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BRIEF REPORT

In Vitro Elimination of Highly Multidrug-Resistant Bacteria by the Lactic Acid Bacterial Drug Candidate ILP100

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ABSTRACT

Introduction: Multidrug resistance (MDR) has been identified in wound bacterial isolates from Ukrainian war victims treated in Ukraine and across Europe. ILP100, a drug candidate for the treatment of skin wounds, is composed of a *Limosilactobacillus reuteri* expressing human chemokine CXCL12. In this study, the antimicrobial effects of ILP100 were tested on MDR bacteria isolated from wounds of Ukrainian war victims.

Methods: ILP100 was co-cultured with one of the wound pathogens (*Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus*; 12 non-MDR and 12 MDR isolates) in broth media for 12 h with subsequent survival recovery on agar plates. Additionally, agar plates were precoated with ILP100 at clinical doses (3 vs. 24 h, 1×10^7 CFU/cm^2) followed by co-culture with pathogens inoculated in soft agar (1×10^4 CFU/cm^2). To compare ILP100 with relevant antibiotics, MDR-inoculated soft agar was applied to plates with standardized ILP100 drops and antibiotic-loaded discs, followed by 18–20 h aerobic incubation at 37 °C.

Results: Dose-dependent growth inhibition of all pathogens was demonstrated, as 1000:1 and 100:1 (ILP100/isolate) inhibited pathogenic growth up to log 6.4 and log 4.3 CFU/ml, respectively. Potent antimicrobial effects were demonstrated after precoating with ILP100, as pathogen recovery was only demonstrated after 3 h of precoating, only for 10/18 isolates and then only partially. Benchmarking to relevant antibiotic discs resulted in large cleared zones surrounding the ILP100 spots but not the antibiotic discs, demonstrating potent bacterial killing by ILP100-secreted factors. Interestingly, the MDR pathogens were significantly more sensitive to the ILP100 released factors than the non-MDR isolates.

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Conclusion: ILP100 effectively eliminates MDR wound pathogens, which reveals a promising strategy for the development of new classes of urgently needed antimicrobials.

PLAIN LANGUAGE SUMMARY

The rise in antibiotic resistance already poses a global threat, which is further exacerbated by geopolitical conflicts. As wounded individuals are transferred to hospitals in neighboring countries for treatment, there is an increased risk of these multi-antibiotic-resistant pathogens spreading across borders, putting healthcare systems under additional strain. ILP100, which comprises *Limosilactobacillus reuteri* R2LC that is genetically modified to produce human CXCL12, significantly accelerated wound healing in mice, as well as in a first-in-human double blinded, placebo-controlled randomized clinical study. Here we demonstrate the potent bactericidal effects of ILP100 against multi-antibiotic-resistant pathogens isolated from wounds of Ukrainian war victims using several co-culturing methods. In light of the stagnating antimicrobial pipeline, our findings indicate that antagonistic bacteria like ILP100 with dual actions may offer important therapeutic alternatives for the eradication of multi-antibiotic-resistant bacteria, while shortening the time to complete wound healing. As a result of the global rise in antibiotic resistance, this work is of high significance, particularly during logistically challenging situations including armed conflicts where effective treatments are most urgently needed.

Keywords: Antibiotic resistance; Wound healing; Drug development

Key Summary Points

Why carry out this study?

Multidrug-resistant (MDR) bacteria are on the rise and have been identified in wound bacterial isolates from Ukrainian war victims treated in Ukraine and across Europe. There is a high risk that this will exacerbate the further spread of these MDR bacteria. Few antimicrobial options are left for these patients.

Does ILP100, a drug candidate accelerating healing of skin wounds (composed of a *Limosilactobacillus reuteri* expressing human chemokine CXCL12), have antimicrobial effects on MDR bacteria?

What was learned from the study?

ILP100 may have a better antimicrobial effect than clinically relevant antibiotics.

ILP100 demonstrated potent antimicrobial effects against a range of infectious bacteria, including MDR isolates. This result was confirmed in three different and relevant antimicrobial susceptibility assays.

ILP100 effectively eliminated MDR wound pathogens, which reveals a promising strategy for the development of new classes of urgently needed antimicrobials.

