PRECISION AND FUTURE MEDICINE

Old drug, new findings: colistin resistance and dependence of *Acinetobacter baumannii*

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ABSTRACT

Colistin is an old drug, and its use has recently resurged because of increasing antibiotic resistance in gram-negative bacteria such as *Acinetobacter baumannii*. Although the colistin resistance rates in gram-negative bacteria are currently not high, many colistin-resistant isolates are being identified and the possibility of horizontal transmission of colistin resistance has increased because of the plasmid-borne colistin resistance gene *mcr-1* (mobilized colistin resistance). In this review, we have discussed colistin resistance in *A. baumannii*. In addition, we have reviewed an abnormal phenomenon called colistin dependence in *A. baumannii*.

Keywords: Gram-negative bacteria; PmrAB; Polymyxins

INTRODUCTION

Acinetobacter was most probably first described as *Diplococcus mucosus* in 1908. In 1954, Brisou and Prévot proposed the genus *Acinetobacter* to indicate that the bacteria were non-motile because they lacked flagella: the Greek "akineto" means "immobile" [1]. *Acinetobacter* spp. are glucose-non-fermentative, non-motile, non-fastidious, catalase-positive, oxidative-negative, aerobic, and gram-negative coccobacilli [2]. The genus *Acinetobacter* includes 55 species (as of July 18, 2017; http://www.bacterio.net/acinetobacter.html), and the number of species is increasing [3]. *Acinetobacter baumannii* is the most common species to cause infections, followed by *Acinetobacter nosocomialis* and *Acinetobacter pittii* [4,5]. *Acinetobacter lwoffii, Acinetobacter haemolyticus, Acinetobacter johnsonii, Acinetobacter junii, Acinetobacter ursingii, Acinetobacter schindleri, Acinetobacter calcoaceticus*, and *Acinetobacter seifertii* have occasionally been reported in humans [5,6]. *A. baumannii, A. calcoaceticus, A. nosocomialis*, and *A. pittii* have very similar biochemical traits and could be separated well; they were grouped into the so-called "*A. calcoaceticus-A. baumannii* (Acb) complex" [7]. *A. seifertii* is also closely related to the species of the Acb complex [8,9].

  Acinetobacter spp., including *A. baumannii*, have long been known as colonizers in humans, but they do not cause severe infections [10]. However, *A. baumannii* causes infections in immunosuppressed patients, patients with serious underlying diseases, and those subjected to invasive procedures and treated with broad-spectrum antibiotics; it may be a pathogen that

has adapted the most to the hospital environment in the 21st century [11]. *A. baumannii* has become a representative pathogen that threatens human health. It is a member of the ESKAPE group, which is the main bacterial group that causes infections in humans [12], and has been recently ranked as a bacterium that poses the greatest health threat by World Health Organization [13]. In the United States, *A. baumannii* has been estimated to cause more than 2% of the healthcare-associated infections [14]. *Acinetobacter* spp. are the seventh most-isolated bacteria in Korean hospitals [15], and they are the most frequent isolates from adults with hospital-acquired pneumonia or ventilator-associated pneumonia in Asian countries, including South Korea [16].

Although β-lactam antibiotics are the preferred choice for susceptible *A. baumannii* infections, carbapenems have become the main therapeutic option because of an increase in resistance [2,6]. However, imipenem-resistant isolates were found in the early 1990s [17], and the rate of carbapenem resistance in *A. baumannii*, mainly due to OXA-type (oxacillin-hydrolysing) carbapenemases, has increased rapidly [7,11]. In South Korea, more than two-thirds of the *A. baumannii* isolates were resistant to imipenem on the basis of several surveillance studies [4,15,18]. Most of the carbapenem-resistant *A. baumannii* isolates showed multidrug resistance (MDR) or extreme drug resistance (XDR), which is defined as resistance to all available antibiotics, except for one or two agents [19]. Current treatment options for XDR *A. baumannii* infections remain quite limited. In addition to tigecycline, a recently developed antibiotic—an old drug, colistin—is often the last resort for treating XDR *A. baumannii* [6,20].

COLISTIN

Polymyxin antibiotics include colistin (also known as polymyxin E), and polymyxin B is an antimicrobial polypeptide that was originally isolated in 1947 from the soil bacterium *Paenibacillus polymyxa* [21]. Colistin differs from polymyxin B by only one amino acid at position 6 in the peptide ring: a leucine in colistin and a phenylalanine in polymyxin B [22]. Although they have similar antimicrobial spectra and resistance mechanisms, the method for administration is different: while polymyxin B is administered directly in the active form, colistin is administered in the form of an inactive prodrug, colistin methanesulphonate (also known as colistimethate [CMS]). CMS itself lacks antibacterial activity, but it is converted into colistin after administration [23,24]. Although colistin has shown significant activity against a wide

variety of gram-negative pathogens, its use was stopped in the 1970s because of its nephrotoxicity and neurotoxicity [25]. However, the emergence of MDR or carbapenem-resistant gram-negative bacterial pathogens and the lack of new antibiotics to treat them have led to the resurgence of colistin [26,27].

