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Clinical Use of Colistin Induces Cross-Resistance to Host Antimicrobials in *Acinetobacter baumannii*

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**ABSTRACT** The alarming rise in antibiotic resistance has led to an increase in patient mortality and health care costs. This problem is compounded by the absence of new antibiotics close to regulatory approval. *Acinetobacter baumannii* is a human pathogen that causes infections primarily in patients in intensive care units (ICUs) and is highly antibiotic resistant. Colistin is one of the last-line antibiotics for treating *A. baumannii* infections; however, colistin-resistant strains are becoming increasingly common. This cationic antibiotic attacks negatively charged bacterial membranes in a manner similar to that seen with cationic antimicrobials of the innate immune system. We therefore set out to determine if the increasing use of colistin, and emergence of colistin-resistant strains, is concomitant with the generation of cross-resistance to host cationic antimicrobials. We found that there is indeed a positive correlation between resistance to colistin and resistance to the host antimicrobials LL-37 and lysozyme among clinical isolates. Importantly, isolates obtained before and after treatment of individual patients demonstrated that colistin use correlated with increased resistance to cationic host antimicrobials. These data reveal the overlooked risk of inducing cross-resistance to host antimicrobials when treating patients with colistin as a last-line antibiotic.

**IMPORTANCE** Increased use of the cationic antibiotic colistin to treat multidrug-resistant *Acinetobacter baumannii* has led to the development of colistin-resistant strains. Here we report that treatment of patients with colistin can induce not only increased resistance to colistin but also resistance to cationic antimicrobials. This worrisome finding likely represents an example of a broader trend observed in other bacteria against which colistin is used therapeutically such as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Furthermore, these data suggest that the possible future use of an array of cationic antimicrobial peptides in development as therapeutics may have unintended negative consequences, eventually leading to the generation of hypervirulent strains that are resistant to innate host defenses. The potential for the induction of cross-resistance to innate immune antimicrobials should be considered during the development of new therapeutics.

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Due to overuse and misuse of antibiotics and the ability of bacteria to rapidly gain resistance, we are now in an age of multidrug-resistant (MDR) and pan-drug-resistant (PDR) bacterial pathogens and face a possible return to the pre-antibiotic era. The burden of antimicrobial resistance has increased the length of hospital stays, patient mortality, and health care costs (1–5). *Acinetobacter baumannii* is a Gram-negative and highly antibiotic-resistant bacterial pathogen (6, 7). Due to its ability to persist on surfaces for weeks, its ability to colonize humans, and its increasing rate of antibiotic resistance, *A. baumannii* has become an emerging health care problem (6, 8).

Polymyxin antibiotics, including colistin, are currently used as last-line drugs to treat MDR *A. baumannii* infections (9). Colistin is a cationic antimicrobial peptide that disrupts both the outer and inner membranes of Gram-negative bacteria (10), a trait shared by the host cationic antimicrobials LL-37 and lysozyme (11–16). LL-37 is a human antimicrobial peptide typically found at sites of inflammation, where it is a primary defense against Gram-negative bacteria (17). Lysozyme is a host antimicrobial found within multiple immune cells as well as in secretions such as tears, breast milk, and mucus and is important for their activity against invading microbes. Importantly, the highly cationic, nonenzymatic, C-terminal portion of lysozyme has very potent antimicrobial activity (12–14).

Given the increasing prevalence of colistin resistance among *A. baumannii* clinical isolates (9), we set out to test whether there was a correlation with cross-resistance to host cationic antimicrobials. We assembled a panel of *A. baumannii* isolates (CI-1, CI-2, CI-3, 17978, CI-4, ARLC, MU134, MU215, MU181, and MU52) and first determined their colistin MICs (see Table S1 in the sup-
plemental material) and susceptibilities to multiple other antibiotics (Table S2). Bacterial suspensions were prepared using a PROMPT (3M Company, St. Paul, MN) inoculation system, and antibiotic susceptibilities were determined using Neg Breakpoint Combo Panel type 41 on a Microscan WalkAway Plus automated system (Siemens Healthcare Diagnostics Inc., West Sacramento, CA). Additionally, the MICs for colistin were measured using Etest strips (bioMérieux, Durham, NC), following the inoculation and reading instructions of the manufacturer. The strains exhibited a range of MIC values. Using Clinical and Laboratory Standards Institute (CLSI) interpretive criteria, we found that strains CI-1, CI-2, CI-3, and 17978 were sensitive to colistin whereas CI-4, ARLC, MU134, MU215, MU181, and MU52 were resistant (Table S1).

We treated the colistin-sensitive and -resistant isolates with LL-37 or lysozyme to determine their sensitivities, as previously described (18). Briefly, overnight cultures were grown from frozen stock in lysogeny broth (LB; also known as Luria broth) (BD Biosciences, Sparks, MD) at 37°C with aeration and then diluted to a final concentration of ~10^6 CFU/ml in 25% LB. Bacteria