INTRODUCTION

The development of antimicrobial resistance and the increased incidence of multidrug-resistant (MDR) healthcare-associated infections are exacerbated by geopolitical conflicts [1]. In agreement with this, extreme MDR Gram-negative infectious bacteria, including hypervirulent pan-resistant (PDR) isolates, were detected among wound isolates from hospitalized patients in Ukraine following the Russian invasion [2, 3]. The spread of MDR strains throughout Europe has been confirmed, as identical carbapenem-resistant clones of *Acinetobacter baumannii* found at Ukrainian military hospitals were detected in

injured Ukrainian soldiers treated in German hospitals [4–6].

Difficult wounds have greater risks of acquiring infections that require antibiotic treatment. There are today limited means to accelerate wound healing. In fact, there are no suitable treatments to wounds inflicted during armed conflicts, as available therapies are based on growth factors or stem cells with very limited shelf life requiring an unbroken cold chain. ILP100 (emilimogene sigulatibac) is a first-in-class drug candidate with a demonstrated favorable and well-tolerated safety profile, and accelerated healing of 6–10 days in the different cohorts of a first-in-human, double blinded, randomized controlled clinical trial [7]. ILP100 comprises genetically modified, freeze-dried *Limosilactobacillus reuteri* and has a good stability and shelf life at 25 °C, making it suited for wide use especially during logistically challenging situations. The drug candidate is currently being evaluated in a phase 2 clinical trial in diabetic foot ulcers, and the mechanism of action involves enhanced restorative abilities of wound macrophages following bacterial onsite production of the human chemokine CXCL12 [8]. Lactic acid bacteria including *L. reuteri* have previously been reported to exert antimicrobial effects by production of organic acids, antimicrobial metabolites, and polyketides, which mainly have been utilized in food fermentations [9–11]. The aim of the current study was to investigate if inherent antimicrobial effects of ILP100 are clinically relevant for wounds infected with MDR bacteria.

Ethical approval was not required for this study.

METHODS

Bacterial Strains

The soft-tissue bacterial isolates KR6000–6200, including non-MDR and MDR isolates, originated from war victims in Ukraine (Tables 1 and 2). The term multidrug-resistant (MDR) is selected as the common feature of all pathogens listed and tested in this study (although highly resistant, all isolates are still resistant to at least two classes of antibiotics). *Staphylococcus aureus* JE2, DA28823 [12], was a kind donation from Professor Dan Andersson, Uppsala University, Sweden.

Media and Reagents

Lactobacillus susceptibility testing media (LSM; [90% IsoSensitest and 10% MRS; Oxoid]) was used in most cases [13].

When soft agar was required, the LSM broth was supplemented with 0.7% bacteriological agar (VWR). The media supported growth of both ILP100 and the different pathogenic isolates when grown alone. De Man–Rogosa–Sharpe substrate (MRS; Merck or Oxoid) agar was used for recovery of ILP100 after co-culturing. A simulated wound fluid substrate (WFS) was produced using cell medium RPMI (Gibco) with the following supplements: 1% yeast extract (FBS substitute), trace manganese sulfate (0.003%), and 10% sucrose (simulating the addition of the lyoprotectant from the drug product).

Antibiotic discs (Mast Group [Nordic Biolabs]) included piperacillin/tazobactam PZT-30/6 µg, ceftazidime/avibactam CZA-30/20 µg, meropenem/vaborbactam MEV-20/10 µg, ceftolozane/tazobactam C/T-40 µg, imipenem/relebactam IMR-10/25 µg, and cefiderocol FDC-30 µg.

Co-culture in Liquid Growth Media

Pure colonies from fresh streaks of each pathogen isolate on LSM agar were used to inoculate LSM broth and then incubated overnight at 37 °C with shaking. ILP100 was resuspended from lyophilized drug product vial in 1 mL LSM and mixed with the pathogen-inoculated LSM broth in a ratio of either 100:1 (ILP100/pathogenic isolate; 1/10 of 10 × diluted vial) or 1000:1 (ILP100/pathogenic isolate; 1/10 of resuspended vial). The treatment ratio ILP100 to pathogen was 1000:1(10^9) and 100:1(10^8), whereas the desired pathogen starting concentration was set to approximately $0.5–1 \times 10^6$. The co-cultures were incubated at 37 °C without shaking for 12 h and were sampled at 0, 3, 6, and 12 h