Colistin mediates bactericidal activity by interacting with the lipid A component of the lipopolysaccharide (LPS) present on gram-negative pathogens, including *A. baumannii* [28]. Because of an electrostatic interaction between the positively charged colistin on one side and phosphate groups of the negatively charged lipid A membrane on the other side, divalent cations such as Ca^{2+} and Mg^{2+} are displaced from the membrane lipids. This destabilizes LPS and, consequently, increases the permeability of the membrane, leading to outer membrane disruption and cell death [26]. Other action mechanisms of colistin have been proposed: endotoxin effect, inhibition of vital respiratory enzymes, and hydroxyl radical production [28,29].

Colistin exhibits bactericidal activity in a concentration-dependent manner against gram-negative bacteria, including *A. baumannii*, with a minimal post-antibiotic effect [30]. However, re-growth with time has frequently observed [31], and the inoculum effect, a phenomenon of decreasing efficacy of an antibiotic with increasing bacterial density, has been reported [30]. Colistin has a relatively narrow *in vitro* bacteria-killing spectrum. It is active against gram-negative bacilli, such as *Acinetobacter* spp., *Pseudomonas aeruginosa, Escherichia coli, Klebsiella* spp., and *Enterobacter* spp. However, it has shown inactivity against some gram-negative bacilli, such as *Burkholderia cepacia, Proteus* spp., *Providencia* spp., and *Serratia* spp., as well as against gram-negative and gram-positive cocci, gram-positive bacilli, anaerobes, fungi, and parasites [32]. In addition, some *Acinetobacter* species, such as *A. seifertii* and *Acinetobacter colistiniresistens*, have exhibited very high colistin resistance rates or seem to be intrinsically resistant to it [3,4].

COLISTIN RESISTANCE

Colistin resistance in gram-negative bacteria is known to occur via several mechanisms. The main mechanism is the addition of a cationic group, such as 4-amino-4-deoxy-L-arabinose (L-Ara4N) or phosphoethanolamine (pEtN) to the lipid A moiety of LPS, which results in a decrease in the net negative charge of the bacterial outer membrane [33-37]. In most gram-negative bacteria, the addition of cationic groups is
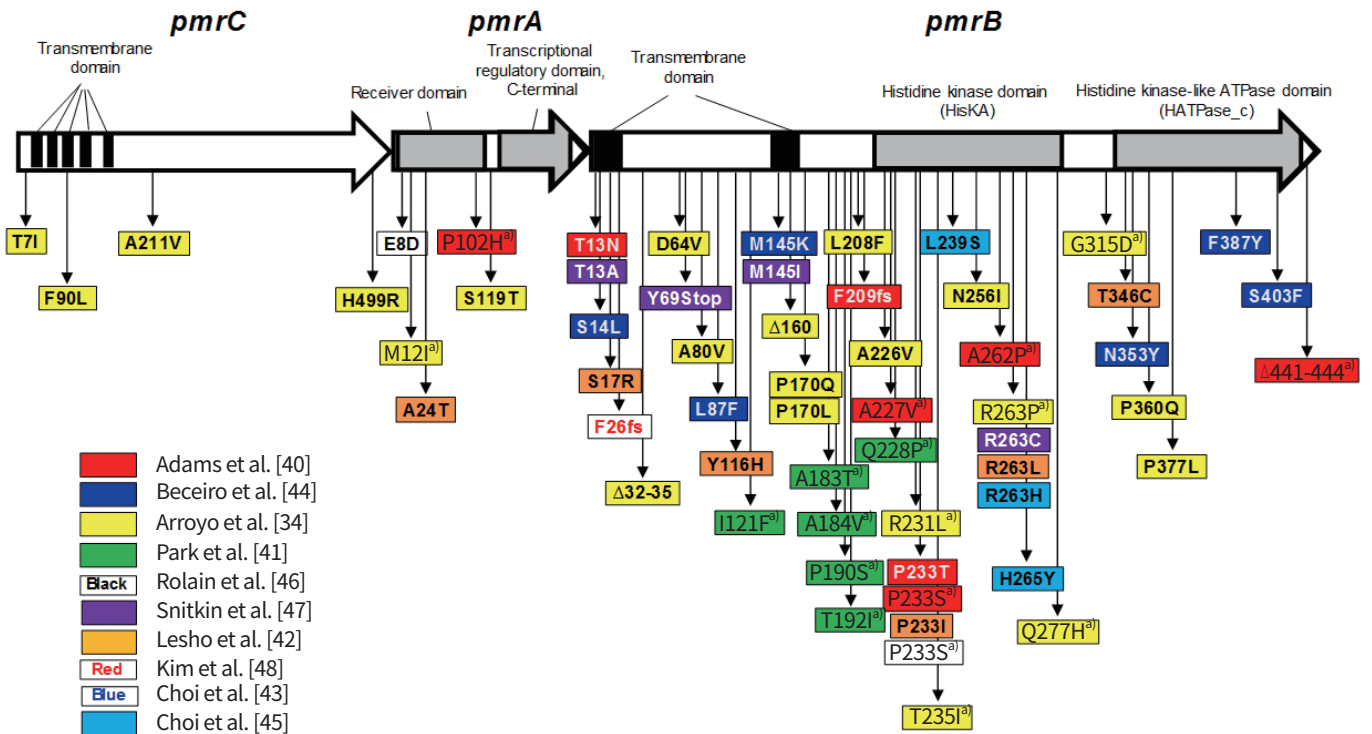
Fig. 1. Overview of amino acid substitutions associated with colistin resistance in the polymyxin resistance (*pmr*) operon in *Acinetobacter baumannii*. Amino acid alterations are indicated with different colors, according to the references. Location of domains within the *pmr* operon was predicted using the SMART (simple, modular, architecture, research, tool) program (http://smart.embl-heidelberg.de/). fs, frameshift; Δ, deletion. [a]On the top right of the letter indicates that an amino acid change found *in vitro* induced a colistin-resistant mutant.

