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A systematic review of implications, mechanisms, and stability of in vivo emergent resistance to colistin and tigecycline in *Acinetobacter baumannii*.

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Abstract

The potential of *A. baumannii* for acquired resistance to last resort antibiotics (colistin and tigecycline) during treatment has important clinical implications, especially when dealing with patients failing to improve despite treatment with an active antimicrobial. However, the relevant literature remains scattered. Therefore, a systematic search was conducted in PubMed and Scopus. Several studies reported emergence of resistance to colistin or tigecycline during treatment, in most cases (86%) resulting in persistent or recurrent infections, especially in cases of emergent resistance without fitness cost. Lipopolysaccharide modification in the case of colistin and overexpression of efflux pumps in the case of tigecycline were the main mechanisms of resistance. Emergent colistin resistance is often associated with fitness cost resulting in re-emergence of the fitter and more virulent colistin susceptible strain after cessation of antibiotic pressure. Prospective studies are needed to determine the frequency of emergent resistance during treatment and its impact on patient outcomes.

Keywords; Acinetobacter, baumannii, colistin, tigecycline, resistance, in vivo

Introduction

Carbapenem-resistance in *Acinetobacter baumannii* is rising and in many regions, especially in Europe and the Middle East, the majority of *A. baumannii* strains are resistant to carbapenems [1, 2]. The main treatment options for carbapenem-resistant *A. baumannii* include colistin, tigecycline and more recently cefiderocol if available [3]. Other options are aminoglycosides, trimethoprim-sulfamethoxazole and sulbactam but resistance to these agents is common [3]. Furthermore, resistance even to colistin and tigecycline is rising, and pandrug-resistant isolates are increasingly being reported [4].

Even with in vitro active treatment with the above antimicrobials mortality remains high [3]. The genomic plasticity and heteroresistance of *A. baumannii* facilitate the emergence of resistance to last resort antimicrobials during treatment [5], which could explain clinical/microbiological failure in some cases, but has not to my knowledge been previously reviewed, and the relevant literature remains scattered.

Of note is that resistant *A. baumannii* mutants developed in vitro may differ significantly from *A. baumannii* strains that develop resistance in vivo during treatment. For example, colistin resistance developed in vitro is usually mediated by lipopolysaccharide (LPS) loss (compared to lipid A modifications with in vivo emergence of resistance), which is associated with higher fitness costs and often reversal of susceptibility to other antibiotics [6, 7]. Therefore, studies of in vivo emergence of resistance during treatment are more clinically relevant.

Considering the above, the aim of this review is to systematically search and consolidate the literature on; a) the occurrence of in vivo emergence of resistance in *A. baumannii* to colistin and tigecycline, b) the mechanisms of emergent resistance, c) whether such resistance is transient and associated with fitness cost, d) the presence of cross-resistance between last resort antibiotics, and d) the impact of emergent resistance on treatment failure.

Methods

Search strategy; The following search was conducted in MEDLINE (PubMed) and Scopus, from inception to April 2020; baumannii AND (serial OR sequential OR subsequent OR successive OR consecutive OR succeeding OR “same patient” OR “same patients” OR “single patient” OR "in vivo emergence" OR “during treatment” OR “during therapy” OR isogenic OR paired OR pair OR pairs) AND (resistance OR resistant). Retrieved articles were initially screened based on their

titles and abstracts. The full texts of potentially eligible articles were then reviewed. The search was supplemented by reference tracking of included papers.

Eligibility criteria; Any study reporting emergence of resistance to colistin and/or tigecycline in sequential *A. baumannii* isolates from the same patient was eligible. In vitro studies, studies in animals and articles written in languages other than English were not eligible for this review.

Data items; The following data were collected from each study; method of antimicrobial susceptibility testing, exposure to colistin/tigecycline before emergence of resistance, mechanisms of emergent resistance, whether emergent resistance was associated with reduced fitness or virulence, the presence of cross-resistance between last resort antibiotics (colistin, tigecycline, minocycline, amikacin, gentamicin, tobramycin, trimethoprim/sulfamethoxazole, ampicillin/sulbactam), availability of molecular analyses to confirm the same origin (versus cross-infection by new strain) of paired isolates from the same patient, stability of resistance after withdrawal of antimicrobials, effect of emergent resistance on treatment failure and in-hospital mortality. Extraction of these data from eligible studies was performed twice.

