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Translational deficiencies in antibacterial discovery and new screening paradigms

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Abstract

An impending disaster is currently developing in the infectious disease community: the combination of rapidly emerging multidrug-resistance among clinically-relevant bacterial pathogens, together with an unprecedented withdrawal from industrial dedication to this disease area, is jeopardizing human health on a societal level. For those who remain focused and dedicated to identifying solutions to this growing problem, additional challenges await when *in vitro* activity does not correlate with *in vivo* efficacy. Thus the development of more effective translational assays will greatly improve and streamline the process of identifying novel antibacterial agents that can stand the test of preclinical and clinical development. Here we describe recent examples of research that justify the need for such assays.

The need for new antibiotics that avoid the action of clinically-relevant resistance mechanisms is continuing to grow. Sadly, it comes at a time when the gap between the spread of resistance and the pharmaceutical investment in developing novel antibacterial agents is widening at an alarming rate. The challenges of identifying novel chemotypes that offer safe and effective antibiotic activity, coupled with a limited return on investment relative to other therapeutic areas, has positioned us for a disastrous, pre-antibiotic era-type scenario. Further complicating matters, recent experiences that describe unexpected challenges during pre-clinical and clinical development have further dampened enthusiasm for novel antibacterial discovery, as unexpected failures are never rewarded and are rarely tolerated by large pharmaceutical organizations, particularly when operating under our current set of economic circumstances. Therefore avoiding such surprises should be considered vital to the continued interest and investment of large research groups. Here we describe multiple case studies that demonstrate the importance of having a fundamental understanding of the physiological context in which target bacterial pathogens exist during an infection. As these examples suggest, appreciating the *in vivo* environment by developing

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and utilizing relevant *in vitro* assays for screening and characterization will improve antibacterial drug discovery and development in two ways. First, through the reduction in the number of efficacy-related failures in preclinical and clinical studies, and second, by expanding our view of new antibiotic targets and pathways that could be exploited due to their importance *in vivo*, despite a lack of demonstrated importance *in vitro* when tested under traditional conditions.

Siderophore conjugation represents a recently revisited attempt by antibacterial researchers to outsmart target Gram-negative pathogens by tricking them into importing toxic compounds via outer membrane receptors used for normal iron acquisition processes. As iron is an essential micronutrient for bacterial survival, the rationale to target this pathway as a means to circumvent antibiotic resistance mediated by decreases in cell permeability via reduction in porin expression was well justified. Indeed, early [1,2] and more contemporary studies [3], [4], [5] demonstrating the *in vitro* activity of different siderophore conjugates provided convincing evidence of this strategy's potential, and multiple pharmaceutical companies made significant investment into this approach as a result. Unfortunately, these novel entities were screened in *in vitro* susceptibility assays using methods compliant with those established by the Clinical and Laboratory Standards Institute (CLSI; [6], [7]), as is the standard practice for new antibacterial drug discovery and development. Included in these traditional methods, however, is the use of nutrient replete media (such as cation-adjusted Mueller Hinton broth), which do not mimic the physiological environments encountered by pathogenic bacteria during an infection in a mammalian host. This is a particularly critical disconnect when investigating the utility of siderophore conjugates, which require active iron acquisition for uptake and functionality. Since the concentration of free iron in these media is substantially higher (i.e. ~5 μM as reported in [8]) than the levels reported *in vivo* (reported as 10^{-24} M in [9]), the significant reduction in antibacterial activity associated with increases in endogenous siderophore production was not accounted for, and the ability of native siderophores to shift the minimum inhibitory concentration (MIC) of lead siderophore conjugates was catastrophic to further development of these compounds [10].

Figure 1 depicts the translational deficiencies for MB-1, a siderophore-monobactam conjugate that was in pre-clinical development at Pfizer for the treatment of multidrug-resistant (MDR) Gram-negative pathogens. With encouraging *in vitro* activity against organisms like *Pseudomonas aeruginosa* ($\text{MIC}_{90}=1$ $\mu\text{g/ml}$), and a spontaneous resistance emergence frequency equivalent to those possessed by current, standard-of-care antibiotics (8.1×10^{-7} at 4X MIC), enthusiasm was high as this compound successfully passed pharmacokinetic (PK) and toxicity evaluations. It was after a comprehensive study for *in vivo* efficacy, however, that the liability of testing using iron-replete conditions *in vitro* became apparent. Using a panel of 9 contemporary *P. aeruginosa* clinical isolates, which varied in their antibiotic resistance profiles against marketed agents yet all appeared to be MB-1-susceptible in CLSI-compliant *in vitro* assays, a lack of widespread efficacy was demonstrated, despite the prediction of broad-spectrum activity across all 9 isolates (Figure 1). This issue with consistency prompted the evaluation of alternative *in vitro* assays, which strayed from the conventional testing guidelines, in an attempt to elucidate the cellular

