1   **Potential risks of antibiotic resistant bacteria and genes in bioremediation**

2   **of petroleum hydrocarbon contaminated soils**

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4   **Colin J. Cunningham[a], Maria S. Kuyukina[* b, c], Irena B. Ivshina [b, c], Alexandr I. Konev[b],**

5   **Tatyana A. Peshkur[d], Charles W. Knapp[d]**

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7   [a] Sustainability Fife Ltd, Glenrothes, UK

8   [b] Microbiology and Immunology Department, Perm State University, 15 Bukirev Street,

9   614990 Perm, Russia

10   [c] Institute of Ecology and Genetics of Microorganisms, Ural Branch of the Russian Academy

11   of Sciences, 13 Golev Street, 614081 Perm, Russia

12   [d] Department of Civil and Environmental Engineering, University of Strathclyde, Glasgow,

13   UK

14

15   *Corresponding author.

16   Professor Maria S. Kuyukina

17   Microbiology and Immunology Department

18   Perm State University, 15 Bukirev Street, 614990 Perm, Russia

19   Tel.: (+7)3422808114, Fax: (+7)3422809211

20   *E-mail address*: kuyukina@iegm.ru

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23   **Abstract**

24   Bioremediation represents a sustainable approach to remediating petroleum hydrocarbon

25   contaminated soils. One aspect of sustainability includes the sourcing of nutrients used to

26   stimulate hydrocarbon-degrading microbial populations. Organic nutrients such as animal

27   manure and sewage sludge may be perceived as more sustainable than conventional inorganic

28   fertilizers. However, organic nutrients often contain antibiotic residues and resistant bacteria

29   (along with resistance genes and mobile genetic elements). This is further exacerbated since

30   antibiotic resistant bacteria may become more abundant in contaminated soils due to co-

31   selection pressures from pollutants such as metals and hydrocarbons. We review the issues

32   surrounding bioremediation of petroleum-hydrocarbon contaminated soils, as an example,

33   and consider the potential human-health risks from antibiotic resistant bacteria. While

34   awareness is coming to light, the relationship between contaminated land and antibiotic

35   resistance remains largely under-explored. The risk of horizontal gene transfer between soil

36   microorganisms, commensal bacteria and/or human pathogens needs to be further elucidated,

37   and the environmental triggers for gene transfer need to be better understood. Findings of

38   antibiotic resistance from animal manures are emerging, but even fewer bioremediation

39   studies using sewage sludge have made any reference to antibiotic resistance. While

40   resistance mechanisms, including those to antibiotics, have been considered by some authors

41   to be a positive trait to enhance population, or community, resilience in strains intended for

42   bioremediation, nevertheless recognition of the potential risks associated with antibiotic

43   resistant bacteria and genes in contaminated soils appears to be increasing and requires

44   further investigation. Careful selection of bacterial candidates for bioremediation possessing

45   minimal antibiotic resistance as well as pre-treatment of organic wastes to reduce selective

46   pressures (e.g., antibiotic residues) are suggested to prevent environmental contamination

47   with antibiotic-resistant bacteria and genes.

Introduction

Bioremediation has generally been considered a more sustainable approach to managing petroleum hydrocarbon contaminated soils than alternatives such as disposal to landfill or thermal desorption.[1, 2] There has been an increasing focus on 'green' and 'sustainable' remediation[3] and an international standard ISO18504:2017 *Soil quality-Sustainable remediation* was recently published (2017). Green remediation aims to reduce the demand placed on the environment by remediation activities and to conserve natural resources.[4]

One aspect of sustainability for bioremediation of petroleum hydrocarbon contaminated soils is the choice of nutrients, principally nitrogen and phosphorous, to support growth (biostimulation) of the hydrocarbon degrading microbial population. Studies have compared biostimulation approaches using organic (e.g., animal manure) and inorganic (e.g., agricultural NPK fertilisers). For example, Cunningham and Philp[5] found horse manure to be equally effective as NPK fertiliser in a field pilot scale *ex-situ* bioremediation of diesel contaminated soil from a UK railway siding. A number of specialist biostimulation products have also been developed over the years, such as the oleophilic fertiliser Inipol EAP22—an oil-in-water microemulsion providing emulsified urea, oleic acid and lauryl phosphate.[6]

While the use of animal manures for biostimulation may be considered a more sustainable option than conventional inorganic agricultural fertilisers, many sources of animal manure contain veterinary antibiotics, antibiotic resistant bacteria (ARB) and their genes (ARGs) that impact the microbial resistome following land application.[7] Another organic nutrient source used for biostimulation is sewage sludge or processed sewage sludge known as biosolids. However, municipal wastewater treatment plants (WWTPs) have been identified as

72 'hotspots' for ARB, and the sewage sludge may contain significant amounts of

73 pharmaceutical and personal-care product (PPCP) residues, complexed metals, and ARGs.[8]

74

75 The development of ARB poses a growing global threat to human health by reducing

76 treatment options for bacterial infections.[9] A recent report for the UK government estimated

77 global deaths arising from antimicrobial resistance could rise from 700,000 per annum to 10

78 million by 2050 at a cost to the global economy of US$100 trillion.[10] It has been further

79 estimated that between 2010 and 2030, global consumption of antibiotics will rise from

80 63,151 ± 1,560 tons to 105,596 ± 3,605 tons, an increase of 67%.[11]. It was proposed that a

81 third of this increase will come from a shifting of production practices in middle-income

82 countries towards larger-scale intensive farming operations that routinely use antibiotics at

83 sub-therapeutic doses to promote animal growth.

84

85 The majority of the antibiotics for human and veterinary use have been derived from soil

86 microorganisms,[12] and ARGs pre-date the introduction of antibiotics as they have been

87 discovered in microbial DNA extracted from 30,000-year-old permafrost sediments[13] and

88 from a cave in New Mexico that has been isolated for over 4 million years.[14] The abundance

89 of ARGs in soils has increased since the introduction of antibiotics in the 1940s. Analysis of

90 samples from historical soil archives in The Netherlands (1940-2008) showed a significant

91 increase in ARGs to all major classes of antibiotics.[15] In another soil-archive study, similar

92 trends were mitigated following effective antibiotic-used policies,[16] suggesting that antibiotic

93 resistance represents a significant impact of the Anthropocene.[17]

94

95 Resistance traits have been observed to emerge in the environment and the clinical settings

96 rather simultaneously;[16] whether coincidental or causal, it remains yet to be determined.

97   However, ARGs are capable of moving from soil bacteria to pathogens, and vice versa,

98   through a variety of gene transfer processes giving the potential for emerging resistant

99   pathogens from soils.[18] A key mechanism for acquired resistance is through horizontal gene

100   transfer mediated by mobile genetic elements including bacteriophages, plasmids,

101   transposons, integrons and insertion sequences. Soils have been determined as a key reservoir

102   (i.e., resistome) from which pathogenic bacteria may acquire resistance.[19] It must be

103   concluded that there are many novel ARGs yet to be discovered, and our knowledge of

104   resistance possibilities may be limited. For example, over thirty new ARGs were recently

105   recovered from experimental farm soil plots in Canada that had been exposed to antibiotics.[20]

106

107   However, not only soils exposed to antibiotics, for example via application of animal manure,

108   serve as potential sources of ARGs. Contaminated soils present a selection pressure to soil

109   microbial communities, and co-selection of resistance to antibiotics and pollutants, such as

110   metals, has been well described.[21] This exhibits as co-resistance if resistance determinants for

111   metals and antibiotics are located on the same mobile genetic element or cross-resistance if

112   the same genetic determinant confers resistance to both metals and antibiotics. Many ARGs

113   have positively correlated with levels of metals in Australian and archived Scottish soils.[22,23]

114   These studies suggest that soil geochemical landscapes may be a useful tool to estimate the

115   baseline ARG presence on local, regional and global scales within epidemiological risk

116   studies examining potential transmission of antibiotic resistance from the environment.

117

118   Action is required to reduce the risks posed by ARGs from the environment including

119   identifying critical control points, reliable surveillance and risk assessment procedures as well

120   as technological solutions to prevent environmental contamination with antibiotic resistant

121   bacteria and genes.[24] Studies are now examining the diversity and abundance of ARGs in the

122 environment arising from diverse sources.[25] Additionally, efforts are emerging that include

123 removal of ARGs from contaminated soil as a remedial goal. For example, Ye et al.[26]

124 described a novel remediation process combining multiple soil washing steps using a solution

125 of powered salmon DNA with ultrasonication. They obtained soil samples from farming land

126 contaminated by a nearby abandoned electronic waste disposal plant and a poultry farm that

127 had regularly disposed of poultry manure and waste antibiotics on the land. A lab scale

128 feasibility trial of the novel remediation process removed 80% of polybrominated diphenyl

129 ethers (brominated flame retardants), 60% of copper, and 100% of tetracycline and

130 sulfadiazine antibiotics as well as markedly decreasing the abundance of ARGs.

131

132 We proposed that ARB and ARGs are likely to become more prevalent on contaminated sites

133 than is currently understood by remediation practitioners, regulators and researchers.

