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Is pandrug-resistance in *A. baumannii* a transient phenotype? Epidemiological clues from a 4-year cohort study at a tertiary referral hospital in Greece

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Abstract

Pandrug-resistant *A. baumannii* (PDRAB) is increasingly being reported but remains rare. Several case studies show that *A. baumannii* can acquire resistance to last resort antibiotics during treatment by single-step chromosomal mutations. However, re-emergence of the ancestral susceptible strain after withdrawal of antibiotics has been described, possibly due to fitness cost associated with acquired resistance. Therefore, PDRAB may be a transient phenotype. Epidemiological data to show this process in larger cohorts are currently lacking. In this study of 91 hospitalized patients with PDRAB we showed the frequent (60%) isolation of non-PDRAB, often susceptible only to colistin, aminoglycosides and/or tigecycline, preceding and/or following PDRAB isolation. However, the isolation of PDRAB in two outpatients, 25 and 36 days after their discharge from the hospital, suggests the potential of some PDRAB strains to persist even in the absence of antimicrobial pressure.

Keywords: Acinetobacter baumannii, resistance, pandrug-resistant, PDR, epidemiology

Introduction

A. baumannii can acquire resistance to last resort antibiotics during treatment by single-step chromosomal mutations, as has been demonstrated by several case studies, predominantly for colistin [1, 2], but also aminoglycosides [3] and tigecycline [2]. As a result, pandrug-resistant *A. baumannii* (PDRAB) is increasingly being reported worldwide [4]. Following withdrawal of antibiotics re-emergence of the ancestral susceptible strain has been described, probably due to fitness cost associated with emergent resistance [2, 5-7].

Therefore, we hypothesized that PDRAB can be a transient phenotype, i.e. emerging from extensively drug-resistant *A. baumannii* (XDRAB) under antibiotics pressure, followed by re-emergence of the fitter ancestral strain after withdrawal of antibiotics. Although case studies support this hypothesis epidemiological data from larger cohorts are lacking. Therefore, we reviewed the antimicrobial susceptibility patterns of sequential *A. baumannii* isolates in a relatively large cohort of hospitalized patients with PDRAB isolation.

Methods

Settings and study design

This cohort study, conducted in the University Hospital of Heraklion (Crete, Greece) from January 2016 to December 2019, comprised all hospitalized patients with PDRAB isolated from a clinical sample. The hospital's Research Ethics Committee approved the study and waived the requirement for patient informed consent.

Outcomes

We assessed the proportion of patients that had non-PDR *A. baumannii* (non-PDRAB) isolated before, concurrently (from another culture) and/or after first PDRAB isolation. The length of

hospital stay before/after first PDRAB isolation was also considered. Too short intervals could decrease the chance of detecting non-PDRAB before PDRAB isolation (considering the lack of systematic screening cultures) or may not allow sufficient time for the re-emergence of non-PDRAB. The antimicrobial susceptibility patterns of non-PDRAB isolates were analyzed. Isolation of PDRAB in outpatients presenting to the Emergency Department was also examined as an indication of the potential for persistence of PDRAB in the outpatient setting, i.e. in the absence of exposure to last resort antibiotics.

Microbiology

Species identification and susceptibility testing were conducted with VITEK-2 (bioMérieux), based on the CLSI breakpoints. Susceptibility to tigecycline was defined as an MIC ≤2mg/ml. Colistin resistance was also confirmed by disk diffusion (50μg colistin disk, cut-off for susceptibility ≥15mm). The list of antibiotics tested includes ampicillin/sulbactam, cotrimoxazole, ciprofloxacin, levofloxacin, aztreonam, imipenem, meropenem, amikacin, gentamicin, tobramycin, tetracycline, minocycline, colistin and tigecycline. PDRAB (non-susceptible to all antimicrobial agents listed) and XDRAB (non-susceptible to ≥1 agent in all but ≤2 categories) were defined according to consensus criteria [8] based on the above list of antibiotics. Tigecycline was grouped together with tetracyclines. Susceptibility to newer antibiotics, such as cefiderocol or eravacycline (neither of which were available during the study period), was not evaluated.