mechanism(s) by which these isolates resisted MB-1 activity. It was ultimately determined that elevations in the production of pyoverdine, one of the endogenous siderophores used by *P. aeruginosa* to acquire iron, was contributing to the transient recalcitrance of *P. aeruginosa* strains, and a predictive *in vitro* assay was developed to predict the performance of MB-1 *in vivo* [10]. In hindsight, a more thorough appreciation for the contextual environment where MB-1 efficacy would be required, particularly as it pertains to free iron levels in this case, would have likely prompted the evaluation of native siderophore production on the activity of MB-1 prior to advancing to *in vivo* efficacy studies.

Another contemporary strategy for novel antibacterial drug discovery is the exploitation of the essentiality of lipid A biosynthesis for the production of lipopolysaccharide (LPS) by Gram-negative bacteria. LPS is involved in maintaining the cellular architecture of the outer membrane of these organisms, but also provides critical protection against host defenses such as complement deposition and phagocytosis. Basic research uncovered the genetic pathway responsible for the biosynthesis of lipid A, identifying the biochemical step mediated by LpxC, a UDP-3-*O*-(*R*-3-hydroxymyristoyl)-*N*-acetylglucosamine deacetylase, as the first committed step in the pathway. Small molecule LpxC inhibitors have since been identified and characterized [11-13], with several showing broad-spectrum antibacterial activity against multiple Gram-negative pathogens, including those that are multidrug-resistant. Interestingly, *Acinetobacter baumannii* strains, which display similar levels of resistance and susceptibility to comparator antibiotics as other Gram-negative bacteria tested, have shown complete recalcitrance to the activity of various LpxC inhibitors. It should be noted here, however, that these *in vitro* susceptibility assays were performed in accordance with the same CLSI guidelines described earlier. While inclusion of *A. baumannii* is important in a novel antibacterial agent's spectrum of activity, failure of LpxC inhibitors to affect this pathogen was overshadowed by their coverage of *P. aeruginosa* and multiple members of the *Enterobacteriaceae*.

An examination of the inhibitory activity of lead LpxC compounds on the isolated enzyme revealed significant differences in the IC₅₀ for the *A. baumannii* enzyme versus that for the *P. aeruginosa* ortholog [14], prompting researchers to believe that this was the primary reason for poor antibacterial activity. Others postulated that efflux-mediated resistance could be contributing to this phenotype, and susceptibility assays were performed in the presence of efflux pump inhibitors such as phenyl-Arg-β-naphthylamine (PAβN) to test this, but their results did not support this hypothesis (unpublished data). It was only after a report was published in 2010 [15], however, which provided the first evidence of the non-essential nature of lipid A biosynthesis in *A. baumannii*, that a new hypothesis emerged. In contrast to the standard dogma of Gram-negative cellular biology that LPS production was required for viability, Moffatt *et al* demonstrated that inactivating mutations in multiple genes within the lipid A pathway, including within *lpxC*, were tolerated by *A. baumannii* cells when grown under standard laboratory conditions. From this critical research, the concept of *A. baumannii* being resistant to LpxC inhibitors as a function of the non-essential nature of the target emerged.

Testing this newly formulated hypothesis required *in vitro* experimentation that did not rely on bacterial growth as the readout. Fortunately, the *Limulus* Amebocyte Lysate (LAL) assay,

which provides a quantitative evaluation of bacterial LPS production, proved successful at demonstrating a dose- and time-dependent reduction in the amount of LPS produced by *A. baumannii* when challenged with LpxC-1, a novel LpxC inhibitor that was in pre-clinical development at Pfizer in 2011 [16]. *A. baumannii* mutants harboring mutations in *lpxA* or *lpxC* were confirmed to be devoid of LPS using the LAL assay. More importantly, these mutants were completely avirulent in relevant mouse models of infection (unpublished data), suggesting that despite their ability to survive *in vitro* under standard laboratory conditions, these mutants could not survive the harsh physiological conditions encountered in a mammalian host. From these crucial preliminary findings, a comprehensive evaluation of LpxC-1 *in vivo* efficacy was conducted, which included both prophylactic and therapeutic administration of this inhibitor against a wild-type, MDR *A. baumannii* strain in both immune-competent and immunocompromised mice [17]. The results of this assessment, as shown in Figure 2, demonstrated significant decreases in *A. baumannii* burden across multiple organs, a significant reduction in the amount of LPS produced by the cells that were recovered from the blood of infected mice, and the widespread reduction in inflammatory cytokines produced by the host when treated with LpxC-1. Collectively, these benefits conferred complete survival of mice throughout the course of the study.