regulated mainly by both PhoPQ and polymyxin resistance (*pmr*) PmrAB, which are two-component regulatory systems [28,38]. However, the *phoPQ* genes have not been found in the genome of *Acinetobacter* spp. [39]; thus, lipid A modification in *A. baumannii* is mediated by mutations in PmrAB [33,34,40-43]. Mutations in the *pmrA* or *pmrB* genes cause upregulation of the *pmrCAB* operon, leading to the synthesis and addition of pEtN, which is responsible for colistin resistance in *A. baumannii*. Amino acid alternations in PmrCAB of *A. baumannii* reported to date are presented in Fig. 1 [34,40-48]. As shown in Fig. 1, most amino acid substitutions associated with colistin resistance have been found in PmrB, a membrane-bound histidine kinase. However, it has not been verified experimentally if most variations are really responsible for colistin resistance in *A. baumannii*. Colistin-resistant mutants with no mutations in the *pmrA* and *pmrB* genes have also been identified, implying that the amino acid changes in the PmrAB two-component system are not essential for *A. baumannii* colistin resistance [41].

In addition to lipid A modification of LPS, loss of LPS has been reported to be associated with colistin resistance in *A.*

baumannii [49]. Alterations in the lipid A biosynthesis genes (*lpxA*, *lpxC*, and *lpxD*) by amino acid substitutions, deletions, or insertion of IS*Aba1* are responsible for the loss of LPS [49,50]. A recent metabolomic study revealed that an LPS-deficient, colistin-resistant *A. baumannii* strain showed perturbation in specific amino acid and carbohydrate metabolites, particularly pentose phosphate pathway and TCA (tricarboxylic acid) cycle intermediates [37]. In addition, depletion of peptidoglycan metabolites was observed in LPS-deficient strains. Several studies have reported increased susceptibility to some antibiotics rather than polymyxins in LPS-deficient, colistin-resistant *A. baumannii* strains [51,52], which has been postulated to be due to an increase in the passive diffusion of antibiotics. Decreased virulence in LPS-deficient strains has also been observed, which is compared with no change in the virulence of colistin-resistant strains due to *pmrAB* mutations [44,53]. To date, colistin resistance through the loss of LPS has not been detected in bacteria other than *Acinetobacter* spp.

Other colistin resistance mechanisms have been suggested in other gram-negative bacteria: overproduction of the cap-

sule polysaccharide (CPS) and efflux pumps [54,55]. For CPS production, reduced production of CPS in colistin-resistant mutants was observed in *Klebsiella pneumoniae*, which is a contradictory finding [56]. While an efflux pump inhibitor, carbonyl cyanide 3-chlorophenylhydrazone (CCCP) increased colistin susceptibility in *A. baumannii*, other efflux pump inhibitors, including phenylalanine-arginine β-naphthylamide (PAβN) did not show such an effect [31]. Thus, the roles of CPS overproduction and efflux pumps in colistin resistance should be further investigated.

