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Discovery and development of new antibacterial drugs

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1 **Discovery and development of new antibacterial drugs:**

2 **learning from experience?**

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15 Running title: Discovery of new antibiotics

16 **Synopsis (241 words)**

17 Antibiotic (antibacterial) resistance is a serious global problem and the need for new

18 treatments is urgent. The current antibiotic discovery model is not developing new agents at

19 a rate that is sufficient to combat present levels of antibiotic resistance. This has led to fears

20 of the arrival of a 'post antibiotic era'. Scientific difficulties, an unfavourable regulatory

21 climate, multiple company mergers and the low financial returns associated with antibiotic

22 drug development led to the withdrawal of many pharmaceutical companies from the field.

23 The regulatory climate has now begun to improve, but major scientific hurdles still impede

24 the discovery and development of novel antibacterial agents. To facilitate discovery activities

25 there must be increased understanding of the scientific problems experienced by

26 pharmaceutical companies. This must be coupled with addressing the current antibiotic

27 resistance crisis so that compounds and ultimately drugs are delivered to treat the most

28 urgent clinical challenges. By understanding the causes of the failures and successes of the

29 pharmaceutical industry's research history, duplication of discovery programmes will be

30 reduced so increasing the productivity of the antibiotic drug discovery pipeline by academia

31 and small companies. The most important scientific issues to address are getting molecules

32 into the Gram-negative bacterial cell and avoiding their efflux. Hence screening programmes

33 should focus their efforts on whole bacterial cells rather than cell-free systems. Despite

34 falling out of favour with pharmaceutical companies, natural product research still holds

35 promise for providing new molecules as a basis for discovery.

Introduction

Antibiotic resistance is a serious global problem and the need for new treatments is urgent. Antibacterial drugs have revolutionised our ability to control bacterial disease, and their clinical availability has led to dramatic decreases in morbidity and mortality.[1] As such, these therapeutics underpin modern medicine. Despite the integral role of antibiotics in the maintenance of our modern lifestyle, they are undervalued in both cost and significance by society. Over the past century, their use has provided a strong selective pressure on micro-organisms, leading to preferential survival and spread of those harbouring antibiotic resistance mechanisms. Multi-drug resistance (MDR) is now commonplace amongst bacterial pathogens and antibiotic resistance now affects all antibiotic classes.[2] This is particularly worrisome in the case of Gram-negative bacteria, (e.g. *Pseudomonas aerugino*sa and *Acinetobacter baumannii*) for which treatment options are already limited.[3] The "broken" economics of antibacterial research and development (R&D) is often quoted as the main reason for a lack of new therapies but the truth is it is hard to discover new antibacterial drugs, and the science is not sufficiently advanced to enable efficient and effective antibacterial drug discovery. This has led to fears of a 'post antibiotic era'. It has been proposed that between 5 and 20 novel antibacterial drugs need to enter the clinical development pipeline in order to effectively contend with the current resistance problem. However, given the attrition rate within the existing drug discovery model, a minimum of 200 discovery programmes would optimistically be needed in order to achieve this outcome. Hence, new approaches to antibiotic discovery are needed.

The antibiotic pipeline is not what it once was.[4] Pharmaceutical companies that were once the main provider of novel antibiotic molecules withdrew from the late 1990s to the present day because of their lack of success and low financial returns in delivering new antibacterial drugs to the market.[5] The environment of discovering and developing new antibiotics was different during the so called 'golden era' of drug discovery. Antibiotics worked remarkably well because resistance was low and physicians had access to a variety of efficacious antibiotics. The objectives of antibiotic R&D programmes tended to be around improved

64 pharmacology to achieve less frequent dosing e.g. once a day, rather than innovative new

65 antibiotics. Natural product screening strategies tended to result in rediscovery of rather than

66 new compounds. There was also no need to take on the speculative improvement of natural

67 products with undesirable properties, such as toxicity. Today, only a few large companies,

68 including GlaxoSmithKline, Novartis, Merck and Roche actively research and develop

69 antibiotics, with many of the historically major antibiotic providers (Bristol-Myers Squibb,

70 Bayer and Eli Lilly), having left the area.

71 Industry has discovered few new antibiotics, and increasingly this activity is performed by

72 academia and the private sector in the form of small companies (small medium enterprises;

73 SMEs) (Table 1).[6,7] Furthermore, programmes that have advanced to late stage clinical

74 evaluation or have had marketing approval have emerged from projects that had originated

75 in large companies and subsequently licensed to SMEs (e.g. ceftazidine-avibactam).

