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A comprehensive and contemporary "snapshot" of β-lactamases in carbapenem resistant *Acinetobacter baumannii*

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ABSTRACT

Successful treatment of *Acinetobacter baumannii* infections require early and appropriate antimicrobial therapy. One of the first steps in this process is understanding which β-lactamase (*bla*) alleles are present and in what combinations. Thus, we performed WGS on 98 carbapenem-resistant *A. baumannii* (CR *Ab*). In most isolates, an acquired bla_{OXA} carbapenemase was found in addition to the intrinsic bla_{OXA} allele. The most commonly found allele was bla_{OXA-23} (n = 78/98). In some isolates, bla_{OXA-23} was found in addition to other carbapenemase alleles: bla_{OXA-82} (n = 12/78), bla_{OXA-72} (n = 2/78) and $bla_{OXA-24/40}$ (n = 1/78). Surprisingly, 20% of isolates carried carbapenemases not routinely assayed for by rapid molecular diagnostic platforms, i.e., bla_{OXA-82} and $bla_{OXA-172}$; all had IS*Aba*1 elements. In 8 CR *Ab*, bla_{OXA-82} or $bla_{OXA-172}$ was the only carbapenemase. Both $bla_{OXA-24/40}$ and its variant bla_{OXA-72} were each found in 6/98 isolates. The most prevalent ADC variants were bla_{ADC-30} (21%), $bla_{ADC-162}$ (21%), and $bla_{ADC-212}$ (26%). Complete combinations are reported.

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1. Introduction

Multidrug-resistant (MDR) *Acinetobacter baumannii* (*Ab*) pose a significant challenge to modern medicine. The World Health Organization categorizes MDR *Ab* as the "highest priority pathogen" for which antibiotic development is urgently needed (Who-Publishes-List, 2020), and studies that aid our understanding of this organism are certain to have significant impact on this crisis. Currently, very few therapeutic agents exist or are in development that can be used to treat MDR *Ab* infections, including those of the bloodstream. In efforts to address this persistent dilemma, multiple pharmaceutical companies have undertaken different approaches to enhance our current therapeutic arsenal. These efforts include the development of a novel fluorocycline (eravacycline), siderophore β-lactams (e.g., cefiderocol), and more effective β-lactam β-lactamase inhibitors (e.g.,

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sulbactam/ETX2514 and cefepime/WCK4234) (Choi and McCarthy, 2018; Durand-Reville et al., 2017; Ito et al., 2018; Mushtaq et al., 2017; Papp-Wallace et al., 2018; Shapiro et al., 2017; Zhanel et al., 2016). In studies performed to date, these therapeutics are showing significant promise, but the fear is ever present that even these will fall short as universal agents to reliably treat MDR *Ab*.

β-lactamase inhibition is key in preserving efficacy of our current armamentarium of antibiotics against *Ab*. However, many times the focus of that inhibition is on a single class of β-lactamase. Clearly, in inhibitor design, we need to take into account all β-lactamases occurring in an individual organism, based upon our understanding of currently circulating alleles in isolates. In addition to OXA carbapenemases that *Ab* can acquire (e.g., OXA-23 [originally called ARI-1] and OXA-24/40), they possess an intrinsic OXA and AmpC (*Acinetobacter* Derived Cephalosporinase, ADC) β-lactamase, that can confer resistance to carbapenems and cephalosporins, respectively (Bou et al., 2000; Donald et al., 2000; Hujer et al., 2005). Therefore, class C and D β-lactamases are important targets for intervention as they are the major contributors of β-lactam resistance in *Ab*.

With these goals in mind, we performed whole genome sequencing (WGS) on 98 carbapenem-resistant *Ab* isolates (CR *Ab*, resistant to doripenem, imipenem, and meropenem) (Evans et al., 2017) and assessed the various β-lactamase combinations contained therein. This WGS database, and others like it, will be important in providing context to future work in the area of β-lactamase inhibitor design and provides a comprehensive "snapshot" of recently circulating β-lactamase genes (*bla* genes) in CR *Ab*. A better understanding of how to treat infections caused by opportunistic organisms like *Ab* will potentially be crucial in the fight for patients in the ICU on ventilators that are at an increased risk of hospital-acquired and ventilator-associated pneumonia caused by CR *Ab*. As CR *Ab* is a leading cause of hospital-acquired pneumonia, and also reported to be a pathogen of community-acquired Gram-negative pneumonia (Chung et al., 2011; Cilloniz et al., 2019; Lescure et al., 2020; Mohd Sazlly Lim et al., 2019; Ozgur et al., 2014; Serota et al., 2018; Wong et al., 2017). This could be especially significant during the COVID-19 pandemic (Lescure et al., 2020).