Table 1 Co-cultivation with ILP100 dose-dependently inhibits the growth of MDR and non-MDR pathogens

	Non-MDR isolates used in co-culture: values used from 12 h delta log_{10}				MDR isolates used in co-culture: values used from 12 h delta log_{10}				Extra information for MDRs
	Non-MDR strain	1000:1	100:1	ctrl*	MDR strain	1000:1	100:1	ctrl*	Antibiotics tested [2]
ILP100:*Pseudomonas aeruginosa*	KR6004	− 5.18	− 4.17	2.64	KR6013	− 6.28	− 4.93	3.13	(R)—PZT, CT, CZA, MEV, IMR, FDC, CIP, GEN, TOB, TSU (S) COL
	KR6008	− 5.61	− 1.33	2.68	KR6017	− 5.85	− 3.26	2.96	
	KR6020	− 5.59	− 2.11	2.59	KR6101	− 5.86	− 4.67	2.83	–
	Average (± SD)	− 5.46 (± 0.25)	− 2.53 (± 1.47)	2.64 (± 0.05)	Average (± SD)	− 6.00 (± 0.25)	− 4.29 (± 0.89)	2.97 (0 ± 0.15)	–
ILP100:*Enterobacter cloacae*	KR6027	− 5.8	− 1.37	2.86	KR6106	− 6.23	− 1.96	3.08	(R)—PZT, CT, CZA, MEV, IMR FDC. CIP, TOB, TSU (S) GEN, COL
	KR6036	− 6.44	− 1.51	3.15	KR6023	− 6.27	− 2.15	2.98	
	KR6088	− 6.63	− 1.6	3.25	KR6049	− 6.36	− 2.1	3.23	R)—PZT, CT, CZA, MEV, IMR FDC. CIP, TSU (S) GEN, TOB, COL
	Average (± SD)	− 6.29 (± 0.43)	− 1.49 (± 0.12)	3.09 (± 0.20)	Average (± SD)	6.29 (± 0.07)	− 2.07 (± 0.10)	3.10 (± 0.13)	–

Table 1 continued

	Non-MDR isolates used in co-culture: values used from 12 h delta log_{10}				MDR isolates used in co-culture: values used from 12 h delta log_{10}				Extra information for MDRs
	Non-MDR strain	**1000:1**	**100:1**	**ctrl***	**MDR strain**	**1000:1**	**100:1**	**ctrl***	**Antibiotics tested [2]**
ILP100:*Acinetobacter baumannii*	KR6082	− 4.98	− 2.34	2.51	KR6038	− 5.71	− 4.51	2.63	(NA)—PZT, CT, CZA, MEV, IMR (R)—MER, IMI, FDC, GEN, TOB, TSU, LEV, AMI (S) COL, TG, AMP/SUL
	KR6133	− 5.35	− 2.12	2.97	KR6155	− 5.7	− 3.29	2.66	
	KR6140	− 5.7	− 5.45	3.26	KR6156	− 5.81	− 2.68	2.54	
	Average (± SD)	− 5.34 (± 0.25)	− 3.3 (± 1.86)	2.91 (± 0.38)	Average (± SD)	− 5.74 (± 0.06)	− 3.49 (± 0.9)	2.61 (± 0.06)	

Table 1 continued

	Non-MDR isolates used in co-culture: values used from 12 h delta log_{10}				MDR isolates used in co-culture: values used from 12 h delta log_{10}				Extra information for MDRs
	Non-MDR strain	1000:1	100:1	ctrl*	MDR strain	1000:1	100:1	ctrl*	Antibiotics tested [2]
ILP100:*Klebsiella pneumoniae*	KR6165	− 5.94	− 1.39	3.01	KR6135	− 6.37	− 1.62	3.32	(R)—PZT, CT, CIP, TOB, TSU: (S)—CZA, MEV, IMR, GEN, FDC, COL
	–	–	–	–	KR6080	− 6.39	− 1.49	3.29	(R)—PZT, CT, CZA, MEV, IMR, FDC, CIP, GEN, TOB, TSU (S) COL
	–	–	–	–	KR6084	− 6.49	− 1.28	3.37	(R)—PZT, CT, MEV, IMR, CIP, GEN, TOB, TSU (S)—CZA, FDC, COL
	Average (± SD)	–	–	–	Average (± SD)	− 6.42 (± 0.06)	− 1.46 (± 0.17)	3.32 (± 0.04)	–
ILP100:*Proteus mirabilis*	KR6091	− 6.2	− 1.52	3.12	–	–	–	–	–