76 Successful strategies include semi-synthetic natural products such as dalbavancin, novel

77 natural product based chemistry e.g. omadacycline, eravacycline, and plazomycin, novel

78 lactamase inhibitor chemistry, e.g. vaborbactam and fast-following approaches e.g. tedizolid

79 and cadazolid. What is clear is that innovative chemistry is a key contributor to success.

80 During the last two decades, antibacterial R&D has suffered from changing clinical and

81 investor priorities as the focus moved from MRSA to *C. difficile* and most recently to Gram-

82 negative bacteria. The changing regulatory advice also created uncertainty and additional

83 financial risks. The recent regulatory focus for antibiotics and a collective will to create

84 innovative regulatory pathways for antibacterial drugs should create an environment that will

85 stimulate discovery, research and development. The community now needs to address other

86 barriers to success.

87 SMEs and academia will continue to lead future antibiotic drug discovery efforts[6] but they

88 can only advance new therapies so far. The clinical development capabilities of

89 pharmaceutical companies and their supply chain are essential components in delivering

90 new therapies and patient benefits. The future delivery of new therapies will require effective

91 partnerships between all stakeholders. By learning from its past failures and successes,

92   pharmaceutical companies should work with academia, charities and SMEs to provide a

93   more effective antibiotic discovery model.

94   It has become clear that antibacterial innovation is needed now and in the long-term.

95   Discovering new antibiotics that are immune to resistance development is unlikely. Training

96   and infrastructure must be put in place to create the capabilities and capacities required to

97   deliver new antibacterial therapies regularly over decades and centuries. This generation

98   may be the last to benefit from cheap antibiotics. This is a critical time and stakeholders'

99   actions now will be judged by historians. We should endeavour to create a solid foundation

100   for future generations to continually respond and innovate as they face their antimicrobial

101   resistance (AMR) challenges.

102

103   **Which antibacterials are needed?**

104   As antibacterial discovery shifts towards an academia/SME-driven discovery activity there is

105   a risk that research funding (called 'push') rather than the clinical need (called 'pull') will

106   define the active programmes. Research-led programmes without consideration of clinical

107   use, manufacturing, regulatory practices, feasibility of clinical study designs and

108   reimbursement, are inefficient and probably futile activities. Recently, the WHO published a

109   list of bacteria for which new antibiotics are urgently needed.[8] The next step is to provide

110   internationally agreed target product profiles (TPPs) that will define what the properties of

111   suitable antibacterial therapies are. Pharmaceutical companies have detailed descriptions of

112   what they consider ideal and acceptable characteristics for new antibacterials. These include

113   indication, patient identification, potency, efficacy, pharmacology, toxicology, safety and

114   dosage etc. These TPPs could be used by other researchers to ensure that their research is

115   aligned with the most urgent medical needs. TPPs could also be used by funders and

116   investors to select projects most likely to have clinical impact. If this is not done, there will

117   continue to be research on new antibiotics and their development that does not address the

118   most urgent needs.

119

Targets for monotherapy

The emergence and spread of antibiotic resistant bacteria is responsible for the dwindling number of effective antibacterials. If the success of a new drug is to be ensured, the potential to develop resistance and the consequences of resistance must be determined. Basic studies are needed to estimate the potential for developing resistance such as determining the MIC, resistance frequencies, concentrations for preventing mutation selection and exploring the consequences of resistance mechanisms. These should be researched in the early stages of drug discovery.[9] In the past, many had hoped that lack of the emergence of resistance in animal models of infection might indicate that resistance may not be an issue in the clinic, but this does not always prove to be the case (e.g. GSK2251052/AN3365).[10]

