MAJOR ARTICLE

Colistin-Resistant *Acinetobacter baumannii*: Beyond Carbapenem Resistance

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(See the Editorial Commentary by Pogue, Cohen, and Marchaim on pages 1304–7.)

Background. With an increase in the use of colistin methansulfonate (CMS) to treat carbapenem-resistant *Acinetobacter baumannii* infections, colistin resistance is emerging.

Methods. Patients with infection or colonization due to colistin-resistant *A. baumannii* were identified at a hospital system in Pennsylvania. Clinical data were collected from electronic medical records. Susceptibility testing, pulsed-field gel electrophoresis (PFGE), and multilocus sequence typing (MLST) were performed. To investigate the mechanism of colistin resistance, lipid A was subjected to matrix-assisted laser desorption/ionization mass spectrometry.

Results. Twenty patients with colistin-resistant *A. baumannii* were identified. Ventilator-associated pneumonia was the most common type of infection. Nineteen patients had received intravenous and/or inhaled CMS for treatment of carbapenem-resistant, colistin-susceptible *A. baumannii* infection prior to identification of colistin-resistant isolates. The 30-day all-cause mortality rate was 30%. The treatment regimen for colistin-resistant *A. baumannii* infection associated with the lowest mortality rate was a combination of CMS, a carbapenem, and ampicillin-sulbactam. The colistin-susceptible and -resistant isolates from the same patients were highly related by PFGE, but isolates from different patients were not, suggesting evolution of resistance during CMS therapy. By MLST, all isolates belonged to the international clone II, the lineage that is epidemic worldwide. Phosphoethanolamine modification of lipid A was present in all colistin-resistant *A. baumannii* isolates.

Conclusions. Colistin-resistant *A. baumannii* occurred almost exclusively among patients who had received CMS for treatment of carbapenem-resistant, colistin-susceptible *A. baumannii* infection. Lipid A modification by the addition of phosphoethanolamine accounted for colistin resistance. Susceptibility testing for colistin should be considered for *A. baumannii* identified from CMS-experienced patients.

Keywords. *Acinetobacter baumannii*; carbapenem resistance; colistin resistance; molecular typing; lipid A.

Acinetobacter baumannii is a major hospital-associated pathogen that causes a spectrum of diseases including respiratory tract, bloodstream, urinary tract, surgical site, and wound infections [1]. *Acinetobacter baumannii* has a propensity to acquire resistance to multiple classes of antimicrobial agents, and treatment of infection by highly resistant strains can be extremely difficult [2, 3]. For this reason, the Infectious Diseases Society of America has included *A. baumannii* among the 6 antimicrobial-resistant pathogens responsible for high morbidity and mortality in patients [4].

A rise in infections due to multidrug-resistant (MDR) *A. baumannii* strains (resistant to at least 3 different classes of antimicrobial agents) has been reported in the last 2 decades [3, 5]. Carbapenems have been considered to be appropriate agents to treat infections due to MDR *A. baumannii* strains [6, 7]. However, a worldwide surge in carbapenem resistance has been observed recently, primarily driven by the spread of several international clones [8, 9]. In the United States, the rates of carbapenem resistance among *A. baumannii* clinical

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strains range from 33% to 58% [10–12]. Therapy of carbapenem-resistant *A. baumannii* infection often requires the use of colistin methansulfonate (CMS). CMS is given intravenously as an inactive prodrug, which is converted in the blood to the active drug colistin sulfate [13]. More recently, however, resistance to colistin has been reported among *A. baumannii* clinical strains [14–17]. Indeed, a surveillance study of US hospitals revealed that 5.3% of all *Acinetobacter* strains were resistant to colistin [18]. Despite the potential magnitude of the problem, data regarding the clinical, microbiological, and molecular characteristics of colistin-resistant *A. baumannii* infections remain scarce to date. The objectives of the present study were therefore to (1) evaluate the clinical characteristics and outcomes of patients with infections due to colistin-resistant *A. baumanii*, (2) determine the molecular epidemiology of the strains, and (3) elucidate the mechanism underlying colistin resistance in *A. baumannii* strains.

MATERIALS AND METHODS

Patients and Bacterial Isolates

Patients colonized or infected with colistin-resistant *A. baumannii* were identified at the University of Pittsburgh Medical Center between 2007 and 2014. Colistin susceptibility testing was performed at the request of the treating physician by broth macrodilution. Colistin minimum inhibitory concentrations (MICs) >2 µg/mL were considered resistant [19]. The colistin-resistant isolates and earlier colistin-susceptible isolates from the same patients were collected through the clinical microbiology laboratory. The study was approved by the institutional review board at the University of Pittsburgh (PRO13030021).