Table 1 continued

	Non-MDR isolates used in co-culture: values used from 12 h delta log_{10}				MDR isolates used in co-culture: values used from 12 h delta log_{10}				Extra information for MDRs
	Non-MDR strain	1000:1	100:1	ctrl*	MDR strain	1000:1	100:1	ctrl*	Antibiotics tested [2]
ILP100:*Staphylococcus aureus*	JE2	− 6.06	0.11	2.9	–	–	–	–	Cured variant of USA300 [12]

*ctrl = control of isolate growth without the presence of ILP100; used to generate total log reduction values for treatment scenarios 1000:1 and 100:1 (shown in Fig. 1). – data not applicable or not used in study. (R) = resistant, (S) = sensitive, *PZT* piperacillin/tazobactam, *CT* ceftolozane/tazobactam, *CZA* ceftazidime/avibactam, *MEV* meropenem/vaborbactam, *IMR* imipenem/avibactam, *FDC* cefiderocol, *CIP* ciprofloxacin, *GEN* gentamicin, *TOB* tobramycin, *TSU* trimethoprim/sulfamethoxazole, *MER* meropenem, *IMI* imipenem, *LEV* levofloxacin, *AMI* amikacin, *COL* colistin, *TG* tigecycline, *AMP/SUL* ampicillin/sulbactam

for serial dilution and plating on two types of media in order to optimally recover ILP100 bacteria (MRS agar; anaerobically incubated for 48 h at 37 °C) and the pathogenic isolate (LSM agar; aerobically incubated for 24 h at 37 °C), respectively. The co-culturing performed in WFS was identical to the LSM experiments for all conditions, except that the 100:1 ratio was not tested. In all these experiments, the inoculation of the pathogens was approximately log 6 CFU/mL, with growth potential of around log 2–3 CFU/mL. The experimental limit of detection was log 3.

Co-culture on Agar Plates

ILP100 was precoated on LSM agar and incubating anaerobically at 37 °C for 3 or 24 h, where clinical (1×10^7 CFU/cm^2 [5×10^8 CFU]) and subclinical (1×10^6 CFU/cm^2 [5×10^7 CFU]) doses of ILP100 were used. The pathogens were thereafter applied onto the precoated agar surface by adding 5 mL of a soft agar (LSM; 0.7%) containing the pathogens (an amount resulting in 1×10^4 CFU/cm^2). The overlay (resulting co-culture) was incubated 24 h at 37 °C and analyzed. The plates that had no visible growth were incubated for an additional 24 h, 48 h in total. Plates were assessed and classified as either "totally cleared", "partially cleared", or "totally overgrown [by the MDR pathogen]". The data analysis was performed either by visual inspection in the "totally cleared" and "totally overgrown" cases, or using standardized imaging and image analysis (ImageJ) with a similar method as for the zone inhibition method (see below). The partially overgrown area was measured in millimeters squared and was subtracted from the total plate surface area to obtain the "partially cleared" area.

Zone of Inhibition

The method was developed and standardized for spots of ILP100 to be able to demonstrate visually its antimicrobial activity and to limit the cumulative killing effect (i.e., severe clear-zone merging) as observed when spot-drops were located too close to each other or when

Table 2 Prevention of pathogen growth by ILP100-precoating in two doses (sc, subclinical dose: 1×10^6; C, clinical dose: 1×10^7 CFU/cm^2)