134 Contaminated sites may have higher baselines of resistance into which antibiotics, ARB and

135 ARGs might further contribute via biostimulation using organic nutrients such as animal

136 manure or sewage sludge. It is not just the addition of resistance genes and/or bacteria that

137 remains concerning, rather environmental conditions and stress may promote the

138 dissemination may promote the dissemination of genes. We use the example of

139 bioremediation of petroleum-contaminated soils to consider potential risks of antibiotic

140 resistant bacteria in the context of sustainable remediation.

141

142 **Antibiotic resistant bacteria and bioremediation of petroleum hydrocarbon**

143 **contaminated sites**

144 The presence of petroleum hydrocarbons in soils is known to result in the co-selection of

145 ARGs in bacteria. Aono et al.[27] were among the first to report the relationship between

146 bacterial tolerance of hydrocarbons and antibiotic resistance. Co-selection of antibiotic

147   resistance was demonstrated for common contaminants such as hexane and toluene[28] and

148   polycyclic aromatic hydrocarbons (PAHs).[29,30] Namely, the presence of naphthalene and

149   phenanthrene in coastal seawater significantly enhanced the abundance of class I integrase

150   gene (*intI1*), sulfanilamide resistance gene (*sulI*), and aminoglycosides resistance gene

151   (*aadA2*) in the microbial community presumably as a result of conjugative transfer mediated

152   by class I integrons.[30] A metagenomic study of Chen et al.[29] revealed the prevalence of

153   *Proteobacteria* carrying the efflux pump-encoding ARGs associated with aromatic antibiotics

154   in PAHs-contaminated soils, thus suggesting that these structurally similar compounds could

155   be pumped out by the same efflux system. So, similar to the antibiotic- and metal resistance,

156   the co-selection of ARB and ARGs in petroleum hydrocarbon contaminated soils can be

157   achieved through horizontal gene transfer and cross-resistant mechanisms. However, there

158   have been relatively few reports in the published literature that consider antibiotic resistance

159   during bioremediation of petroleum hydrocarbon contaminated sites (Table 1).

160

161   Bello-Akinosho et al.[31] recently reported on the isolation of *Pseudomonas* sp. strain 10–1B

162   capable of degrading PAHs as well as solubilizing phosphate and fixing atmospheric

163   nitrogen, properties related to plant growth promoting rhizobacteria (PGPR). Green and

164   sustainable remediation was the key driver for isolation of a more efficient bioremediation

165   strain that required less exogenous nutrient input. However, their study did not consider

166   ARGs or antibiotic resistance traits of *Pseudomonas* sp. strain 10–1B. It would be prudent to

167   consider the knowledge coming from agriculture in this respect. PGPR are used to colonise

168   the roots of crop plants with a key function being to increase the supply or availability of

169   nutrients and therefore reduce soil inputs of inorganic agricultural fertilisers.[32] A recent study

170   by Kang et al.[33] found that all of the PGPR strains they examined, including several of the

171   genus *Pseudomonas*, possessed multiple ARGs. These authors proposed that careful attention

172   should be given to potential intensification of ARGs in soils through the deliberate

173   introduction of PGPR to improve crop sustainability.

174

175   Some bioremediation studies have urged caution with respect to antibiotic resistant bacteria

176   (Fig. 1). Multiple antibiotic resistances identified in hydrocarbon degrading strains of

177   *Pseudomonas aeruginosa* led Kaszab et al.[34] to propose "as a preventive measure, pathogen

178   microorganisms such as *P. aeruginosa* ought to be eliminated from bioremediation processes

179   as efficiently as possible". They detected *P. aeruginosa* in 62% of the twenty-six

180   hydrocarbon contaminated soils they studied in Hungary, with eight of the sites producing

181   multi drug-resistant strains. More recently, Kaszab et al.[35] further cautioned against the use of

182   *P. aeruginosa* based on phylogenetic profiling that revealed a strong correlation between two

183   environmental multi-drug resistant strains and those known to cause infection in humans,

184   notably in those with cystic fibrosis.

185

186   Similar concerns over resistant strains of *P. aeruginosa* have also been reported from

187   agricultural studies. The presence of antibiotic resistant traits halted further development of a

188   naturally occurring endophytic *P. aeruginosa* strain PaBP35 as a biocontrol agent for

189   *Phytophthora* rot and other plant diseases in food crop production.[36] Genotyping and

190   functional analysis had revealed resistance to multiple antibiotics and similar virulence as

191   clinical *P. aeruginosa* type strains. Others have also identified environmental multi-drug

192   antibiotic resistant strains of *P. aeruginosa* from agricultural soils.[37]

193

194   The monitoring and management of ARGs during the bioremediation process has been

195   proposed to be crucial by Sun et al.[38] They considered four ARGs corresponding to

196   tetracycline and sulfonamide resistance as co-contaminants of soils alongside PAHs in soil

197 located near an abandoned poultry farm and steel plant. No tetracycline and sulfonamide was

198 detected in the soils but the corresponding ARGs had persisted. In their microcosm study,

199 inoculation with a type strain *Sphingobium* sp. PHE3 along with addition of sorphorolipid

200 biosurfactant not only significantly reduced pyrene concentrations but also resulted in a

201 significant decline in the abundance of ARGs.

202

203 Buyukunal and Tas[39] surveyed Gram-negative bacterial diversity and antibiotic resistances in

204 heavily polluted soil, sludge and water samples from around the Sır Dam Lake in Turkey.

205 Among the multi-drug resistant isolates identified were strains of *Escherichia coli*, *Klebsiella*

206 *oxytoca* and a single *Acinetobacter* strain that were together considered of relevance to public

207 health. The second most predominant isolates were strains of *Acidovorax temperans* that

208 didn't exhibit antibiotic resistances. It was proposed that the *A. temperans* strain might have

209 potential for biodegradation of hydrocarbons and may be beneficial for protecting public

210 health from transmission of antibiotic resistance during bioremediation processes. This

211 precautionary approach has been reported elsewhere. For example, Saranya et al.[40] isolated a

212 strain of *Vibrio fluvalis*, with high resistance to mercury but little resistance to antibiotics,

213 which removed 60% of mercury from aqueous solution from a starting concentration of

214 250 μg/ml.

215

216 Perhaps identification of strains with low antibiotic resistance should become a future

217 direction of bioremediation research, although there remains always the potential for such

218 strains to acquire antibiotic resistance from the environment. Many petroleum hydrocarbon

219 contaminated sites are also co-contaminated with metals either as trace elements of crude oil

220 and its derivatives or from other industrial activity. Metal contamination in soils also

221 produces co-selection for bacterial genes conferring antibiotic resistance and the relationship

222   between metal and antibiotic resistance in bacteria is very well established.[21,41,42] Even low

223   concentrations of metals found in residential soils, assumed to be have been free of antibiotic

224   exposure, showed a greater relative abundance of ARGs.[22]

225

226   Máthé et al.[43] sought to obtain strains of hydrocarbon degrading bacteria for bioaugmentation

227   of diesel and fuel oil contaminated sites in Romania that would also be resistant to copper,

228   lead and zinc. They assessed antibiotic resistance and identified several multi drug resistant

229   *Pseudomonas* strains but made no reference to potential risks from ARGs. Continuing this

230   work, Benedek et al.[44] developed an inoculum for bioremediation of hydrocarbon and metal

231   co-contaminated sites based on two of the isolated strains, *Rhodococcus qingshengii* and *P.*

232   *fluorescens*, making no further reference to antibiotic resistance.

233

234   Alternative bacteria may exist and be considered to perform bioremediation without carrying

235   the additional risk to environmental- and public health. Members of the genus *Rhodococcus*

236   are widely used for bioremediation of hydrocarbon-contaminated soil due to their broad

237   catabolic versatility and cellular adaptations to assimilate hydrophobic substrates.[45,46] The

238   environment safety of rhodococcal inocula intended for bioremediation is supported by the

239   lack of pathogenicity within this genus (except for *R. hoagii* and *R. fascians* associated with

240   pathogenicity for animals and plants, correspondingly), the lack of mutagenicity or

241   ecotoxicity.[45,47] While most environmental *Rhodococcus* isolates tested were sensitive to

242   antibiotics, a non-specific increase in antibiotic resistance was registered in the cultures

243   grown on hydrocarbons, presumably determined by changes in the cell envelope lipid

244   composition.[48]

245

246   On the contrary, the propensity of bacteria to acquire and/or maintain antibiotic resistance has

247   even been considered positively by some authors in strains for bioremediation. For example,

248   co-resistance to metals and antibiotics in strains isolated from contaminated sites in Nigeria

249   was stated by Oyetibo et al.[49] to be advantageous in developing inocula for bioremediation of

250   metal co-contaminated sites that would "compete with antibiotic producing flora". In this

251   study, five of the twenty-two metal resistant strains isolated also showed resistance to all of

252   the eighteen antibiotics they tested. Contaminated land can represent stressful conditions

253   (e.g., toxic) towards microorganisms, and resistance traits (the resistome) and stress-response

254   factors play crucial roles in ecosystem responses to stress along with community structure

255   (composition) and function (activity). Certain resistance traits can possibly aid in community

256   resilience (e.g., hydrocarbon catabolism, and metal resistance). This does not contradict the

257   argument against antibiotic resistance, but further emphasizes the importance of better

258   understanding the resistome in bioremediation.

