_{90} of 2

462 µg/ml for a clinical panel of 88 Enterobacterales isolates expressing ESBLs, KPCs and MBLs

463 (153) and no single-step mutants were selected from 12 β-lactamase-expressing

464   Enterobacterales exposed to the drug at 8 x MIC, though mutants were selected from 2/12

465   strains, neither of which expressed MBLs, at 4 x MIC (155).

466   A randomized evaluator-blinded multi-center phase 2 trial (NCT03354754) to evaluate

467   pharmacokinetics, clinical responses, safety, and tolerability of BOS-228 in cIAI commenced in

468   2018. The drug is being administered as IV monotherapy (without metronidazole) q6h for at

469   least 5 days and compared to standard of care, with outcomes evaluated at day 28. A

470   randomized controlled evaluator-blinded multi-center trial (NCT03377426) in cUTI has also

471   been initiated.

472

473   **Cephalosporins or carbapenems combined with triple-action DBOs**

474   **Zidebactam and nacubactam.** Unlike with cyclic boronates (see below), it has not been possible

475   to discover DBOs that directly inhibit MBLs. However, nacubactam and zidebactam are DBO

476   analogs that combine inhibition of SBLs with direct antibacterial activity by inhibiting PBP2

477   (156). When combined with PBP3-targetted β-lactams, this attack on PBP2 leads to an

478   'enhancer' effect, with further β-lactamase-inhibition-independent synergy observed (156,

479   157). Consequently, cefepime-zidebactam and cefepime- or meropenem- nacubactam

480   combinations are active *in vitro* against >75% of MBL-producing Enterobacterales and, in

481   cefepime-zidebactam's case, also against many MBL-producing *P. aeruginosa* (158).

482   Although the direct antibacterial activity of nacubactam and zidebactam is readily lost

483   via mutations compensating for inhibition of PBP2 (159), the enhancer effect is retained, with

484   many of the mutants consequently remaining susceptible to e.g. cefepime-zidebactam or

485   meropenem-nacubactam at low concentrations (156, 157). Cefepime-zidebactam is currently

486   the most advanced of these combinations, with a phase 3 trial due to commence (160).

487

488   **Direct inhibitors of MBLs.**

489   **Cyclic boronates - VNRX-5133 (taniborbactam) and QPX7228**. Inhibitors that target both SBLs

490   and MBLs are of great interest but have proved difficult to obtain owing to structural and

491   functional differences between and among these enzymes. This combination of inhibitory

492   activities nonetheless has recently been achieved with several cyclic boronates, notably

493   taniborbactam and QPX7228. These mimic the tetrahedral anionic intermediate common to

494   SBL and MBL catalysis (161) and additionally inhibit some penicillin-binding proteins (e.g. PBP-

495   5, which is non-essential) by the same mechanism (162). They represent a considerable

496   expansion in spectrum over vaborbactam, their progenitor, which inhibits only few class A β-

497   lactamases, notably KPC types (163).

498   Taniborbactam (VenatoRx) is the more advanced of these two 'second-generation'

499   boronates, and is in Phase III trials combined with cefepime (164). It irreversibly inhibits class

500   A, C, and D SBLs, and is a reversible competitive inhibitor of VIM and NDM MBLs, though not

501   of IMP types (165). Safety has been established in healthy volunteers (NCT02955459), and the

502   FDA has allowed cefepime-taniborbactam to proceed via fast track pathway for the clinical

503   indications of cUTI and cIAI.   QPX7728 (QPEX) likewise inhibits both SBLs and MBLs: 50%

504   inhibitory concentrations [IC_{50}], for KPC enzymes are around 2.9 ± 0.4 nM, compared with 22

505   ± 8 nM for the class C cephalosporinase of *E. cloacae* P99, 55 ± 25 nM for the NDM-1 MBL and

506   14 ± 4 nM for VIM-1 enzyme. As with taniborbactam, the IC_{50} for IMP-1 enzyme is considerably

507   higher, at 610 ± 70 nM) (166). An IV combination of QPX7728 with meropenem is being

508   explored. This significantly lowered bacterial counts in murine thigh and lung infection models

509   with carbapenem-resistant *K. pneumoniae*, *P. aeruginosa* and *A. baumannii* when compared

510   to meropenem alone, although strain genotypes were not reported. Unlike taniborbactam,

511　QPX7228 is orally bioavailable and combinations with ceftibuten and tebipenem were

512　evaluated *in vitro* against CPE, including those with MBLs (167).