Statistical analysis

Data were processed and analyzed in IBM SPSS software. Categorical variables are presented as number (%) whereas continuous variables as median (interquartile range, IQR).

Results

Study population

A total of 152 PDRAB isolates were detected in 91 patients. Typically, PDRAB was isolated from middle-aged or elderly patients (median age 67 years, IQR 54-77), after lengthy hospital stays (median 23 days, IQR 13-41), during (56%) or after (29%) intensive care unit stay. In 2 cases PDRAB was isolated at presentation to the Emergency Department following prolonged hospitalizations (Figure 1).

Isolation of non-PDRAB before/after PDRAB

Non-PDRAB was isolated from 60% (55/91) of the patients with PDRAB; before the first PDRAB isolation in 34 patients (37%), on the same day in 12 patients (13%), after the first PDRAB isolation in 34 patients (37%), and both before and after in 16 patients (18%).

Documentation of non-PDRAB was more likely in patients with longer hospital stays. Non-PDRAB was documented before PDRAB in 45% (33/74) and 56% (19/34) of patients with pre-PDRAB length of stay >10 and >30 days, respectively. Non-PDRAB was documented after PDRAB in 59% (32/54) and 78% (25/32) of patients with post-PDRAB length of stay >10 and >30 days, respectively. Non-PDRAB isolation was documented both before and after PDRAB isolation in 33% (15/45) and 41% (7/17) of patients with >10 and >30 days of stay both before and after PDRAB isolation, respectively.

In most cases non-PDRAB was XDRAB, most often only susceptible to colistin and/or tigecycline/minocycline (Table 1). All non-PDRAB were carbapenem-resistant and non-susceptible to all antibiotics except at least one of the following: colistin, tigecycline/minocycline, amikacin/tobramycin and/or cotrimoxazole (Supplementary Tables 1 and 2). None of these isolates was reported as susceptible to ampicillin/sulbactam.

Discussion

Non-PDRAB, typically susceptible to only 1 or 2 antimicrobials, predominantly colistin and tigecycline (the main agents used for the treatment of XDRAB/PDRAB infections in our institution [9, 10]), was found in many patients before and/or after PDRAB isolation, despite the lack of systematic screening cultures. This is supportive of our hypothesis that *A. baumannii* may be able to temporarily acquire resistance to last resort antibiotics (Supplementary Figure 1).

The potential for emergence of PDRAB from non-PDRAB has important clinical implications. PDRAB infections can result in significant excess mortality [11] and treatment options are very limited [12]. Furthermore, transient emergence of PDRAB could be missed in the absence of screening cultures but may have the potential to result in PDRAB outbreaks, which has important infection control implications. Another important implication of our hypothesis is that PDRAB may be outcompeted by fitter non-PDRAB in the absence of continued antimicrobial pressure. Therefore, limiting unnecessary use of last resort antibiotics can result in reduction of the PDRAB burden. However, the isolation of PDRAB in 2 outpatients, suggests that some PDRAB strains may persist without exposure to last resort antimicrobials.

Our findings are in agreement with several studies reporting emergence of colistin or tigecycline resistance during treatment resulting from single-step chromosomal mutations [1, 2]. Notably, resistance mediated by upregulation of efflux pumps may result in cross-resistance to >1 last resort antibiotics affected by the same efflux pumps, including tigecycline, minocycline, aminoglycosides, trimethoprim [2, 13, 14], and even colistin [15]. This suggests potential for single-step emergence of PDRAB from *A. baumannii* susceptible to >1 last resort antimicrobials, which could explain some of the findings of this study.

Several mechanisms can explain the re-emergence of susceptible strains; a) re-emergence of the fitter ancestral strain [7], b) loss of resistance by additional compensatory mutations [5, 7], c) resistance mediated by unstable gene amplification [5], d) outcompeting of the resistant strain by a susceptible strain [7]. Persister *A. baumannii* cells, i.e. viable but non-dividing cells that can survive lethal doses of antibiotics and can re-emerge after cessation of antibiotics [16], in combination with fitness cost associated with resistance [5], can explain the first mechanism. However, resistance can emerge without any fitness/virulence cost [2].