Definitions; Emergent resistance was considered to be responsible for treatment failure when associated with persistent or recurrent symptoms and/or sings of infection necessitating further treatment. Cross-resistance was defined as simultaneous emergence of resistance to >1 last resort antimicrobial and mediated by a common mechanism.

Synthesis of results; A qualitative presentation and synthesis of the characteristics and findings of retrieved studies was conducted.

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Results

Study selection and study characteristics

The flow chart of the review is depicted in Figure 1. The main characteristics and findings of included studies (n=39 [8-46]) are summarized in Table 1 and Supplementary Table 1. The strains reported in 7 studies [14, 15, 18, 20, 21, 24, 41] were duplicates of strains reported in prior studies [13, 17, 19, 23, 42] but these studies were still included because complementary data relevant to the review were provided in the later studies. Excluding duplicates, in vivo emergence of resistance to colistin was reported in n=55 patients [11, 16-20, 22-29, 31-38, 40-44, 46] and to tigecycline in n=16 patients [8-10, 12-15, 23, 30, 32, 33, 39, 45].

Exposure to colistin/tigecycline before emergent resistance

In most cases (colistin n=52/55, tigecycline n=9/16) resistance emerged during or after treatment with the same antibiotic [8, 10, 11, 13-15, 17-30, 32-38, 40-43, 45, 46]. In one case, resistance to both tigecycline and tobramycin emerged during treatment with tobramycin, associated with overexpression of adeB efflux pump [12]. In few cases (tigecycline n=6, colistin n=2) it was not reported if treatment with colistin/tigecycline preceded the emergence of resistance. In one case a colistin-resistant *A. baumannii* strain emerged without prior exposure to colistin [44].

Cross-resistance between last resort antibiotics

Colistin resistance was not associated with cross-resistance to other last resort antibiotics (Supplementary Table 1). Emergent resistance to tigecycline may be associated with decreased susceptibility to aminoglycosides [8, 9, 12, 39] and minocycline [39], potentially mediated by overexpression of adeB efflux pump [9, 12, 39] (Supplementary Table 1).

In 2 cases resistance to both colistin and tigecycline emerged during treatment [23, 33]. In one of these cases exposure to both tigecycline and colistin preceded emergence of resistance [33], while in the other case whether exposure to tigecycline or aminoglycosides preceded the emergence of resistance was not clarified [23]. Mutation in *pmrB* (conferring colistin resistance) were found in both cases, but the potential mechanisms of tigecycline resistance were not evaluated [23, 33].

In contrast to colistin-resistant mutants developed in vitro [29], colistin resistance emerging in vivo did not generally result in increased susceptibility to other antibiotics (Supplementary Table 1), although reversal of susceptibility to ampicillin/sulbactam was noted in 4 cases [17, 23, 41].

Mechanisms of emergent resistance

The mechanism of emergent colistin resistance was evaluated in n=49 cases (Table 1). In most cases (n=44) a variety of mutations were noted in the *pmrCAB* locus (predominantly *pmrB*) [16, 18, 20, 23, 25-29, 31-33, 36, 38, 40, 41, 44, 46], resulting in upregulation of *pmr* genes [16, 23, 25, 27, 36, 44, 46]. The locus regulates a phosphoethanolamine (pEtN) transferase coded by *pmrC*, that by modifying the lipid A component of LPS reduces its negative charge and decreases the binding of the positively charged colistin. Upregulation of *eptA*, a *pmrC* homologue, which also codes for a pEtN transferase, was reported in 2 cases [37, 44]. Mutations in genes essential for synthesis of lipid A component of LPS, often observed in colistin-resistant *A. baumannii* developed in vitro [6, 7, 29], were reported in only one case in vivo [36].

The mechanism of tigecycline resistance was evaluated in n=11 cases [9, 12, 14, 15, 30, 39, 45]. Upregulation of adeABC efflux pumps was found in 8 cases, caused by mutations in regulatory genes *AdeS* [9, 14, 15, 39], *AdeN* [39], and *AdeR* [12]. Other mechanisms, not involving the adeABC pumps, were proposed in 3 other cases [30, 45] (Table 1).

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Is in vivo emergence of resistance transient and associated with fitness cost?