2. Methods and materials

This group of isolates was used in the Primers III study and a description of the isolates can be found there (Evans et al., 2017; Hujer et al., 2006; Perez et al., 2010). In short, the isolates used in this study consisted of 94 carbapenem resistant *Ab* collected between 2007 and 2013 from a 6-hospital healthcare system in Northeast Ohio: including 5 community hospitals and a facility serving as both as a long-term care unit and a long-term acute care hospital. Four additional *Ab* were collected from patients at the Walter Reed Army Medical Center between March 2003 and February 2005. All *Ab* clinical isolates in this study displayed an MDR phenotype and were resistant to all carbapenems tested.

The genomes of all *Ab* clinical isolates were sequenced using paired-end NexteraXT libraries by Illumina NextSeq (2×150 bp) to ~100-fold coverage. Reads were assembled using SPAdes (Bankevich et al., 2012), annotated using NCBI's Prokaryotic Genome Annotation Pipeline (Tatusova et al., 2016) and deposited in the NCBI SRA and GenBank WGS repositories (BioProjects PRJNA384060 and PRJNA384065). In addition, the DNA sequence for each isolate was analyzed using ResFinder at the Center for Genomic Epidemiology website. β-lactamase genes were manually extracted if not a 100% match with 100% coverage of a known β-lactamase gene.

For PCR analysis of IS*Aba*1 upstream of $bla_{\text{OXA-82}}$ and $bla_{\text{OXA-172}}$, the following primer set was used: 5' TGGATTGCACTTCATCTTGG 3' (OXA-51) and 5' CACGAATGCAGAAGTTG 3' (IS*Aba*1) (Segal et al., 2005; Woodford et al., 2006). This combination of primers produces an approximate 1200 bp product when IS*Aba*1 is proximal to the bla_{OXAs}.

3. Results

From the WGS data, the combination of β-lactamases found in each isolate was determined, along with the Pasteur and Oxford Multilocus Sequence Types (Bartual et al., 2005; Diancourt et al., 2010). This was done for each of the 98 CR *Ab*. The predominant Pasteur sequence type was the ST2 group, which is also known as international clonal lineage 2 (IC2). There were 79 CR *Ab* that were ST2, and 19 that were non-ST2 according to the Pasteur Scheme. Using the Oxford scheme ST1626 and ST1631 accounted for 40 and 23 isolates, respectively. Table 1 summarizes the combination of β-lactamases found in this collection, as well as which STs they were found in.

3.1. bla_{OXAs}

In most isolates, an acquired bla_{OXA} carbapenemase was found in addition to the intrinsic or chromosomal bla_{OXA} allele. However, in 18 ST2 isolates, the intrinsic bla_{OXA} acquired a single point mutation that converted $bla_{\text{OXA-66}}$ (the variant associated with IC2 isolates) to $bla_{\text{OXA-82}}$ (Table 1) (Zander et al., 2013). $bla_{\text{OXA-82}}$ was also found as the sole carbapenemase allele in 6% of isolates. In addition, $bla_{\text{OXA-172}}$ was found in two ST1088 isolates. Both OXA-82 (OXA-66 variant with L167V), and OXA-172 (OXA-66 variant with I129V and W222L) are carbapenemases (Mitchell and Leonard, 2014; Schroder et al., 2016; Zander et al., 2013), and were found in combination with IS*Aba*1 upstream of the bla_{OXA} gene as evidenced by PCR analysis.

By far, the most commonly found acquired OXA carbapenemase allele was $bla_{\text{OXA-23}}$ (Table 1) (Donald et al., 2000). This *bla* gene was detected in 78 of the 98 CR isolates. In 81% (n = 63/78) of the isolates containing $bla_{\text{OXA-23}}$, it was the only carbapenemase present. However, in some isolates it was found in addition to other carbapenemase alleles, i.e., in addition to $bla_{\text{OXA-82}}$ (n = 12/78), $bla_{\text{OXA-72}}$ (n = 2/78) and $bla_{\text{OXA-24/40}}$ (n = 1/78). The carbapenemase genes $bla_{\text{OXA-24/40}}$ (Bou et al., 2000) and its variant $bla_{\text{OXA-72}}$ were each found in 6/98 isolates (both alone and in combination with $bla_{\text{OXA-23}}$), but never found together in a single isolate (Table 1). While the $bla_{\text{OXA-58}}$ allele (Poirel et al., 2005) was found in 2 isolates as the sole carbapenemase gene.