Our study has some limitations. Cross-infection by different strains might explain the isolation of non-PDRAB before/after PDRAB in some patients [7]. Nevertheless, in a recent review most cases of emergent colistin and tigecycline resistance during treatment were confirmed to represent in vivo emergence of resistance rather than cross-infection based on comparison of sequential isolates with molecular methods [2].

Another potential limitation is that broth microdilution, the currently recommended method for colistin susceptibility testing, was not available during the study period. Nevertheless, false resistance is rare [17, 18]. Therefore, detection of the PDRAB phenotype was reliable. False susceptibility is more common and likely reflects lower sensitivity of other methods in detecting resistant subpopulations compared to broth microdilution [19, 20], but the clinical relevance of this is unclear [1]. Considering that all isolates were tested with the same methodology, any differences reflect an actual change in the susceptibility pattern rather than discrepancy attributable to different susceptibility testing methods.

Despite these limitations, our findings add to the available evidence supporting in vivo emergence of resistance in *A. baumannii* to last resort agents and the potential reversibility of this resistance after withdrawal of antibiotic pressure. However, further research is needed to confirm this hypothesis and the extent to which it occurs in vivo. This would require prospective

studies conducting systematic screening cultures from multiple sites to detect *A. baumannii* before, during and after exposure to last resort antimicrobials, ideally supplemented by genomic analyses to confirm the clonal relatedness of sequential isolates and identify mechanisms of emergent resistance and mechanisms of re-emergence of susceptible strains.

Declarations

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

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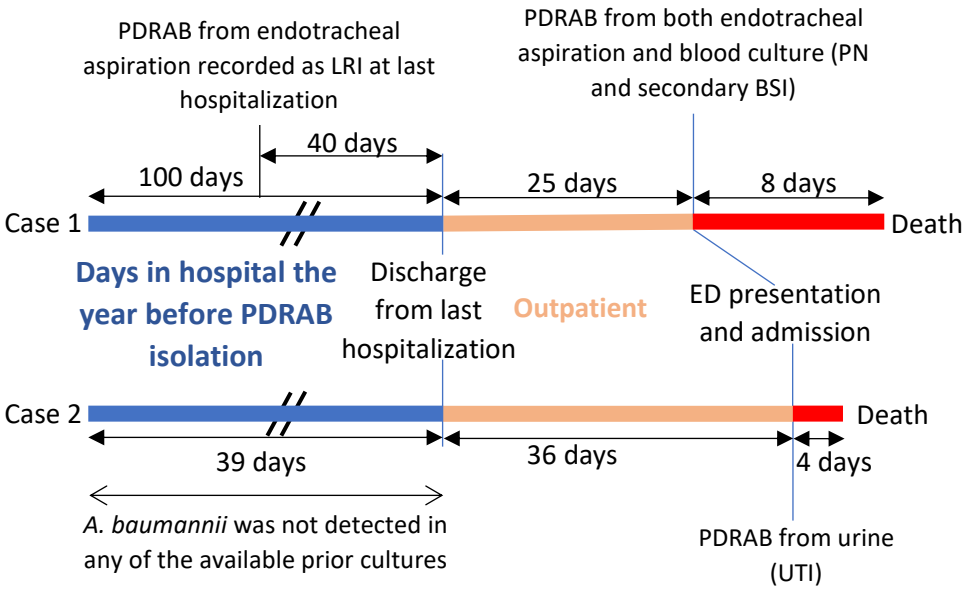
Figure 1; Course of the 2 cases of PDRAB isolation at the ED



Abbreviations; BSI= bloodstream infection, ED= emergency department, LRI = lower respiratory tract infection, PDRAB= pandrug-resistant *A. baumannii*, PN= pneumonia, UTI= urinary tract infection.

Table 1; Antimicrobial susceptibility pattern of non-PDR *A. baumannii* isolates preceding or following PDRAB isolation.

