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Brooke A. Napier, Emory University
Victor Band, Emory University
Eileen Burd, Emory University
David S Weiss, Emory University

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Colistin Heteroresistance in *Enterobacter cloacae* Is Associated with Cross-Resistance to the Host Antimicrobial Lysozyme

Brooke A. Napier,a,⁎ Victor Band,a Eileen M. Burd,c,d David S. Weissa,b,c

Department of Microbiology and Immunology,a Emory Vaccine Center,b Division of Infectious Diseases, Department of Medicine,c and Department of Pathology and Laboratory Medicine,d Emory University School of Medicine, Atlanta, Georgia, USA

Here, we describe the first identification of colistin-heteroresistant *Enterobacter cloacae* in the United States. Treatment of this isolate with colistin increased the frequency of the resistant subpopulation and induced cross-resistance to the host antimicrobial lysozyme. This is the first description of heteroresistance conferring cross-resistance to a host antimicrobial and suggests that clinical treatment with colistin may inadvertently select for bacteria that are resistant to components of the host innate immune system.

**Antibiotic-resistant pathogens are responsible for 2 million infections and at least 23,000 deaths each year in the United States alone (1). The problem of increasing antibiotic resistance is compounded by the lack of new drugs in development, together threatening a return to the preantibiotic era. Colistin is often the only therapeutic option to treat infections caused by Gram-negative bacteria that are resistant to most or all other antibiotics (2–4). This cationic antimicrobial peptide disrupts both the outer and inner membranes of Gram-negative bacteria (5), acting similarly to several host antimicrobials, including the cationic C-terminal portion of lysozyme (6–8). Like colistin, this nonenzymatic portion of lysozyme exerts a potent antimicrobial action against a variety of Gram-negative bacteria by crossing the outer membrane via self-promoted uptake and forming pores within the inner membrane (6–8). Unfortunately, resistance to colistin has emerged, rendering infections by some strains essentially untreatable.**

Several types of resistance to colistin have been identified, including heteroresistance, which has been observed in several

![FIG 1](aac.asm.org) Frequency of the colistin-resistant subpopulation in a novel *Enterobacter cloacae* clinical isolate. (A) Image of *Enterobacter cloacae* isolate colR/S plated on MH with a colistin Etest strip. Resistant colonies are present within the zone of clearing. (B) The frequency of the colistin-resistant subpopulation of colR/S was quantified after growth in MH broth for 16 h, subsequent treatment with a sublethal dose of colistin (200 μg/ml; +colistin) for 2 h or 12 h, and subsequent passage in MH without colistin (−colistin).
Gram-negative pathogens (9–11). Heteroresistance is broadly defined as the presence of an antibiotic-resistant subset of microbes within a larger population that is susceptible to the antibiotic (12–14). Heteroresistance can complicate assessment of the MIC to a specific antibiotic and may promote resistance to antibiotics in vivo, thereby affecting diagnostic tests and patient treatment (12).

We recently isolated a colistin-heteroresistant strain (colR/S) of *Enterobacter cloacae* from a bronchoalveolar lavage specimen from a kidney transplant patient. This is the first identification of colistin-heteroresistant *E. cloacae* in the United States and only the second description worldwide (15). *E. cloacae* is a Gram-negative intestinal commensal bacterium that colonizes 40 to 80% of the human population (16) and has previously been identified as an opportunistic nosocomial pathogen (17–19). Treatment of infection by *E. cloacae* can be complicated by its natural resistance to many antibiotics and its ability to acquire resistance to others after exposure (20, 21).

The colR/S strain was highly antibiotic resistant (see Table S1 in the supplemental material) and displayed heteroresistance to colistin as determined using Etest strips (bioMérieux, Durham, USA).
lates of the nosocomial bacterium E. cloacae, whose frequency is significantly increased upon colistin treatment similarly induced colR/S prevented killing upon subsequent exposure to lysozyme. colR/S prevented killing upon subsequent exposure to lysozyme, whether or not they had been pretreated with colistin (Fig. 2F and G). These data indicate that, among the colistin-heteroresistant E. cloacae isolates tested here, pretreatment with colistin induces an increased frequency of colistin resistance as well as cross-resistance to the host antimicrobial lysozyme. This is the first demonstration that heteroresistance to an antibiotic can confer cross-resistance to a component of the host innate immune system.

The data presented here suggest that E. cloacae heteroresistance to colistin may impact the outcome of clinical infection, since exposure to this antibiotic can lead to increased resistance to host innate immune defenses. This is likely a phenomenon that occurs broadly and is relevant to a range of pathogens for which colistin-heteroresistant strains have been isolated, including Acinetobacter baumannii and Klebsiella pneumoniae (9–11). Use of susceptibility testing methods capable of identification of heteroresistance may be essential in guiding optimal patient treatment, to avoid unknowingly inducing resistance to the host innate immune system.

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