Target validation plays a central role in the development of a successful therapeutic. The traditional view of antibacterial target validation was that an essential protein or process is a good target. Target essentiality is now viewed as the beginning of the validation process, as opposed to the end. To develop novel drugs, there needs to be a focused effort to understand the biology of the target and impact of target inhibition. This will provide insights into how resistance could occur or how essentiality could be bypassed when that target is inhibited. For instance, before screening candidate inhibitors against a potential target, genetic studies to assess the mutability of a drug-binding pocket should be undertaken. Such studies would reveal how likely mutations that alter the drug target and confer resistance will occur. Studies should also be carried out to determine whether changes to the drug target affect the fitness of the bacterium and its ability to cause infection.

Considerable advances have been made over the last decade in identifying gene products that are important or essential to bacterial physiology and pathogenic attributes. As a result, there have been numerous suggestions in the literature that such factors could comprise novel targets for new antibiotics. However, there is a considerable gap between identifying an essential or important bacterial factor, and inhibitors that are able to form the basis for developing a new drug. This is because a drug discovery programme needs to identify

148 inhibitors that are amenable to medicinal chemistry which can provide the basis of a new

149 drug.

150 Academia can contribute towards the basic understanding of bacterial cellular processes,

151 pathogen biology and pathways that may influence resistance development. A better

152 understanding in this area could help to avoid some of the problems encountered in the past

153 regarding target validation and resistance. It is probable that both small compounds and

154 natural products that provide a good basis for antibacterial drug monotherapies have been

155 identified. Any new targets will require extensive validation before being developed further.

156 Good monotherapies comprise a single compound that targets multiple essential protein

157 activities and for which multiple mutations to the gene encoding the target, or the evolution of

158 target modifying enzymes, antibiotic degrading enzymes, efflux pumps, or all of these are

159 needed to develop clinically relevant resistance. Inhibiting the products of single genes,

160 whether they are essential or conditionally essential e.g. virulence or pathogenicity factors, is

161 unlikely to lead to effective treatment by a drug containing only one small compound or

162 natural product.

163

164 **Screening: Overcoming the Gram-negative permeability barrier**

165 The discovery of novel, broad and narrow spectrum inhibitors of Gram-negative bacteria has

166 proven difficult. The last broad-spectrum class of antibacterial agents to enter the clinic was

167 the quinolones, discovered in the 1960s.[11] This is due to their intrinsic resistance to many

168 different drugs. This is largely attributed to the architecture of the Gram-negative cell

169 envelope and multi-drug efflux pumps. The outer membrane and the efflux machinery work

170 together to reduce the intracellular concentration of many different types of antibiotic so that

171 the bacterium resists the action of a range of structurally diverse, antibacterial compounds.[12]

172 The differences in antibiotic activity between Gram-positive and Gram-negative bacteria is

173 rarely (e.g. daptomycin)[13] due to target differences between the two groups of organisms,

174 but instead is a result of the additional permeability and efflux barrier which Gram-negative

175 bacteria possess.[9,14]

176 There remains a fundamental lack of understanding regarding the physiology and
177 permeability properties of the Gram-negative cell envelope. Academia play a pivotal role
178 increasing knowledge in this area, driving new basic research on how to avoid efflux and
179 ensure the entry of drugs to the bacterial cytoplasm. The generation of 'rules of entry',
180 regarding the chemical properties that are required of compounds to accumulate within the
181 cytoplasm of Gram-negative bacteria and reach their respective intracellular targets will
182 greatly aid the development of novel broad-spectrum antibiotics. The recent findings of
183 Richter et al[15], will help generate these rules. There has been some progress improving
184 activity of the oxazolidinone class of drugs against *Escherichia coli* and identifying the
185 structural properties required to penetrate cells.[16] Furthermore, a complete understanding of
186 the orientation and binding of lipopolysaccharide molecules (LPS) on the outer monolayer of
187 the Gram-negative outer membrane could facilitate the development of cationic molecules to
188 disrupt it. To successfully develop a new antibiotic to treat infections by Gram-negative
189 bacteria, the ability of the drug and whether it is susceptible to efflux mechanisms must be
190 tracked throughout the drug optimisation process. This can be achieved by including whole-
191 cell screening assays comparing activities in wild type and in efflux mutants. However, care
192 over the choice of efflux mutants is essential; point mutations inactivating the transporter
193 process whilst maintaining the presence of the protein should be used rather than deletion
194 mutants.[17] Recent clinical isolates should be included during optimization programmes to
195 ensure compounds are effective against those bacteria giving current clinical problems.
196 The importance of overcoming the barriers to antibiotic entry in Gram-negative pathogens
197 has also been highlighted in the, 'Scientific Roadmap for Antibiotic Discovery', from the Pew
198 Charitable Trust.[18] The primary objectives outlined for antibiotic drug development include
199 overcoming the permeability barrier of particularly impermeable, Gram-negative bacteria and
200 subsequently tailoring chemical matter for this discovery process.