3.2. $blaADC_{\text{s}}$

The most prevalent *Acinetobacter* Derived Cephalosporinase (ADC) variants were $bla_{\text{ADC-30}}$ (n = 21/98 isolates), $bla_{\text{ADC-162}}$ (ADC-30 variant with an A220E mutation in the Ω loop, n = 21/98), and $bla_{\text{ADC-212}}$ (ADC-25 variant with A200D, P219L, and an alanine insertion in the Ω loop, n = 25/98; Tables 1 and 2) (Kuo et al., 2015). Also, of note, $bla_{\text{ADC-33}}$ and $bla_{\text{ADC-219}}$ (ADC-33 with G222D) were found in 8/98 isolates (Rodriguez-Martinez et al., 2010). Other ADC variants (21/98) were found in all but 2 isolates (Table 1). Numbering of the amino acids in the ADC variants is based on the *SANC* numbering scheme (Mack et al., 2019).

Many combinations of β-lactamases were found in individual isolates. However, certain combinations were more frequent than others. The 3 most frequently found combinations were: OXA-23/OXA-66/ADC-162 with or without TEM-1 (n = 21); OXA-23/OXA-66/ADC-212 (n = 21); and OXA-23/OXA-66/ADC-30 with or without TEM-1 (n = 10) (Table 1). The high prevalence of OXA-66 reflects the prevalence of ST2 isolates circulating in hospitals (Adams et al., 2019; Wright et al., 2014; Wright et al., 2016).

4. Discussion

The current prevalence of multidrug- and pandrug-resistant strains of *Ab*, combined with the lack of new antibiotics, underscores

Table 1
β-lactamase combinations found within the 98 carbapenem resistant *Acinetobacter baumannii*.

	All β-lactamases present				
Pasteur ST - (# of Isolates)	Carbapenemase	Intrinsic OXA	ADC	Other	Oxford ST
ST2 - (21)	OXA-23	OXA-66	ADC-212		ST1631
ST2 - (16)	OXA-23	OXA-66	ADC-162	TEM-1	ST1626 - (13), ST1660 - (1), ST1661 - (1), ST1676 - (1)
ST2 - (9)	OXA-23	OXA-66	ADC-30	TEM-1	ST1626
ST2 - (5)	OXA-23	OXA-66	ADC-162		ST1626
ST2 - (1)	OXA-23	OXA-66	ADC-143	TEM-1	ST1626
ST2 - (1)	OXA-23	OXA-66	ADC-213		ST1631
ST2 - (1)	OXA-23	OXA-66	ADC-30		ST1626
ST2 - (3)	OXA-23, OXA-82[a]		ADC-56	TEM-1	ST1626
ST2 - (3)	OXA-23, OXA-82[a]		ADC-33		ST1637
ST2 - (3)	OXA-23, OXA-82[a]		ADC-219		ST1637
ST2 - (3)	OXA-23, OXA-82[a]		ADC-30		ST1626
ST2 - (1)	OXA-82[a]		ADC-56		ST1626
ST2 - (1)	OXA-82[a]		ADC-219		ST1637
ST2 - (2)	OXA-82[a]		ADC-30		ST1626
ST2 - (1)	OXA-82[a]		ADC-33		ST1637
ST2 - (1)	OXA-23[c], OXA-82[a]		ADC-212		ST1631
ST2 - (3)	OXA-72	OXA-66	ADC-30		ST1626 - (1), ST1628 - (2)
ST2 -(1)	OXA-72[c]	OXA-66	ADC-217		ST1628
ST2 - (2)	OXA-23, OXA-72	OXA-66	ADC-30		ST1628 - (1), ST1632 - (1)
ST2 - (1)	OXA-58	OXA-66	ADC-30	TEM-1	ST1626
ST250 - (3)	OXA-23	OXA-407	ADC-216		ST1646
ST10 - (2)	OXA-23	OXA-68	ADC-76		ST447
ST406 - (2)	OXA-23	OXA-71	ADC-212		ST1635
ST1 - (1)	OXA-23	OXA-69	ADC-176[c]	TEM-1	ST1663
ST25 - (1)	OXA-23	OXA-64	ADC-26		ST993
ST79 - (1)	OXA-23, OXA-24/40	OXA-65	ADC-218		ST1629
ST79 - (1), ST93 - (1)	OXA-24/40	OXA-65		TEM-1	ST1348 - (1), ST1629 - (1)
ST79 - (1)	OXA-24/40	OXA-65	ADC-218		ST1629
ST406 - (1)	OXA-24/40	OXA-71	ADC-212		ST1635
ST79 - (1)	OXA-24/40	OXA-65	ADC-214	TEM-1	ST1629
ST32 - (1)	OXA-58	OXA-100	ADC-79		ST1627
ST406 - (1)	OXA-72	OXA-223	ADC-220		ST1635
ST1088 - (2)	OXA-172[b]		ADC-215		ST1656 (1), ST1669 (1)