The impact of emergent colistin resistance on fitness and/or virulence was examined in n=17 cases [18, 21, 24, 25, 27-29, 33, 37, 38, 44]. In 10 cases (59%) emergence of colistin resistance was associated with fitness cost and/or lower virulence based on in vitro growth [18, 21, 24, 25, 27] and/or in vivo infection models [18, 21, 24].

The cost associated with colistin resistance may result in re-emergence of colistin-susceptible *A. baumannii* after withdrawal of colistin, as demonstrated in 7 patients [25, 28, 32, 41]. The mechanisms of loss of colistin resistance were examined in one study by whole genome sequencing and comparison between sequential *A. baumanni* isolates from the same patients (susceptible before exposure to colistin, resistant during treatment, and susceptible after withdrawal of colistin) [25]. In all cases emergent resistance was due to mutations in *pmrB*. The loss of resistance following withdrawal of colistin resulted from; a) additional compensatory mutations in pmr genes (1 case), b) re-emergence of the ancestral susceptible strain based on an identical *pmr* genotype (2 cases), c) cross-infection by a different strain based on a different pulsotype (1 case).

However, colistin resistance may often emerge without apparent fitness cost (based on growth curves [25, 28, 29, 33, 37, 38, 44]) or with unaffected [28] or even increased [24, 33, 44] virulence (based on in vivo infection models [24, 28, 44], or in vitro studies of virulence [33]). For example, a stably resistant strain was described in one study and its transmission between patients was demonstrated [25].

Generally, *pmrB* mutations (the predominant mechanism responsible for in vivo emergence of resistance) are associated with less fitness cost compared to LPS loss (typically observed only in mutants developed in vitro) [6, 7, 38]. It has also been hypothesized that in the long term

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fitness-compensatory mutations may increase the stability and persistence of colistin-resistant *A. baumannii* strains [19, 25, 28]. For example, increasing fitness and virulence was demonstrated in one patients with multiple sequential colistin-resistant *A. baumannii* isolates [24].

Re-emergence of susceptibility to tigecycline was not reported in any of the reviewed studies. The effect of emergent tigecycline resistance on fitness and virulence (based on the *G. mellonella* model) was evaluated in 2 cases; the susceptible strain outcompeted its susceptible counterpart in one case, while the opposite was found in the second case [15]. Regardless of fitness/virulence differences both tigecycline-susceptible and tigecycline-resistant strains were pathogenic [15].

Does emergent resistance explain treatment failure?

The impact of emergent resistance was evaluable in 28 cases; in 86% (24/28) emergent resistance was associated with persistent or recurrent infection [8, 11, 12, 17, 22, 23, 28, 32, 37, 38, 40, 43, 46] (Table 1), while in 14% (4/28) emergent resistance did not require any further treatment [13, 19, 27, 43], possibly due to reduced virulence of the resistant strains [15, 19, 27]. Treatment failure appears to be more common when emergent resistance is not associated with reduced fitness/virulence, but even strains with reduced fitness may be associated with treatment failure (Supplementary Table 2). In-hospital mortality was 42% (14/33) among the 33 cases evaluable for this outcome (Table 1).

Cross-infection by new strain versus in vivo emergence of resistance

Isolation of a resistant strain from a patient previously colonized/infected by a susceptible strain has too possible explanation; a) emergence of resistance in the same strain, or b) cross-infection by a different resistant strain. The fact that in most cases resistance to colistin/tigecycline

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emerged during/after treatment, in combination with molecular methods available in most of the included studies (Table 1), support that in most cases resistance emerged from the same strain as a result of antibiotic pressure.

Discussion

Summary of the main findings

Several case studies reported the emergence of resistance to colistin or tigecycline during treatment of *A. baumannii* infections, which was in most cases associated with persistent or recurrent infection. Cross-resistance was not observed between colistin and other last resort antibiotics, but tigecycline may be associated with cross-resistance to aminoglycosides and minocycline. If resistance is associated with fitness cost, which is common for colistin, the ancestral susceptible strain may re-emerge after withdrawal of antibiotic pressure. Based on the limited available data treatment failure appears more common when resistance is not associated with reduced fitness/virulence, but this observation requires confirmation in larger cohorts.

How common is emergent resistance during treatment?

Considering that this review was based on case reports or small series the frequency of emergent resistance during treatment could not be investigated. Based on cohorts of patients being treated for *A. baumannii* infections emergent resistance appears to be uncommon (5% [47], 0% [48], 6.4% [49], 13.5% [25]).