513

514　**Thiol-containing MBL inhibitors and chelating agents**.　Small molecules that bind and/or

515　chelate zinc ions include thiols, dicarboxylates, hydroxamates, and tetrazoles; these are widely

516　reported to inhibit MBLs, but human metallo-proteases are vulnerable too, so toxicity may

517　preclude clinical development.

518　　　Thiol-containing compounds inhibit all MBL subtypes (B1, B2 and B3) (168), with strong

519　competitive inhibition of IMP-1 by thioester derivatives first reported in 1999 (169). The

520　dipeptide L-captopril deserves mention in context. It is used as an ACE inhibitor in the

521　treatment of hypertension and is reported also to inhibit MBLs by chelating the active site zinc

522　ions via its thiol group (170); the corresponding D- stereoisomer is a more potent inhibitor and

523　can potentiate meropenem against strains with VIM-2 MBLs (170). Both captopril isomers act

524　via zinc chelation and repurposing is attractive given the known safety of the L-isomer at its

525　licensed dose; however the economic model for development is yet to be established and

526　safety issues for the D-isomer need exploration. Other thio-carbonyl compounds, such as

527　thiomandelic acid, exhibit synergy with meropenem against Enterobacterales with VIM, NDM,

528　and IMP enzymes (171).

529　　　Bisthiazolidines are carboxylate-containing bicyclic compounds, considered to be

530　penicillin analogs that inhibit MBLs through a zinc-bridging thiol group and a carboxylate that

531　interacts with K224 (172). The orientation of the carboxylate and thiol moieties create diverse

532　binding that is observed on X-ray crystal structures and has been shown to inhibit all MBL types

533　(173). The bisthiazolidine scaffold inhibits NDM-1 enzymes *in vitro*, with K_i values in the low

534   micromolar range (from 7 ± 1 to 19 ±3 μM); they restore imipenem activity against *E. coli*

535   producing NDM-1 (172).

536   The divalent cation chelator EDTA has raised interest, too, both as an inhibitor of MBLs,

537   and also because it disrupts the gram-negative outer membrane and neutralizes various

538   bacterial enzymes and toxins (174, 175). It is widely used in identification tests for MBLs.

539   Sodium calcium EDTA, which is licensed for use for treatment of lead poisoning, reportedly

540   restored imipenem's activity *in vivo* against *P. aeruginosa* producing IMP- and VIM- enzymes

541   and against *E. coli* producing NDM-1 enzyme (176, 177), raising the issue of whether it might

542   be used to potentiate carbapenems in human infections. Elores®, which is marketed in India,

543   combines ceftriaxone, sulbactam and EDTA (178, 179) and reportedly achieved cures of

544   infections due to MBL producers in multiple patients, with no serious adverse events (178).

545   However, prospective and controlled studies are lacking, the dose of EDTA is low, and there

546   remains uncertainty (above) about the function of NDM-1 enzyme *in vivo*. More negatively,

547   the FDA has placed strict limits on the amount of EDTA permissible even in food (180) and

548   sodium calcium EDTA is capable of producing toxic effects that can be fatal (181). High

549   concentrations of EDTA are likely to strip divalent cations from human metalloenzymes,

550   including matrix metalloproteinases, carbonic anhydrase and carboxypeptidases, thus limiting

551   clinical applicability.

552   Aspergillomarasmine A (AMA) is a fungal natural product discovered in the 1960s (182),

553   and re-evaluated in the 1980s as an inhibitor of the human metalloproteinase angiotensin-

554   converting enzyme (ACE). AMA inhibits MBLs via a metal ion sequestration mechanism and

555   displays rapid and potent inhibition of NDM-1 and VIM-2 enzymes *in vitro* (183). It restored

556   the activity of meropenem against a *K. pneumoniae* strain expressing NDM-1 enzyme in an

557   intraperitoneal murine infection model (184). Again, the hazard of inhibiting human metallo-

558   enzymes requires careful investigation.

559

560   **Challenges for the development of inhibitors of MBLs**

561   One of the biggest challenges in designing MBL inhibitors is the diversity among these enzymes,

562   which share less than one third sequence identity at their active sites. Thus, for example,

563   taniborbactam and QPX7728 target NDM and VIM enzymes, but not IMP types (185).

564   Development of inhibitors that bind remotely from the active site might overcome this

565   limitation, but possible target areas also vary within class B1 and seem even better able to

566   tolerate mutations than the active site (29). Another challenge is the shallow binding site in B1

567   enzymes, meaning that inhibitors can only make limited interactions (29). Specificity for

568   bacterial MBLs is a further recurring challenge; interactions with human metallo-enzymes and

569   contingent toxicity are major concerns. Molecules that solely inhibit MBLs are limited by the

570   fact that many MBL producers also co-produce SBLs, including carbapenemases, meaning that

571   the partner β-lactam must evade these enzymes, that the inhibitor must inactivate both MBLs

572   and SBLs, or that a second inhibitor is required.