Clinical Data

Patient demographics, underlying medical conditions, types of infection, antimicrobial agents given before and after isolation of colistin-resistant *A. baumannii* isolates, intensive care unit (ICU) admission, Acute Physiology and Chronic Health Evaluation II (APACHE II) score at the time of identification of colistin-resistant *A. baumannii*, clinical outcomes at 30 days, and recurrence of infection within 90 days were extracted from electronic medical records. The types of infection were defined according to standardized definitions by the Centers for Disease Control and Prevention/National Healthcare Safety Network [20]. For pneumonia, the PNU2 (pneumonia with specific laboratory findings) and PNU3 (pneumonia in immunocompromised patients) categories were applied as appropriate. Patients who did not receive specific treatment for *A. baumannii* were considered colonized only. Clinical response to treatment was classified as success for patients who had resolution of signs and symptoms that defined the infection, and failure for patients who had persistence or deterioration of symptoms and signs of colistin-resistant *A. baumannii* infection. For pneumonia, improvement of hypoxemia, leukocytosis, fever, and reduction in secretions was considered success. For bacteremia, resolution of symptoms and clearance of blood cultures defined success. Hospital records and the Social Security Death Index were assessed to determine mortality at 30 days from the onset of colistin-resistant *A. baumannii* infection. Death was attributed to infection when the patient had persistent infection at the time of death.

Susceptibility Testing

MICs of colistin were confirmed by standard agar dilution methods [21]. MICs of tigecycline and minocycline were determined by Etest (bioMérieux, Durham, North Carolina). MICs of other antimicrobial agents were determined by broth microdilution using Sensititre GNX3F plates (TREK Diagnostic Systems, Oakwood Village, Ohio). Results were interpreted according to the Clinical and Laboratory Standards Institute susceptibility breakpoints [19]. Tigecycline MICs were interpreted using the breakpoints for Enterobacteriaceae defined by the US Food and Drug Administration.

Pulsed-Field Gel Electrophoresis and Multilocus Sequence Typing

Genetic relatedness of colistin-susceptible and -resistant isolates from the same patients was determined by pulsed-field gel electrophoresis (PFGE) using a CHEF DR III system (Bio-Rad, Hercules, California) using the *Apa*I restriction enzyme [22] and interpreted according to the criteria proposed by Tenover et al [23]. The genetic relatedness among the colistin-resistant isolates from all patients was assessed by the unweighted-pair group method using Bionumerics version 6.01 (Applied Maths, Austin, Texas). To determine the clonal lineages, the sequence types (STs) of the colistin-resistant isolates were determined by multilocus sequence typing (MLST) [24].

Detection of Carbapenemase-Encoding Genes

Detection of the intrinsic $bla_{\text{OXA-51-like}}$ carbapenemase gene was performed by polymerase chain reaction (PCR) using primer sets and conditions described previously [25]. A multiplex PCR was conducted to detect the $bla_{\text{OXA-23}}$, $bla_{\text{OXA-40}}$, and $bla_{\text{OXA-58}}$ genes, the 3 major groups of acquired carbapenemase genes [26].

Analysis of Lipid A

Lipid A was extracted using an ammonium hydroxide/isobutyric acid–based procedure [27]. Once extracted, 1 µL of the concentrate was spotted on a matrix-assisted laser desorption/ionization–time of flight (MALDI-TOF) plate followed by 1 µL of norharmane matrix (Sigma-Aldrich, St Louis, Missouri) and then air-dried [16]. The samples were analyzed on a Bruker AutoFlex mass spectrometer (Bruker Daltonics, Billerica, Massachusetts) in the negative-ion mode.

RESULTS

Twenty unique patients with colistin-resistant *A. baumannii* were identified. Nineteen of them had colistin-susceptible *A. baumannii* isolates identified prior to the onset of colistin resistance, and the susceptible isolates were available for further analysis in 18 patients. The remaining patient presented directly with infection due to colistin-resistant *A. baumannii*. Taken together, 38 isolates (18 pairs of colistin-resistant and -susceptible isolates, and 2 colistin-resistant isolates without accompanying susceptible isolates) were available for analysis.

