SUPPLEMENTARY METHODS

Sample and metadata collection

Fecal samples were collected from November of 2013 until April of 2015 from all acute leukemia patients hospitalized at the Hospital La Fe (Valencia, Spain) who agreed to participate in this study. Sampling was performed weekly during every hospitalization period that each patient underwent, allowing us to obtain several samples for each patient. Samples were kept at 4ºC for less than 24 h. Subsequently, fecal samples were resuspended in autoclave-sterilized PBS 15% glycerol (~200mg/ml) in order to preserve viability of bacteria upon freezing and kept at -80ºC until further processing. Notably, no differences in the intestinal MRE levels were detected if fecal samples were cultured immediately or after thawing from being frozen at -80ºC (N= 30 samples tested; p= 0,412; two-tailed paired t-test).

Patient related metadata was prospectively collected and recorded in a computerized database in Access®. The metadata collected included antibiotic and antifungal treatments, neutropenia status, mucositis development, parenteral feeding, type of leukemia, reason of hospital admission (i.e. chemotherapy, transplant, infection, graft versus host disease), gender and age. Blood was drawn daily, during the time a patient was hospitalized, and neutrophils were counted in order to determine if patient was neutropenic. Neutropenia was defined as having less than 500 absolute neutrophil counts per μl of blood.

Due to the type of analysis performed (dynamics of MRE levels across time), we included only patients from which we had collected 2 or more samples during the same hospitalization period. Five patients were excluded due to the lack of metadata required for the analysis leaving us with a total of 133 patients.

Determination of MRE colonization levels

In order to quantify the levels of MRE, fecal samples stored at -80ºC were thawed, 10-fold diluted in PBS and plated in Brilliance ESBL Agar plates (Oxoid), which contain a 3rd generation cephalosporin as selective agent. These plates allow for isolation of Extended Spectrum Beta-Lactamase producing organisms, while inhibiting the growth of non-ESBL Enterobacteriaceae and most AmpC organisms, allowing for identification of ESBL-producing *E. coli* and the *Klebsiella, Enterobacter, Serratia* and *Citrobacter* group (known as KESC). Brilliance ESBL Agar plates were chosen for isolation and quantification of MRE due to the clinical relevance of acquisition of bacterial resistance to 3^{rd} generation cephalosporins. Besides 10-fold dilutions, 100 μl of the original non-diluted sample (resuspended in PBS 15% glycerol) was also plated in these types of plates. Plates were incubated for 24 h at 37°C. If no growth was observed, the plate was left for additional 24 h at 37°C in order to confirm the negative result. The number of colonies in each plate was normalized by dilution and fecal weight in order to calculate the levels of colonization of MRE per gram of feces.

Characterization of MRE isolates from patients' fecal samples

In order to confirm that the detected colonies were MRE, 5 isolated colonies from each sample were subjected to taxonomic identification through MALDI-TOF MS, including, if present, colonies that had a different color and/or morphology. In addition, the antibiotic resistant pattern was determined through the Vitek 2 system, using the antimicrobial susceptibility testing cards for Enterobacteriaceae. Antibiotics tested include amikacin, amoxicillin-clavulanic acid, ampicillin, cefepime, cefotaxime, cefoxitin, ceftazidime, cefuroxime, ciprofloxacin, gentamicin, imipenem, ertapenem, piperacillin-tazobactam, tigecycline, and trimethoprim-sulfamethoxazole (cotrimoxazole). The susceptibility was determined according to the Clinical and Laboratory Standards Institute Guidelines (2016). In addition, resistance to meropenem was evaluated using ETEST antibiotic gradient strips (bioMérieux) in strains isolated from patients that had received meropenem (before and after treatment initiation).

An isolate was considered multidrug resistant (MRE) if it was non-susceptible to at least 1 agent in 3 or more antimicrobial categories defined by Magiorakos and co-workers (1), not taking into account those antibiotics for which the bacterium is intrinsically resistant. All characterized isolates fulfilled the definition of MRE.