573   Preclinical development is challenging, too, because it is difficult to establish reliable

574   animal models in which MBL-mediated resistance is expressed, perhaps owing to the already-

575   mentioned lack of essential zinc at infection sites. Moreover bacteria are prone to lose MBL-

576   encoding plasmids, or fail to reliably express them, in murine models, resulting in

577   pharmacodynamic data that suggest meropenem susceptibility (186, 187). Consequently it is

578   difficult to establish the efficacy of candidate MBL-stable drugs or inhibitor combinations. It is

579   unclear if the same phenomena occur in patients (188), and this requires further research.

580   Irrespective of this aspect, it is also challenging to find and recruit the required number of

581   patients with MBL-producing pathogens to clinical trials. Rapid diagnostics should help, but

582   their use is complicated by cost and the need to deploy them to all trial sites, including in

583   countries where they are not licensed or are licensed only to inform infection control, not

584   treatment.

585

586   **Conclusion**

587   MBLs are disseminating internationally, particularly in Asia, and often are produced by gram-

588   negative bacteria with extremely broad spectra of *in vitro* resistance. Unlike for KPC and OXA-

589   48-like carbapenemases, producers are typically not susceptible to recently licensed β-

590   lactamase inhibitor combinations such as ceftazidime-avibactam, meropenem-vaborbactam,

591   imipenem-relebactam, although cefiderocol may be a potential answer. The ability of MBLs to

592   confer resistance to carbapenems may not be so great *in vivo* as *in vitro*, though this is

593   uncertain and may vary by enzyme type even within MBL subclasses.

594   Inhibitors are known, and the developmental boronates, taniborbactam and QPX7728

595   are of particular interest. Nonetheless, the quest for effective inhibitors is complicated by

596   differences in active site structure and zinc ligand interactions among MBLs, and by difficulties

597   in the design of appropriate preclinical and clinical trials. Non-boronate inhibitors face toxicity

598   issues, particularly if they interact with other metallo-enzymes or are general chelators. Other

599   approaches to overcoming MBLs include, avibactam-protected aztreonam; stable β-lactam,

600   notably BOS-228 as well as cefiderocol, and combinations of β-lactams with-triple action DBOs,

601   notably cefepime-zidebactam and meropenem-nacubactam.

602   And *that* is the positive aspect on which to close: there is now a diverse and exciting

603   pipeline of potential agents for the treatment of infections caused by bacteria that produce

604   MBLs. It remains to be seen what will be the most effective of these agents.

619

620

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1276   187.   Wiskirchen DE, Nordmann P, Crandon JL, Nicolau DP. 2014. In vivo efficacy of human

1277   simulated regimens of carbapenems and comparator agents against NDM-1-producing

1278   Enterobacteriaceae. Antimicrob Agents Chemother 58:1671–7.

1279   188.   MacVane SH, Crandon JL, Nichols WW, Nicolau DP. 2014. Unexpected in vivo activity

1280   of ceftazidime alone and in combination with avibactam against New Delhi metallo-β-

1281   lactamase-producing Enterobacteriaceae in a murine thigh infection model.

1282   Antimicrob Agents Chemother 58:7007–9.

1283

1284   Table 1 Examples of chromosomal and plasmid-associated MBLs (11)

1285

Chromosomal MBLs			Plasmid-associated MBLs	
Species	Enzyme	Subclass	Enzyme	Subclass
Bacillus cereus	BcII	**B1**	Verona integron-encoded (VIM)	**B1**
Chryseobacterium indologenes	IND	**B1**	New-Delhi metallo-β-lactamase (NDM)	**B1**
Elizabethkingia meningoseptica	BlaB	**B1**	Imipenemase (IMP)	**B1**
Myroides odoratimimus	MUS/ MYO	**B1**	Sao Paulo metallo-β-lactamase (SPM)	**B1**
Bacteriodes fragilis*	CfiA / CcrA	**B1**	German imipenemase (GIM)	**B1**
Aeromonas spp.	CphA	**B2**	KHM	**B1**
Stenotrophomonas maltophilia	L1	**B3**	Dutch imipenemase (DIM)	**B1**
Elizabethkingia meningoseptica	GOB	**B3**	Adelaide Imipenemase (AIM)	**B1**