Unlike the chromosome-related colistin resistance mechanisms described above, the plasmid-borne resistance gene mobilized colistin resistance (*mcr-1*) has been recently reported from *E. coli* isolates in China [57]. Since the first report, *mcr-1*, which encodes pEtN transferase, has been detected in dozens of countries worldwide, including South Korea [58,59]. Although it has been reported in diverse bacterial species, such as *E. coli*, *K. pneumoniae, Enterobacter cloacae, Enterobacter aerogenes, Salmonella* spp., and *Shigella sonnei*, it has not been found in *Acinetobacter* spp. isolates [60]. However, a *mcr-1*-carrying plasmid could be introduced into *A. baumannii*, and reduced susceptibility to colistin was observed, highlighting the risk of horizontal transfer of colistin resistance in *A. baumannii* [61].

It is estimated that colistin preserves its activity against many gram-negative pathogens, including *Acinetobacter* spp. An antimicrobial susceptibility study based on worldwide collection during 2006 and 2009 exhibited that colistin showed potent *in vitro* activities against *Acinetobacter* spp. (MIC$_{90}$, 1 mg/L; 98.6% susceptibility) [62]. Recent SENTRY Antimicrobial Surveillance Program data also show that more than 95% of *Acinetobacter* spp. isolates from Europe, China, and the United States are susceptible to colistin [63,64]. In South Korea, colistin resistance rates among *A. baumannii* isolates have been estimated to be 7.0% and 2.4% [4,8]. A recent study has also shown a colistin resistance rate of 8.6% among *Acinetobacter* spp. clinical isolates [65]. However, it did not delineate *Acinetobacter* spp. and may have overestimated the colistin resistance rate in *A. baumannii* because of high colistin resistance rates in other species of the Acb complex, *A. seifertii* and *A. pittii* [4,8]. While colistin resistance in *A. baumannii* seems to occur readily by simple mutation in both laboratories and patients [45,66], a genotyping study revealed that colistin-resistant *A. baumannii* isolates did not disseminate clonally [67].

COLISTIN DEPENDENCE

Several investigators have reported heteroresistance to colistin in *A. baumannii*, which has been supposed to cause the emergence of colistin resistance by exposure to colistin [68-70]. Heteroresistance is generally defined as a case in which subpopulations of antibiotic-susceptible bacteria show resistance to certain antibiotics [71]. The heteroresistant subpopulations survive at high antibiotic concentrations in a ratio of ~10^{-6} in a population analysis profiling (PAP) or appear as dis-