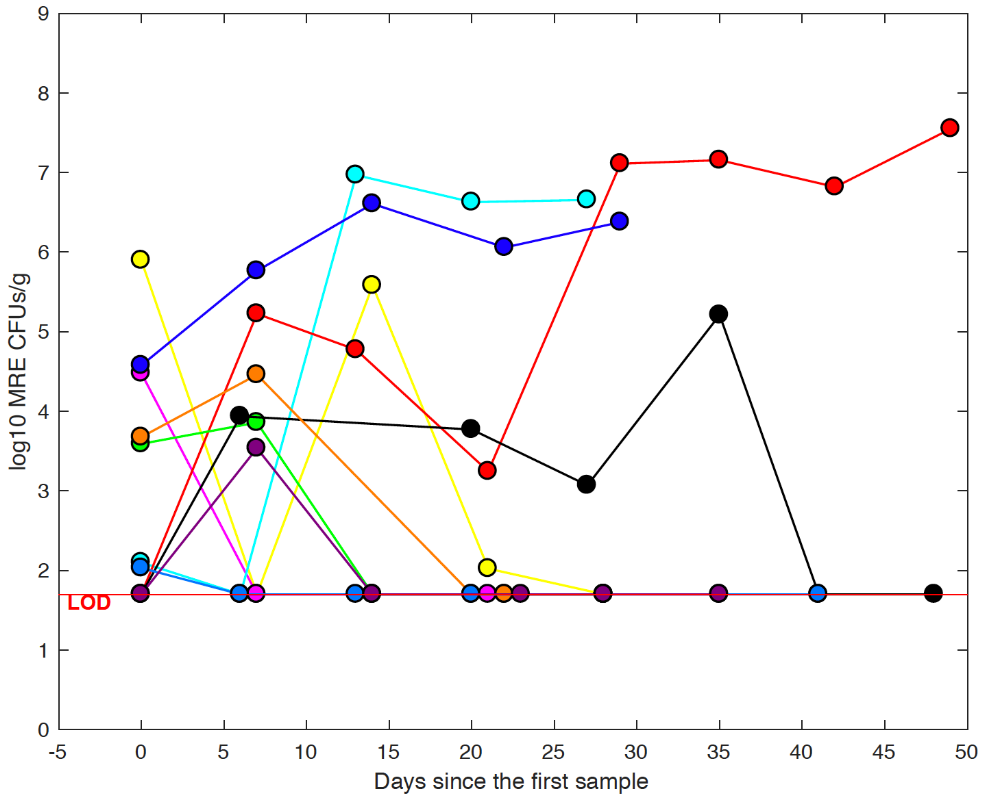
Production of extended spectrum beta-lactamases was confirmed in a subset of MRE isolates (Supplementary Table 11, Figure 4) through Double Disk Synergy Test (DDST) as described in the EUCAST guidelines ("Antimicrobial susceptibility testing EUCAST disk diffusion method") and interpreted according to the "EUCAST guidelines for the detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance". When DDST was insufficient for the verification of ESBL phenotype, a genotypic confirmation test was performed as previously described (2).

Multivariate regression analysis

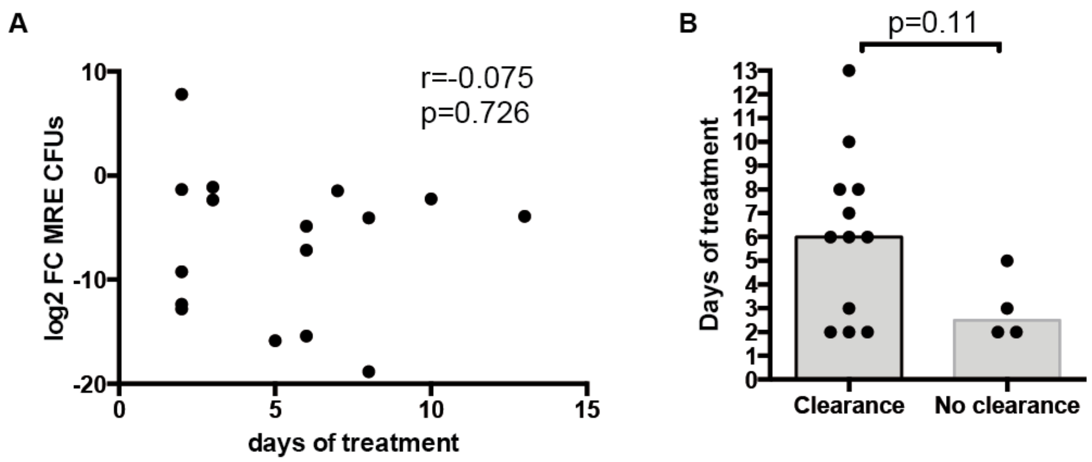
For confirming the independent association between MRE levels and the clinical variables under study, multivariate regression analysis was performed on all variables collected from patients (i.e. antibiotics, antifungals, neutropenia, parenteral nutrition, mucositis, type of admission, gender, age and type of leukemia). Lasso regression implemented in Matlab with positive penalty term λ was applied in order to achieve a sparse solution. This allowed selection of those variables that are independently associated with the change in MRE levels.