However, several factors could have underestimated the frequency of emergent resistance in these studies; (a) lack of culture after emergence of resistance due to patient death before culture could be obtained, (b) lack of culture after emergence of resistance due to patient

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improvement (patients may improve despite persistent colonization and emergence of resistance), (c) potentially low sensitivity of cultures in pneumonia and lack of sampling of other potential sites of *A. baumannii* colonization, (d) false colistin susceptibility (for example 20% of the isolates in one of the above trials initially classified as colistin-susceptible according to Vitek-2 or E-test were then found to be resistant using broth microdilution [50]).

To evaluate the frequency and impact of emergent resistance during treatment prospective studies are needed with repeated cultures from the site of infection (and ideally from all potential sites of *A. baumannii* colonization) before, during and after treatment. Such studies should ideally incorporate molecular methods to determine the mechanisms of resistance and the clonal relatedness of sequential isolates from each patient, as well as methods to examine the effect of emergent resistance on fitness and virulence and correlate this with treatment outcomes.

Potential implications of transient resistance

Rapid emergence of colistin resistance from a heteroresistant population during treatment may allow *A. baumannii* to survive under antibiotic pressure. When antibiotic pressure ceases the fitter and more virulent ancestral susceptible strain may re-emerge [25], potentially resulting in re-infection in the same patient or transmission to other patients. The development of persister cells (viable but non-dividing cells that can survive lethal doses of antibiotics and are able to re-emerge after cessation of antibiotic pressure) during treatment may explain this observation [51]. Of note is that colistin heteroresistance is likely to be missed by methods such as Vitek 2 or E-test [25, 35, 50] and may even be missed with broth microdilution [32, 52]. Therefore, the true prevalence of in vivo colistin resistance may be underestimated and its impact under-recognized.

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Limitations

The main limitation of this review is that it is based solely on case reports or small case series. Furthermore, despite the fact that resistance emerged during treatment and the availability of molecular methods to support the same origin of sequential isolates, the possibility of cross-infection or mixed infection by more than one strain cannot be completely ruled out. Moreover, the method of susceptibility testing was not reported in some studies or unreliable methods were used (e.g. E-test for colistin) (Table 1). Finally, comparison of the genomic and transcriptomic signatures between resistant and susceptible *A. baumannii* strains is more complex than presented here [30, 36, 44] but beyond the scopes of this review.

Conclusions

Several studies have described the in-vivo emergence of resistance to colistin or tigecycline during treatment of *A. baumannii* infections, often resulting in persistent or recurrent infection. The frequency and impact of emergent resistance during treatment requires further study in appropriately designed prospective studies, as discussed above. This has important clinical implications, especially when dealing with patients not responding to apparently active (based on initial antimicrobial susceptibility results) antibiotics. Furthermore, the role of combination therapy for *A. baumannii* may require reconsideration, despite negative results of recent meta-analyses and trials which combined colistin with in-vitro inactive agents (meropenem, sulbactam, fosfomycin, rifampicin) [53-56]. Combination with a second active agent (e.g. tigecycline [57]) or the potential of other synergistic combinations [34, 58-60] requires further study.

Declarations

Conflict of interest; I have no conflict of interest to declare

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Figure 1; Flow chart of the review