	Cleared 3 h pre-incubation with ILP100	Partially cleared (%)*	Overgrown	Cleared 24 h pre-incubation with ILP100
Isolate	*Pseudomonas aeruginosa*			
KR6004	C	–	sc	sc C
KR6006	C	–	sc	sc C
KR6008	C	–	sc	sc C
KR6020	C	–	sc	sc C
KR6024	C	–	sc	sc C
	Enterobacter cloacae			
KR6027	–	C (71)	sc	sc C
KR6036	–	C (76)	sc	sc C
KR6040	–	C (77)	sc	sc C
KR6041	–	C (61)	sc	sc C
KR6088	–	C (84)	sc	sc C
	Acinetobacter baumannii			
KR6082	C	–	sc	sc C
KR6132	–	C (85)	sc	sc C
KR6133	–	C (50)	sc	sc C
KR6140	C	–	sc	sc C
	Klebsiella pneumoniae			
KR6100	–	C (66)	sc	sc C
KR6165	–	C (85)	sc	sc C
	Proteus mirabilis			
KR6091	–	C (88)	sc	sc C
	Staphylococcus aureus			
JE2	C	–	sc	sc C

*Partially cleared area of the plate was measured as a percentage of the area cleared in relation to the entire plate area

the effect was very potent. Triplicate, spot-drops (10 µl) of ILP100 suspensions (1×10^8, 5×10^7, or 5×10^6 CFU/drop) were evenly distributed on LSM agar plates. The plates were anaerobically incubated at 37 °C for 22 ± 2 h. Following incubation, LSM soft agar (5 ml; 0.7%) was inoculated with pathogenic bacteria in three different concentrations (1×10^6, 1×10^5, and 1×10^4) for each isolate and was immediately poured onto the agar surface

containing the pre-incubated drops of ILP100. This was followed by another 18–20 h of aerobic incubation at 37 °C. Each plate was visually inspected and then imaged and analyzed. Final measurement results can be found in Supplemental Material (SM) Tables S1–S3.

The area of the zone of inhibition was measured using ImageJ (version 1.53/4) and analyzed. The diameter of the plate (90 mm) was used as a reference to set the scale and define the size of a pixel. The areas of the cleared zone (including the drop/antibiotic disc region) and the drop/antibiotic disc were then measured. The areas of the drops/discs were then subtracted from the cleared zone area. For each condition, the average cleared area (MAC) of triplicates was used. Measurements varied generally by less than 10%. The antibiotic disc itself was measured for every MAC calculated for the disc and 100 measurements were used to calculate the accuracy and precision (30.72 ± 0.73).

Plate Imaging and Statistical Analysis

Following incubation, all plates were inspected visually, and images was acquired by utilizing a Nikon D5600 camera set in automatic mode and without a flash. Images were acquired with a resolution of 24.2 megapixels. To ensure optimal contrast and clarity, a black background was incorporated during the imaging process. Moreover, a controlled light source was deployed to facilitate image acquisition.

Data are primarily presented as mean values ± standard deviations, except where otherwise specified. Differences between ILP100 treatments in the MDR and non-MDR groups, with respect to colony-forming units (CFU) at various cultivation time points during co-cultivation, as well as the size (MAC) of the clear zones on the plates, were evaluated using Welch's *t* test, unless otherwise indicated. A significance threshold of $p < 0.05$ was applied. Fisher's exact test was employed to assess differences in the number of MDR and non-MDR pathogens killed after 12 h of co-cultivation with ILP100.

RESULTS

Co-culture in Liquid Growth Media

The antimicrobial effect of ILP100 in doses used in clinical studies was assessed against six different wound infectious bacterial species: *P. aeruginosa*, *A. baumannii*, *E. cloacae*, *K. pneumoniae*, *P. mirabilis*, and *S. aureus* (the first five reported in [2]). In total, 24 isolates were included, of which 12 were characterized as MDRs (Table 1). ILP100 was co-cultured with pathogenic isolates in a ratio of 100:1 or 1000:1 (ILP100/pathogen) in LSM broth. The co-cultures were sampled at 0, 3, 6, and 12 h, whereafter ILP100 and the pathogen were quantified on separate agar plates. In control experiments, growth of each pathogen alone for the 12-h time period resulted in an increase of log 2.5–3.4 CFU/mL, which reflects an infection with optimal growth [14] (Table 1, SM Fig. S1). In contrast, co-culturing for 12 h with ILP100 resulted in a dose-dependent growth inhibition of all pathogens. The 1000:1 concentration ratio inhibited growth by between log 5.3 to 6.4 CFU/mL, while the 100:1 ratio resulted in an inhibition of up to log 4.3 CFU/mL (Fig. 1c, Table 1). Additionally, all pathogens were dose-dependently reduced at the earlier time points (3 h and 6 h) (Fig. 1a, b). Importantly, this dramatic growth reduction was observed for all pathogens, including the MDR isolates (Fig. 1a–c, Table 1). Similar reductions in pathogen growth were observed when using simulated wound fluid substrate, which mimics growth conditions within wounds (Fig. 1a–c).