P values lower than 0.05 were considered statistically significant.

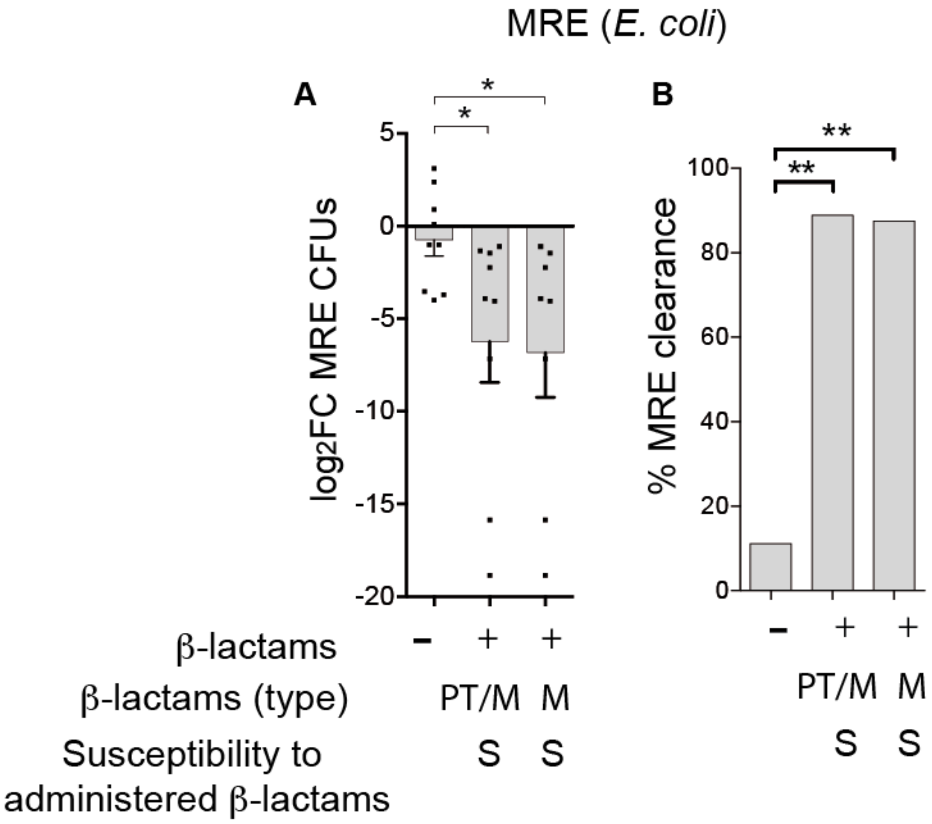
SUPPLEMENTARY FIGURES



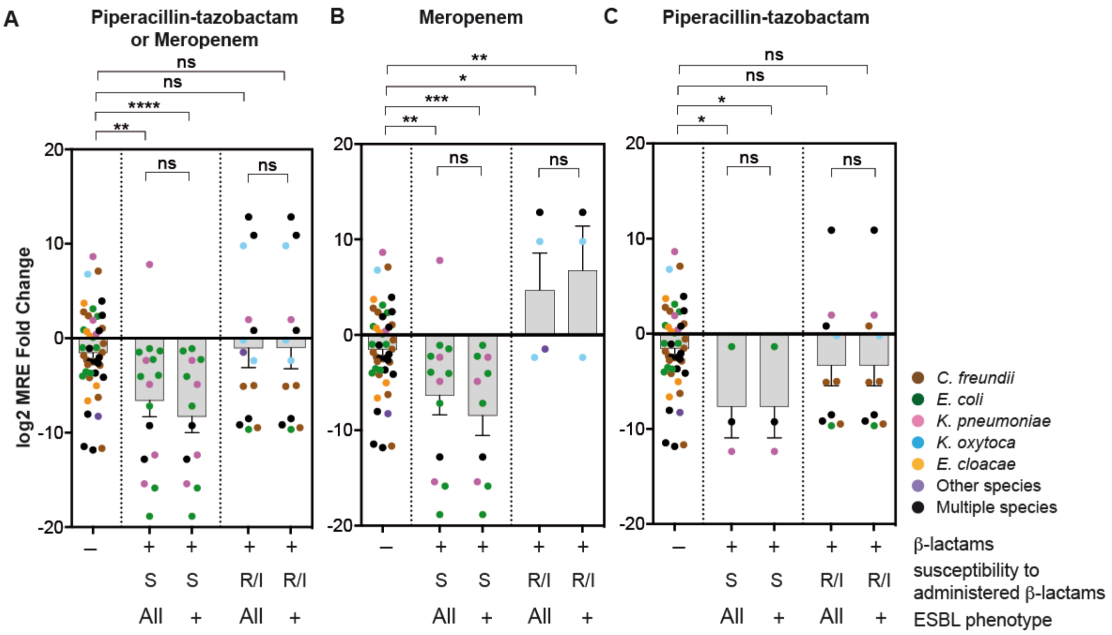
Supplementary Figure 1. Changes in MRE levels in acute leukemia patients during hospitalization. MRE levels in samples collected during a patient's hospital admission are shown. Levels from the same patient are connected with a line. Different patients are labeled with different colors. The figure shows only patients with more than 4 samples collected during the same hospital admission period after MRE detection. LOD= limit of detection. N= 10 patients.