Clinical Characteristics of Patients With Colistin-Resistant *A. baumannii* Infections

The clinical features and outcomes of all patients are summarized in Table 1. Overall, the patients were critically ill with a median APACHE II score of 19.5 (range, 10–28), and all patients but one were in an ICU at the time of isolation of colistin-resistant *A. baumannii*. The types of infection included ventilator-associated pneumonia (VAP) (13 [65%]), bacteremia (2 [10%]), mediastinitis (1 [5%]), and hospital-acquired pneumonia (1 [5%]). The source of bacteremia was presumed to be VAP in 2 patients. All 19 patients initially infected with colistin-susceptible *A. baumannii* received therapy with intravenous CMS, inhaled CMS, or both, prior to isolation of colistin-resistant *A. baumannii*; 18 (95%) received therapy with intravenous CMS for a median duration of 12.5 days (range, 2–76), and 16 (84%) received therapy with inhaled CMS for a media duration of 10.5 days (range, 5–84). The median interval between the isolation of the colistin-susceptible *A. baumannii* isolate and the colistin-resistant *A. baumannii* isolate was 20 days (range, 4–99).

Of the 20 patients, 17 were treated for colistin-resistant *A. baumannii* infections, whereas 3 patients were asymptomatic, did not receive treatment against colistin-resistant *A. baumannii,* and were thus classified as colonization. All 3 colonized patients had received CMS for prior infections due to colistin-susceptible *A. baumannii*. Specifically, the first patient completed treatment for VAP due to colistin-susceptible *A. baumannii*, and at the time of colistin-resistant *A. baumannii* detection, the patient demonstrated improved clinical and radiographic characteristics. The second patient had a mucous plugging event that improved with bronchoscopy, and otherwise lacked signs of infection at the time of the culture. The last patient had colistin-resistant *A. baumannii* isolated from a sputum culture in the absence of any signs or symptoms of infection. Among 17 patients who were treated for colistin-resistant *A. baumannii* infections, 15 received various CMS-based combination regimens. The most common regimen was a combination of CMS, a carbapenem, and ampicillin-sulbactam (n = 7). None of these 7 patients died within 30 days of the infection, compared with 6 of 10 (60%) patients who received other antimicrobial regimens ($P = .03$ by Fisher exact test). All-cause mortality was 30% (6/20) at 30 days. Of the 6 deaths, 4 were likely attributable to *A. baumannii* infection. Two patients had a recurrence of infection within 90 days. They were both treated with a combination of CMS and a carbapenem at the time of recurrence; 1 patient survived and 1 died during the hospital stay.

Antimicrobial Susceptibility and Carbapenemase-Encoding Genes

MICs of colistin-resistant *A. baumannii* isolates are shown in Table 2. All isolates were nonsusceptible to piperacillin-tazobactam, gentamicin, imipenem, meropenem, doripenem, and ciprofloxacin, and most isolates were nonsusceptible to trimethoprim-sulfamethoxazole (95%), tobramycin (85%), amikacin (80%), and ampicillin-sulbactam (70%). Fifty percent and 20% were nonsusceptible to minocycline and tigecycline, respectively.

Among the colistin-susceptible *A. baumannii* isolates, all were nonsusceptible to meropenem and doripenem, and all except 1 were nonsusceptible to imipenem (Supplementary Table). They were nominally more resistant to ampicillin-sulbactam (94.4% nonsusceptible) and tigecycline (50% nonsusceptible) compared with the colistin-resistant isolates. Apart from these agents, no differences were observed in the MICs between the colistin-susceptible and -resistant isolates. All 38 *A. baumannii* isolates (20 colistin-resistant and 18 colistin-susceptible) were positive for $bla_{\text{OXA-51-like}}$, the intrinsic carbapenemase gene in *A. baumannii*. Additionally, all 38 isolates were positive for $bla_{\text{OXA-23}}$ by multiplex PCR, accounting for the carbapenem resistance. None of the isolates was positive for the $bla_{\text{OXA-40}}$ and $bla_{\text{OXA-58}}$ genes.

Molecular Typing

PFGE was performed on all 38 isolates. Within the 18 pairs of colistin-susceptible and -resistant isolates from the same patients, 12 pairs shared indistinguishable restriction profiles (0 band difference), 4 pairs were within a 3-band difference (considered closely related), and 2 pairs had 5- and 6-band differences (considered possibly related). Using a cutoff of 80% similarity, the 20 colistin-resistant isolates were grouped into 9 clusters (Figure 1). In contrast with the high level of relatedness observed between the susceptible and resistant isolates from the same patients, there was considerable interpatient variability of the restriction profiles.

By MLST, 16, 3, and 1 isolates belonged to ST92, ST282, and ST451, respectively. All these STs belong to clonal complex 92 (CC92; CC2 by the alternative MLST protocol proposed by Diancourt et al [28]), which corresponds to part of the international clone II and is commonly observed among carbapenem-resistant *A. baumannii* in hospitals worldwide [29].