Co-culture on Agar Plates

To simulate topical application of ILP100, LSM agar plates were precoated with ILP100 at clinical (1×10^7) and subclinical doses (1×10^6 CFU/cm^2) prior to pathogen (non-MDRs) application in a layer of inoculated soft agar (1×10^4 CFU/cm^2). Whereas all isolates tested recovered after 3 h inoculation with the subclinical ILP100 dose, the clinical dose prevented recovery of eight isolates in the subsequent assay, whereas the remaining 10 isolates were only partially recovered (Table 2). After 24 h of inoculation, none of the isolates

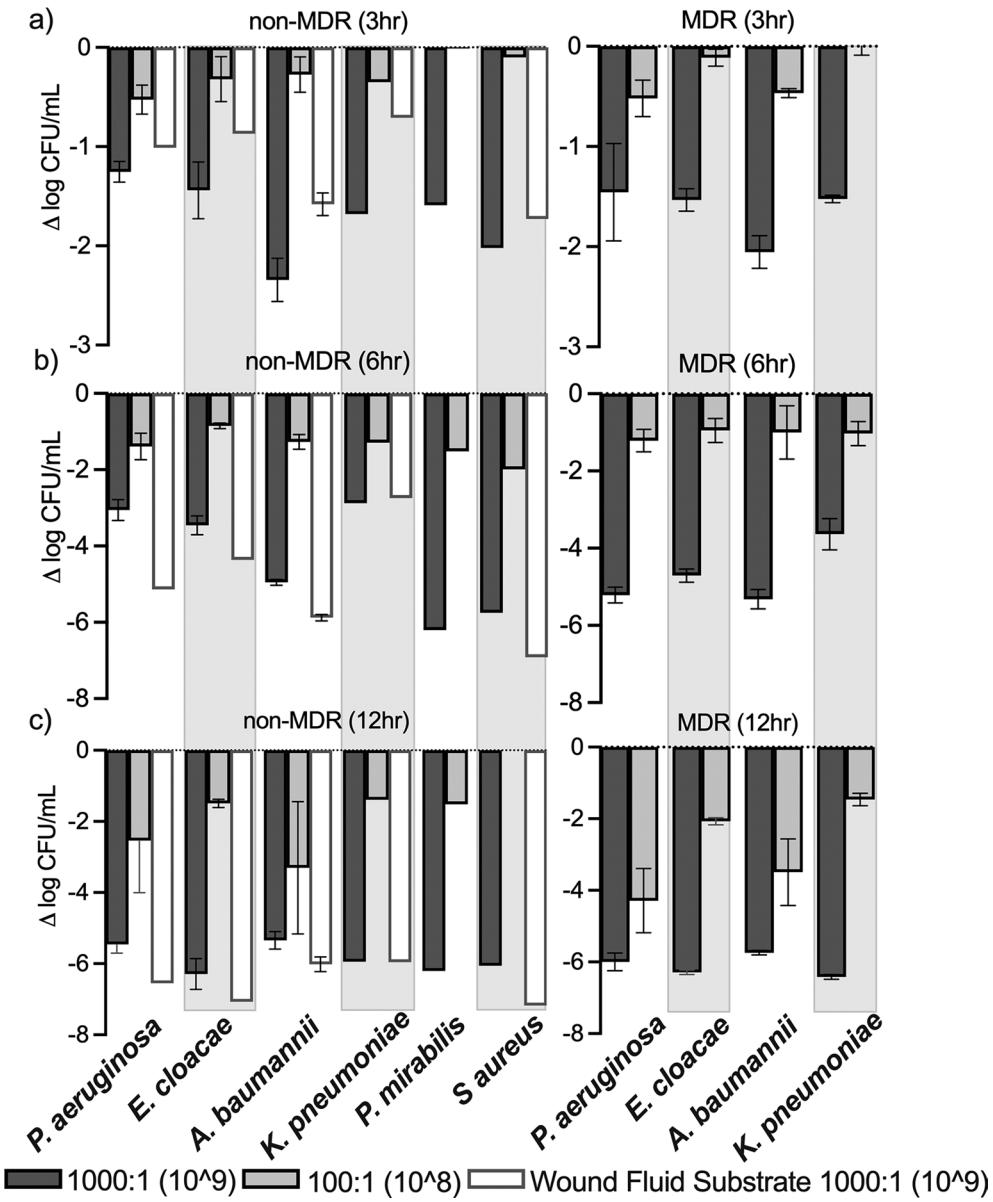

Fig. 1 Co-cultivation with ILP100 inhibits the growth of both MDR and non-MDR pathogens in a dose-dependent manner. Growth inhibition of the all isolates following 3 h (**a**), 6 h (**b**), and 12 h (**c**) recovery. Results are presented as co-culture CFU minus the untreated CFU for the different time points normalized to starting CFU. Each MDR bar is an average of three tested isolates; however, for the non-MDR of *K. pneumoniae*, *P. mirabilis*, and *S. aureus*, only one isolate per species was tested. White bars (simulated wound fluid substrate) show one isolate per species but two for *A. baumannii*. Results are presented as mean ± SD