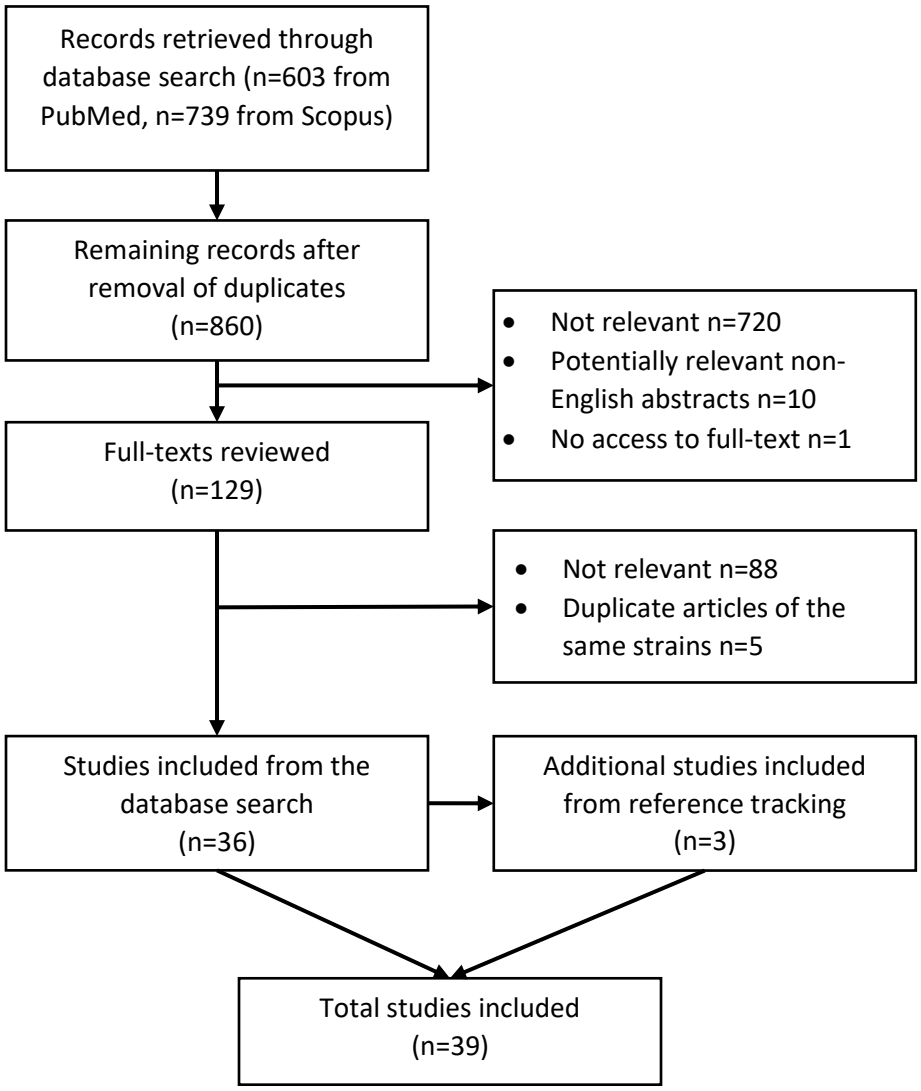

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Table 1; Summary of characteristics and findings of included studies

First author, Year	Emergent resistance to (n[a]);	Method of susceptibility testing [b]	Mechanisms of resistance [c]	Fitness cost	Molecular methods [d]	Effect on treatment failure [e]	Outcome
Reid GE, 2007 [8]	TGC (n=1) [f]	E-test	NR	NR	No	Yes	Discharged
Ruzin A, 2007 [9]	TGC (n=1) [g]	E-test	Disruption of *AdeS* by ISAba1 resulting in overexspression of adeABC efflux pumps	NR	Ribotyping	NR	NR
Anthony KB, 2008 [10]	TGC (n=1) [f]	E-test	NR	NR	No	Unclear [h]	Discharged
David MD, 2008 [11]	COL (n=1) [f]	agar dilution	NR	NR	No	Yes	Died
Higgins PG, 2010 [12]	TGC (n=1) [i]	E-test	*adeR* mutation (D20N) resulting in overexpression of adeB efflux pump	NR	PFGE, rep-PCR	Yes	Died
Hornsey M, 2010 [13-15]	TGC (n=2) [f]	agar dilution, E-test	*adeS* mutations (A98V, S8R) resulting in overexspression of adeABC efflux pumps	Yes (1/2)	PFGE, WGS, SNPs	Yes (1/2)	Discharged (2/2)
Beceiro A, 2011[16]	COL (n=1) [g]	agar dilution, E-test	*pmrB* mutation (L87F)	NR	PFGE	NR	NR
López-Rojas, 2011 R [17, 18]	COL (n=1) [f]	NR	*pmrA* mutation (M12K)	Yes	rep-PCR	Yes	Discharged
Rolain JM, 2011 [19-21]	COL (n=1) [f]	NR	*pmrA* mutation (E8D)	Yes	PGFE, WGS, SNPs, MLST	No	Discharged
Shields RK, 2011 [22]	COL (n=3) [f]	E-test	NR	NR	No	Yes (3/3)	Died (3/3)