Table 1. Characteristics and Outcomes of Patients With Colistin-Resistant *Acinetobacter baumannii*

Patient	Age	Sex	Underlying Diseases	Culture Site	Type of Infection	ICU	APACHE II Score	Prior Intravenous CMS, d[a]	Prior Inhaled CMS, d[a]	Treatment of Colistin-Resistant Infection	Clinical Response	30-d Mortality	Death Attributable to Infection	90 d Recurrence
1	55	F	Lung transplant	Sputum	VAP	Yes	21	16	16	CMS, TIG, AMS	Failure	Yes	Yes	. . .
2	63	M	Heart transplant	Mediastinal fluid	Mediastinitis	Yes	25	8	None	CMS, TIG	Failure	Yes	Yes	. . .
3	43	M	Lung transplant	BAL	VAP	Yes	19	76	84	AMS, TIG, RIF	Failure	Yes	No[b]	. . .
4	53	M	Renal transplant	Sputum	VAP	Yes	20	5	None	CMS, DOR, AMS	Success	No	. . .	No
5	84	F	Dementia, recurrent pneumonia	Tracheal aspirate	VAP	Yes	20	14	14	CMS, DOR	Success	No	. . .	Yes
6	76	F	CVA	BAL	VAP	Yes	28	15	9	AMS	Failure	Yes	No[b]	. . .
7	36	M	Morbid obesity, liver cirrhosis	BAL	VAP	Yes	25	10	11	CMS, DOR	Failure	No	. . .	. . .
8	68	M	Lung transplant	Sputum	Colonization	Yes	22	4	7	None	. . .	No	. . .	No
9	61	M	Heart and lung transplant	Sputum	HAP	No	15	5	9	CMS, DOR, AMS	Success	No	. . .	Yes
10	52	F	Liver transplant	BAL	VAP	Yes	20	11	10	CMS, DOR, AMS	Success	No	. . .	No
11	62	M	Lung transplant	Bronchial wash	VAP	Yes	12	14	14	CMS, DOR, AMS	Success	No	. . .	No
12	71	M	Lung transplant	Bronchial wash	VAP	Yes	17	None	9	CMS (inhaled only), DOR	Success	No	. . .	No
13	62	F	Mental retardation, Parkinson's disease	BAL	VAP	Yes	13	28	28	CMS, DOR	Failure	Yes	Yes	. . .
14	66	F	CVA	BAL	VAP	Yes	20	32	15	CMS, DOR	Failure	Yes	Yes	. . .
15	63	M	CVA	BAL	Colonization	Yes	15	2	None	None	. . .	No	. . .	No
16	77	M	Lung transplant	Sputum	Colonization	Yes	17	7	7	None	. . .	No	. . .	No
17	63	F	Lung transplant	BAL	VAP	Yes	10	30	6	CMS, DOR, AMS	Success	No	. . .	No
18	25	F	Toxic epidermal necrolysis	Pleural fluid	VAP	Yes	19	21	21	CMS, MEM	Success	No	. . .	No
19	73	M	Lung transplant	Blood	Bacteremia	Yes	19	None[c]	None[c]	CMS, DOR, AMS	Success	No	. . .	No
20	57	M	COPD, tonsillar carcinoma	Blood	Bacteremia	Yes	27	7	5	CMS, DOR, AMS	Success	No	. . .	No

Abbreviations: AMS, ampicillin-sulbactam; APACHE II, Acute Physiology and Chronic Health Evaluation II; BAL, bronchoalveolar lavage specimen; CMS, colistin methansulfonate; COPD, chronic obstructive pulmonary disease; CVA, cerebrovascular accident; DOR, doripenem; F, female; HAP, hospital-acquired pneumonia; ICU, intensive care unit; M, male; MEM, meropenem; RIF, rifampin; TIG, tigecycline; VAP, ventilator-associated pneumonia.

[a] Days of therapy between isolation of colistin-susceptible and colistin-resistant isolates.

[b] Subsequent aspiration event and bowel ischemia were deemed to be the causes of their deaths, respectively.

[c] The patient did not have a prior colistin-susceptible isolate, so did not receive CMS before the onset of bacteremia with the colistin-resistant isolate.