[a] OXA-82 is a single amino acid variant of the intrinsic OXA-66 with carbapenemase activity.
[b] OXA-172 is a double amino acid variant of OXA-66 with carbapenemase activity.
[c] Partial sequence.

the critical need for the development of novel therapeutic strategies to combat this pathogen. The end goal of studies like this is to facilitate the design of inhibitors that target the most prevalent combinations of β-lactamases occurring in circulating CR *Ab*, and the first step in this process is to determine which β-lactamase alleles are present in contemporary isolates.

Building upon our work with PRIMERS III, an evaluation of rapid molecular diagnostics to identify carbapenem susceptibility and resistance in *Acinetobacter* spp. (Evans et al., 2017), our group performed an extensive analysis of the WGS of 98 CR *Ab* strains used in that study, with particular attention being paid to the β-lactamase combinations contained within each isolate. Based on these results:

Table 2
New ADC variants in this study.

New ADC number	ADC-like	Reference number
ADC-212	ADC-25 A200D, P219L, Ala219a ins btw P219L and A220	OTN05897.1
ADC-213	ADC-25 A200D, ins of SLA that replaces AP btw D217 and A220	OTR53589.1
ADC-214	ADC-52 G222S, N320T	OTR85897.1
ADC-215	ADC-170 G214A, P219S, S320T	OTT53070.1
ADC-216	ADC-25 G75A, D86N, S143P, P169S, N206K, T279P	OTT57830.1
ADC-217	ADC-30 V262E	OTT60833.1
ADC-218	ADC-30 A200D, P219L, Ala219a ins btw P219L and A220, K362E	OTU52329.1
ADC-219	ADC-33 G222D	OTU79690.1
ADC-220	ADC-25 Q120K, A200D, P219L	OVN99777.1

Table 1 summarizes β-lactamase alleles present; Table 2 describes the new ADCs in this collection, Table 3 presents what is already known about the key properties and structure/function basis of the OXA carbapenemases and ADC β-lactamases found herein, and Table 4 compares the ability of various rapid molecular diagnostic (RMD) platforms to detect the carbapenemases found in this collection, as determined from the platform's product literature.

OXA-23 has been shown to be a major driver of carbapenem resistance in *Ab*, and OXA-24/40, an enzyme similar to OXA-23 in structure and specificity, is also extensively found. Both are acquired high affinity carbapenemases that have a common structural feature thought to be primarily responsible for this property. This structural feature is a hydrophobic bridge of two residues that stretches across the top of the active site (Table 3) (Kaitany et al., 2013; Santillana et al., 2007; Schneider et al., 2011; Smith et al., 2013; Stewart et al., 2019). It is important to note that both of these acquired carbapenemase genes are found on highly transmissible mobile genetic elements, this is particularly worrisome from an infection control standpoint, because not only can horizontal transmission of these common resistance elements occur between *Acinetobacter* strains and plasmids, but also interspecies plasmid transfer can occur (Grosso et al., 2012).

Of note, we have identified OXA variants in this collection with substitutions that expand their substrate profile. OXA-82 is one such variant, a L167V substitution in OXA-66 greatly enhances the hydrolytic efficiency of that enzyme toward carbapenems (Table 3) (Zander et al., 2013; Mitchell and Leonard, 2014). In most isolates, an acquired bla_{OXA} carbapenemase allele was found in addition to the intrinsic or chromosomal bla_{OXA} allele. However, in 18 isolates it appears that the intrinsic $bla_{\text{OXA-66}}$ was converted by mutation to

Table 3
Class C and D β-lactamases most prevalent in the clinical isolates of carbapenem-resistant *A. baumannii*.

