

Supplemental Material

Table S1 - MIC determination of gallium citrate against multiple bacterial species

Bacteria	Number of Strains	MIC* (ug/mL)	MIC range (ug/mL)	MIC* (ug/mL)	MIC range (ug/mL)	MIC* (ug/mL)	MIC range (ug/mL)
Gram Negative		Gallium Citrate		Tobramycin		Aztreonam	
<i>P. aeruginosa</i>	160	0.5	0.12 - 8	4	1 - 256	2	1 - 64
<i>Klebsiella pneumonia</i> (20 ESBL strains)	31	0.5	0.03 - 2	>8	1 - >256	16	<0.03 - >256
<i>K. pneumonia</i> NDM-1 strain**	1	-	0.125	-	>256	-	64
<i>E. coli</i> (17 ESBL strains)	29	8	<0.025 - 32	>8	1 - >32	2	<0.03 - 32
<i>Stenotrophomonas maltophilia</i>	10	0.5	0.25-1	16	4 - >32	32	4 - >32
<i>Acinetobacter baumannii</i> (Includes 30 multidrug resistant strains)	40	8	0.02 - 32	-	-	-	-
<i>Burkholderia cepacia</i> & <i>cenocepacia</i>	15	8	1-32	>32	0.25 - >32	>32	64 - >256
Gram Positive		Gallium Citrate		Tobramycin		Vancomycin	
<i>Streptococcus pneumoniae</i>	10	32	4-64	16	4 - 32	-	-
<i>MRSA & MSSA</i>	24	16	4 - >32	16	0.25 - >32	<0.25	<0.25-0.5

*Values are mean MIC, where >10 strains were tested, the MIC values are MIC₅₀
**New Delhi metallo-beta-lactamase (NDM-1) *K. pneumonia* strain from the ATCC® BAA-2146™
All MIC testing was performed in iron-deficient BM-2 broth media

Table S2 - Antibiogram of KP4640 was determined using the Phoenix (Becton, Dickinson and Co., Franklin Lakes, NJ) automated system according to the manufacturer's instructions. STS is an acronym for sterile tissue site. Strain is considered sensitive (S), resistant (R) or intermediate (I) to each given antibiotic.

Strain	Isolation site	Antibiotic	MIC (µg/mL)	Sensitivity
4640	STS	Amikacin	≤8	S
4640	STS	Amoxicillin-Clavulanate	>16/8	R
4640	STS	Ampicillin	>16	R
4640	STS	Ampicillin-Sulbactam	>16/8	R
4640	STS	Aztreonam	>16	R
4640	STS	Cefazolin	>16	R
4640	STS	Cefepime	>16	R
4640	STS	Ceftazidime	>16	R
4640	STS	Ceftriaxone	>32	R
4640	STS	Ciprofloxacin	>2	R
4640	STS	Ertapenem	>4	R
4640	STS	Gentamicin	>8	R
4640	STS	Imipenem	8	I
4640	STS	Levofloxacin	>4	R
4640	STS	Moxifloxacin	>4	R
4640	STS	Nitrofurantoin	>64	R
4640	STS	Piperacillin-Tazobactam	>64/4	R
4640	STS	Tetracycline	4	S
4640	STS	Tobramycin	>8	R