Table 2. Antimicrobial Susceptibility and Molecular Types of Colistin-Resistant *Acinetobacter baumannii* Isolates

			MIC, µg/mL													
Patient	Sequence Type	OXA Carbapenemase	CST	AMS	PTZ	AMK	GEN	TOB	CIP	TMP-SMX	IPM	MEM	DOR	MIN	TIG	pEtN[a]
1	92	51-like, 23	>256	16/8	>64/4	>32	>8	>8	>2	>4/76	>8	>8	>4	6	3	+
2	92	51-like, 23	>256	**8/4**	>64/4	>32	>8	>8	>2	>4/76	4	8	>4	**2**	**1.5**	+
3	92	51-like, 23	>256	16/8	>64/4	>32	>8	>8	>2	>4/76	>8	>8	>4	12	4	+
4	92	51-like, 23	4	16/8	>64/4	>32	>8	>8	>2	>4/76	>8	>8	>4	12	3	+
5	92	51-like, 23	128	16/8	>64/4	>32	>8	>8	>2	>4/76	>8	>8	>4	8	**2**	+
6	282	51-like, 23	128	**≤4/2**	>64/4	**≤4**	>8	**≤1**	>2	>4/76	4	8	>4	**1.5**	**2**	+
7	92	51-like, 23	64	32/16	>64/4	>32	>8	>8	>2	>4/76	>8	>8	>4	**1.5**	**0.25**	+
8	92	51-like, 23	>256	16/8	>64/4	>32	>8	>8	>2	>4/76	8	>8	>4	**2**	**1.5**	+
9	92	51-like, 23	>256	16/8	>64/4	>32	>8	>8	>2	>4/76	>8	>8	>4	8	4	+
10	92	51-like, 23	>256	16/8	>64/4	>32	>8	>8	>2	>4/76	8	8	>4	6	**2**	+
11	92	51-like, 23	32	16/8	>64/4	>32	>8	>8	>2	>4/76	>8	8	>4	**0.75**	**1**	+
12	92	51-like, 23	256	16/8	>64/4	>32	>8	>8	>2	>4/76	>8	>8	>4	**0.25**	**0.25**	+
13	92	51-like, 23	>256	**8/4**	64/4	>32	>8	>8	>2	>4/76	8	8	4	8	**2**	+
14	282	51-like, 23	32	**8/4**	>64/4	**≤4**	>8	**≤1**	>2	>4/76	>8	>8	>4	**1.5**	**2**	+
15	92	51-like, 23	16	32/16	>64/4	>32	>8	>8	>2	>4/76	>8	>8	>4	8	**2**	+
16	92	51-like, 23	4	16/8	>64/4	>32	>8	>8	>2	>4/76	>8	>8	>4	6	**2**	+
17	92	51-like, 23	64	**≤4/2**	64/4	>32	>8	>8	>2	>4/76	>8	8	>4	**1**	**1.5**	+
18	282	51-like, 23	16	**8/4**	>64/4	**≤4**	>8	**≤1**	>2	>4/76	>8	8	>4	**1.5**	**2**	+
19	92	51-like, 23	16	16/8	>64/4	**16**	8	8	>2	**≤0.5/9.5**	>8	>8	>4	**2**	**2**	+
20	451	51-like, 23	>256	64/32	>64/4	>32	>8	>8	>2	>4/76	8	>8	>4	6	**1.5**	+

Colistin MICs were obtained with the agar dilution method, and minocycline and tigecycline MICs were obtained with Etest. The other MICs were obtained with the broth microdilution method. MICs in susceptible ranges according to the Clinical and Laboratory Standards Institute breakpoints are shown in bold.

Abbreviations: AMK, amikacin; AMS, ampicillin-sulbactam; CIP, ciprofloxacin; CST, colistin; DOR, doripenem; GEN, gentamicin; IPM, imipenem; MEM, meropenem; MIC, minimum inhibitory concentrations; MIN, minocycline; OXA, oxacillinase; pEtN, phosphoethanolamine; PTZ, piperacillin-tazobactam; TIG, tigecycline; TMP-SMX, trimethoprim-sulfamethoxazole; TOB, tobramycin.

[a] Absent in the lipid A of all corresponding colistin-susceptible isolates.

Lipid A Profiles of Colistin-Resistant and -Susceptible Isolates

To determine the presence or absence of this lipid A modification, MALDI-TOF mass spectrometry was performed on all 38 isolates (20 colistin-resistant and 18 colistin-susceptible). The lipid A from colistin-resistant isolates typically showed 2 major $[M\text{-}H]^-$ ions at a mass-to-charge ratio (m/z) of 1910 and 2034 (Figure 2). The most prominent ion at m/z 1910 corresponds to a bisphosphorylated hepta-acylated lipid A. The ion at m/z 2034 corresponds to the hepta-acylated lipid A (m/z 1910) modified with phosphoethanolamine addition. The ion at m/z 1910 was present in all 38 isolates. The ion at m/z 2034 was present in all 20 colistin-resistant isolates, but in none of the colistin-susceptible isolates (Table 2).