201

202 **Sources of antibacterial compounds**

203 Natural products dominate the existing antibacterial compendium, with around 75% of
204 available antibiotics being of natural origin.[19] The importance of the natural world as a source
205 of antibacterial drugs is also evident from the history of the antibiotic pipeline, which has
206 continued to be re-stocked with semi-synthetic derivatives of established, natural product
207 classes. However, despite previous successes, the labour intensive, low-throughput nature
208 of natural product drug discovery and diminishing returns eventually caused the
209 pharmaceutical industry to stop active research in this area. During the late 1990s, the focus
210 of attention shifted to synthetic compound libraries, which were utilised in high-throughput
211 screens to search for novel, target specific inhibitors *in vitro*.[9] This method of drug discovery
212 did not prove fruitful as it did not discover novel antibacterial compounds amenable to drug
213 discovery.[5] The failures of the genomic era to deliver novel drug targets and scaffolds,
214 coupled with the threat of a 'post-antibiotic era' have prompted a revival of natural product
215 drug discovery in both academia and the biotechnology sector. As pharmaceutical
216 companies are less active in this area, they cannot offer a sustainable contribution to natural
217 product discovery on their own. It is likely that many readily accessible sources of potent,
218 broad-spectrum antibacterial compounds have already been exhausted by past discovery
219 efforts by pharmaceutical companies. Therefore, natural product sources should be
220 investigated as a source for potential, untapped leads, especially when combined with novel
221 assays.
222 Slow-growing, uncultivable environmental organisms may represent a large potential
223 untapped resource of novel antibiotics, and recent innovations could allow natural product
224 discovery to be carried out in a sustainable manner. For instance, the development of the *in*
225 *situ* culture device, the iChip, has allowed the high throughput cultivation of environmental
226 microorganisms.[20] The merit of this device can be seen from the discovery of teixobactin, a
227 compound of a novel antibiotic class which possesses activity against Gram-positive
228 bacteria but hits a well characterized target – the bacterial cell wall biosynthesis machinery.[21]
229 Alternatively, cryptic biosynthetic pathways could be activated (which lead to the production
230 of novel secondary metabolites with antibiotic activity).[22] Metagenomics (analysis of

231 genomes from DNA from microorganisms in environmental samples) could be used to

232 investigate the secondary metabolite diversity of non-cultivatable environmental organisms.

233 Lastly, a key process in natural product drug discovery is the inclusion of de-replication

234 techniques such as high-resolution LC-MS/MS, which ensures the elimination of previously

235 characterised compounds from further study.

236 It is possible that all the antibacterial molecules amenable to drug discovery have been

237 identified and that the search for novelty may not pay off. In this case, substantial investment

238 into innovative chemistry on and around the known molecules would be prudent to

239 determine the advances that can be made. This less speculative, directed chemistry is

240 surprisingly difficult to fund and yet is a successful strategy to overcome resistance and or

241 side effects.

242 It may be that all the good targets for single drug therapy have been identified. Therefore, to

243 find alternative chemical classes to inhibit these targets investment in innovative chemistry is

244 required.