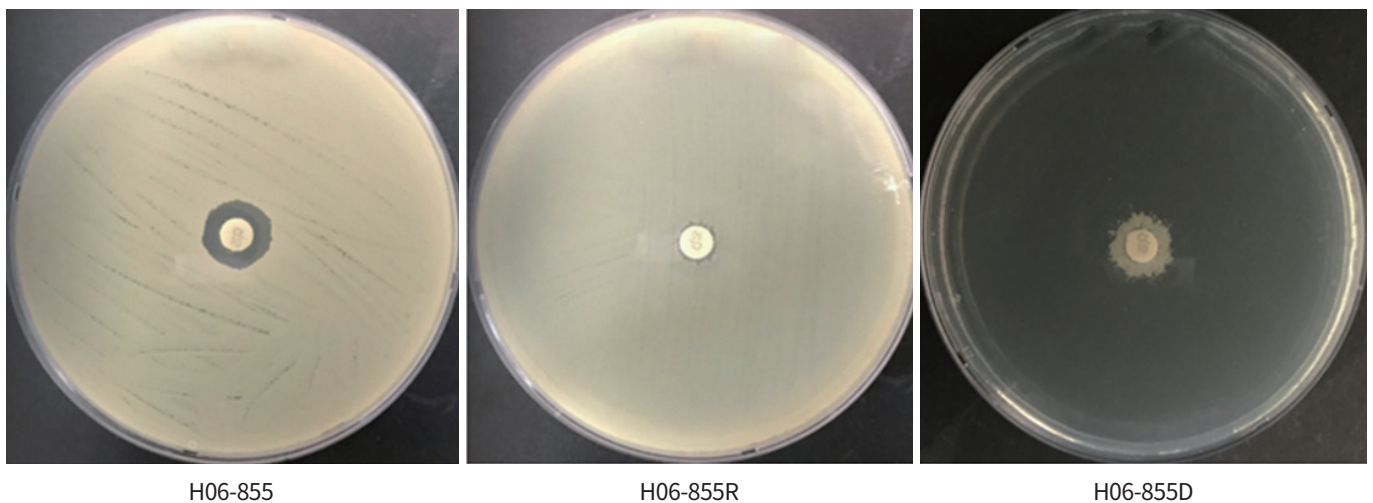H06-855   H06-855R   H06-855D

Fig. 2. Results of the disc diffusion assay for the colistin-susceptible, colistin-resistant, and colistin-dependent phenotypes. H06-855R and H06-855D are the colistin-resistant and colistin-dependent mutants, respectively, that originated from the colistin-susceptible *Acinetobacter baumannii* strain H06-855. The colistin-dependent mutant was obtained from colonies that survived 10 mg/L of colistin during the population analysis. While the colistin-resistant mutant grew throughout the plate, irrespective of the colistin disc, the colistin-dependent mutant grew only around the colistin disc.

tinct colonies growing within the clear zone of inhibition in the disc or E-test assay [71].

Unusually, some surviving *A. baumannii* subpopulations at high colistin concentrations in PAP exhibit the colistin dependence phenomenon. That is, when surviving colonies at ≥ 8 mg/L colistin during PAP were plated on solid agar with discs of 10 mg colistin, the bacteria grew only near the disc (Fig. 2). Such colistin dependence was first identified in an *Acinetobacter* sp. isolate from a calcaneus bone specimen of a patient with calcaneal osteomyelitis and bacteremia by Hawley et al. [72] during a population analysis. It was the first report on colistin dependence, and they identified the isolate as *A. baumannii-A. calcoaceticus* because they did not identify it to the species level.

The antibiotic dependence phenomenon was first reported in vancomycin-dependent *Enterococcus faecalis* in 1994 [73]. Vancomycin dependence in enterococci has subsequently been identified [74-76]; it may not be rare and may occur regardless of the use of vancomycin [77]. Because vancomycin-dependent isolates lack ligase activity because of mutations in the D-alanine D-alanine ligase (*ddl*) gene encoding the D-Ala-D-Ala ligase protein, they require glycopeptide antibiotics for cell-wall synthesis [78]. Although linezolid-dependent *Staphylococcus epidermidis* and β-lactam-dependent *Staphylococcus saprophyticus* have been reported [79,80], antibiotic dependence has been rarely identified in gram-negative bacteria.

After Hawley et al. [72], Garcia-Quintanilla et al. [81] identified partial colistin dependence by using the E-test assay. They found that some LPS-deficient, colistin-resistant *A. baumannii* strains with mutations in *lpxA, lpxC,* and *lpxD* showed partial colistin dependence. However, colistin resistance through LPS modification due to mutations in the PmrAB did not convert into colistin dependence. Although they proposed the loss of LPS as a colistin dependence mechanism, they did not address why a colistin-resistant isolate with the loss of LPS converted into colistin dependence. Thus, the mechanism underlying colistin dependence in *A. baumannii* is unclear.

Recently, we reported the development of colistin dependence in clinical colistin-susceptible *A. baumannii* isolates after exposure to colistin [82]. In that study, development of colistin dependence was not rare; 32.9% of 149 colistin-susceptible isolates developed colistin dependence. Genotypic analyses revealed that colistin dependence originated from the corresponding susceptible parental isolates, and no evidence of clonal dissemination of the isolates that developed colistin dependence was found. Colistin-dependent mutants have shown increased susceptibility to several antibiotics, such as carbapenems [72,81,82], which is the feature of LPS-deficient, colistin-resistant isolates [51,52]. Of note, patients with colistin-dependent strains have shown higher 3- and 7-day treatment failure than the patients without colistin-dependent strains [82]. Thus, the development of colistin-dependent mutants may have clinical significance, and it should be investigated. A recent study showed that the colistin-dependent phenotype may arise from the loss of LPS or defects in its structure, resulting from the disruption of LpxC [83]. In that study, transition of colistin dependence into colistin resistance was also demonstrated in the absence of antibiotic selection pressure [83].

CONCLUSION

The need for new antibiotics is growing in this era of antibiotic resistance; however, the development of new antibiotics, particularly for MDR gram-negative bacteria, has slowed down. Thus, the importance of older drugs, such as colistin, is increasing. However, the colistin resistance rate seems to be increasing, and information on the colistin resistance mechanism is limited. In addition to colistin resistance, abnormal phenomena such as colistin dependence have been found; however, there are few studies on colistin dependence. To cope with the antibiotic resistance era and use colistin effectively, a wide range of studies on colistin resistance and dependence should be performed.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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