DISCUSSION

Colistin, or its prodrug CMS, is a key therapeutic option for treatment of carbapenem-resistant *A. baumannii*, alone or in combination with other agents such as tigecycline, ampicillin-sulbactam, rifampin, and carbapenems [8]. Nevertheless, increased exposure has led to the emergence of colistin resistance, further limiting the therapeutic options against this pathogen [18]. Our study involved 20 unique patients with infection or colonization due to colistin-resistant *A. baumannii*. To our knowledge, this study represents the largest series describing detailed clinical and molecular characteristics of colistin-resistant *A. baumannii*. Our data highlight an emerging clinical problem that may be underappreciated by centers not routinely performing colistin susceptibility testing against *A. baumannii*.

A distinguishing factor associated with isolation of colistin-resistant *A. baumannii* among patients at our center was prior drug exposure. Indeed, all patients except 1 received CMS therapy (intravenous and/or inhaled) prior to the identification of a colistin-resistant isolate. This finding is consistent with a recent report of colistin-resistant *A. baumannii* from the US military health system [14] and is supported by the genetic relatedness of colistin-susceptible and -resistant isolates by PFGE. Moreover, only 2 pairs of patients (in 2007 and 2010, respectively) resided in the same ICU for overlapping periods of time in our study. There were no identifiable transmission

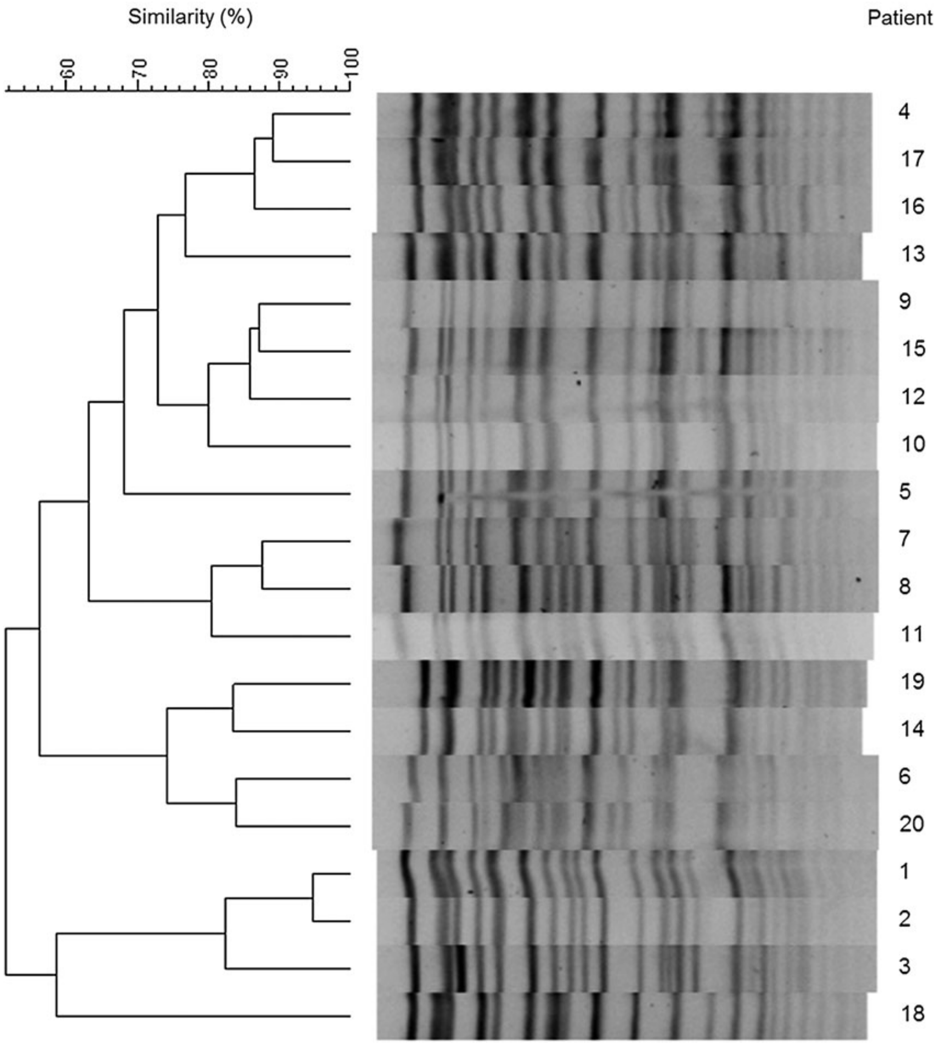

Figure 1. Pulsed-field gel electrophoresis dendrogram of colistin-resistant *Acinetobacter baumannii* isolates from 20 patients. The isolates were grouped into 9 clusters with a cutoff of 80%, demonstrating substantial diversity.