259

260   Nevertheless, recognition of the potential risks associated with ARB and ARGs in

261   contaminated soils appears to be growing (see Fig. 1). *Acinetobacter*, commonly associated

262   with petroleum hydrocarbon contaminated soils, are increasingly being identified as potential

263   sources of novel human pathogens with multi-drug resistances.[50] Recently, Tayabali et al.[51]

264   has proposed that all commercial microbial bioremediation products should be examined for

265   pathogenic potential and susceptibility to antibiotics prior to commercial use.

266

267   **Antibiotic resistant bacteria associated with organic nutrients**

268   The use of a variety of animal manures for biostimulation has been reported in the scientific

269   literature. The extent to which these reflect current industry practice in different countries is

270   difficult to assess. As well as providing slow release nitrogen and phosphorous for

271 biostimulation, animal manures also serve as a source of organic matter and sometimes an

272 inoculum. In addition to containing antibiotics and pathogenic bacteria, animal manures are

273 likely to contain metals including copper and zinc from feed supplementation. Nevertheless,

274 in many cases it is presented as being an environmentally friendly practice utilising wastes

275 and benefitting from lower cost than manufactured inorganic fertilisers. Pre-treatment such as

276 composting of cattle manure has been shown to reduce antibiotic residues, pathogenic

277 organisms and ARGs.[52]

278

279 We found that none of the published studies on biostimulation using organic nutrients, even

280 from recent years, considered the issue of antibiotic resistance. In developing countries, the

281 cost and availability of mineral fertilisers may be the primary driver for using animal manures

282 in bioremediation[53] A wide range of other organic nutrient sources including corn residues,

283 sugarcane bagasse, banana skin, yam peel, saw dust, spent brewing grain, rice husk, and

284 coconut shell have been used for biostimulation.[54]

285

286 Poultry litter is a mixture of manure and bedding material. Gupta and Tao[55] proposed this as a

287 useful inoculum as well as source of nutrients for bioremediation. They noted the abundance

288 of microorganisms in poultry litter resulting in an 80% increase in total bacterial counts

289 following amendment of gasoline (petrol) contaminated soil. Using poultry litter for

290 biostimulation was considered to be a useful niche outlet for excess materials from intensive

291 production in the United States. An inoculum was developed through serial enrichment of the

292 microorganisms present in the litter with diesel as the sole carbon source. A combination of

293 poultry litter and the enriched consortia was the most successful treatment in terms of diesel

294 reduction observed in a field scale study over several weeks.[56]

295

296   Others have used poultry litter primarily for biostimulation. For example, in a study from

297   India, Rahman et al.[57] air-dried and sieved the poultry litter prior to application to diesel-

298   contaminated soils along with an exogenous bacterial consortium and *Pseudomonas*

299   rhamnolipid biosurfactant. The majority of publications on use of litter/manure alone or in

300   combination with other nutrient sources come from Nigeria and focus on crude oil

301   bioremediation.[58–61] The issue of ARB in poultry litter in Nigeria has been examined. Hemen

302   et al.[62] reported finding multi-drug resistant bacteria at many of the 480 sites they sampled. It

303   is thought to be particularly problematic in Nigeria due to the challenges of regulating

304   antibiotic use in a very large number of small producers.[63]

305

306   Studies from a number of other countries have reported on bioremediation of hydrocarbons

307   using various animal manures. From China, Liu et al.[64] used pig manure in a field study on an

308   oily sludge contaminated soil. From Romania, Bina et al.[65] found poultry manure resulted in

309   the highest reduction in diesel-spiked topsoil compared with pig and cattle manures. From

310   Thailand, Naowasarn and Leungprasert[66] also found poultry manure appropriate for

311   biostimulation in a laboratory study on used lubricating oil spiked soil.

312

313   Sewage sludge from wastewater treatment plants (WWTPs) has also been used for

314   biostimulation. Antibiotics enter the sewerage system in human urine and faeces. Many of

315   these drugs and their metabolites will pass untreated through WWTPs and become

316   disseminated via sewage sludge or treated effluent entering the water environment.[28] Given

317   the intensive biological treatment processes and complex mixtures of contaminants entering

318   WWTPs, sewage sludge is increasingly well recognised as an abundant source and 'hotspot'

319   of ARB and ARGs.[8] In their recent review, Bondarczuk et al.[67] stated an urgent need for

320   greater understanding of the risks of spreading antibiotic resistance through application of

321 sewage sludge to agricultural soils. This area has received growing attention in recent years

322 examining the effects of pre-treatments such as anaerobic-digestion, aerobic-digestion, heat-

323 treatment and pelletization on the abundance and persistence of ARGs in biosolids amended

324 soils.[68,69]

325

326 Similar to our findings for animal manures, bioremediation studies using sewage sludge have

327 made little or no reference to antibiotic resistance. In a study from Spain, Gallego et al.[70]

328 considered sewage sludge as "a cheaper disposable fertiliser" than inorganic mineral nutrients

329 for bioremediation of diesel contaminated soil. Biodegradation efficiency after 45 days in

330 laboratory scale bioreactors was 90% with addition of inorganic nitrogen, phosphorus and

331 magnesium and 65% using sewage sludge as a nutrient source but. Several diesel-degrading

332 isolates were tested for antibiotic resistance and found to be sensitive to all antibiotics

333 screened for except fosfomycin and cephalothin. However, antibiotic resistance wasn't

334 considered in the context of dissemination of resistance to soil via the sewage sludge.

335

336 A South Korean study by Namkoong et al.[71] used laboratory scale bioreactors to examine the

337 efficacy of dewatered sewage sludge for bioremediation of 10,000 mg/kg of spiked diesel in

338 topsoil. Removal of total petroleum hydrocarbons was 99% after 30 days and the sewage

339 sludge was considered an effective and inexpensive inoculant and nutrient source.

340 Supplementation of carbon was proposed as the reason why pelletized sewage sludge

341 (biosolids) was found be more effective than inorganic fertilizer in a US laboratory study of

342 petroleum hydrocarbon degradation in a diesel contaminated soil.[72] This study reported that

343 an additional benefit of using biosolids was an absence of toxicity to soil microflora observed

344 following application of inorganic fertiliser.

345

346   A field study on the efficacy of sewage sludge for landfarming of oil refinery waste in Spain

347   was performed by Ros et al.[73] Over an 8-month period they studied the influence of fresh and

348   composted sewage sludge on hydrocarbon degradation and microbial community structure.

349   Fresh sludge was associated with a 46% reduction in total hydrocarbon degradation compared

350   to 36% for composted sludge and 31% in the unamended treatment. A Malaysian laboratory

351   study by Agamuthu et al.[74] on biodegradation of lubricating oil contaminated soil reported

352   94% and 82% removal for cow dung and sewage sludge amendments respectively. Most

353   recently, Jakubauskaite et al.[75] from Lithuania, emphasised the sustainability of using of

354   dewatered sewage sludge in bioremediation of petroleum hydrocarbon contaminated soils as

355   "one waste product is used for the management of the other waste".

356

357   Another possible source of nutrients and inoculum from a WWTP is activated sludge. Juteau

358   et al.[76] found that activated sludge from a Canadian oil refinery WWTP enhanced

359   hydrocarbon biodegradation in soils and alkane biodegradation in particular when compared

360   to inorganic fertiliser. Activated sludge from an Australian municipal WWTP was used as a

361   source of inoculum for hydrocarbon contaminated soils in slurry phase bioreactors.[77] This

362   study made no reference to ARGs or antibiotics despite sourcing activated sludge from the

363   largest WWTP in South Australia.

364

365   As we found almost no consideration of ARB and ARGs in the context of biostimulation

366   using organic nutrients on contaminated soils, the potential risks of disseminating ARB and

367   ARGs appear to have been overlooked. There is evidence that ARGs reduce over time

368   following application of animal manure to soil[16,78] and that highly diverse microbial

369   communities may resist the spread of ARGs.[79] It is difficult to generalise giving the

370   complexity and site specificity of biotic and abiotic factors in contaminated soils. However,

371   as hydrocarbon-contaminated soils may exhibit reduced microbial diversity,[80] microbial

372   communities in such soils may therefore be more susceptible to the introduction of ARGs.

373   The most competitive and successful hydrocarbon degraders may be those with multiple

374   resistances including antibiotic resistance. For example, Table 2 summarizes ARGs found in

375   the published genomes of known important hydrocarbon-degrading *Actinobacteria*. It seems

376   that many actinobacterial genera relevant for bioremediation, e.g. *Arthrobacter*,

377   *Corynebacterium*, *Dietzia*, *Gordonia*, *Mycobacterium*, *Nocardia*, and *Rhodococcus* could be

378   potential hosts for antibiotic resistance mechanisms. Moreover, not only pathogenic (e.g. *C.*

379   *diphtheria*, *G. terrae*, *M. tuberculosis*, *N. brasiliensis*, *R. hoagii*) and opportunistic species,

380   but also typical soil inhabitants, not associated with pathogenicity, possess ARGs (Table S1).