Figure S1

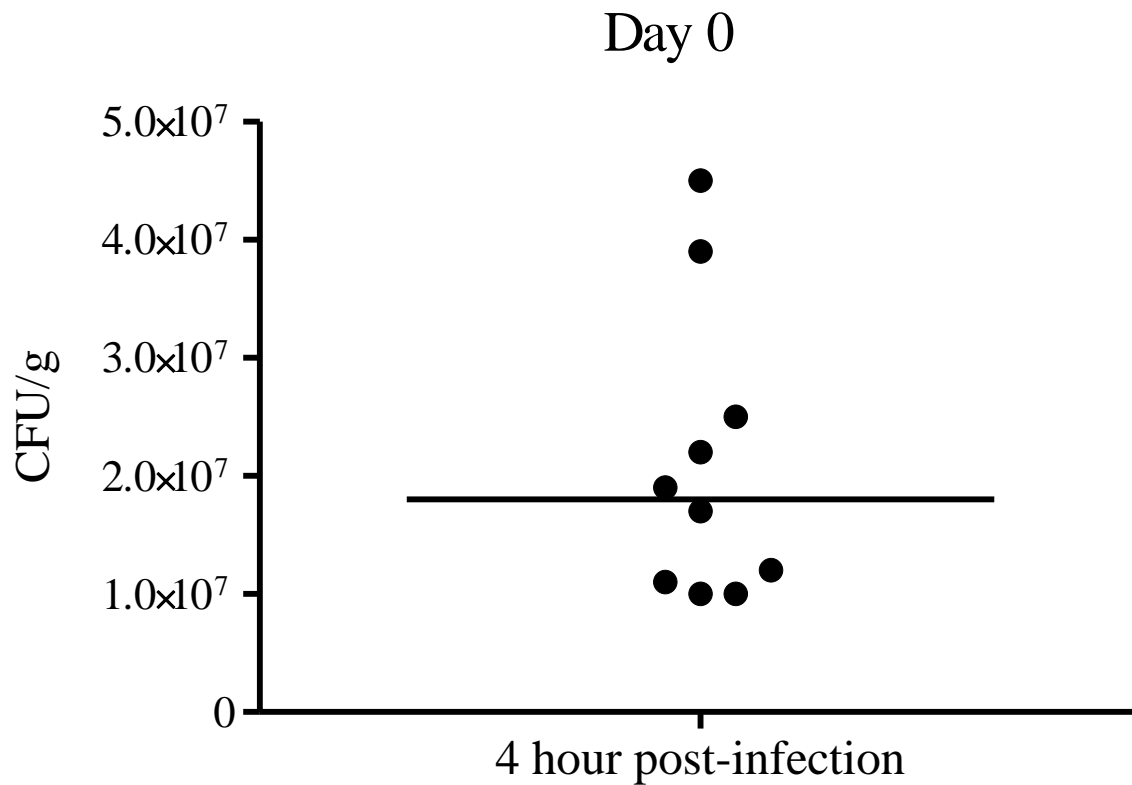


Figure S1 - Bacterial burden in the whole bed at 4 hours post-inoculation

Bacterial burden of KP4640 in the entire wound bed (not just 4 mm² punch biopsy) of mice was measured at 4 hours post-inoculation. Individual mice are represented by black dots. The mean is represented by the horizontal line. The results are from two biological replicates of 5 mice each.