1286

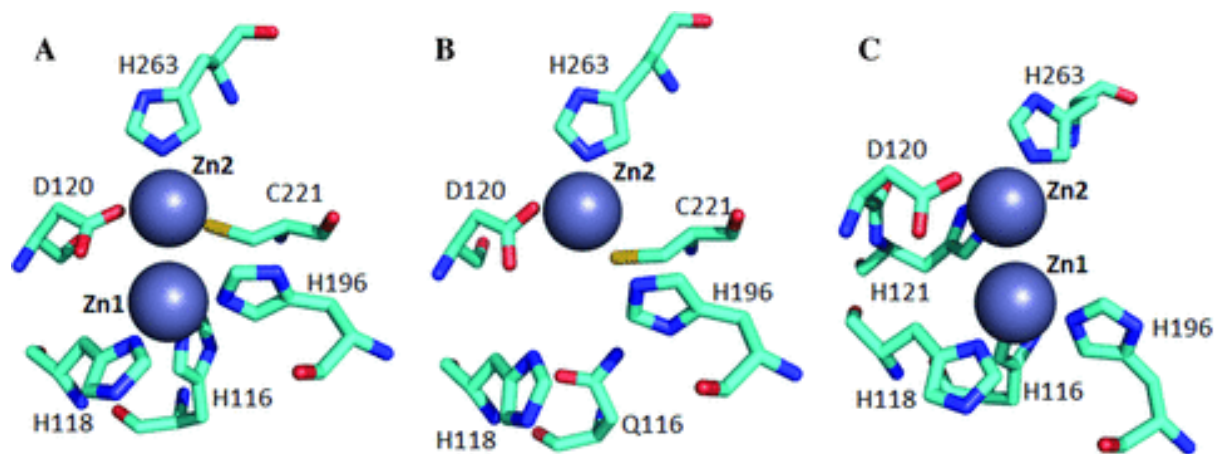1287   *Unlike most other chromosomal MBLs, the *Bacteroides fragilis* enzyme is rare in the species

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1289

Figure 1. Structure of amino acid residues in metallo-β-lactamase enzyme subclasses (8)

(Reproduced with permission from John Wiley and Sons Publishers, sourced from Palzkill T et al. 2013. Metallo-β-lactamase structure and function. Ann N Y Acad Sci 1277:91–104)

Figure 1 illustrates the amino acid residues that bind zinc at the active sites of B1, B2, and B3 MBLs. Crystal structures of B1 enzymes, including IMP-, VIM-, NDM-, and *B. fragilis* CcrA, (panel A) reveal two zinc-binding sites (Zn1 and Zn2). The Zn1 site contains three histidine residues (His116, His118, and His196), whereas the ligands for the Zn2 site are aspartic acid (Asp120), cysteine (Cys221), and histidine (His263). There is only one zinc ion in the active site of the *A. hydrophila* enzyme (subclass B2, panel B), and two in the active site of the *S. maltophilia* enzyme (subclass B3, panel C).
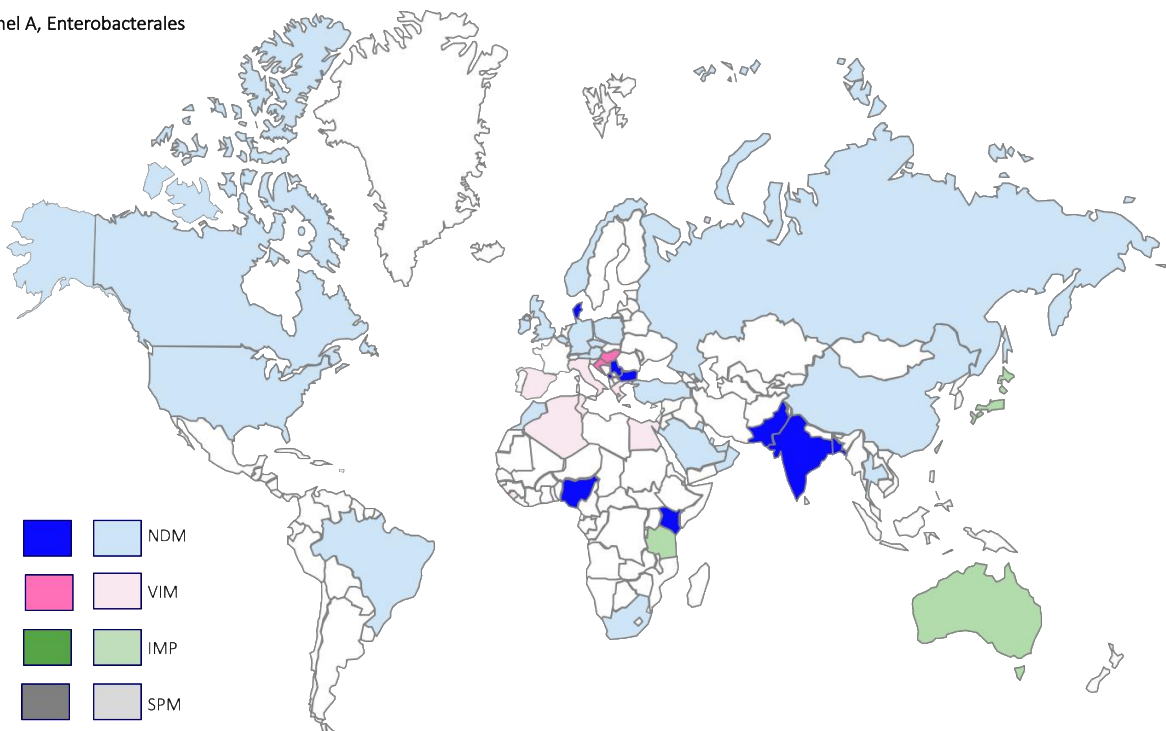
Figure 2. Global distribution of acquired metallo-β-lactamases