381   Most abundant ARGs (found in more than 50% of species) belong to antibiotic efflux pump

382   families (CARD; https://card.mcmaster.ca) and some of these pumps are associated with

383   multiple drug resistance (MDR). Many of these actinobacterial genomes contain several

384   efflux pumps, thus indicating their ancestral origins; moreover their over-expression can be

385   triggered by the presence of toxic hydrocarbons or stressful environmental conditions.[81]

386   Other type ARGs predominantly found in hydrocarbon-degrading *Actinobacteria* represent

387   main resistance mechanisms (antibiotic inactivation, target protection, replacement and

388   alteration), including *aph* - aminoglycoside phosphotransferase, *sul1* and *folP* which confer

389   resistance to sulfonamide and sulfone antibiotics, *msrA* and *carB* - ribosomal protection

390   protein confers resistance to MLSB, and *pbp* - penicillin-binding protein mutations conferring

391   resistance to beta-lactam antibiotics (Table S1).

392

393   **Concluding remarks**

394   Soils are known reservoirs of ARGs, with pollution by hydrocarbons, metals and other

395   contaminants contributing co-selection pressures on soil microbial populations. Emerging

396   reports begin to recognize ARGs as a co-contaminant along with conventional chemical

397   pollutants. However, the awareness of antibiotic resistance in the context of bioremediation

398   of petroleum-contaminated soils remains widely varied. This ranges from no recognition to

399   reports urging caution when using environmental isolates with well characterized multidrug

400   resistance traits. A few authors have balanced the needs of bioremediation with the context of

401   protecting public health by considering bacterial isolates with few antibiotic resistance traits.

402

403   The application of animal manure and/or sewage sludge containing antibiotics, ARB and

404   ARGs for biostimulation may represent an unacceptable risk given their potential to facilitate

405   the transfer of resistance traits to human pathogens. This would clearly be an undesirable and

406   unintended consequence of an otherwise green bioremediation project. The potential risks of

407   horizontal gene transfer from soil microorganisms to commensal bacteria and/or human

408   pathogens need to be quantified and mechanisms for transfer better understood.[82] Attention is

409   being given to ARB and ARGs by regulators,[28] not specifically around remediation of soils,

410   but there is recognition that not all potentially relevant pathways and drivers for antibiotic

411   resistance are being tackled by action plans currently considered.

412

413   Given the global importance of antibiotic resistance to human health and the economy,

414   consideration of ARB and ARGs may become an essential component of remediation

415   projects (Fig. 2). This represents only a small part of the overall global challenge and the

416   need for co-ordinated effort was most clearly expressed by Graham et al.[16] who stated "…one

417   cannot address broader problems of increasing AR [antibiotic resistance] by employing only

418   medical, agricultural or environmental solutions because acquired AR (regardless of where it

419   emerges) readily migrates across sectors".

420

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Table 1. List of ARB and ARGs found in hydrocarbon contaminated soils & sediments

Soil samples	ARB	Resistance to antibiotics	Reference
Heavy oil contaminated alpine soil	*Gammaproteobacteria* (genera *Pseudomonas* and *Serratia*), *Alphaproteobacteria* and, to a lesser extent, *Actinobacteria*	Penicillin, chloramphenicol, rifampicin, streptomycin, tetracycline	Hemala et al., 2014
PAH contaminated soil near the wastewater pond of petrochemical plant	Mostly *Proteobacteria*	Fluoroquinolones (ampicillin, ceftriaxone), beta-lactams (penicillin, cefoxitin, cefazolin), nitrofurantoin	Chen et al., 2017
Petroleum hydrocarbon and heavy metal contaminated soils near diesel-oil storage units	*Gammaproteobacteria* (*Pseudomonas fluorescens*, *Pseudomonas syringae* and *Pseudomonas veronii*)	Cephalosporins (cefuroxime sodium, cefoperazone, cefotaxime, ceftazidime, cefaclor, moxalactam); penicillins (piperacillin, amoxicillin, piperacillin+tazobactam); aminocumarins (novobiocin); quinolones (nalidixic acid); carbapenems (imipenem)	Máthé et al., 2012
Hydrocarbon contaminated rhizosphere of willow from a former petrochemical plant site	*Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria* and *Acidobacteria*	Increased normalized mRNA abundance for ARGs	Yergeau et al., 2014
Oily sludge	*Bacillus*, *Lysinibacillus*, *Rhodococcus*, *Shewanella*, *Aeromonas*, *Pseudomonas* and *Klebsiella*	Ampicillin, kanamycin	Stancu et al., 2011
Hydrocarbon contaminated sediments of Red Sea lagoons	Mostly *Proteobacteria* and *Bacteroidetes*	68-73% of bacterial DNA sequences associated with antibiotic resistance enzymes	Al-Amoudi et al., 2016
Lagoon sediments chronically contaminated	*Gammaproteobacteria*, mostly *Pseudomonas* and *Acinetobacter*	Penicillin, amoxicillin, oxacillin, cefoxitin, streptomycin, tetracyclin, cotrimoxazole	Ben Said et al., 2008

with PAHs and other pollutants			
Hydrocarbon-impacted soil from industrial sites	*Pseudomonas aeruginosa*	Ticarcillin, clavulanic acid, imipenem	Youenou et al., 2014
Hydrocarbon-contaminated soil and groundwater at former military sites	*Pseudomonas aeruginosa*	Cephalosporins (cefotaxime, ceftazidime, ceftriaxone, cefepime), penicillins (piperacillin), imipenem, ofloxacin, gentamicin	Kaszab et al., 2010
Soil and groundwater near crude oil and crude condensate pipeline breaks	*Pseudomonas aeruginosa*	Penicillins (penicillin, ampicillin, piperacillin), cefems (cefotaxime, ceftriaxone, ceftazidime), aminoglycosides (gentamicin, tobramycin, kanamycin, streptomycin), imipenem, ofloxacintetracycline, trimethoprim, chloramphenicol	Kaszab et al., 2016
Crude oil flow station saver pit	*Pseudomonas aeruginosa*	Nitrofurantoin, cephalotine, cephtriaxone, ampicillin, trimetoprin-sulfametoxazol, cefotaxime, netilmicine, pefloxacine, gentamicine, carbeniciline, chloramphenicol, amikacine	Okoh, 2003
Oil contaminated soils	*Pseudomonas aeruginosa*	Chloramphenicol, streptomycin, erythromycin, ceftazidime, ciprofloxacin, ofloxacin, norfloxacin	Alonso et al., 1999
Hydrocarbon contaminated soils	*Pseudomonas aeruginosa*	Ticarcillin, ticarcillineclavulanic acid, imipenem, minocycline and trimethoprimesulfamethoxazole	Deredjian et al., 2011
Petroleum contaminated soils	*Pseudomonas* sp.	Tetracycline, ampicillin, streptomycin, and kanamycin	Dayana and Abraham, 2011
Oil-polluted soil	*Pseudomonas* sp.	Erythromycin and nalidixic acid	Pyrchenkova et al., 2006
Petroleum contaminated soil at motor service stations	*Pseudomonas* sp., *Enterobacter* sp.	Streptomycin, ampicillin	Batool et al., 2017
Technosol rich in	*Acinetobacter baumannii*	Carbapenems (meropenem, imipenem),	Henovic et al., 2017

		fluoroquinolones (ciprofloxacin, levofloxacin), aminoglycosides (amikacin), penicillins/β-lactamase inhibitors (ticarcillin/clavulanic acid, piperacillin/tazobactam), folate pathway inhibitors (trimethoprim/sulfamethoxazole)	
petroleum hydrocarbons and heavy metals			
Soil contaminated with herbicide DALAPON (2,2-dichloropropionate)	*Xanthomonas maltophilia*, *Comamonas acidovorans*, *Alcaligenes xylosoxidans*	Ampicillin, kanamycin, rifampicin, streptomycin, sulfonamide, tetracyclin	Schwarze et al., 1997
Plants grown in PAH−contaminated soils near an oil refinery	Endophytic *Enterobacter* sp.	Kanamycin, streptomycin, ampicillin, rifampicin and spectinomycin	Sheng et al., 2008
Alopecurus aequalis Sobol grown in PAH contaminated soil near a petrochemical plant	Endophytic *Massilia* sp.	Ampicillin and chloramphenicol	Liu et al., 2014
Plants grown in PAH−contaminated soil near a petrochemical plant	Endophytic *Acinetobacter* sp. and *Kocuria* sp.	Ampicillin, gentamicin, kanamycin, erythromycin, chloromycetin, spectinomycin	Sun et al., 2014
Petroleum contaminated soil in oil refinery wastewater irrigation zone	Plasmids captured directly from soil using *E. coli* strains as recipient and donor	One of four isolated plasmids associated with resistance to chloramphenicol, spectinomycin and tetracycline	Li et al., 2016

Table 2. Occurrence of ARGs in most important hydrocarbon-degrading *Actinobacteria* genera