![Fig 1](image-url)
were treated with host antimicrobials (LL-37, 6.25 µg/ml; lyso-
some, 2.5 mg/ml) and incubated with aeration at 37°C, and ali-
quots were plated at 0 h, 1 h, and 2 h for enumeration of CFU. The
colistin-sensitive clinical isolates CI-1 (Fig. 1A) and CI-2 (Fig. 1B)
were inhibited from replicating to wild-type levels in the presence
of LL-37, while CI-3 (Fig. 1C) and 17978 (Fig. 1D) were killed. In
contrast, the colistin-resistant clinical isolates CI-4 (Fig. 1E),
ARLC (Fig. 1F), MU134 (Fig. 1G), MU215 (Fig. 1H), MU181
(Fig. 1I), and MU52 (Fig. 1J) each replicated roughly 100- to 200-
fold after 2 h in the presence of LL-37. These data suggest that
colistin resistance correlates with increased resistance to the host
cationic antimicrobial peptide LL-37, which was further clearly
demonstrated when results from 4 experiments performed with
the aforementioned strains were pooled (Fig. 1K). We observed
similar phenotypes following lysozyme treatment, with the excep-
tion of the colistin-susceptible 17978 isolate (Fig. 1D), which was
able to persist in the presence of lysozyme instead of being killed,
and CI-2 (Fig. 1B), which exhibited very limited replication. These
results were again further illustrated when data from several ex-
periments were pooled (Fig. 1L) and suggest that colistin resis-
tance is highly correlated with resistance to lysozyme.

We next investigated the basis for the resistance phenotypes
observed. The A. baumannii PmrAB two-component regulatory
system induces phosphoethanolamine modifications to the lipid
A component of lipopolysaccharide (LPS) in response to the pres-
ence of polymyxins (19, 20). Arroyo et al. showed that point mu-
tations within the PmrB periplasmic domain, histidine kinase
(HisK) dimerization/phosphoacceptor domain, or C-terminal
ATP binding domain (ATPB) can confer constitutive polymyxin
resistance through these lipid A modifications (20). We sequenced
the pmrB genes in the colistin-resistant isolates used in this study
and found that each harbored nonsynonymous mutations in the
sequences encoding one or more of these three domains com-
pared to the pmrB sequence from the colistin-sensitive 17978
strain (see Table S1 in the supplemental material). In contrast,
only synonymous mutations were identified in the sequences of
pmrB from the colistin-sensitive strains. Nonsynonymous point
mutations conferring resistance can be selected rapidly and likely
explain the ability of some of the colistin-sensitive strains to eventu-
ally acquire resistance during extended exposure to LL-37 or
lysozyme (see Fig. S1A to D in the supplemental material) (20).
Taken together, these data suggest that mutations within pmrB
confer not only colistin resistance but also cross-resistance to host
cationic antimicrobial peptides.

Since the clinical isolates used here were very diverse (isolated
from different anatomical sites, from different areas of the United
States, and exhibiting differing antibiotic susceptibility patterns;
see Table S1 and S2 in the supplemental material), it is not sur-
prising that there was heterogeneity in their patterns of resistance
to host antimicrobials; specifically, the colistin-sensitive CI-2 iso-

FIG 2 Clinical treatment with colistin can induce increased resistance to host cationic antimicrobials. Sequential A. baumannii isolates were collected from 2
patients (Ab6266 and Ab6267, patient 1; Ab3527 and Ab3941, patient 2) pre-colistin treatment (Ab6266 and Ab3527) and post-colistin treatment (Ab6267 and
Ab3941). Isolates were treated with 6.25 µg/ml of LL-37 (A and C) or 2.5 mg/ml of lysozyme (B and D). Percent changes from time zero to 2 h after treatment
with host antimicrobials from 3 experiments with 3 to 4 samples of each isolate are represented. Data were analyzed for significance using the unpaired Student’s
t test. *, P < 0.05; **, P < 0.001. Error bars represent the standard deviations of the results determined for triplicate samples.
late displayed low-level resistance to LL-37 and lysozyme (Fig. 1B) that was not observed with the other colistin-sensitive isolates (Fig. 1A and C to D). In order to overcome the complications of comparing diverse strains, and to directly determine whether colistin treatment can induce cross-resistance to host cationic antimicrobials, we obtained 2 pairs of *A. baumannii* clinical isolates from patients pre- and post-colistin treatment (21). Each pair of serial isolates (Ab6266 and Ab6267, and Ab3527 and Ab3941) was from a distinct patient, under 35 years of age, who had sustained severe trauma overseas (21). Ab6267 was collected after 3 weeks of patient treatment with colistin, and Ab3941 was collected after 6 weeks of patient treatment with colistin. Both pre-treatment isolates (Ab6266 and Ab3527) were colistin sensitive, while the post-treatment isolates (Ab6267 and Ab3941) displayed increased resistance to colistin (see Table S1 in the supplemental material). In addition, the transition to increased colistin resistance was not accompanied by changes in susceptibility to the other antibiotics tested (Table S2). While we cannot specify the event(s) (an induced mutational event, selection for a preexisting mutant, or reselection) that led to the selection for the post-treatment resistant isolates, the pre- and post-treatment isolates were shown to be highly similar by pulse-field gel electrophoresis (PFGE), using a 90% similarity cutoff, compared to 1,500 clinical isolates CI-1, CI-2, CI-3, CI-4, and ARIC. The MU134, MU215, MU181, and MU52 isolates were collected by the Georgia Emerging Infections Program (GEIP) as part of the active population-based surveillance for multidrug-resistant Gram-negative bacteria. Additionally, we would like to thank Chui-Yoke Chin, Phil Rather, Tim Sampson, and William Shafer for critical review of the manuscript.

The findings and conclusions in this report are ours and do not necessarily represent the official position of the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry.

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