As a result of these findings, a working model for the mechanism behind LpxC-1 activity against *A. baumannii* was developed. In this model, challenging *A. baumannii* cells with this inhibitor results in the loss of lipid A biosynthesis and, by extension, the production of LPS. This loss does not carry bactericidal consequences when grown *in vitro*, and the lack of target expression allows MIC values to skyrocket to unattainable levels. When the physiological environment is switched from *in vitro* growth medium to *in vivo* models, however, this deficiency is clearly exploited. Again, as was the case with the siderophore conjugates described earlier, in retrospect the strict adherence to conventional guidelines for new antibacterial screening proved misleading in terms of translation to *in vivo* efficacy. Unlike the previous example, however, this disconnect resulted in a much more favorable outcome, with the potential therapeutic utility of LpxC inhibitors being expanded to include MDR *A. baumannii*.

With these recent experiences in mind, it is clear that novel screening approaches are needed in order to comprehensively evaluate new chemical matter as potential antibiotics. And while many in the field believe that new sources of compounds are required to successfully identify new drug candidates [18], a parallel strategy that should be implemented is the rescreening of existing compound libraries using non-traditional screens with target bacteria grown in alternative media types. Specifically, if the environmental conditions encountered by bacterial pathogens during an active infection could be mimicked, then presumably the physiological changes that result could reveal new antibiotic targets and pathways that could be exploited for new drug discovery. The vast majority of large-scale, whole-cell antibacterial screens have been conducted in a nutrient-replete medium such as tryptic soy broth (TSB) or Mueller Hinton Broth (MHB). By doing so, bacteria are profiled as being susceptible/resistant to compound challenge under a fixed, and clinically-irrelevant set of circumstances. If treatment with a particular compound inhibits a metabolic pathway making the organism auxotrophic, but the missing metabolite is included in the medium, then the

action of that compound would be completely missed as a consequence of the screening parameters. As a result, new screening conditions that more closely resemble those experienced by bacteria *in vivo* could identify novel chemical scaffolds that target unprecedented cellular targets.

A recent example of this described the exploitation of the glyoxylate shunt in Gram-negative bacteria [19]. This two-enzyme bypass of the tricarboxylic acid (TCA) cycle is used during conditions where carbon molecules, normally given off in the form of CO₂, must be conserved due to the nutrient-limiting nature of the environment the bacterium is experiencing. Expression of the genes encoding these two enzymes, an isocitrate lyase (ICL) and malate synthase (MS), is minimal when organisms such as *P. aeruginosa* are grown in carbohydrate-rich media types such as those commonly used for antibacterial screening. Conversely, when *P. aeruginosa* is provided with lipids as its sole carbon source, expression of ICL and MS increases substantially. These increases are consistent with those seen when *P. aeruginosa* is grown in/recovered from the pulmonary environment of a mammalian system, where lung surfactant serves as the primarily food source. Surfactant, made up primarily of phospholipids, can be utilized by *P. aeruginosa* and other bacteria through the production of phospholipases, lipases, long-chain fatty acid receptors, and the iterative use of β -oxidation to reduce these moieties into acetyl-CoA molecules, which can then be shuttled into the TCA cycle and used in the glyoxylate shunt. When the genes constituting the glyoxylate shunt are inactivated via mutation, the ability of *P. aeruginosa* to grow using carbohydrates is unaffected relative to wild-type strains. When the primary carbon source is switched to acetate, however, the mutant is completely unable to grow. Additionally, a double ICL MS mutant was demonstrated to be completely avirulent in a pulmonary mouse model of *P. aeruginosa* infection [19].