Genus (number of species*)	ARGs (number of ARG containing species)	Number of ARGs
Actinomyces (51)	*acrA* (19), *ampC* (2), *aph* (26), *bacA* (15), *carB* (31), *emrB/gacA* (16), *folA* (11), *folP* (12), *lmrA* (1), *marR* (43), *mdtA* (1), *mdtH* (1), *msrA* (39), *pbp* (2), *penA* (2), *rarD* (31), *sul1* (4), *tetM* (1), *tetR/acrR* (41), *tetW* (1), *vanB* (1)	21
Arthrobacter (23)	*acrA* (4), *acrB* (13), *ampC* (2), *aph* (13), *carB* (19), *emrB/gacA* (6), *folA* (12), *folP* (16), *marR* (21), *mdtA* (1), *mdtG* (1), *msrA* (12), *oprD* (1), *pbp* (3), *rarD* (20), *sul1* (17), *tetD* (1), *tetR/acrR* (17), *tolC* (1), *vgb* (1)	20
Corynebacterium (116)	*aadA1* (2), *aadA2* (1), *aadA9* (1), *acrA* (7), *acrB* (5), *ampC* (14), *aph* (55), *aphA1* (2), *carB* (91), *catA1* (1), *cfiA* (1), *cmr* (3), *cmx* (9), *cphA* (1), *dfrA* (1), *emrB/gacA* (33), *ermA* (4), *folA* (46), *folP* (78), *lmrA* (3), *marR* (100), *mdtG* (1), *mdtH* (14), *mdtL* (5), *mepA* (11), *msrA* (98), *pbp* (67), *pbp2a* (9), *penA* (3), *qacB* (1), *strA* (4), *strB* (4), *tetA* (7), *tetB* (8), *tetC* (3), *tetD* (1), *tetM* (3), *tetR/acrR* (93), *tetW* (1), *tnpA* (12), *vanA* (3), *vanB* (3)	45
Dietzia (13)	*ampC* (5), *aph* (5), *bacA* (1), *carB* (7), *folP* (4), *lmrA* (1), *marR* (8), *mdtL* (1), *msrA* (8), *rarD* (8), *sul1* (7), *tetD* (1), *tetR/acrR* (12)	13
Gordonia (30)	*ampC* (10), *aph* (7), *bacA* (2), *carB* (27), *emrB/gacA* (24), *folA* (24), *folP* (26), *fosB* (1), *marR* (29), *mdtH* (10), *mdtL* (2), *msrA* (28), *pbp* (24), *rarD* (11), *sul1* (26), *tetA* (2), *tetC* (1), *tetR/acrR* (30), *tnpA* (1), *vanA* (4), *vanB* (1)	21
Micrococcus (4)	*acrA* (1), *acrB* (3), *aph* (2), *carB* (4), *emrB/gacA* (2), *folA* (1), *folP* (3), *marR* (4), *mdtH* (1), *mecA* (1), *msrA* (2), *msrC* (1), *penA* (1), *rarD* (3), *sul1* (3), *tetA* (1), *tetD* (1), *tetR/acrR* (3), *tnpA* (1)	20
Micromonospora (52)	*acrA* (22), *acrB* (44), *ampC* (9), *aph* (48), *bacA* (6), *carB* (49), *emrB/gacA* (10), *folA* (35), *folP* (17), *marR* (52), *mdtH* (7), *msrA* (44), *rarD* (51), *sul1* (41), *tetM* (2), *tetR/acrR* (52), *tolC* (2), *tnpA* (1), *vanB* (51), *vgb* (30)	20
Mycobacterium (77)	*aac* (19), *acrA* (2), *acrB* (6), *ampC* (55), *aph* (48), *bacA* (10), *bla1* (2), *carB* (73), *ceoA* (1), *cfiA* (1), *cmr* (4), *cphA* (6), *emrB/gacA* (19), *ermB* (3), *emrD* (1), *fabK* (2), *floR* (48), *folA* (24), *folP* (39), *fosB* (6), *marR* (76), *mdtA* (2), *mdtE* (1), *mdtG* (5), *mdtH* (5), *mdtL* (2), *mepA* (1), *msrA* (15), *msrC* (1), *oprD* (3), *penA* (3), *rarD* (9), *sul1* (63), *sulA* (1), *tetA* (3), *tetC* (7), *tetD* (1), *tetM* (1), *tetR/acrR* (76), *tolC* (2), *vanB* (25)	42
Nocardia (77)	*acrB* (1), *ampC* (56), *aph* (70), *bacA* (1), *carB* (48), *cfiA* (1), *cphA* (1), *emrB/gacA* (30), *ermA* (1), *ermB* (1), *ermC* (1), *folA* (52), *folP* (43), *fosB* (1), *lmrA* (1), *marR* (78), *mdtG* (1), *mdtH* (36), *mdtL* (3), *mepA* (1), *msrA* (31), *msrC* (3), *penA* (4), *rarD* (66), *strB* (1), *sul1* (62), *sul2* (1), *tetA* (2), *tetC* (2), *tetD* (1), *tetL* (1), *tetM* (1), *tetO* (1), *tnpA* (4), *vanB* (2), *vanYB* (1), *vatD* (4), *vgb* (6)	39
Nocardioides	*acrA* (2), *acrB* (2), *ampC* (8), *carB* (19), *cmr* (1), *cphA* (2), *emrB/gacA* (10), *folA* (12), *folP* (8), *marR* (21), *mdtH* (1),	20

(29)	*msrA* (21), *pbp* (20), *rarD* (18), *sul1* (17), *tetA* (1), *tetC* (1), *tetR/acrR* (21), *vanB* (2), *vgb* (7)	
Pseudonocardia (11)	*aac* (2), *acrA* (1), *ampC* (3), *aph* (9), *bacA* (1), *carB* (8), *cphA* (1), *emrB/gacA* (2), *folA* (4), *folP* (6), *fosB* (1), *marR* (9), *mdtH* (4), *mdtL* (1), *msrA* (5), *msrC* (2), *pbp* (8), *rarD* (8), *sul1* (8), *tetA* (1), *tetC* (1), *tetD* (1), *tetR/acrR* (9), *tnpA* (2), *vanB* (5), *vgb* (2)	26
Rhodococcus (33)	*acrA* (1), *acrB* (1), *ampC* (4), *aph* (13), *bacA* (1), *carB* (17), *catA1* (5), *cmrA* (1), *emrB/gacA* (11), *emrD* (1), *folA* (16), *folP* (16), *fosB* (1), *fosB 2* (1), *lmrA* (1), *marR* (30), *mdtG* (2), *mdtH* (15), *mdtL* (4), *msrA* (23), *pbp5* (1), *penA* (3), *pmrA* (2), *rarD* (26), *sul1* (18), *tetA* (2), *tetC* (2), *tetD* (2), *tetR/acrR* (27), *vanA* (1), *vanYB* (1), *vatD* (2)	33
Streptomyces (335)	*aac* (20), *aacC* (3), *aacC4* (1), *aac(6')-II* (2), *acrA* (55), *acrB* (173), *ampC* (67), *aph* (277), *bacA* (9), *carB* (120), *cmr* (1), *cmx* (1), *cphA* (4), *emrB/gacA* (159), *fabK* (6), *floR* (1), *folA* (172), *folP* (190), *fosB* (18), *lmrA* (9), *marR* (314), *mdtA* (6), *mdtG* (7), *mdtH* (179), *mdtL* (11), *mecA* (236), *mepA* (3), *msrA* (200), *nisB* (1), *oprD* (6), *pbp* (314), *pbp5* (94), *penA* (7), *picA* (1), *pikR1* (1), *pikR2* (1), *pmrA* (15), *rarD* (262), *spcN* (3), *strA* (12), *strB* (3), *sul1* (253), *tetA* (3), *tetC* (2), *tetD* (8), *tetM* (67), *tetO* (7), *tetR/acrR* (317), *tnpA* (130), *tolC* (7), *vanA* (7), *vanB* (27), *vatD* (7), *vgb* (130), *yceL* (1)	56