β-lactamase	Variant	Property	Structure/function rationale	Ref.
Class D carbapenemases	OXA-23 and OXA-24/40	High-affinity carbapenemases	Similar bridge residues in OXA-24/40 (Y112 and M223) and OXA-23 (F110 and M221) suggest that both use the same mechanism to achieve tight substrate binding in order to compensate for a slow turnover rate of carbapenems, thus resulting in clinical resistance. Structural analyses of OXA-24/40, with and without doripenem bound, revealed that the hydrophobic bridge across the top of the active site helps hold onto carbapenems that possess extended nonpolar side chains. However, penicillins that possess bulky side chains are sterically prohibited in the active site.	Kaitany et al. (2013), Santillana et al. (2007), Schneider et al. (2011), Smith et al. (2013), Stewart et al. (2019)
	OXA-72	High-affinity carbapenemase	OXA-24/40 with G224D substitution in the $\beta5\beta6$ loop causes no loss of activity for carbapenems. A crucial role of the $\beta5$-$\beta6$ loop in carbapenemase activity of class D β-lactamases has been demonstrated.	Schneider et al. (2011), Lu et al. (2009), De Luca et al. (2011)
	OXA-82	Carbapenem gain-of-function	As demonstrated with molecular modeling, OXA 51/66 with a L167V substitution makes room for the rotation of the side-chain of I129; this in turn removes the steric clash of isoleucine with the hydroxyethyl group of carbapenems and affinity is greatly increased.	Zander et al. (2013), Mitchell and Leonard (2014)
	OXA-172	Carbapenem gain-of-function	Substitutions, I129V and W222L in OXA-66. Result in tighter binding of doripenem and imipenem in the active site.	Schroder et al. (2016)
Class C β-lactamases	ADC-30	Sulbactam resistance	21/98 of ADCs isolated from multidrug-resistant *Ab* were ADC-30; overexpression of ADC-30 contributes to sulbactam resistance in *Ab*.	Kuo et al. (2015)
	ADC-162	Not determined	ADC-30 with A220E, which is a substitution in the Ω loop region.	
	ADC-56	Cefepime resistance	ADC-30 with an R148Q substitution. Molecular modeling demonstrated that the R148Q substitution in ADC-56 disrupts hydrogen bonds with Q267, E272, and I291 providing the H-10 helix more flexibility, likely allowing for better binding and turnover of cefepime.	Tian et al. (2011)
	ADC-33	Increased hydrolysis of ceftazidime, cefepime, and aztreonam	In ADC-33, an alanine insertion allowed for the hydrolysis of ceftazidime, cefepime, and aztreonam at high levels.	Rodriguez-Martinez et al. (2010)
	ADC-219	Not determined	ADC-33 with G222D, which is a substitution in the Ω loop region.	
	ADC-212	Not determined	ADC-25 with A200D/P219L and an alanine insertion between P219L and A220. In ADC-212, the P219L followed by an alanine insertion might confer the same phenotype as in ADC-33.	Rodriguez-Martinez et al. (2010)

$bla_{\text{OXA-82}}$. Another such variant in this collection was OXA-172, found in 2 ST1088 isolates. It also appears to have evolved from $bla_{\text{OXA-66}}$, as no other intrinsic bla_{OXA} was found, and it differs from OXA-66 by 2 substitutions, I129V and W222L. It has been shown that these substitutions result in tighter binding of the enzyme to doripenem and imipenem. In fact, OXA-172 displayed carbapenem K_S values comparable to those of OXA-23 and OXA-24/40 (Schroder et al., 2016). IS*Aba*1 was found upstream of $bla_{\text{OXA-82}}$ and $bla_{\text{OXA-172}}$ (both intrinsic bla_{OXAs}), as previously reported in other isolates; this is of utmost importance, as the presence of this insertion element is necessary for a clinically CR phenotype, because these bla_{OXAs} are weak carbapenemases and the IS element provides a strong promoter for overexpression (Zander et al., 2013; Zander et al., 2012). Also of note, the wild-type OXA-66 does not display the carbapenemase activity of its point mutation derivative OXA-82 (Zander et al., 2013). Lastly, OXA-72, which is OXA-24/40 with a G224D substitution, was present in 6 isolates. OXA-72 retains its carbapenemase activity and has been associated with numerous outbreaks (Barnaud et al., 2010; Franolic-Kukina et al., 2011; Lee et al., 2009; Lu et al., 2009).