Figure S2

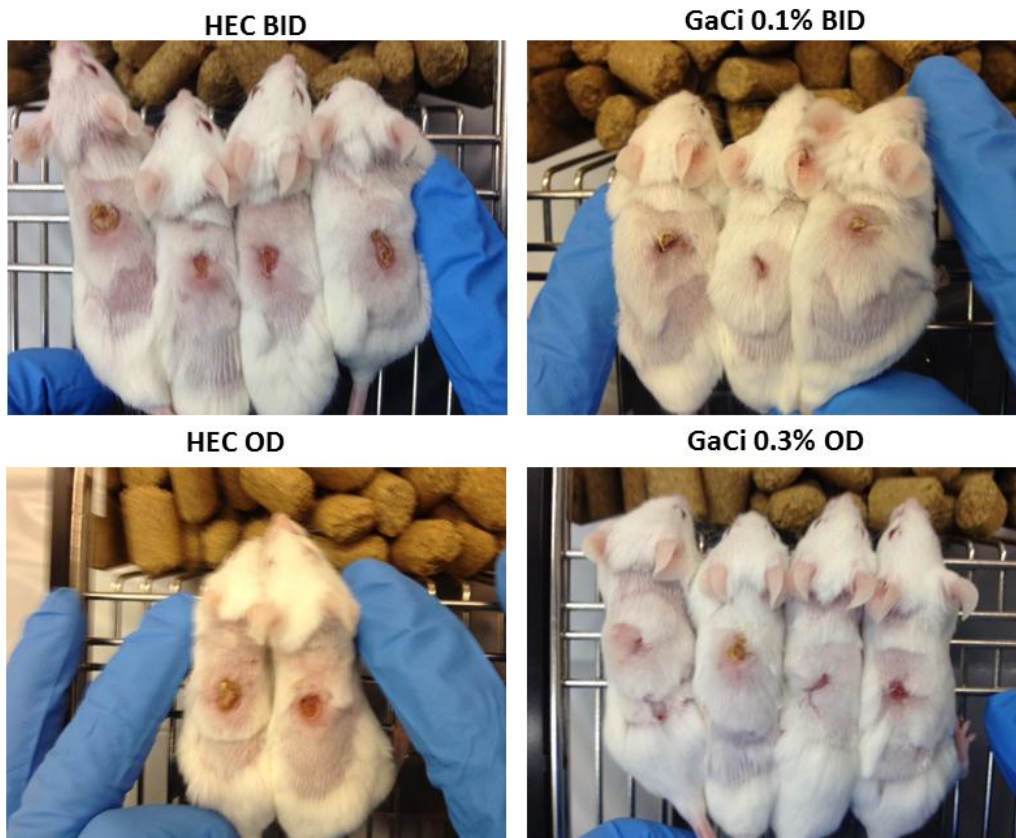
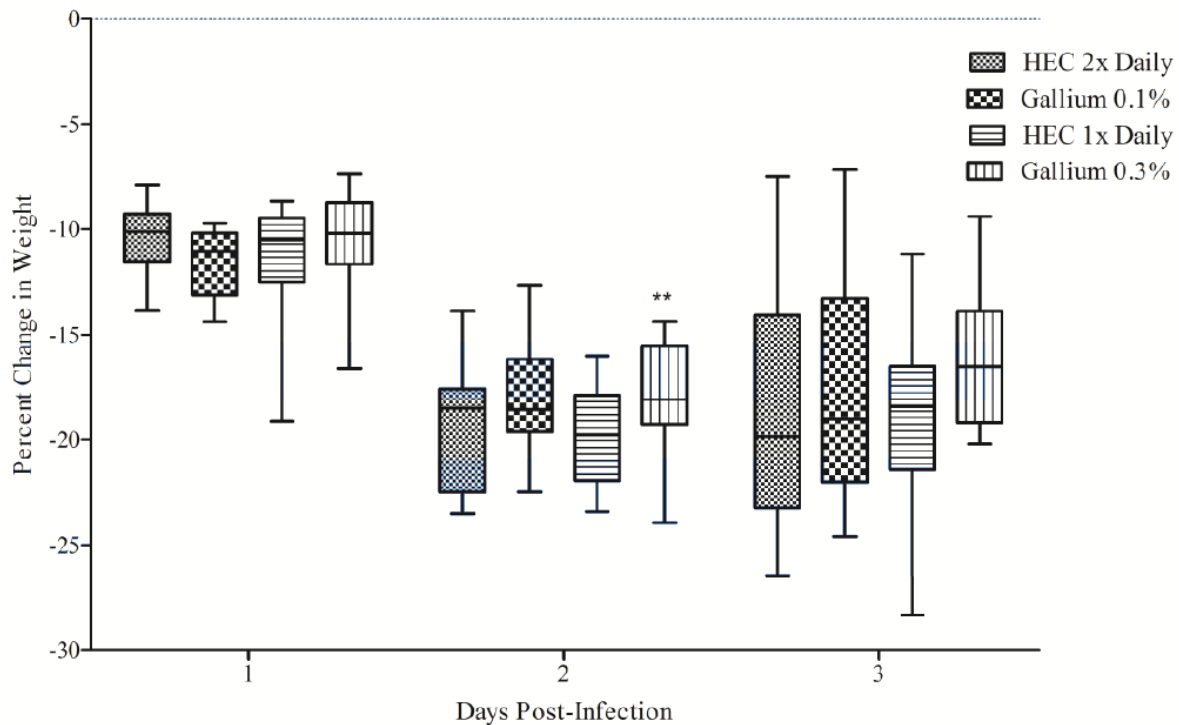


Figure S2 – Day 20 Gross Pathology

Photographs of gross pathology of full-thickness wound over time in representative BALB/c mice. Panels show full dorsal wounds on Day 20. These images are representative of treated animals with HEC BID or OD (negative controls) or HEC with GaCi 0.1% BID or GaCi 0.3% OD from two separate experiments with 12 mice per group.