*Number of species with genomes available in NCBI/GenBank. Most abundant ARGs found in >50% species are underlined.
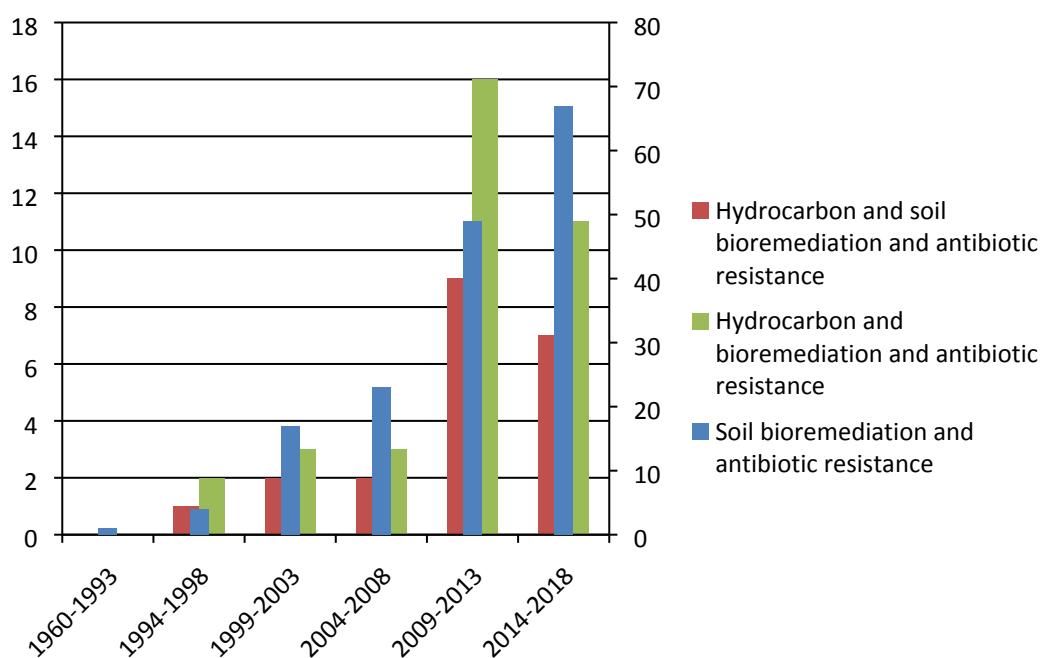
Figure 1. An increased number of research articles concerned to bioremediation and antibiotic resistance (according to http://www.scopus.com). Queries: Title/Abstract/Keywords. Non-relevant papers were removed from the query results.
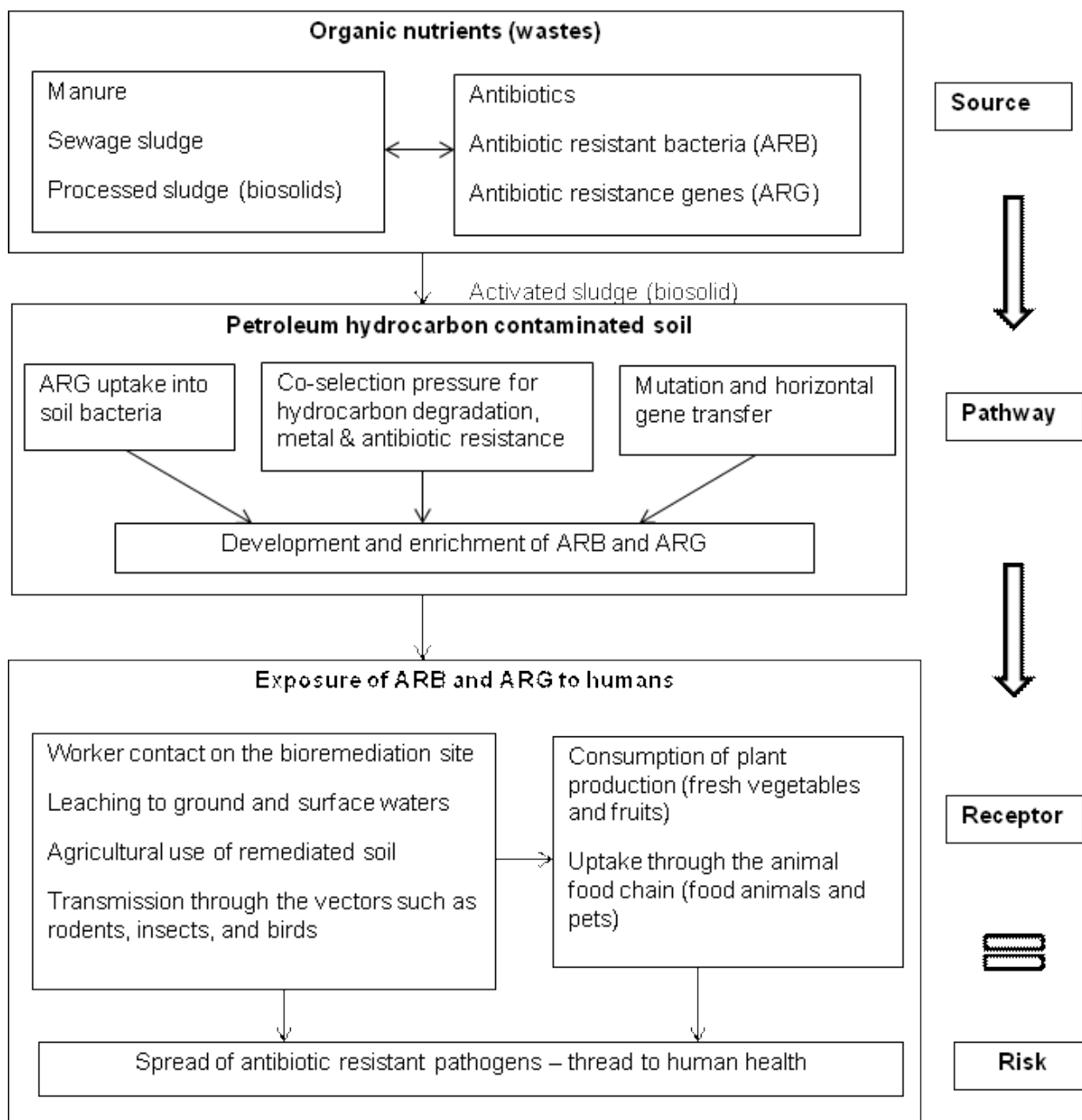
Figure. 2. Risk and pathway for ARB and ARGs during bioremediation of petroleum hydrocarbon-contaminated soil.

Supplementary Material

Potential risks of antibiotic resistant bacteria and genes in bioremediation of petroleum hydrocarbon contaminated soils

Colin J. Cunningham[a], Maria S. Kuyukina* [b, c], Irena B. Ivshina [b, c], Alexandr I. Konev[b], Tatyana A. Peshkur[d], Charles W. Knapp[d]

[a] Sustainability Fife Ltd, Glenrothes, UK

[b] Microbiology and Immunology Department, Perm State University, 15 Bukirev Street, 614990 Perm, Russia

[c] Institute of Ecology and Genetics of Microorganisms, Ural Branch of the Russian Academy of Sciences, 13 Golev Street, 614081 Perm, Russia

[d] Department of Civil and Environmental Engineering, University of Strathclyde, Glasgow, UK

*Corresponding author.

Professor Maria S. Kuyukina

Microbiology and Immunology Department

Perm State University, 15 Bukirev Street, 614990 Perm, Russia

Tel.: (+7)3422808114, Fax: (+7)3422809211

E-mail address: kuyukina@iegm.ru

Table S1. Abundance of ARGs in most important hydrocarbon-degrading *Actinobacteria* genera (totally 13 genera, 851 species)

ARGs	Target antibiotics	Resistance mechanism/gene family	*Actinobacteria* genera – number of ARG-carrying species	Total number of ARG-carrying species
marR	Multidrug	Antibiotic target alteration, antibiotic efflux/resistance-nodulation-cell division (RND) antibiotic efflux pump	*Actinomyces* – 43; *Arthrobacter* – 21; *Corynebacterium* – 100; *Dietzia* – 8; *Gordonia* – 29; *Micrococcus* – 4; *Micromonospora* – 52; *Mycobacterium* – 76; *Nocardioides* – 21; *Nocardia* – 78; *Pseudonocardia* – 9; *Rhodococcus* – 30; *Streptomyces* – 314	785
acrR (tetR)	Multidrug	Antibiotic target lteration, antibiotic efflux/resistance-nodulation-cell division (RND) antibiotic efflux pump	*Actinomyces* – 41; *Arthrobacter* – 17; *Corynebacterium* – 93; *Dietzia* – 12; *Gordonia* – 30; *Micrococcus* – 3; *Micromonospora* – 52; *Mycobacterium* – 76; *Nocardioides* – 21; *Pseudonocardia* – 9; *Rhodococcus* – 27; *Streptomyces* – 317	698
rarD	Chloramphenicol	Antibiotic efflux/predicted chloramphenicol resistance permease	*Actinomyces* – 31; *Arthrobacter* – 20; *Corynebacterium* – 74; *Dietzia* – 8; *Gordonia* – 11; *Micrococcus* – 3; *Micromonospora* – 51; *Mycobacterium* – 9; *Nocardioides* – 18; *Nocardia* – 66; *Pseudonocardia* – 8; *Rhodococcus* – 26; *Streptomyces* – 262	587
aph	Aminoglycosides	Antibiotic inactivation/ aminoglycoside *O*-phosphotransferase (APH)	*Actinomyces* – 26; *Arthrobacter* – 13; *Corynebacterium* – 55; *Dietzia* – 5; *Gordonia* – 7; *Micrococcus* – 2; *Micromonospora* – 48; *Mycobacterium* – 48; *Nocardia* – 70; *Pseudonocardia* – 9; *Rhodococcus* – 13; *Streptomyces* – 277	573
sul1	Sulfonamides and sulfones	Antibiotic target replacement/ sulfonamide resistant dihydropteroate synthase	*Actinomyces* – 4; *Arthrobacter* – 17; *Corynebacterium* – 54; *Dietzia* – 7; *Gordonia* – 26; *Micrococcus* – 3; *Micromonospora* – 41; *Mycobacterium* – 63; *Nocardioides* – 17; *Nocardia* – 62; *Pseudonocardia* – 8; *Rhodococcus* – 18; *Streptomyces* – 253	573
msrA	MLSB	Antibiotic target protection/ABC-F ATP-binding cassette ribosomal protection protein	*Actinomyces* – 39; *Arthrobacter* – 12; *Corynebacterium* – 98; *Dietzia* – 8; *Gordonia* – 28; *Micrococcus* – 2; *Micromonospora* – 44; *Mycobacterium* – 15; *Nocardioides* – 21; *Nocardia* – 31; *Pseudonocardia* – 5; *Rhodococcus* – 23;	541