It is an extremely troubling finding that 20% of isolates contained $bla_{\text{OXA-82}}$ and $bla_{\text{OXA-172}}$ carbapenemase genes, as currently available RMD platforms do not assay for these resistance determinants, nor

Table 4
Comparison of RMDs ability to detect carbapenemase genes found in this study.[a]

Platform	OXA-23	OXA-24/40	OXA-58	OXA-72	OXA-82	OXA-172
Verigene BC-GN	Yes	Yes	Yes	Yes[b]	No	No
BioFire Film Array	No	No	No	No	No	No
Xpert® Carba-R Assay	No	No	No	No	No	No
Acuitas AMR Gene Panel	No	No	No	No	No	No
ePlex BCID-GN Panel	Yes	No	No	No	No	No
Check-points CT 103XL	yes	Yes	Yes	Yes[b]	No	No

[a] Data as determined from platform's product literature.
[b] Predicted to be detected based on in silico analysis, would call it OXA-24/40.

do they detect the presence of IS*Aba*1insertion elements upstream of the genes. It is important to keep in mind that without the IS element upstream, these bla_{OXAs}, including bla_{OXA-23}, do not confer carbapenem resistance. Even WGS can miss IS*Aba*1-associated *bla* genes due to the IS*Aba*1insertion sequences being present in multiple locations of the chromosome and plasmids, leading to misassembles and fragmented contigs when only using short Illumina reads. It has been our experience that besides closing genomes by inclusion of long reads, standard PCR amplifications are needed to accurately determine the IS*Aba*1 positions relative to a gene of interest, such as bla_{OXA-82} and $bla_{OXA-172}$ (Zander et al., 2013; Zander et al., 2012).

Table 4 compares the ability of various RMD platforms to detect the carbapenemase genes found in this collection of *Ab* isolates. As can be seen, none of the currently available platforms are able to detect bla_{OXA-82} and $bla_{OXA-172}$. BioFire Film Array, Xpert® Carba-R Assay, and Acuitas AMR Gene Panel do not even detect bla_{OXA-23}, which was by far the most commonly found OXA carbapenemase allele (80% of isolates).

ADCs are chromosomally encoded class C β-lactamases, found in *Ab* and other *Acinetobacter* spp., that are responsible for resistance to penicillins, cephalosporins, and BL/BLI combinations (Hujer et al., 2005). Being among the first laboratories to recognize the importance of this β-lactamase, our early work showed that ADC-7 β-lactamase demonstrates a remarkably high turnover rate for first-generation cephalosporins and relatively low affinity for the commercially available BLIs (Hujer et al., 2005).

The ADC allele most commonly found in this collection was $bla_{ADC-212}$ (n = 25/98 isolates). It encoded an ADC-25-like β-lactamase with the following substitutions: A200D, P219L, and an Ala219a insertion between P219L and A220 (*SANC* numbering) (Table 2). ADC-33 (ADC-30-like, with a P213R substitution and the same insertion of an Ala residue inside the Ω loop) hydrolyzes ceftazidime, cefepime, and aztreonam, but not carbapenems (Rodriguez-Martinez et al., 2010). We speculate that for ADC-212, the Ala219a (between P219L and A220) might confer an extended-spectrum resistance phenotype similar to ADC-33 (Tables 2 and 3). In efforts to understand the importance of these substitutions in the larger context of structure-activity relationships, we generated preliminary data that suggests the alanine insertion between P219L and A220 in the Ω loop increases ceftazidime MICs from 16 mg/L to greater than 512 mg/L (unpublished data).

Other studies of ADC variants found within this collection demonstrate that subtle changes in the active site can lead to alterations in substrate turnover. ADC-30 was shown to contribute to sulbactam resistance when overexpressed in *Ab* (Kuo et al., 2015). Within our collection, bla_{ADC-30} (n = 21/98 isolates) and $bla_{ADC-162}$ (n = 21/98 isolates) were also frequently found. ADC-162 is ADC-30 with an A220E substitution in the Ω loop region. Additionally, ADC-56 present in some of our clinical isolates, is a variant of ADC-30 containing a single mutation at R148Q that confers the ability to hydrolyze cefepime (Table 3) (Tian et al., 2011). Even more worrisome is ADC-68, an ADC that has gained the ability to hydrolyze carbapenems. ADC-68 was not identified in this study, but is worthy of mention given its carbapenemase activity (Jeon et al., 2014).

In conclusion, we believe that bla_{OXA-82} and $bla_{OXA-172}$ are currently underappreciated as causative agents of, and contributors to, carbapenem resistance in *Ab*, as they were present in 20% of the CR *Ab*. Additionally, we anticipate that focusing our efforts of inhibition on targeting the various combinations of β-lactamases found in currently circulating, clinical isolates of *Ab* will enable us to truly assess whether newly synthesized inhibitors will be effective in the clinic. Our innovative approach considers all the β-lactamases occurring in a single organism based on the WGS combinations observed in this contemporary group of isolates from the US. Inhibitors need to effectively inhibit all of these β-lactamases simultaneously, and it is critical to counter bacterial resistance caused by the expansion and expression of multiple β-lactamases. We believe this a possibility, and that the impact of these studies may have broader implications for other bacterial pathogens.