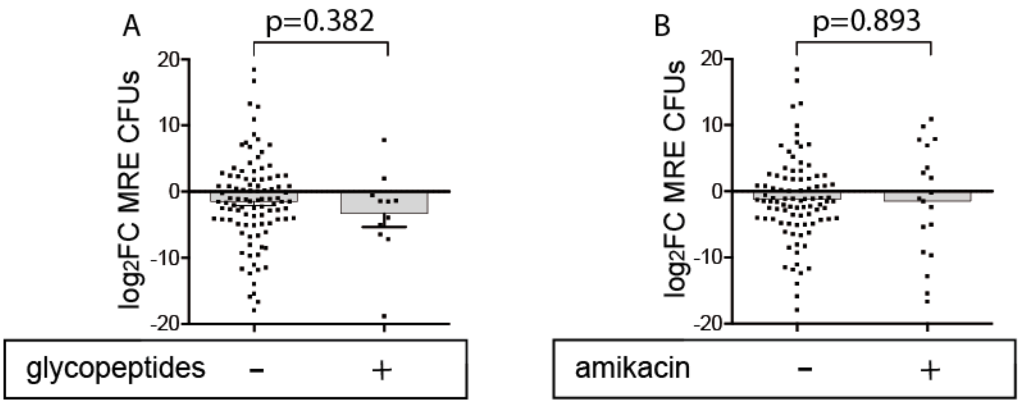
Supplementary Figure 2. Effect of Meropenem/PTZ treatment length on the changes in MRE levels of susceptible strains. (A) Spearman correlation analysis between (i) the MRE Log2 Fold change between consecutive samples of the pair, collected before and after the initiation of a Meropenem and/or PTZ treatment and (ii) the length of the treatment (i.e. from the date of initiation until the date of collection of the second sample, since all treatments were administered beyond the second sample was collected). Note that only pairs of samples containing exclusively strains that are susceptible to the administered beta-lactams are included in the analysis. N=16 pairs of samples from 15 patients. (B) Comparison (two-sided Wilcoxon test) of treatment length between pairs of samples included in Figure A in which MRE strains could be detected in the second sample of the pair (not clearance) or could not be detected (clearance). N= 4-12 pairs of samples per group.