First author, Year	Emergent resistance to (n[a]);	Method of susceptibility testing [b]	Mechanisms of resistance [c]	Fitness cost	Molecular methods [d]	Effect on treatment failure [e]	Outcome
Lesho E, 2013 [23, 24]	COL (n=2) [f] COL+TGC (n=1) [g]	broth microdilution	*pmrA* mutation (E8D) *pmrB* mutations (S17R, Y116H) mechanism of TGC resistance was note evaluated	Yes (1/1)	PFGE, optical genome mapping, SNPs phylogenetic analysis	Yes (2/2)	"successfully treated" (2/2)
Snitkin ES, 2013 [25]	COL (n=4) [f]	broth microdilution	*pmrB* mutations (P233S, indel AAT at 69, R263C, T187P, A226T, L271R) *pmrC* mutation (R109C)	Yes (3/4)	WGS, Mauve alignment, PFGE	NR	NR
Kim Y, 2014 [26]	COL (n=3) [f]	E-test	*pmrB* mutations (A227V, P233S, frame shift after Phe26)	NR	PFGE	Yes (3/3)	Died (2 of 3 patients)
Pournaras S, 2014 [27]	COL (n=2) [f]	broth microdilution	*pmrB* mutations (P170L, P233S)	Yes (2/2)	PFGE, MLST	No (2/2)	Discharged (2/2)
Durante-Mangoni E, 2015 [28]	COL (n=1) [f]	broth microdilution	*pmrB* mutation (P233S)	No	PFGE, MLST	Yes	Died
Lim TP, 2015 [29]	PMB (n=2) [f]	broth microdilution	*pmrB* mutations (P233S, R263H)	No	WGS, SNPs phylogenetic analysis, MLST	NR	NR
Liu L, 2016 [30]	TGC (n=2) [f]	broth microdilution	Unlcear [j]	NR	WGS, SNPs phylogenetic analysis, MLST	NR	Discharged (2/2)
Wright MS, 2016 [31]	COL (n=1) [g]	broth microdilution	*pmrB* mutation (P154T)	NR	WGS, SNPs phylogenetic analysis	NR	NR

First author, Year	Emergent resistance to (n[a]);	Method of susceptibility testing [b]	Mechanisms of resistance [c]	Fitness cost	Molecular methods [d]	Effect on treatment failure [e]	Outcome
Choi HJ, 2017 [32]	COL (n=1) [f] TGC (n=1) [f]	broth microdilution	*pmrB* mutations (H265Y, L239S, R263H) [k] mechanism of TGC resistance was note evaluated	NR	PFGE	Yes [l]	Died [l]
Dahdouh E, 2017 [33]	COL (n=1) [f] COL + TGC (n=1) [f]	E-test (COL) Vitek-2 (TGC)	*pmrB* mutations (P233S, ΔI19), mechanism of TGC resistance not reported	Yes (1/2)	MLTS, RAPD	Yes (2/2)	Died (1/2)
Lenhard JR, 2017 [34]	COL (n=1) [f]	broth microdilution	NR	NR	PFGE	NR	NR
Savari M, 2017 [35]	COL (n=1) [f]	E-test	Unclear [m]	NR	IC, MLST, MLVA	NR	NR
Cafiso V, 2018 [36]	COL (n=2) [f]	broth microdilution	*pmrB* mutations (L208F, R263H) *lpxC* mutation (S171Y) *lpxD* mutation (truncated protein at the 292 amino acid)	NR	WGS, PFGE, MLST, SNPs phylogenetic analysis	NR	NR
Deveson Lucas D, 2018 [37]	COL (n=1) [f]	broth microdilution	Insertion of IS*Aba125* resulting in inactivation of the regulatory gene *hns* and upregulation of *eptA* (a *pmrC* homologue)	No	WGS, SNPs phylogenetic analysis	Yes	Died
Farshadzadeh Z, 2018 [38]	COL (n=2) [f]	broth microdilution	*pmrB* mutations (P233S, T232I)	No (2/2)	MLST, MLVA	Yes (2/2)	Died (1/2)