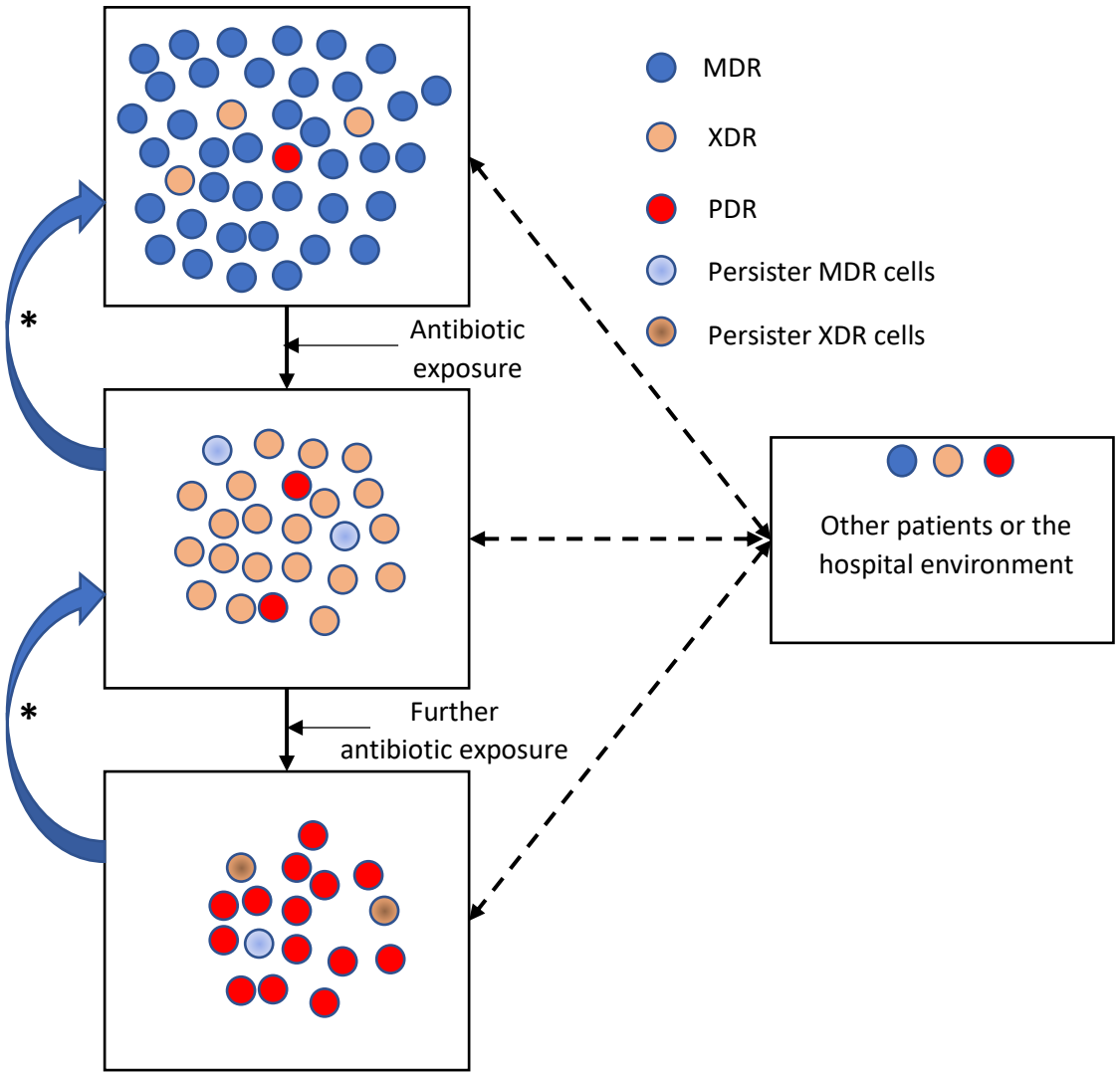
	Antimicrobial susceptibility pattern of non-PDRAB [#]					
Timing of isolation of non-PDRAB compared to the first PDRAB isolation	XDR n (%)*	Susceptible to only 1 antibiotic group n (%)*	Susceptible only to colistin n (%)*	Susceptible only to tigecycline/ minocycline n (%)*	Susceptible only to aminoglycoside n (%)*	Susceptible only to cotrimoxazole n (%)*
▪ Before (n=34)	30 (88%)	19 (56%)	17 (50%)	4 (12%)	2 (6%)	2 (6%)
▪ Same day (n=12)	11 (92%)	9 (75%)	6 (50%)	2 (17%)	1 (8%)	0 (0%)
▪ After (n=34)	30 (88%)	23 (68%)	13 (38%)	6 (18%)	4 (12%)	1 (3%)