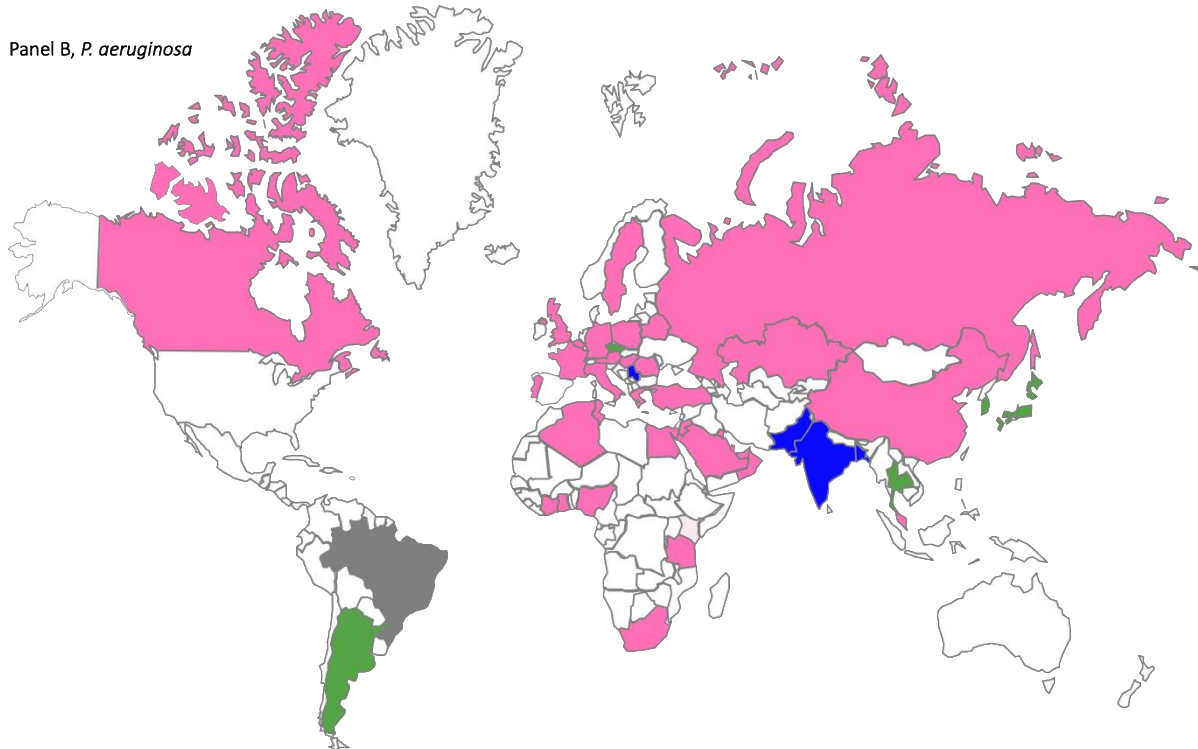Panel A, Enterobacterales

NDM
VIM
IMP
SPM

Full-tone color is used when the indicated MBL is the most prevalent carbapenemase in the country. The

lighter tone is used to indicate the most prevalent MBL group in countries where serine carbapenemases

(KPC or OXA-48-like) are more prevalent. Panel A, Enterobacterales; Panel B, *P. aeruginosa*

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Panel B, *P. aeruginosa*

*In the USA there are just a few reports of *P. aeruginosa* with either IMP or VIM MBLs

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