tested could be recovered from plates precoated with either ILP100 dose (Table 2).

Zone of Inhibition

To benchmark ILP100 against clinically relevant antibiotics, disc diffusion experiments

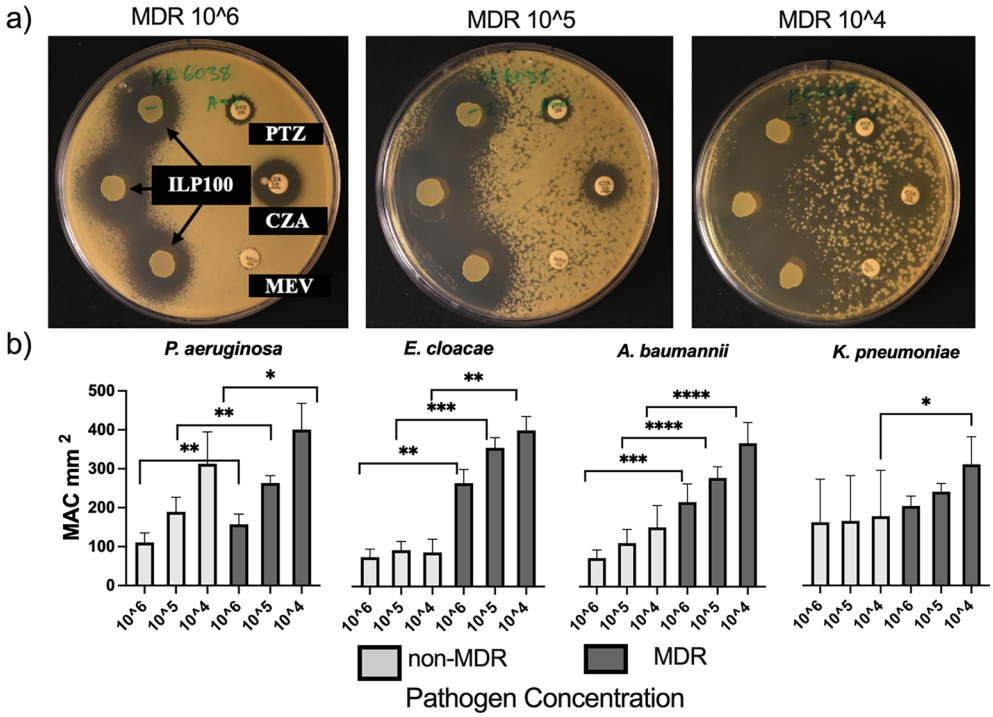

Fig. 2 ILP100 dose-dependently inhibits growth of MDR and non-MDR pathogens. **a** Representative images of disc diffusion experiments of MDR *A. baumannii* at three different concentrations with clinically relevant dosing of ILP100 and antibiotics (PTZ, piperacillin-tazobactam 30/6 µg; CZA, ceftazidime-avibactam 30/20 µg; MEV, meropenem-vaborbactam 20/10 µg). **b** Effects of ILP100 (5×10^7 CFU/drop) tested against MDR and non-MDR isolates presented as the mean area cleared zone (MAC, mm^2) directly following 18–20 h aerobic incubation, 37 °C. Results are mean ± SD, and differences were evaluated using Welch's *t* test, where $p < 0.05$ (*) was significant [$p < 0.005$ (**), $p < 0.0005$ (***), $p < 0.0001$ (****)]