opportunities among the remaining 16 patients. Taken together, we hypothesize that colistin resistance predominantly emerges under selective pressure during CMS therapy in individual patients, rather than through patient-to-patient transmission in the hospital. Identification of prior CMS exposure should be considered in selecting appropriate therapy for patients with *A. baumannii* infection. Overall, 30% of patients died by 30 days; however, mortality rates were lower among patients receiving a 3-drug combination of CMS, a carbapenem, and ampicillin-sulbactam compared with other regimens. These data support recent in vitro data that demonstrated rapid bactericidal activity of the combination by time-kill analysis against colistin-resistant *A. baumannii* [30]. Thus, in treating patients with prior exposure to CMS, colistin susceptibility testing should be considered to best guide effective therapy. In addition, future studies should focus on how to best utilize CMS to minimize the risk of developing resistance.

The dissemination of carbapenem-resistant *A. baumannii* in hospitals worldwide is now understood as a highly clonal process, with the international clone II being the most prevalent clone [31]. Within the international clone II, CC92, as defined by the original MLST protocol [24], has been shown to have global distribution [32]. We previously documented the predominance of CC92 among carbapenem-resistant *A. baumannii* isolates identified in US hospitals [33]. All the colistin-resistant *A. baumannii* isolates in our study belonged to CC92. This makes our findings on the development of colistin resistance relevant to locales where carbapenem-resistant CC92 isolates are widespread.

Finally, lipid A analysis provided insights into the mechanism of colistin resistance. Colistin is a cationic amphiphilic