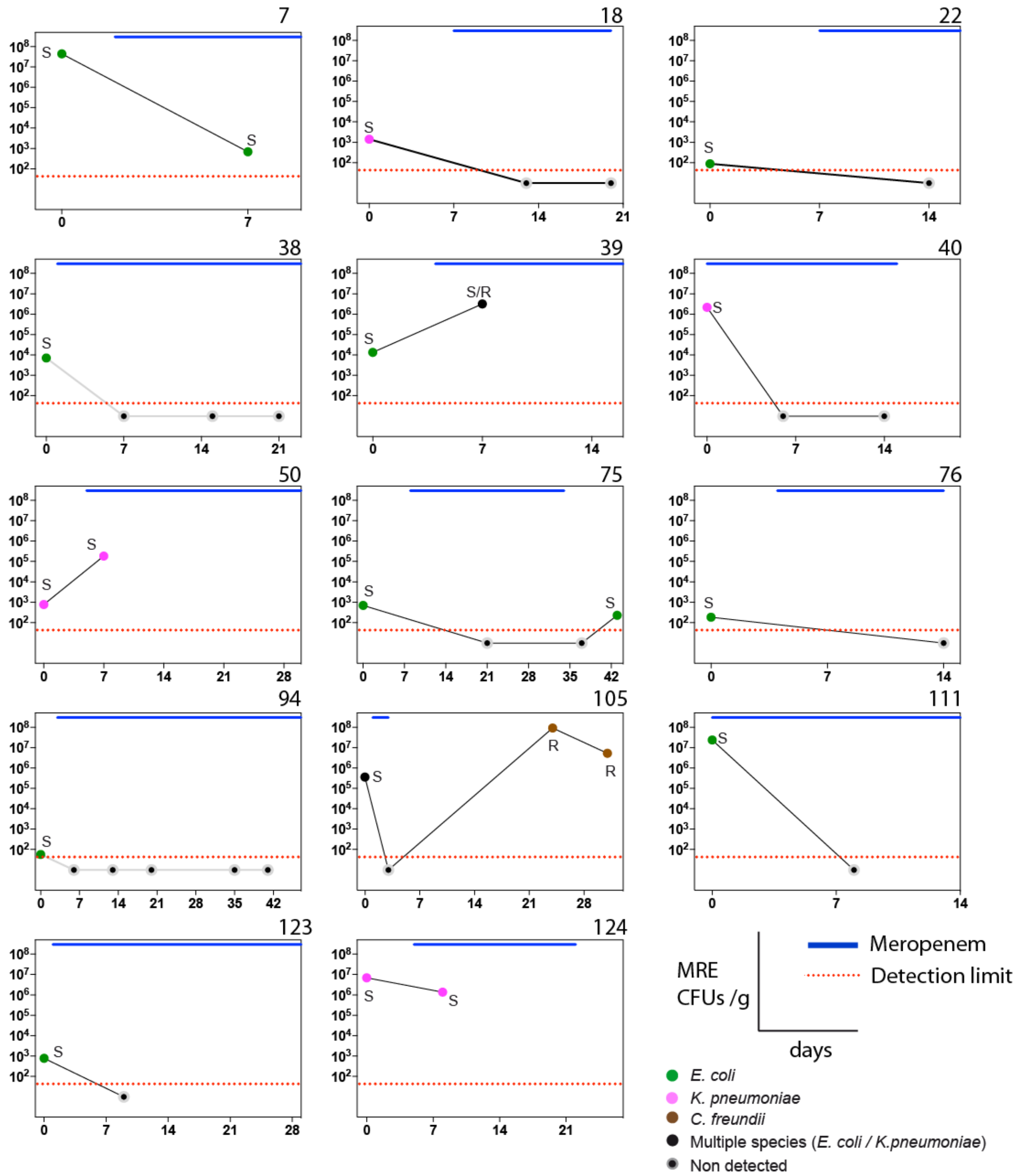
Supplementary Figure 3. Beta-lactams (i.e. piperacillin-tazobactam, meropenem) reduce the fecal levels of multidrug-resistant *E. coli* susceptible to the antibiotic administered. **(A)** Multidrug-resistant *E. coli* $\log_2$FC among pairs of consecutive samples in which a beta-lactam (i.e. piperacillin-tazobactam (PT) and/or meropenem (M)) was administered (+) or not (-). Only pairs of samples containing exclusively *E. coli* strains susceptible to the administered beta-lactam are included in the groups PT/M or M. Pairs of samples in which exclusively the beta-lactam meropenem was administered are shown in the M group. Due to the low number of pairs of samples (N=1), the individual effect of piperacillin-tazobactam on MRE (*E. coli*) levels could not be evaluated. Only pairs of samples exclusively colonized with MREs classified as *E. coli* are included in the analysis. **(B)** % of pairs of samples shown in (A) in which MRE could not be detected in the second sample of the pair. * $p<0.05$; ** $p<0.01$; Two-tailed t-test (A) or Fischer test (B) comparing with the group of samples in which a beta-lactam was not administered. The number of pairs of samples (S) and patients (P) included in each group are: no beta-lactam (S=9, P=7); susceptible to beta-lactams (S=9, P=9); susceptible to meropenem (S=8, P=8).