245

246 **Efficacy**

247 Animal models of bacterial infection can be highly predictive of efficacy in clinical use.

248 Marketed antibiotics perform well in these models and researchers have come to expect

249 high levels of bacterial kill by candidate drugs. However, some compounds with modest

250 potency in *in vivo* studies may have been overlooked or de-prioritized in optimization

251 programmes. The community does not know what level of animal model efficacy is the

252 minimum necessary to deliver clinical benefit for a monotherapy. Until recently a three-log

253 reduction in bacterial burden was considered the necessary level of efficacy to continue

254 research and development in a pharmaceutical company. Many now consider a two-log

255 reduction adequate and indicative of potential clinical utility.[23] Is a one-log reduction or just

256 bacteriostasis sufficient? Research on this area is urgently required.

257

258 **Resistance**

259 The community urgently requires evidence-based guidelines from regulators on what levels

260 of *in vitro* evolution to give drug resistance are acceptable for antibiotics in development.

261 Current target product profiles for monotherapy products vary by orders of magnitude from

262 $<10^{-8}$ to $<10^{-12}$. The metric may depend on the consequences of resistance, what increase in

263 MIC of a drug resistant mutant provides, and whether the mutant is attenuated in infection

264 models. Understanding all aspects of resistance and transmission of drug-resistant bacteria

265 is essential if new drugs are to have longevity.[24]

266

267 A key metric of an antibiotic in considering it as a new monotherapy is the mutant prevention

268 concentration (MPC). This is the drug concentration at which no mutants survive. When a

269 culture of drug-susceptible susceptible bacteria is exposed to a new antibacterial compound,

270 pre-existing rare point mutations that confer resistance to the compound may be selected.[25]

271 The activity of the compound against these insusceptible mutants is likely to be less than for

272 wildtype bacteria and a multiple of the MIC of the compound may be required to kill or inhibit

273 a mutant's growth. To suppress resistance development in clinical use, bacteria must be

274 exposed to a concentration of the antibiotic which kills both the susceptible and first step

275 mutants of the species. Typically, bacteria require two or more mutations to become

276 insusceptible at the MPC and this happens rarely *in vitro*, but is not uncommon once a drug

277 has been licensed. One example of this is with the fluoroquinolone drugs (note, mutations

278 have been found in the same and different genes).[26]

279 If the MIC of a strain with a first-step mutation does not greatly increase, only a modest

280 increase in drug concentration is required to achieve the MPC. If there is a big increase in

281 the MIC of the first step mutation a much higher dose is required to achieve the MPC. To

282 stop resistance developing in clinical use, bacteria at the site of infection must be exposed to

283 free-drug concentrations above the MPC for a significant period of the dosing interval (e.g. 8

284 hours). In practice, this means that antibacterials have to be potent and well tolerated to

285 achieve these exposures. Too few antibacterial drug R&D programmes demonstrate

286 understanding of the pharmacology of managing resistance and fail to build this into their

287 programmes. When thoroughly analysed, many of the novel target – new compound

288 programmes fail to adequately address resistance because sufficient exposure to doses

289 above the MPC cannot be achieved.

290

291 **Combinations**

292 As monotherapies have proven so challenging to discover and develop, much focus has

293 turned towards antibacterial combinations and it here that academia has much to offer. This

294 approach is much like those adopted for the treatment of HIV or tuberculosis, where different

295 drugs with different modes of action are used as part of a combination treatment. When

296 used, current combinations of antibiotics, such as those used to treat patients with sepsis,

297 focus is on covering Gram-positive and Gram-negative bacteria as well as ensuring

298 adequate drug concentration at the probable site of infection.[27]

299 There is much literature on *ad hoc* combinations of antibiotics and their effects on laboratory

300 strains and clinical isolates; this has led to suggestions of novel combinations that could be

301 used to treat Gram-negative bacterial infections. However, definitive large-scale studies

302 have been lacking. This area would be enabled by widespread open access to well

303 characterized drug-resistant and multi-drug resistant isolates. Double, triple and quadruple

304 combinations that are able to inhibit challenging strains may be feasible and might be

305 unpredictable. As resources are the only barrier, exhausting combination opportunities now

306 from drugs already available for human use should be investigated. Unfortunately, such

307 studies are rare; the focus on resolving the crisis of AMR has focused on establishing

308 economic incentives to stimulate pharmaceutical companies to stay (or return) to this field.