			Streptomyces – 200	
carB	MLSB	Antibiotic target alteration/23S ribosomal RNA methyltransferase	*Actinomyces* – 31; *Arthrobacter* – 19; *Corynebacterium* – 91; *Dietzia* – 7; *Gordonia* – 27; *Micrococcus* – 4; *Micromonospora* – 49; *Mycobacterium* – 73; *Nocardioides* – 19; *Nocardia* – 48; *Pseudonocardia* – 8; *Rhodococcus* – 17; *Streptomyces* – 120	513
folP	Sulfonamides	Antibiotic target alteration/antibiotic resistant dihydropteroate synthase	*Actinomyces* – 12; *Arthrobacter* – 16; *Corynebacterium* – 78; *Dietzia* – 4; *Gordonia* – 26; *Micrococcus* – 3; *Micromonospora* – 17; *Mycobacterium* – 39; *Nocardioides* – 8; *Nocardia* – 43; *Pseudonocardia* – 6; *Rhodococcus* – 16; *Streptomyces* – 190	458
pbp	β-Lactam antibiotics	Antibiotic target alteration/penicillin-binding protein	*Actinomyces* – 2; *Arthrobacter* – 3; *Corynebacterium* – 67; *Gordonia* – 24; *Nocardioides* – 20; *Pseudonocardia* – 8; *Streptomyces* – 314	438
folA	Trimethoprim	Antibiotic target alteration/ antibiotic resistant dihydrofolate reductase	*Actinomyces* – 11; *Arthrobacter* – 12; *Corynebacterium* – 46; *Gordonia* – 24; *Micrococcus* – 1; *Micromonospora* – 35; *Mycobacterium* – 24; *Nocardioides* – 12; *Nocardia* – 52; *Pseudonocardia* – 4; *Rhodococcus* – 16; *Streptomyces* – 172	409
emrB/qacA	Multidrug	Antibiotic efflux/ major facilitator superfamily (MFS) antibiotic efflux pump	*Actinomyces* – 16; *Arthrobacter* – 6; *Corynebacterium* – 33; *Gordonia* – 24; *Micrococcus* – 2; *Micromonospora* – 10; *Mycobacterium* – 19; *Nocardioides* – 10; *Nocardia* – 30; *Pseudonocardia* – 2; *Rhodococcus* – 11; *Streptomyces* – 159	322
mdtH	Multidrug	Antibiotic efflux/ major facilitator superfamily (MFS) antibiotic efflux pump	*Actinomyces* – 1; *Corynebacterium* – 14; *Gordonia* – 10; *Micrococcus* – 1; *Micromonospora* – 7; *Mycobacterium* – 5; *Nocardioides* – 1; *Nocardia* – 36; *Pseudonocardia* – 4; *Rhodococcus* – 15; *Streptomyces* – 179	273
acrB	Multidrug	Antibiotic efflux/resistance-nodulation-cell division (RND) antibiotic efflux pump	*Arthrobacter* – 13; *Corynebacterium* – 5; *Micrococcus* – 3; *Micromonospora* – 44; *Mycobacterium* – 6; *Nocardioides* – 2; *Nocardia* – 1; *Rhodococcus* – 1; *Streptomyces* – 173	248
mefA	MLSB	Antibiotic efflux/ major facilitator	*Micrococcus* – 1; *Nocardia* – 2; *Streptomyces* – 236	239

		superfamily (MFS) antibiotic efflux pump		
mecA	Penams (penicillins)	Antibiotic target replacement/ methicillin resistant penicillin-binding protein	*Micrococcus* – 1; *Streptomyces* – 236	237
ampC	Cephalosporins, p enams (penicillins)	Antibiotic inactivation/beta-lactamase	*Actinomyces* – 2; *Arthrobacter* – 2; *Corynebacterium* – 14; *Dietzia* – 5; *Gordonia* – 10; *Micromonospora* – 9; *Mycobacterium* – 55; *Nocardioides* – 8; *Nocardia* – 56; *Pseudonocardia* – 3; *Rhodococcus* – 4; *Streptomyces* – 67	235
vgb	Streptogramins	Antibiotic inactivation/ virginiamycin B lyase	*Arthrobacter* – 1; *Micromonospora* – 30; *Nocardioides* – 7; *Nocardia* – 6; *Pseudonocardia* – 2; *Streptomyces* – 130	176
vanB	Glycopeptide antibiotics	Antibiotic target alteration/vancomycin resistant ligase	*Actinomyces* – 2; *Corynebacterium* – 3; *Gordonia* – 1; *Micromonospora* – 51; *Mycobacterium* – 25; *Nocardioides* – 2; *Nocardia* – 2; *Pseudonocardia* – 5; *Streptomyces* – 27	118
acrA	Multidrug	Antibiotic efflux/resistance-nodulation-cell division (RND) antibiotic efflux pump	*Actinomyces* – 19; *Arthrobacter* – 4; *Corynebacterium* – 7; *Micrococcus* – 1; *Micromonospora* – 22; *Mycobacterium* – 2; *Nocardioides* – 2; *Pseudonocardia* – 1; *Rhodococcus* – 1; *Streptomyces* – 55	114
pbp5	β-Lactam antibiotics	Antibiotic target alteration/ penicillin-binding protein	*Rhodococcus* – 1; *Streptomyces* – 94	95
tetM	Tetracyclines	Antibiotic target protection/ ribosomal protection protein	*Actinomyces* – 1; *Corynebacterium* – 3; *Micromonospora* – 2; *Mycobacterium* – 1; *Nocardia* – 1; *Streptomyces* – 67	75
floR	Phenicols	Antibiotic efflux/ major facilitator superfamily (MFS) antibiotic efflux pump	*Mycobacterium* – 48; *Streptomyces* – 1	49
tnpA	Multidrug	ISNCY family-transposase	*Corynebacterium* – 12; *Gordonia* – 1; *Micrococcus* – 1; *Micromonospora* – 1; *Nocardia* – 4; *Rhodococcus* – 9; *Streptomyces* – 21	49
bacA	Peptide antibiotics	Antibiotic target alteration/undecaprenyl	*Actinomyces* – 15; *Dietzia* – 1; *Gordonia* – 2; *Micromonospora* – 6; *Mycobacterium* – 10; *Nocardia* – 1;	46

		pyrophosphate phosphatase	*Pseudonocardia* – 1; *Rhodococcus* – 1; *Streptomyces* – 9	
aac	Aminoglycosides	Antibiotic inactivation/ aminoglycoside acetyltransferase	*Mycobacterium* – 19; *Pseudonocardia* – 2; *Streptomyces* – 20	41
mdtL	Multidrug	Antibiotic efflux/ major facilitator superfamily (MFS) antibiotic efflux pump	*Corynebacterium* – 5; *Dietzia* – 1; *Gordonia* – 2; *Mycobacterium* – 2; *Nocardia* – 3; *Pseudonocardia* – 1; *Rhodococcus* – 4; *Streptomyces* – 11	29
fosB	Fosfomycin	Antibiotic inactivation/thiol transferase	*Gordonia* – 1; *Mycobacterium* – 6; *Nocardia* – 1; *Pseudonocardia* – 1; *Rhodococcus* – 1; *Streptomyces* – 18	28
penA	β-Lactam antibiotics	Antibiotic target alteration/ penicillin-binding protein	*Actinomyces* – 2; *Corynebacterium* – 3; *Micrococcus* – 1; *Mycobacterium* – 3; *Nocardia* – 4; *Rhodococcus* – 3; *Streptomyces* – 7	23
tetA	Tetracyclines	Antibiotic efflux/ major facilitator superfamily (MFS) antibiotic efflux pump	*Corynebacterium* – 7; *Gordonia* – 2; *Micrococcus* – 1; *Mycobacterium* – 3; *Nocardioides* – 1; *Nocardia* – 2; *Pseudonocardia* – 1; *Rhodococcus* – 2; *Streptomyces* – 3	22
tetC	Tetracyclines	Antibiotic efflux/ major facilitator superfamily (MFS) antibiotic efflux pump	*Corynebacterium* – 3; *Gordonia* – 1; *Mycobacterium* – 7; *Nocardioides* – 1; *Nocardia* – 2; *Pseudonocardia* – 1; *Rhodococcus* – 2; *Streptomyces* – 2	19
mdtG	Fosfomycin	Antibiotic efflux/ major facilitator superfamily (MFS) antibiotic efflux pump	*Arthrobacter* – 1; *Corynebacterium* – 1; *Mycobacterium* – 5; *Nocardia* – 1; *Rhodococcus* – 2; *Streptomyces* – 7	17
pmrA	Fluoroquinolones	Antibiotic efflux/ major facilitator superfamily (MFS) antibiotic efflux pump	*Rhodococcus* – 2; *Streptomyces* – 15	17
tetD	Tetracyclines	Antibiotic efflux/ major facilitator superfamily (MFS) antibiotic efflux pump	*Arthrobacter* – 1; *Corynebacterium* – 1; *Dietzia* – 1; *Micrococcus* – 1; *Mycobacterium* – 1; *Nocardia* – 1; *Pseudonocardia* – 1; *Rhodococcus* – 2; *Streptomyces* – 8	17
lmrA	MLSB	Antibiotic target alteration, antibiotic efflux/ATP-binding cassette (ABC) antibiotic efflux pump	*Actinomyces* – 1; *Corynebacterium* – 3; *Dietzia* – 1; *Nocardia* – 1; *Rhodococcus* – 1; *Streptomyces* – 9	16
mepA	Multidrug	Antibiotic efflux/multidrug and toxic compound extrusion (MATE) transporter	*Corynebacterium* – 11; *Mycobacterium* – 1; *Nocardia* – 1; *Streptomyces* – 3	16