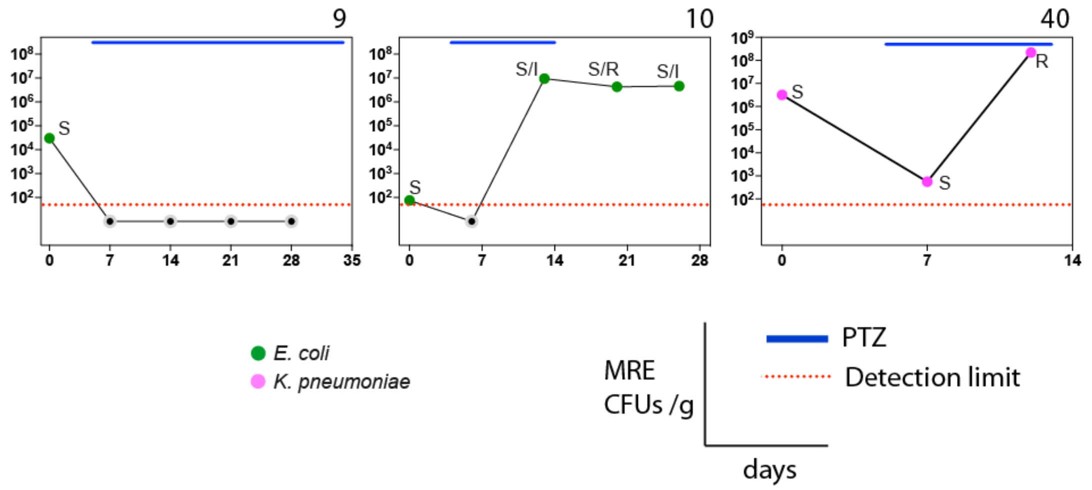
Supplementary Figure 4. Impact of IV beta-lactams (piperacillin-tazobactam and meropenem) on MRE fecal levels that are ESBL producers depends on the MRE resistance profile. (A) MRE $\log_2$FC among pairs of consecutive samples between which a beta-lactam (i.e. piperacillin-tazobactam or meropenem) was administered (+) or not (-). MRE strains detected in the consecutive pairs of samples were either susceptible (S), or non-susceptible (R/I) towards the administered beta-lactam. ESBL phenotype was determined. Data bars are displayed separately for ESBL (+) and both ESBL/non-ESBL strains (All). (B, C) Same as in (A) but only including pairs of samples in which the beta-lactam therapy initiated between the samples of the pair was exclusively meropenem (B) or exclusively piperacillin-tazobactam (C). *P<0.05; **P<0.01; ***P<0.001; ***P<0.0001 two-tailed t-test compared with the group not receiving beta-lactams. The results show that beta-lactams (i.e. meropenem and piperacillin-tazobactam) reduce the levels of MRE strains susceptible to the beta-lactam administered, even if they are producers of ESBL. Colors indicate the taxonomy of the MRE identified in each pair of samples. Detailed taxonomy and antibiotic resistant pattern of all the MREs identified within each pair of samples is shown in Supplementary Table 11. The number of pairs of samples (S) and patients (P) included in each group are: no beta-lactam (S=46, P=27); susceptible to beta-lactams All (S=16, P=15); susceptible to beta-lactams ESBL+ (S=13, P=12); non-susceptible to beta-lactams All (S=14, P=9); non-susceptible to beta-lactams ESBL+ (S=13, P=9); susceptible to meropenem All (S=13, P=13); susceptible to meropenem ESBL+ (S=10, P=10); non-susceptible to meropenem All (S=4, P=3); non-susceptible to meropenem ESBL+ (S=3, P=2); susceptible to PTZ All (S=3, P=3); susceptible to PTZ ESBL+ (S=3, P=3); non-susceptible to PTZ All (S=10, P=8); non-susceptible to PTZ ESBL+ (S=10, P=8).



Supplementary Figure 5. Effect of glycopeptides and aminoglycosides (i.e. amikacin) on MRE intestinal levels. (A) MRE log2FC among pairs of consecutive samples in which glycopeptides (i.e. vancomycin, teicoplanin) were administered (+) or not (-). **(B)** Same as in (A) but for the antibiotic amikacin. P values are indicated; Two-tailed t-test. Bar represents the mean, lines represent the SEM. The number of pairs of samples (S) and patients (P) included in each group are: no glycopeptides (S=99, P=48); glycopeptides (S=11, P=11); no amikacin (S=91, P=46); amikacin (S=19, P=16).