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Authors Contribution

Andrea M. Hujer: Writing-original and final draft presentations; data analysis; and conceptualization. **Kristine M. Hujer:** Reviewing and editing of manuscript; data review. **David A. Leonard:** Conceptualization; reviewing and editing of manuscript. **Rachel A. Powers:** Conceptualization; reviewing and editing of manuscript. **Bradley J. Wallar:** Conceptualization; reviewing and editing of manuscript. **Andrew R. Mack:** Data analysis; reviewing and editing of manuscript. **Magdalena A. Taracila:** Data analysis. **Philip N. Rather:** reviewing and editing of manuscript. **Paul G. Higgins:** Conceptualization; reviewing and editing of manuscript. **Fabio Prati:** Reviewing and editing of manuscript. **Emilia Caselli:** Reviewing and editing of manuscript. **Steven H. Marshall:** Data acquisition, curation, and analysis. **Thomas Clarke:** Data acquisition, curation, and analysis. **Christopher Greco:** Data acquisition, curation, and analysis. **Pratap Venepally:** Data acquisition, curation, and analysis. **Lauren Brinkac:** Data acquisition, curation, and analysis. **Barry N. Kreiswirth:** Reviewing and editing of manuscript. **Derrick E. Fouts:** Supervision; data acquisition and curation; reviewing and editing of manuscript. **Robert A. Bonomo**: Conceptualization; supervision; reviewing and editing of manuscript.

References

http://www.who.int/en/news-room/detail/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed.

Choi JJ, McCarthy MW. Cefiderocol: a novel siderophore cephalosporin. Expert Opin Investig Drugs 2018;27:193–7.

Durand-Reville TF, Guler S, Comita-Prevoir J, Chen B, Bifulco N, Huynh H, et al. ETX2514 is a broad-spectrum beta-lactamase inhibitor for the treatment of drug-resistant Gram-negative bacteria including *Acinetobacter baumannii*. Nat Microbiol 2017;2:17104.

Ito A, Sato T, Ota M, Takemura M, Nishikawa T, Toba S, et al. In vitro antibacterial properties of cefiderocol, a novel siderophore cephalosporin, against gram-negative bacteria. Antimicrob Agents Chemother 2018;62: e01454-17.

Mushtaq S, Vickers A, Woodford N, Livermore DM. WCK 4234, a novel diazabicyclooctane potentiating carbapenems against Enterobacteriaceae, *Pseudomonas* and *Acinetobacter* with class A, C and D beta-lactamases. J Antimicrob Chemother 2017;72:1688–95.

Papp-Wallace KM, Nguyen NQ, Jacobs MR, Bethel CR, Barnes MD, Kumar V, et al. Strategic approaches to overcome resistance against gram-negative pathogens using beta-Lactamase Inhibitors and beta-Lactam Enhancers: activity of three novel diazabicyclooctanes WCK 5153, zidebactam (WCK 5107), and WCK 4234. J Med Chem 2018;61:4067–86.