These results prompted the high-throughput screen of an existing 150,000 compound library that had only been evaluated for antibacterial activity using traditional screening approaches (i.e. TSB-grown bacteria) against an efflux-proficient strain of *P. aeruginosa* grown in minimal medium containing acetate as the sole carbon source. Primary hits were triaged based on their ability to inhibit growth in the same minimal medium containing glucose as the sole carbon source, thereby enriching for compounds that showed selective activity in glyoxylate shunt-requiring conditions. After performing IC₅₀ assays using purified ICL and MS, a total of 8 compounds were demonstrated to have specific activity against these enzymes (Figure 3). MIC testing of these lead inhibitors was conducted in both standard MHB and minimal medium against a panel of MDR Gram-negative pathogens. It was these susceptibility data that clearly demonstrated why these compounds were never identified to have antibacterial activity previously; while their MICs in minimal medium were easily visualized, little, if any, activity was seen against any pathogen tested when screened using MHB. Therefore the mode of screening was proven to be critical to identifying novel targets, pathways, and inhibitors. Furthermore, consideration for the components of the infectious environment when designing new screens should represent a proven strategy to revisit legacy compound collections in an attempt to uncover new leads that previously went unnoticed due to the strict adherence to traditional screening practices.

Appreciation for the host environment is not only pertinent for the identification of new antibacterial targets and inhibitors. Changes in assay conditions can also affect the expression of various resistance determinants, again contributing to a translational disconnect between *in vitro* activity and *in vivo* efficacy. Indeed, recent studies have revealed that *A. baumannii* growth in physiologically relevant salt conditions and/or human serum induce a repertoire of putative drug efflux pumps and confer pump-mediated tolerance to antibiotics at concentrations mimicking those expected in a patient [20]. Such host condition-associated regulated changes in efflux pump expression has been termed adaptive efflux-mediated resistance [21] and activity is hypothesized to temporarily increase the organism's ability to survive antibiotic challenge thereby allowing otherwise CLSI defined antibiotic susceptible strains to resist antibiotic challenge. Such findings suggest that alternative medium conditions should be a consideration in drug discovery campaigns as a means to interrogate the antimicrobial properties of agents in the face of bacterial adaptive efflux resistance (and other) host-condition associated resistance/tolerance conditions that cannot be recapitulated in conventional medium.

In summary, the need for novel antibacterial agents remains high, and a fundamental appreciation for the environmental conditions experienced by target pathogens is imperative. Not only can having such an understanding help to avoid disastrous *in vivo* efficacy consequences, but it can also enable new target discovery and expand the spectrum of antibacterial activity. In the fight against spreading antibiotic resistance, all hope is not lost. New, clinically-useful antibiotic candidates may still exist within current chemical libraries. But it may require researchers to think outside the box and stray from traditional screening paradigms in order to identify these hidden gems, a strategy which may keep society from returning to a pre-antibiotic era.

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

** of outstanding interest

1. Fung-Tomc J, Bush K, Minassian B, Kolek B, Flamm R, Graderski E, Bonner D. Antibacterial activity of BMS-180680, a new catechol-containing monobactam. *Antimicrob Agents Chemother.* 1997; 41:1010–1016. [PubMed: 9145861]
2. Mollmann U, Ghosh A, Dolence EK, Dolence JA, Ghosh M, Miller MJ, Reissbrodt R: Selective growth promotion and growth inhibition of gram-negative and gram-positive bacteria by synthetic siderophore-beta-lactam conjugates. *Biometals.* 1998; 11:1–12. [PubMed: 9450313]
3. Page MG, Dantier C, Desarbre E. In vitro properties of BAL30072, a novel siderophore sulfactam with activity against multiresistant gram-negative bacilli. *Antimicrob Agents Chemother.* 2010; 54:2291–2302. [PubMed: 20308379]
4. Brown MF, Mitton-Fry MJ, Arcari JT, Barham R, Casavant J, Gerstenberger BS, Han S, Hardink JR, Harris TM, Hoang T, et al. Pyridone-conjugated monobactam antibiotics with gram-negative activity. *J Med Chem.* 2013; 56:5541–5552. [PubMed: 23755848]
5. McPherson CJ, Aschenbrenner LM, Lacey BM, Fahnoe KC, Lemmon MM, Finegan SM, Tadakamalla B, O'Donnell JP, Mueller JP, Tomaras AP. Clinically relevant Gram-negative

resistance mechanisms have no effect on the efficacy of MC-1, a novel siderophore-conjugated monocarbam. *Antimicrob Agents Chemother.* 2012; 56:6334–6342. [PubMed: 23027195]