Supplementary Figure 6. Meropenem generally decrease fecal levels of susceptible MRE but occasionally resistant strains emerge. MRE levels and sensitivity pattern to meropenem before and after the initiation of meropenem therapy. Each panel contains the sample collected before meropenem treatment and all the consecutive samples collected from that specific patient during the same hospital admission period. The ID of each patient is indicated in each panel. The period in which the patient received meropenem is indicated with a blue line. Sensitivity to meropenem is indicated when the MRE could be detected. Note that the first sample contains always MRE strains susceptible to meropenem. S= susceptible, R= resistant, I=intermediate phenotype. Colors indicate the taxonomy of the MRE identified in each sample. Days are relative to the date of the first sample included in the figure for each patient. The length of the X axis matches the number of days from the first sample included in the figure until the end of the hospital admission.



Supplementary Figure 7. PTZ decrease fecal levels of susceptible MRE but resistant strains emerge. MRE levels and sensitivity pattern to PTZ before and after the initiation of PTZ therapy. Each panel contains the sample collected before PTZ treatment and all the consecutive samples collected from that specific patient during the same hospital admission period. The ID of each patient is indicated in each panel. The period in which the patient received PTZ is indicated with a blue line. Sensitivity to PTZ is indicated when the MRE could be detected. Note that the first sample contains always MRE strains susceptible to PTZ. S= susceptible, R= resistant, I=intermediate phenotype. Colors indicate the taxonomy of the MRE identified in each sample. Days are relative to the date of the first sample included in the figure for each patient. The length of the X axis matches the number of days from the first sample included in the figure until the end of the hospital admission.

SUPPLEMENTARY TABLES

Supplementary Table 1. Characteristics of 133 acute leukemia patients included in the study[a].

Parameter	No. (%)
Age (years)	
<30	15 (11.3)
30-39	22 (16.5)
40-49	29 (21.8)
50-59	32 (24.1)
>59	35 (26.3)
Gender	
Male	72 (54.1)
Female	61 (45.9)
Type of leukemia	
Myeloid	98 (73.7)
Lymphoid	31 (23.3)
Biphenotypcic	4 (3)

[a] Numbers in parenthesis represent % of total patients.

Supplementary Table 3. Characteristics of the 422 hospital admissions included in the study[a].

Parameter	
Cause of admission	No. (%)
Chemotherapy	226 (53.6)
Transplant	76 (18)
Infection	89 (21.1)
Other[b]	31 (7.3)
Antibiotics received[c]	No. (%)
Quinolones	285 (67.5)
Ciprofloxacin	270 (64)
Other quinolones[d]	21 (5)
Glycopeptides	164 (38.9)
Vancomycin	151 (35.8)
Teicoplanin	25 (5.9)
Beta-lactams	306 (72.5)
Piperacillin-tazobactam	190 (45)
Meropenem	187 (44.3)
Other beta-lactams[d]	36 (8.5)
Aminoglycosides	132 (31.3)
Amikacin	132 (31.3)
Other aminoglycosides[d]	3 (0.7)
Others	120 (28.4)
Linezolid	79 (18.7)
Metronidazole	11 (2.6)
Colistin	25 (5.9)
Tigecycline	12 (2.8)

[a] Numbers in parenthesis represent % of total admissions. Antibiotics variables are not mutually exclusive and do not sum to 100%.

[b] Other causes of admission include: treatment related complications such as graft-versus-host disease, organ toxicities, diagnostic procedures and other diseases non related to leukemia.

[c] Number of hospital admissions in which a specific antibiotic was administered

[d] Other quinolones include levofloxacin and moxifloxacin. Other beta-lactams include ampicillin, aztreonam, amoxicillin/clavulanic acid, cefepime, ceftazidime, ceftriaxone, cefuroxime, cloxacillin, ertapenem and imipenem. Other aminoglycosides include gentamicin and tobramycin.

Supplementary Table 4. Days of therapy (DOT) per 1000 patient days[a].

Antibiotics received	Mean DOT/1000 patient days	Median DOT/1000 patient days
Quinolones	350.75	304.76
Ciprofloxacin	329.5	284.21
Other quinolones[b]	25.32	0
Glycopeptides	132.9	109.09
Vancomycin	114.8	78.01
Teicoplanin	19.81	0
Beta-lactams	562.64	578.95
Piperacillin-tazobactam	252.25	225.81
Meropenem	288.38	230.77
Other beta-lactams[b]	36.49	0
Aminoglycosides	104.38	59.7
Amikacin	103.35	59.7
Other aminoglycosides[b]	1.03	0
Others	122.91	56.6
Linezolid	76.92	0
Metronidazole	14.43	0
Colistin	26.84	0
Tigecycline	9.19	0

[a] Numbers represent mean/median value across patients for days in which a patient received an antibiotic treatment, divided by total days that patient was in hospital and multiplied by 1000.