309 Furthermore, companies have no incentive to support studies on combinations of old drugs

310 and has been generally unsupportive of this approach.

311 There are examples in the literature of antibiotics and non-antibacterial marketed drugs that

312 could be used to potentiate the activity of an antibiotic against insusceptible or drug-resistant

313 bacteria sometimes called 'resistance breakers'.[28] The marketed drug may alter permeability

314 through the bacterial cell membrane, interfere with efflux or act via alternative mechanisms.

315 While the titles of some publications look appealing it is unclear whether any clinically useful

316 new combinations have emerged. Not only does the activity of combinations of drugs for

317 multi-drug resistant clinical isolates need to be established, but the primary pharmacology of

318 the drug to be combined with an antibiotic may not be amenable to clinical use in a co-

319 delivered combination. For example, the dose may be much higher than approved dose.

320 Alternatively, the toxicity and safety at higher doses, plus the requirement for matched or

321 manageable pharmacology of the combination must be considered.

322 Instead of using marketed drugs, some are developing bespoke non-antibiotic and antibiotic

323 combinations that disrupt the bacterial cell membrane and increase antibiotic access (e.g.

324 Spero Therapeutics). Industry, SMEs and academics working on novel targets and

325 chemistries have created programmes that have failed as monotherapies; these may provide

326 options for the creation of novel combination products. While the development may be

327 challenging and risky, partnering the right projects could create useful new therapies. LpxC

328 is an essential enzyme required for LPS biosynthesis in Gram-negative bacteria.[29] As

329 inhibition of LpxC tends to increase susceptibility to other antibacterials, combination of

330 LpxC-inhibitors with antibiotics may be a fruitful line of discovery.

331

332 **Anti-virulence compounds**

333 During the genomic-led antibacterial discovery period the community thought it was limited

334 by the number of targets for antibiotics. As a result, inhibition of conditionally essential

335 single-gene virulence targets was proposed as a way to increase the number of targets

336 available. While there are claims that inhibition of virulence targets will circumvent resistance

337 development, drugs targeting virulence will be subject to evolutionary pressures and it is

338 probable that resistance will develop, particularly where small compounds are used. Anti-

339 virulence monoclonal antibodies, may be less susceptible to the evolution of resistance.

340 This is because of the much larger surface area through which they interact.

341

342 **Funding**

343 Despite spending considerable resources over the last two decades, the pharmaceutical

344 industry has largely failed to discover or deliver new antibacterial drugs. Future discovery

345 programmes will have to work smarter, use effective collaboration and be adequately

346 resourced for a sustained period to have any chance of delivering new antibacterials. Such

347 collaborations have started to emerge such as the Community for Open Antimicrobial Drug

348 Discovery,[30] where they have a screening facility and will take compounds and screen them.

349 What is lacking is a seamless flow from academic discovery to SME and large

350 pharmaceutical companies so that the requisite early discovery hit to lead optimization

351 research can be carried out. Historically, the area of antibiotic drug discovery was

352 considered the domain of large pharmaceutical companies and consequently, the existing

353 funding structure for academia and SME remains inadequate for the task. This is despite the

354 advent of CARB-X,[31] The Global Antibiotic Research and Development Partnership

355 (GARDP),[32] and initiatives by numerous national funding agencies. Addressing AMR

356 requires a sustained and concerted effort with all stakeholders working together to make the

357 case for unprecedented levels of funding and delivering new processes to use that funding

358 effectively.

359

360 **How do we prioritize?**

361 The last two decades have shown that chasing novelty in terms of targets or compound

362 scaffolds has been inefficient and that time establishing firm foundations of science upon

363 which to build future activities is required. We recommend that (1) investment is needed to

364 provide innovative chemistry on and around known clinically effective drug scaffolds; (2)

365 alternative ways to inhibit the function of clinically validated targets; (3) understand

366 resistance mechanisms and how they can be inhibited; (4) understand the utility of animal

367 models and the risks around reducing drug-efficacy hurdles; and (5) establish the levels of *in*

368 *vitro* resistance development that are unacceptable.