Figure S3



2.5

Figure S3 - Weight change

Box and Whisker plots of percent weight change on days 1 through 3 post-inoculum. Boxes show median and interquartile ranges, while whiskers represent 95% CI. Groups were compared each day via Mann-Whitney U-test; ** represent p -value of 0.01. These data are pooled from three separate experiments with 12 mice per group, 48 mice per test condition. Mice were treated with HEC alone OD or HEC + GaCi at 0.3% OD for 3 days, or mice were treated with HEC alone BID or HEC + GaCi at 0.1% BID for 3 days.

Figure S4

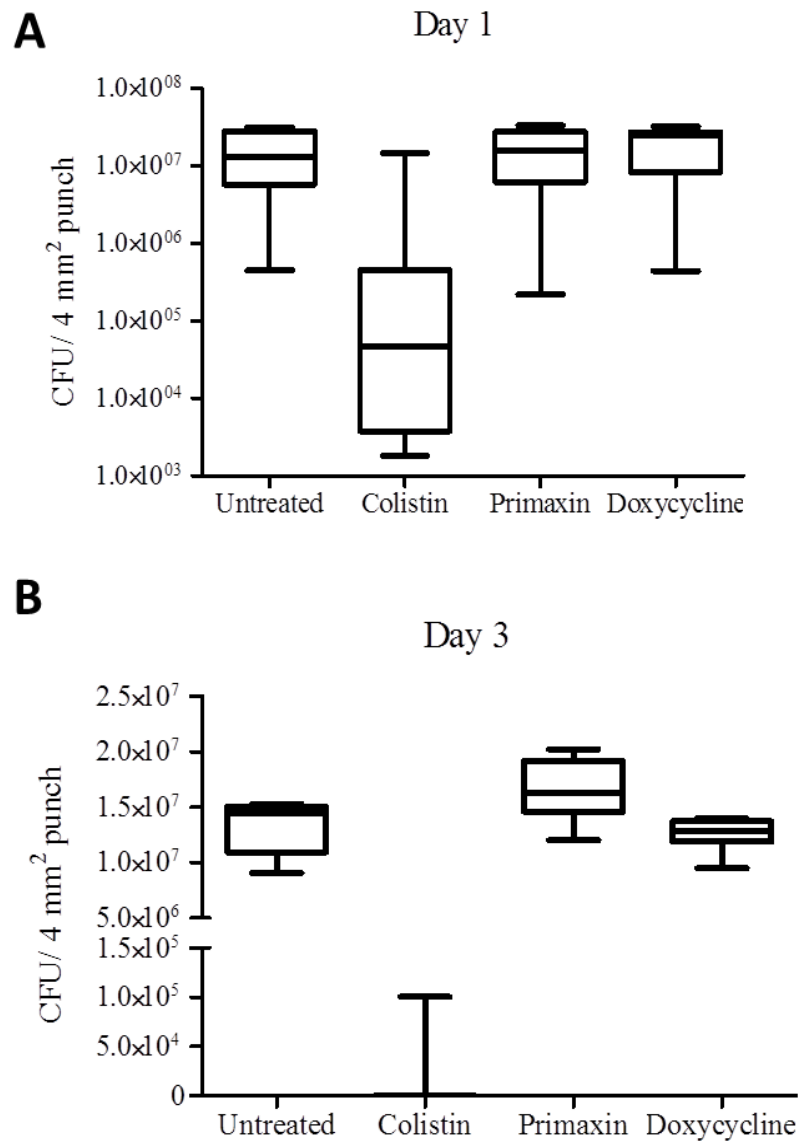


Figure S4 - Comparison of antibiotic treatment of KP4640-infected mice in the murine wound model of infection

Balb/C mice (two groups (biological replicates) of five mice, 10 mice total) were infected with 5.0×10^5 CFU on day 0. At four hours, mice were given colistin (2.5 mg/kg) topically, Primaxin™ (2.5 mg/kg) via IP injection, or doxycycline (25 mg/kg) via IP

injection. Subsequently, mice were treated with the same doses BID for the next three days. On day 1 (A) or day 3 (B), mice were sacrificed and 4 mm² punch of tissue was removed from the wound bed and the bacterial burden was calculated. The colistin results on Day 1 and Day 3 are statistically significant when compared to untreated via the Mann Whitney t-test. On Day 3, the colistin CFU are also statistically significant when compared to all of the other tested groups via Kruskal Wallis (ANOVA) test followed by Dunn's Multiple Comparisons.