PDR= pandrug-resistant, PDRAB= pandrug-resistant *A. baumannii*, XDR= extensively drug-resistant

[#] All non-PDRAB were non-susceptible to all antibiotics except at least one of the following: colistin, tigecycline, minocycline, amikacin, tobramycin, cotrimoxazole. Simultaneous emergence of resistance to >1 of these antibiotics may be possible by overexpression of efflux pumps [2, 13-15]. For more details on antimicrobial susceptibility patterns see Supplementary Tables 1 and 2.

* Number of patients with at least one *A. baumannii* with the described antimicrobial resistance pattern (percentage of patients in each row).

Supplementary Figure 1: Proposed pathways to pandrug-resistant *A. baumannii*



MDR=multidrug-resistant, **XDR=** extensively drug-resistant, **PDR=** pandrug-resistant

The presence of resistant subpopulations within the main population reflects heteroresistance [1, 5]. Heteroresistance may result from spontaneous chromosomal mutations conferring resistance [1, 2, 5] or from mixed infections [5]. Under antibiotic pressure the resistant subpopulations are selected [1, 2, 5]. Several case studies have confirmed this process [1, 2].

*Following withdrawal of antibiotic pressure re-emergence of susceptible strains is possible and has also been described in other studies [2, 7]. This may be possible due to 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 [16]) and unstable heteroresistance (resistance associated with fitness cost or unstable mutations [5]).

The frequent isolation of XDR *A. baumannii* before/after PDR isolation in this cohort is compatible with these pathways.

Supplementary Table 1: Susceptibility patterns of non-PDRAB susceptible to only 2 antibiotic groups

	Susceptible only to					
Timing of isolation of non-PDRAB compared to the first PDRAB isolation	Colistin + tigecycline/ minocycline n (%)*	Colistin + aminoglycoside n (%)*	Colistin + cotrimoxazole n (%)*	Tigecycline/ minocycline + aminoglycoside n (%)*	Tigecycline/ minocycline + Cotrimoxazole n (%)*	Cotrimoxazole + aminoglycoside n (%)*
▪ Before (n=34)	5 (15%)	4 (12%)	2 (6%)	1 (3%)	0 (0%)	0 (0%)
▪ Same day (n=12)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (8%)	0 (0%)
▪ After (n=34)	3 (9%)	4 (12%)	2 (6%)	4 (12%)	1 (3%)	0 (0%)

PDRAB= pandrug-resistant *A. baumannii*

* Number of patients with at least one *A. baumannii* with the described antimicrobial resistance pattern (percentage of patients in each row).

Supplementary Table 2: Susceptibility patterns of non-PDRAB susceptible to only 3 antibiotic groups

	Susceptible only to			
Timing of isolation of non-PDRAB compared to the first PDRAB isolation	Colistin + tigecycline/ minocycline + aminoglycoside n (%)*	Colistin + tigecycline/ minocycline + cotrimoxazole n (%)*	Colistin + aminoglycoside + cotrimoxazole n (%)*	Tigecycline/ minocycline + aminoglycoside + cotrimoxazole n (%)*
▪ Before (n=34)	8 (24%)	4 (12%)	1 (3%)	0 (0%)
▪ Same day (n=12)	1 (8%)	0 (0%)	0 (0%)	0 (0%)
▪ After (n=34)	4 (12%)	3 (9%)	0 (0%)	0 (0%)

PDRAB= pandrug-resistant *A. baumannii*

* Number of patients with at least one *A. baumannii* with the described antimicrobial resistance pattern (percentage of patients in each row).