[b] Other quinolones include levofloxacin and moxifloxacin. Other beta-lactams include ampicillin, aztreonam, amoxicillin/clavulanic acid, cefepime, ceftazidime, ceftriaxone, cefuroxime, cloxacillin, ertapenem and imipenem. Other aminoglycosides include gentamicin and tobramycin.

Supplementary Table 7. Summary of the antibiotic resistant patterns of the isolated MREs

	Total MRE [a]	***E. coli*** [b]	***C. freundii***	***K. pneumoniae***	***K. oxytoca***	***E. cloacae***	***M. morganii***	***C. amalonaticus***
Penicillins [c]								
Ampicillin	344 (100)	108 (100)	IR	IR	IR	IR	IR	15 (100)
Penicillins + b-lactamase inhibitors								
Amoxicillin-clavulanate	312 (90.7)	77 (71.3)	IR	59 (100)	22 (100)	IR	IR	15 (100)
Antipseudomonal penicillins + β-lactamase inhibitors								
Piperacillin-tazobactam	283 (82.3)	67 (62)	90 (100)	47 (79.7)	22 (100)	23 (100)	10 (71.4)	15 (100)
1^{st} and 2^{nd} generation cephalosporins								
Cefuroxime	343 (99.7)	107 (99.1)	90 (100)	59 (100)	22 (100)	23 (100)	IR	15 (100)
3^{rd} and 4^{th} generation cephalosporins								
Ceftazidime	290 (84.3)	67 (62)	90 (100)	54 (91.5)	21 (95.4)	23 (100)	11 (78.6)	15 (100)
Cefotaxime	321 (93.9)	91 (84.3)	89 (100)	57 (96.6)	21 (95.4)	23 (100)	14 (100)	15 (100)
Cefepime	261 (76.3)	64 (59.3)	85 (95.5)	36 (61)	20 (90.9)	22 (95.6)	14 (100)	15 (100)
Cephamycins								
Cefoxitin	257 (74.7)	61 (56.5)	IR	31 (52.5)	21 (95.4)	IR	6 (42.8)	15 (100)
Carbapenems								
Imipenem	167 (48.5)	3 (2,8)	55 (97.8)	13 (22)	21 (95.4)	15 (65.2)	3 (21.4)	15 (100)
Ertapenem	184 (53.5)	11 (10.2)	87 (96.7)	27 (45.8)	20 (90.9)	16 (69.6)	2 (14.3)	15 (100)
Aminoglycosides								

Amikacin	29(8.4)	6 (5.6)	4 (4.4)	9 (15.2)	0	1 (4.3)	3 (21.4)	6 (40)
Gentamicin	191(55, 5)	22 (20.4)	73 (81.1)	46 (78)	20 (90.9)	13 (56.5)	14 (100)	1 (6.7)
Fluoroquinolones								
Ciprofloxacin	298 (86.6)	97 (89.8)	87 (96.7)	48 (81.4)	20 (90.9)	13 (56.5)	14 (100)	15 (100)
Nalidixic acid	310 (90.2)	101 (93.5)	89 (100)	49 (83)	20 (90.9)	17 (73.9)	14 (100)	15 (100)
Levofloxacin	298 (86.6)	97 (89.8)	87 (96.7)	48 (81.4)	20 (90.9)	13 (56.5)	14 (100)	15 (100)
Glycylcyclines								
Tigecycline	84 (24.6)	4 (3.7)	21 (23.6)	14 (23.7)	19 (86.4)	12 (52.2)	IR	0
Folate pathway inhibitors								
Co-trimoxazole	252 (73.5)	84 (78.5)	61 (67.8)	43 (72.9)	21 (95.4)	14 (60.9)	12 (85.7)	11 (73.3)

[a] Number of isolates that have a resistant or intermediate phenotype to the antibiotic indicated. Numbers in parenthesis represent % of total isolates. Those isolates obtained from the same sample that had the same taxonomy and resistant pattern were only considered once in this table.

[b] Percentage of resistance is shown for individual species with > 5 identified isolates. Intrinsic resistance to a certain antibiotic, as defined by Magiorakos and co-workers [1], is indicated as IR

[c] Antimicrobial categories defined by Magiorakos and co-workers are highlighted in grey [1].

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