strA	Aminoglycosides	Antibiotic inactivation/ aminoglycoside phosphotransferase	*Corynebacterium* – 4; *Streptomyces* – 12	16
cphA	Carbapenems	Antibiotic inactivation/beta-lactamase	*Corynebacterium* – 1; *Mycobacterium* – 6; *Nocardioides* – 2; *Nocardia* – 1; *Pseudonocardia* – 1; *Streptomyces* – 4	15
vanA	Glycopeptide antibiotics	Antibiotic target alteration/ vancomycin resistant ligase	*Corynebacterium* – 3; *Gordonia* – 4; *Rhodococcus* – 1; *Streptomyces* – 7	15
vatD	Streptogramins	Antibiotic inactivation/ streptogramin vat acetyltransferase	*Nocardia* – 4; *Rhodococcus* – 2; *Streptomyces* – 7	13
tolC	Multidrug	Antibiotic efflux/many multidrug efflux pumps	*Arthrobacter* – 1; *Micromonospora* – 2; *Mycobacterium* – 2; *Streptomyces* – 7	12
cmx	Phenicols	Antibiotic efflux/major facilitator superfamily (MFS) antibiotic efflux pump	*Corynebacterium* – 9; *Streptomyces* – 1	10
mdtA	Multidrug	Antibiotic efflux/resistance-nodulation-cell division (RND) antibiotic efflux pump	*Actinomyces* – 1; *Arthrobacter* – 1; *Mycobacterium* – 2; *Streptomyces* – 6	10
oprD	Multidrug	Reduced permeability to antibiotic/outer membrane porin	*Arthrobacter* – 1; *Mycobacterium* – 3; *Streptomyces* – 6	10
cmr	Phenicols	Antibiotic efflux/major facilitator superfamily (MFS) antibiotic efflux pump	*Corynebacterium* – 3; *Mycobacterium* – 4; *Nocardioides* – 1; *Streptomyces* – 1	9
pbp2a	Penams (penicillins)	Antibiotic target replacement/methicillin resistant penicillin-binding protein	*Corynebacterium* – 9	9
fabK	Triclosan	Antibiotic target alteration/3-oxoacyl-acyl carrier protein reductase	*Mycobacterium* – 2; *Streptomyces* – 6	8
strB	Aminoglycosides	Antibiotic inactivation/ aminoglycoside phosphotransferase	*Corynebacterium* – 4; *Nocardia* – 1; *Streptomyces* – 3	8
tetB	Tetracyclines	Antibiotic efflux/ major facilitator superfamily (MFS) antibiotic efflux pump	*Corynebacterium* – 8	8
tetO	Tetracyclines	Antibiotic target protection/	*Nocardia* – 1; *Streptomyces* – 7	8

		ribosomal protection protein		
msrC	MLSB	Antibiotic target protection/ABC-F ATP-binding cassette ribosomal protection protein	*Micrococcus* – 1; *Mycobacterium* – 1; *Nocardia* – 3; *Rhodococcus* – 2	7
catA1	Chloramphenicol	Antibiotic inactivation/ chloramphenicol acetyltransferase	*Corynebacterium* – 1; *Rhodococcus* – 5	6
ermA	MLSB	Antibiotic target alteration/23S ribosomal RNA methyltransferase	*Corynebacterium* – 4; *Nocardia* – 1	5
ermB	MLSB	Antibiotic target alteration/23S ribosomal RNA methyltransferase	*Mycobacterium* – 3; *Nocardia* – 1	4
aacC	Aminoglycosides	Antibiotic inactivation/ aminoglycoside acetyltransferase	*Streptomyces* – 3	3
cfiA	Carbapenems	Antibiotic inactivation/beta-lactamase	*Corynebacterium* – 1; *Mycobacterium* – 1; *Nocardia* – 1	3
spcN	Aminoglycosides	Antibiotic inactivation/ aminoglycoside phosphotransferase	*Streptomyces* – 3	3
aphA1	Aminoglycosides	Antibiotic inactivation/ aminoglycoside phosphotransferase	*Corynebacterium* – 2	2
aac(6')-II	Aminoglycosides	Antibiotic inactivation/ aminoglycoside acetyltransferase	*Streptomyces* – 2	2
aadA1	Aminoglycosides	Antibiotic inactivation/ aminoglycoside nucleotidyltransferase	*Corynebacterium* – 2	2
bla1	Penams (penicillins)	Antibiotic inactivation/beta-lactamase	*Mycobacterium* – 2	2
emrD	Multidrug	Antibiotic efflux/ major facilitator superfamily (MFS) antibiotic efflux pump	*Mycobacterium* – 1; *Rhodococcus* – 1	2
qacB	Fluoroquinolones	Antibiotic efflux/ major facilitator superfamily (MFS) antibiotic efflux pump	*Corynebacterium* – 1; *Streptomyces* – 1	2
tetW	Tetracyclines	Antibiotic target protection/ ribosomal protection protein	*Actinomyces* – 1; *Corynebacterium* – 1	2

vanYB	Glycopeptide antibiotics	Antibiotic target alteration/vancomycin resistant ligase	*Nocardia* – 1; *Rhodococcus* – 1	2
aacC4	Aminoglycosides	Antibiotic inactivation/ aminoglycoside acetyltransferase	*Streptomyces* – 1	1
aadA2	Aminoglycosides	Antibiotic inactivation/ aminoglycoside nucleotidyltransferase	*Corynebacterium* – 1	1
aadA9	Aminoglycosides	Antibiotic inactivation/ aminoglycoside nucleotidyltransferase	*Corynebacterium* – 1	1
ceoA	Multidrug	Antibiotic efflux/resistance-nodulation-cell division (RND) antibiotic efflux pump	*Mycobacterium* – 1	1
cmrA	Phenicols	Antibiotic efflux/ major facilitator superfamily (MFS) antibiotic efflux pump	*Rhodococcus* – 1	1
dfrA1	Diaminopyrimidine antibiotic	Antibiotic target replacement/ trimethoprim resistant dihydrofolate reductase	*Corynebacterium* – 1	1
ermC	MLSB	Antibiotic target alteration/23S ribosomal RNA methyltransferase	*Nocardia* – 1	1
fosB2	Fosfomycin	Antibiotic inactivation/thiol transferase	*Rhodococcus* – 1	1
mdtE	Multidrug	Antibiotic efflux/resistance-nodulation-cell division (RND) antibiotic efflux pump	*Mycobacterium* – 1	1
nisB	Nisin	Antibiotic efflux/nisin dehydratase	*Streptomyces* – 1	1
picA	Macrolides	Antibiotic efflux/unknown	*Streptomyces* – 1	1
pikR1	MLSB	Antibiotic target alteration/23S ribosomal RNA methyltransferase	*Streptomyces* – 1	1

pikR2	MLSB	Antibiotic target alteration/23S ribosomal RNA methyltransferase	*Streptomyces* – 1	1
qacH	Fluoroquinolones	Antibiotic efflux/small multidrug resistance (SMR) antibiotic efflux pump	*Corynebacterium* – 1	1
sulA	Sulfonamides	Antibiotic target replacement/ sulfonamide resistant dihydropteroate synthase	*Mycobacterium* – 1	1
sul2	Sulfonamides	Antibiotic target replacement/ sulfonamide resistant dihydropteroate synthase	*Nocardia* – 1	1
tetL	Tetracyclines	Antibiotic efflux/ major facilitator superfamily (MFS) antibiotic efflux pump	*Nocardia* – 1	1
yceL	Fosfomycin	Antibiotic efflux/ major facilitator superfamily (MFS) antibiotic efflux pump	*Streptomyces* – 1	1

MLSB - Macrolide-Lincosamide-Streptogramin B.