369 Currently, too many academic and SME programmes are research-push driven without

370 appreciation of the manufacturing, regulatory and clinical hurdles their approaches present.

371   A substantial and sustained programme of investment in training of the next generation of

372   AMR researchers to equip them to understand how to create feasible projects is required. To

373   our knowledge there are at least three new doctoral training programmes designed to fill this

374   gap.[33-35] More are needed across the world.

375   Society must not assume short term solutions can be found and there is no point in

376   prioritizing programmes that are unlikely to be feasible in the next 10 to 30 years. Investment

377   must be prioritized on the feasible projects and where possible additional funding used for

378   more speculative programmes.

379

380   **Conclusions and future perspectives**

381   There is still much to discover in regards to bacterial physiology that would benefit the field

382   of antibiotic R & D and so academia has an essential role to play. Academic research groups

383   can assist by undertaking a systems biology approach to the understanding of potential

384   targets, and increasing our understanding of the permeability barrier and multi-drug efflux in

385   Gram-negative bacteria. A new paradigm for preclinical research has been proposed.[36] It

386   should be helpful to those engaged in early drug discovery. However, early discovery

387   research should be in partnership with SMEs and large companies and not in isolation in

388   academia. Otherwise, there is the danger of spending considerable time and funding on

389   research that will never deliver a new drug.

390   The natural world remains the largest source of novel chemical drug scaffolds and natural

391   product drug discovery remains a viable option in the search for new antibiotic compounds.

392   Advances in bacterial culture techniques, molecular biology and metagenomics will continue

393   to improve the ease and cost effectiveness of natural product drug discovery, which have

394   been a major limiting factor in the past. Screening procedures must include whole-bacterial

395   cell assays, addressing the issue of bacterial permeability and efflux early in the discovery

396   process.[37] Additionally, the generation of training schemes by and with pharmaceutical

397   companies, in relation to all aspects of the pipeline and including natural product drug

398   discovery, are essential and will ensure that expertise is passed to future researchers.

399 Investment should also be made into the study of previously characterized lead compounds
400 that did not reach the clinic, so called 'old leads'. The reasons that led these compounds to
401 be dropped from further development vary, ranging from financial issues, dosing problems,
402 to trial design and toxicity issues. It may be that there is now sufficiently improved
403 technology and expertise to develop these as efficacious, safe antibacterials, and the study
404 of 'old leads' could provide an additional source of novel antimicrobials. A freely accessible
405 database of antibiotics that were not developed has been recently launched, Antibiotic DB;[38]
406 prevent replication of discovery efforts. Another database comprising 'old natural product
407 leads' would also help the community. However, care must be taken to review all previous
408 research on the compound(s) of interest to ensure that the failures of the past are not
409 repeated.
410

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Table. Source of discoveries, clinical developer and recently approved antibiotics (in alphabetical order and by development phase)

Antibiotic	Discovered by	Developed by and transfer between companies over time	Status
Approved since 2015			
Ceftazidime+avibactam (Avycaz)	Sanofi	Novexel; AstraZeneca-Forest/Actavis	Approved in USA and EU
Ceftobiprole (Zevtera)	Roche	Basilea	Not approved in USA. Approved in13 EU countries plus several others
Ceftolozane+tazobactam (Zerbaxa)	Astellas	Calixa, Cubist=Merck	Approved in USA and EU
Dalbavancin (Xydalba)	**Lepetit Research Center/Vicuron**	Pfizer, Durata, Actavis	Approved in USA and EU
Oritavancin (Orbactive)	Eli Lilly	Intermune, Targanta, The Medicine Company	Approved in USA and EU
Solithromycin, (Cemprex)	**Optimer**	Cempra	Approved in USA and EU

Tedizolid (Sivextro)	**Dong-A**	Trius, Bayer/Cubist=Merck	Approved in USA and EU

New Drug Application (NDA) submitted

Carbavance (vaborbactam+meropenem)	**Rempex**	**Rempex**, The Medicines Company	Phase 3
Delafloxacin	Wakunaga	Abbott, Wakunaga, Rib-X (Melinta Therapeutics)	Phase 3