were performed. ILP100 was administered by spot-dropping (standardized triplicates: 1×10^8, 5×10^7, or 5×10^6 CFU) and cultivated overnight, whereafter antibiotic-loaded discs were added prior to pathogen application in a layer of inoculated soft agar (1×10^6, 10^5, and 10^4). Potent MDR-killing by ILP100-secreted factors was revealed as large cleared zones surrounding the ILP100 spots, whereas small or no zones surrounded the antibiotic discs (Fig. 2a, SM Table S1). Interestingly, when ILP100 (5×10^7 CFU) was tested against both non-MDR and MDR isolates in a separate experiment, the MDR pathogens were significantly more sensitive for the ILP100 secreted factors than the non-MDR isolates were (Fig. 2b, SM Tables S2, S3).

DISCUSSION

Antibiotic resistance poses a global threat, which is further exacerbated by geopolitical conflicts. The MDR strains selected from the conflict zone were carbapenem-resistant *A. baumannii* (CRAB), difficult to treat *P. aeruginosa* (DTR), and carbapenem-resistant *Enterobacterales* (CRE), which were considered as strains of global concern. Additionally, resistance levels to cefiderocol, a siderophore-based antibiotic that is a relatively novel compared to the other antibiotics, were 66.6%, 100%, 100%, and 33.3% for the selected MDR isolates of *P. aeruginosa*, *E. cloacae*, *A. baumannii*, and *K. pneumoniae*, respectively. ILP100 demonstrated a potent, dose-dependent antimicrobial effect against a variety of pathogens, including

MDR strains, in liquid co-culture experiments. The significant growth inhibition observed at both 100:1 and 1000:1 ratios, especially at early time points (3 h and 6 h), highlights its rapid action, which is critical for treating infections in clinical settings. The results from the agar plate co-culture confirm the observations of the antimicrobial effects of ILP100 from the co-cultures in liquid substrates (Fig. 1a–c), and strengthen the observation that ILP100 completely inhibits pathogenic growth.

This strong inhibitory effect was also visualized by co-culture with MDR isolates on agar plates, where large cleared zones surrounded the ILP100 spots but not discs with clinically relevant antibiotics. In fact, the MDR strains were significantly more sensitive to ILP100 exposure than the non-MDR isolates; this may be because the MDRs are less fit due to the cost of carrying the genetic determinants of multiple resistance and may therefore make the MDRs more susceptible to the robust antimicrobial properties produced and secreted by ILP100. The results suggest that ILP100 could be an effective treatment for MDR wound infections, and its effectiveness in simulated wound fluid indicates maintained activity under clinically relevant conditions.

CONCLUSION

Clinically relevant doses of ILP100 potently inhibited the growth and reduced the numbers of wound pathogens with up to log 6.4 CFU after 12 h treatment in two different media. These findings support the potential of ILP100 for wound infection therapy, warranting further studies to explore its mechanism, dosing, and in vivo efficacy. Elimination of MDR bacteria by the addition of ILP100 shows that antagonistic bacteria may provide promising new treatment options in the face of the currently stagnating antimicrobial pipeline.

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Data Availability. All relevant data is included in this communication or in the Supplementary Material.

Declarations

Conflicts of Interests. Hava Lofton-Tomenius, Yanhong Pang, Anton Pallin, Zhanar Myktybekova, Ninus Lelham and Evelina Vågesjö are current or former employees of Ilya Pharma. Mia Phillipson is on the board of directors. Stefan Roos can be considered an uncompensated standing scientific advisor. Employees of Ilya Pharma may have stock options. A patent has been filed related to the antimicrobial activity. Kristian Riesbeck has no competing interests.

Ethics Approval. Ethical approval was not required for this study.

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