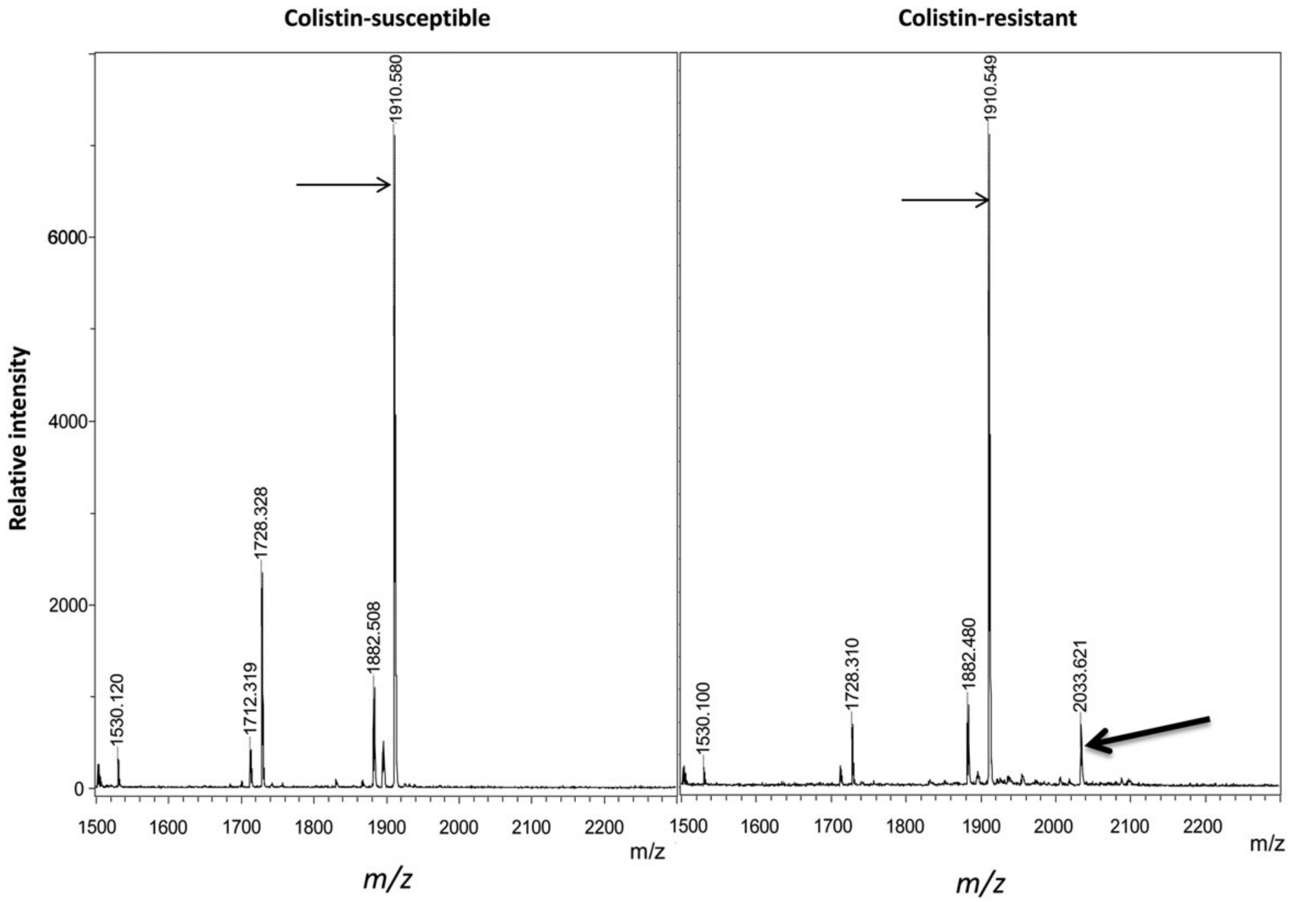

Figure 2. Comparison of matrix-assisted laser desorption/ionization–time of flight analysis of lipid A isolated from colistin-susceptible and -resistant *Acinetobacter baumannii* isolates from patient 2. Lipid A isolated from colistin-resistant strains produced an ion peak at a mass-to-charge ratio (*m/z*) of 2034 on mass spectrometry (bold arrow) that corresponds to modified lipid A with the addition of a phosphoethanolamine group. Thin arrows reveal ion at *m/z* 1910 that corresponds to the bisphosphorylated hepta-acylated lipid A of *A. baumannii.*

antimicrobial agent that interacts with the lipid A component of outer membrane lipopolysaccharide (LPS), resulting in its disruption and thereby causing cell death [34]. Modification of lipopolysaccharide outer membrane by addition of phosphoethanolamine to the hepta-acylated lipid A structure has been suggested as a major mechanism of colistin resistance in *A. baumannii* [16, 35, 36]. We observed this modification in all colistin-resistant *A. baumannii* isolates, but none of the corresponding colistin-susceptible isolates. Our data strengthen the contention that resistance to colistin is strongly associated with lipid A modification by phosphoethanolamine [14, 16]. Colistin resistance among *A. baumannii* may also be attributed to the complete loss of LPS [37]; however, we were able to identify the lipid A species intrinsic to *A. baumannii* (bisphosphorylated hepta-acylated lipid A) in all colistin-resistant isolates. Nevertheless, colistin MICs ranged from 4 µg/mL to >256 µg/mL, suggesting that resistance is likely multifactorial, and other factors cannot be excluded on the basis of our study.

Our data come from a single center in the United States, so the findings may not be generalizable to other institutions. Colistin susceptibility was not routinely tested on all *A. baumannii* isolates; thus, it is possible that some colistin-resistant *A. baumannii* cases were not identified. In addition, the lack of a comparison group with colistin-susceptible *A. baumannii* cases precludes our ability to make definitive conclusions on clinical outcomes. In terms of microbiological investigations, all isolates belonged to the international clone II and produced OXA-23 carbapenemase, which is a common combination observed among carbapenem-resistant *A. baumannii* worldwide [31]. Also, our investigation of colistin resistance mechanism was limited to lipid A profiles, which accounted for colistin resistance categorically, but not the levels of resistance.

In conclusion, colistin-resistant *A. baumannii* occurred almost exclusively among patients who had received CMS therapy for carbapenem-resistant, colistin-susceptible *A. baumannii* infection. Treatment with a combination of CMS, a carbapenem, and ampicillin-sulbactam was associated with lower mortality in comparison to other treatment regimens in this study. However, the numbers of cases were small, and this signal requires confirmation in a larger study. All isolates belonged to the globally epidemic international clone II, and lipid A modification was the mechanism underlying colistin resistance in all isolates. Susceptibility testing for colistin should be considered for *A. baumannii* identified from CMS-experienced patients.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. R. K. S. has received research funding from Astellas Pharma and Merck & Co for studies unrelated to this work. R. K. E. has received research funding from MedImmune for a study unrelated to this work. Y. D. has served on an advisory board for Shionogi Inc, consulted for Melinta Therapeutics, and received research funding from Merck & Co for a study unrelated to this work. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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