First author, Year	Emergent resistance to (n[a]);	Method of susceptibility testing [b]	Mechanisms of resistance [c]	Fitness cost	Molecular methods [d]	Effect on treatment failure [e]	Outcome
Gerson S, 2018 [39]	TGC (n=4) [g]	agar dilution	insertion of IS*Aba1* in *adeS* insertion of IS*Aba125* or IS*Aba1* in AdeN	NR	WGS (<10 allele differences), Mauve alignment, MLST	NR	NR
Jaidane N, 2018 [40]	COL (n=1) [f]	broth microdilution	*pmrB* mutation (S13I14-CILIFSVILG-duplication)	NR	WGS (<25 SNPs)	Yes	Discharged
Mustapha MM, 2018 [41, 42]	COL (n=14) [f]	broth microdilution	*pmrB* mutations (R134S, T235I, A226T, T232A, F267L, L271F, Q277K, L208R, R263C, P233T, L292H, P233S, G315V) *pmrA* mutations (L20F, M12I) [n]	NR	WGS, SNPs phylogenetic analysis, PFGE, MLST	Yes (but unclear in how many of the patients) [o]	Unclear [o]
Saun TJ, 2018 [43]	COL (n=2) [f]	NR	NR	NR	PGFE	Yes (1/2)	Discharged (2/2)
Gerson S, 2019 [44]	COL (n=3) [p]	broth microdilution	*pmrB* mutations (ΔL9-G12, I232T, S17R) insertion of IS*Aba1* upstream of *eptA*	No (2/2)	WGS (<10 allele differences)	NR	NR
Cheng J, 2020 [45]	TGC (n=1) [f]	broth dilution	Unclear (upregulation of efflux pumps among other proposed mechanisms)	NR	WGS, MLST, SNPs phylogenetic analysis	NR	NR
Marano V, 2020 [46]	COL (n=3) [f]	broth microdilution	*pmrB* mutations (E301G, L168K, P233T)	NR	PFGE	Yes (3/3)	Died (2/3)

Abbreviations; COL= colistin, IC= international clonage lineage, MLST= multilocus sequencing typing, MLVA= multi-locus variable number tandem repeat analysis, NR= not reported, PFGE= pulsed-field gel electrophoresis, RAPD= random amplified polymorphic DNA, rep-PCR= repetitive extragenic palindromic polymerase chain reaction, PMB= polymyxin B, SNP= single nucleotide polymorphism, TGC= tigecycline, WGS= whole genome sequencing

[a] The number of patients with paired sensitive and resistant isolates.

[b] The method of susceptibility testing used for the antibiotic to which resistance emerged (colistin or tigecycline).
[c] The resulting amino acid substitutions/deletions are shown in parentheses.
[d] Any molecular method that was used to confirm the same origin (vs cross-infection) of sequential *A. baumannii* isolates from the same patient.
[e] Emergent resistance was considered to be responsible for treatment failure when associated with persistent or recurrent symptoms and/or sings of infection necessitating further treatment.
[f] Exposure to the same antibiotic preceded the emergence of resistance.
[g] Whether the patients had been exposed to colistin/tigecycline before emergence of resistance was not reported.
[h] Patient with ventilator-associated pneumonia and empyema. Tigecycline resistance developed after 14 days of treatment with tigecycline and received treatment for a total of days. The patient received treatment with tigecycline for 49 days and was successfully treated.
[i] Resistance to tigecycline emerged during treatment with tobramycin (potential cross-resistance by overexpression of AdeB efflux pump).
[j] Resistance was attributed to a homologous recombination (including OprD, a sugar-type MFS permease, and a LuxR-type transcriptional regulator).
[k] Multiple colistin-resistant strains isolates from the same patient and with different mutations conferring colistin resistance.
[l] The patient died with persistent colistin-susceptible bacteremia, but all isolates demonstrated colistin-resistant subpopulations and the isolate from the endotracheal aspirate was colistin-resistant.
[m] No *pmrA*/*pmrB* mutations were detected and lipopolysaccharide was intact. Colistin resistance was attributed to acquisition of two genomic regions including among other genes *ispA* and *cadA*, both of which have the potential to modify lipid A or reduce the surface negative charges.
[n] In most cases mutation in either *pmrB* (n=8) or *pmrA* (2) were found. In one case a 2.3 kb deletion involving 94% of *pmrC*, all of *pmrA* and the first 44 amino acids of *pmrB* was found in the colistin-susceptible strain but not in the colistin-resistant strain. In 3 cases no *pmrA* or *pmrB* mutations were described, but an accumulation of mutations was observed in genes involved in surface structures including pilus, capsule and O antigen biosynthesis and transport.
[o] In most cases (based on the first study [42]) colistin-resistant *A. baumannii* required treatment and was associated with high in-hospital mortality but the relevant data could not be extracted for the subset of the 14 patients included in this review [41].
[p] Treatment with colistin preceded the development of colistin resistance in only 2 of the 3 cases.