6. CLSI. Performance standards for antimicrobial susceptibility testing: 19th informational supplement. Clinical Laboratory Standards Institut. 2009:M100.
7. CLSI. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard. Clinical Laboratory Standards Institute (CLSI). 2009:M07.
8. Girardello R, Bispo PJ, Yamanaka TM, Gales AC. Cation concentration variability of four distinct Mueller-Hinton agar brands influences polymyxin B susceptibility results. *J Clin Microbiol.* 2012; 50:2414–2418. [PubMed: 22553247]
9. Gkouvatso K, Papanikolaou G, Pantopoulos K. Regulation of iron transport and the role of transferrin. *Biochim Biophys Acta.* 2012; 1820:188–202. [PubMed: 22085723]
- 10*. Tomaras AP, Crandon JL, McPherson CJ, Banevicius MA, Finegan SM, Irvine RL, Brown MF, O'Donnell JP, Nicolau DP. Adaptation-based resistance to siderophore-conjugated antibacterial agents by *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother.* 2013; 57:4197–4207. [PubMed: 23774440] [This work represents the first description of adaptive resistance to a siderophore-antibiotic conjugate, achieved through outcompetition by endogenous siderophore molecules. It also describes the development and validation of an *in vitro* assay that accurately predicts adaptive resistance to these conjugates.]
11. Mdluli KE, Witte PR, Kline T, Barb AW, Erwin AL, Mansfield BE, McClerren AL, Pirrung MC, Tumey LN, Warrenner P, et al. Molecular validation of LpxC as an antibacterial drug target in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother.* 2006; 50:2178–2184. [PubMed: 16723580]
12. Caughlan RE, Jones AK, Delucia AM, Woods AL, Xie L, Ma B, Barnes SW, Walker JR, Sprague ER, Yang X, et al. Mechanisms decreasing *in vitro* susceptibility to the LpxC inhibitor CHIR-090 in the gram-negative pathogen *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother.* 2012; 56:17–27. [PubMed: 22024823]
13. Brown MF, Reilly U, Abramite JA, Arcari JT, Oliver R, Barham RA, Che Y, Chen JM, Collantes EM, Chung SW, et al. Potent inhibitors of LpxC for the treatment of Gram-negative infections. *J Med Chem.* 2012; 55:914–923. [PubMed: 22175825]
14. Tomaras AP, McPherson CJ, Kuhn M, Carifa A, Mullins L, George D, Desbonnet C, Eidem TM, Montgomery JI, Brown MF, et al. LpxC inhibitors as new antibacterial agents and tools for studying regulation of lipid A biosynthesis in Gram-negative pathogens. *MBio.* 2014; 5:e01551–01514. [PubMed: 25271285]
- 15**. Moffatt JH, Harper M, Harrison P, Hale JD, Vinogradov E, Seemann T, Henry R, Crane B, St Michael F, Cox AD, et al. Colistin resistance in *Acinetobacter baumannii* is mediated by complete loss of lipopolysaccharide production. *Antimicrob Agents Chemother.* 2010; 54:4971–4977. [PubMed: 20855724] [This work describes the capability of *A. baumannii* to avoid the action of colistin by abolishing the production of lipid A and, consequently, lipopolysaccharide (via genetic mutation to the *lpx* biosynthesis operon). As a Gram-negative bacterium, this represents the first description of such an organism maintaining viability in the absence of outer membrane decoration.]
16. Montgomery JI, Brown MF, Reilly U, Price LM, Abramite JA, Arcari J, Barham R, Che Y, Chen JM, Chung SW, et al. Pyridone methylsulfone hydroxamate LpxC inhibitors for the treatment of serious gram-negative infections. *J Med Chem.* 2012; 55:1662–1670. [PubMed: 22257165]
- 17**. Lin L, Tan B, Pantapalangkoor P, Ho T, Baquir B, Tomaras A, Montgomery JI, Reilly U, Barbacci EG, Hujer K, et al. Inhibition of LpxC Protects Mice from Resistant *Acinetobacter baumannii* by Modulating Inflammation and Enhancing Phagocytosis. *MBio.* 2012;3. [This report demonstrates that the primary mechanism of pathogenesis of *A. baumannii* is sepsis-mediated. Additionally, the therapeutic utility of an LpxC inhibitor, despite its lack of *in vitro* activity based on MIC, was proven in terms of mouse survival, bacterial burden reduction, and reduction of cytokine response to infection.]
18. Silver LL. Challenges of antibacterial discovery. *Clin Microbiol Rev.* 2011; 24:71–109. [PubMed: 21233508]