In development

BC-(Lefamulin) 3781	Sandoz/Novartis	Nabriva, Forest/Actavis, Nabriva	Phase 3
Cadazolid	Actelelion	Actelion Pharmaceuticals	Phase 3
Iclaprim	Hoffman LaRoche, Arpida	MotifBio PLC	Phase 3
Imipenem/cilastatin + Relebactam (MK- 7655)	Merck & Co Inc	Merck & Co Inc	Phase 3
Omadacycline	**Paratek**	**Paratek**/Bayer, Paratek/Merck, Paratek Novartis, **Paratek**	Phase 3

Plazomicin	**Isis**	Achaogen		Phase 3
S-649266	Shionogi	Shionogi Inc		Phase 3
Solithera (Solithromycin)	Cempra Inc,			
Taksta (fusidic acid)	Leo Pharmaceuticals	Cempra		Phase 3
Eravacycline TP- 434	**Harvard University**	Tetraphase		Phase 3
Zabofloxacin	Dong Wha	Dong Wha Pharmaceuticals Co Ltd		Phase 3
Aztreonam + avibactam		Actavis, Allergon PLC, Astra-Zeneca, Pfizer		Phase 2
CG400549	Crystal Genomics Inc	Crystal Genomics Inc		Phase 2
Afabicin (Debio 1450)	Debiopharm International SA			
ETX0914	Astra-Zeneca	Entasis Therapeutics Inc		Phase 2
Finafloxacin	Centre for Natural Product Research Singapore-Institute of	Merlion Pharmaceuticals Pte Lted		Phase 2

	Molecular and Cell Biology			
Gepotidacin (GSK2140944)	GSK	GSK		Phase 2
MRX-1	MicuRx Pharmaceuticals Inc			Phase 2
Nemonoxacin	**TaiGen**	Procter & Gamble, Warner Chilcott,TaiGen		Phase 2
Brilacidin PMX-30063	**University of Pennsylvania**	Polymedix, Cellceutix Corporation		Phase 2
POL7080	**University of Zurich**	**Polyphor**, Roche, **Polyphor**		Phase 2
Ramoplanin	Merrell Dow Research Institute	**Nanoterapeutics Inc**		Phase 2
Ridinilazole (SMT19969)	**Summit Therapeutics Inc**			Phase 2
WCK 4873	**Wockhardt Ltd**			Phase 2

CRS3123	Crestone Inc.	Phase 1
ETX2514SUL	Entasis Therapeutics Inc.	Phase 1
GSK*3342830	GlaxoSmithKline PLC (Shionogi licensee)	Phase 1
KBP-7072	KBP BioSciences Pharmaceutical Technical Co. Ltd.	Phase 1
LCB0 1-0371	LegoChem Biosciences Inc	Phase 1
MGB-BP-3	MGB Biopharma Ltd	Phase 1
OP0595 (RG6080)	Meiji Seika Pharma Co. Ltd./Fedora Pharmaceuticals Inc (Roche licensee)	Phase 1

SPR741	**Spero Therapeutics**		
TD-1607	**Theravance Biopharma Inc.**		Phase 1
TP-271	**Tetraphase Pharmaceutials Inc.**		Phase 1
TP-6076	Tetraphase Pharmaceuticals Inc.		Phase 1
WK 771	Wockhardt Ltd		Phase 1
WK 2349	Wockhardt Ltd		Phase 1
Zidebactam + cefepime (WCK 5222)	Wockhardt Ltd		Phase 1

Drugs no longer under development

AFN-1252/Debio 1450	**University of Toronto**	Affinium, Debiopharm SA	

Radezolid (RX-1741)	**Yale University**	Rib-X (Melinta Therapeutics)
Ceftaroline + avibactam		Actavis Allergon PLC, Astra-Zeneca, Pfizer
BAL30072	**Basilea Pharmaceutica Ltd**	
JNJ-(Avarofloxacin) Q2	**J&J (Janssen Pharm.)**	Furiex, Forest/Actavis

Bold font indicated those agents discovered by academia and SMEs.

Adapted from from references.[7,39,40]