19. Fahnoe KC, Flanagan ME, Gibson G, Shanmugasundaram V, Che Y, Tomaras AP. Non-traditional antibacterial screening approaches for the identification of novel inhibitors of the glyoxylate shunt in gram-negative pathogens. *PLoS One*. 2012; 7:e51732. [PubMed: 23240059]
- 20*. Hood MI, Jacobs AC, Sayood K, Dunman PM, Skaar EP. *Acinetobacter baumannii* increases tolerance to antibiotics in response to monovalent cations. *Antimicrob Agents Chemother*. 2010; 54:1029–1041. [PubMed: 20028819] [This work describes the adaptive expression of multidrug efflux pumps in response to environmental signals, demonstrating fundamental differences when bacteria are tested in standard *in vitro* assays versus under *in vivo* relevant conditions.]
21. Fernandez L, Hancock RE. Adaptive and mutational resistance: role of porins and efflux pumps in drug resistance. *Clin Microbiol Rev*. 2012; 25:661–681. [PubMed: 23034325]
22. Chell RM, Sundaram TK, Wilkinson AE. Isolation and characterization of isocitrate lyase from a thermophilic *Bacillus* sp. *Biochem J*. 1978; 173:165–177. [PubMed: 687365]
23. Khan AS, Van Driessche E, Kanarek L, Beeckmans S. Purification of the glyoxylate cycle enzyme malate synthase from maize (*Zea mays* L.) and characterization of a proteolytic fragment. *Protein Expr Purif*. 1993; 4:519–528. [PubMed: 8286948]

Highlights for “Life inside the box: consequences of *in vitro*-to-*in vivo* disconnects and the opportunity for non-traditional antibacterial screening”

- Appreciation for bacterial physiology during infection will impact discovery
- Following CLSI guidelines can prevent accurate prediction of compound utility
- New antibacterial screening strategies may identify new drug targets/pathways

Figure 1.

Efficacy of the siderophore-monobactam conjugate MB-1 against 9 different clinical isolates of *P. aeruginosa* using an immunocompromised mouse thigh model, as reported in [10].

Two hours after intramuscular infection, mice were subcutaneously administered a regimen of MB-1 (50 mg/kg) that resulted in free drug exposures of 4 μ g/ml for the duration of the study. Mouse thighs were removed 24 hours after the initiation of therapy, bacterial burdens were determined, and changes from the initial bacterial burden are shown. *In vitro* MIC values, generated using standard, CLSI-compliant methods, are indicated in parentheses along the X-axis for each bacterial strain.

Figure 2.

Effective LpxC-1 treatment of immunocompetent mice as described in [17]. Briefly, groups of C3H/FeJ mice ($n=15$) were infected with *A. baumannii* HUMC-1, and 100 mg/kg LpxC-1 were administered intravenously at 1 and 24 hours post-infection. Bacterial burdens in blood and various organs (A), serum LPS (B), and cytokine levels (C) were determined for placebo and LpxC-1-treated mice. *, $P < 0.01$ versus results for placebo-treated mice.

	MHB				M9 Acetate					
Compound	AB-3167	EC 1545-08	KP MGH78578	PAO1	AB-3167	EC 1545-08	KP MGH78578	PAO1	ICL IC ₅₀	MS IC ₅₀
1	>64	>64	>64	>64	2	16	8	8	0.69	0.18
2	64	>64	>64	64	2	2	2	4	0.24	1.77
3	32	32	64	32	4	4	4	8	0.17	5.30
4	64	64	64	64	1	1	1	2	0.03	1.29
5	32	32	64	64	8	4	4	16	0.37	2.35
6	64	64	64	64	4	4	4	8	0.09	2.73
7	32	32	64	64	8	4	4	8	0.19	6.93
8	64	64	64	64	8	4	8	16	0.37	8.88

Figure 3.

MIC and IC₅₀ values (in µg/ml and µM, respectively) for 8 potential glyoxylate shunt inhibitors against 4 Gram-negative pathogens using two different growth media, as reported in [19]. Strains of *A. baumannii* (AB-3167), CTX-M-expressing *E. coli* (EC 1545-08), ESBL-expressing *K. pneumoniae* (KP MGH78578), and efflux-proficient *P. aeruginosa* (PAO1) were subjected to susceptibility testing in both standard, CLSI-compliant Mueller Hinton Broth (MHB) or the glyoxylate shunt-requiring M9 minimal medium containing 0.5% acetate as the sole carbon source (M9 Acetate). Both ICL and MS proteins from *P. aeruginosa* were expressed and purified from recombinant *E. coli* strains, and activity assays for each were performed as described previously [22,23]