Shapiro AB, Gao N, Jahic H, Carter NM, Chen A, Miller AA. Reversibility of covalent, broad-spectrum serine beta-Lactamase inhibition by the Diazabicyclooctenone ETX2514. ACS Infect Dis 2017;3:833–44.
Zhanel GG, Cheung D, Adam H, Zelenitsky S, Golden A, Schweizer F, et al. Review of eravacycline, a novel fluorocycline antibacterial agent. Drugs 2016;76:567–88.
Bou G, Oliver A, Martinez-Beltran J. OXA-24, a novel class D beta-lactamase with carbapenemase activity in an *Acinetobacter baumannii* clinical strain. Antimicrob Agents Chemother 2000;44:1556–61.
Donald HM, Scaife W, Amyes SG, Young HK. Sequence analysis of ARI-1, a novel OXA beta-lactamase, responsible for imipenem resistance in *Acinetobacter baumannii* 6B92. Antimicrob Agents Chemother 2000;44:196–9.
Hujer KM, Hamza NS, Hujer AM, Perez F, Helfand MS, Bethel CR, et al. Identification of a new allelic variant of the *Acinetobacter baumannii* cephalosporinase, ADC-7 beta-lactamase: defining a unique family of class C enzymes. Antimicrob Agents Chemother 2005;49:2941–8.
Evans SR, Hujer AM, Jiang H, Hill CB, Hujer KM, Mediavilla JR, et al. Informing antibiotic treatment decisions: evaluating rapid molecular diagnostics to identify susceptibility and resistance to carbapenems against *Acinetobacter* spp. in PRIMERS III. J Clin Microbiol 2017;55:134–44.
Chung DR, Song JH, Kim SH, Thamlikitkul V, Huang SG, Wang H, et al. High prevalence of multidrug-resistant nonfermenters in hospital-acquired pneumonia in Asia. Am J Respir Crit Care Med 2011;184:1409–17.
Cilloniz C, Dominedo C, Torres A. Multidrug resistant gram-negative bacteria in community-acquired Pneumonia. Crit Care 2019;23:79.
Lescure FX, Bouadma L, Nguyen D, Parisey M, Wicky PH, Behillil S, et al. Clinical and virological data of the first cases of COVID-19 in Europe: a case series. Lancet Infect Dis 2020;20:697–706.
Mohd Sazlly Lim S, Zainal Abidin A, Liew SM, Roberts JA, Sime FB. The global prevalence of multidrug-resistance among *Acinetobacter baumannii* causing hospital-acquired and ventilator-associated pneumonia and its associated mortality: a systematic review and meta-analysis. J Infect 2019;79:593–600.
Ozgur ES, Horasan ES, Karaca K, Ersoz G, Nayci Atis S, Kaya A. Ventilator-associated pneumonia due to extensive drug-resistant *Acinetobacter baumannii*: risk factors, clinical features, and outcomes. Am J Infect Control 2014;42:206–8.
Serota DP, Sexton ME, Kraft CS, Palacio F. Severe community-acquired pneumonia due to *Acinetobacter baumannii* in North America: case report and review of the literature. Open Forum Infect Dis 2018;5:ofy044.
Wong D, Nielsen TB, Bonomo RA, Pantapalangkoor P, Luna B, Spellberg B. Clinical and pathophysiological overview of *Acinetobacter* infections: a century of challenges. Clin Microbiol Rev 2017;30:409–47.
Hujer KM, Hujer AM, Hulten EA, Bajaksouzian S, Adams JM, Donskey CJ, et al. Analysis of antibiotic resistance genes in multidrug-resistant *Acinetobacter* sp. isolates from military and civilian patients treated at the Walter Reed Army Medical Center. Antimicrob Agents Chemother 2006;50:4114–23.
Perez F, Endimiani A, Ray AJ, Decker BK, Wallace CJ, Hujer KM, et al. Carbapenem-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae* across a hospital system: impact of post-acute care facilities on dissemination. J Antimicrob Chemother 2010;65:1807–18.
Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 2012;19:455–77.
Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, et al. NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res 2016;44:6614–24.
Segal H, Garny S, Elisha BG. Is IS*Aba*-1 customized for *Acinetobacter*?. FEMS Microbiol Lett 2005;243:425–9.
Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, et al. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. Int J Antimicrob Agents 2006;27:351–3.
Bartual SG, Seifert H, Hippler C, Luzon MA, Wisplinghoff H, Rodriguez-Valera F. Development of a multilocus sequence typing scheme for characterization of clinical isolates of *Acinetobacter baumannii*. J Clin Microbiol 2005;43:4382–90.
Diancourt L, Passet V, Nemec A, Dijkshoorn L, Brisse S. The population structure of *Acinetobacter baumannii*: expanding multiresistant clones from an ancestral susceptible genetic pool. PLoS One 2010;5:e10034.
Zander E, Chmielarczyk A, Heczko P, Seifert H, Higgins PG. Conversion of OXA-66 into OXA-82 in clinical *Acinetobacter baumannii* isolates and association with altered carbapenem susceptibility. J Antimicrob Chemother 2013;68:308–11.
Mitchell JM, Leonard DA. Common clinical substitutions enhance the carbapenemase activity of OXA-51-like class D beta-lactamases from *Acinetobacter* spp. Antimicrob Agents Chemother 2014;58:7015–6.
Schroder EC, Klamer ZL, Saral A, Sugg KA, June CM, Wymore T, et al. Clinical variants of the native class D beta-lactamase of *Acinetobacter baumannii* pose an emerging threat through increased hydrolytic activity against carbapenems. Antimicrob Agents Chemother 2016;60:6155–64.
Poirel L, Marque S, Heritier C, Segonds C, Chabanon G, Nordmann P. OXA-58, a novel class D {beta}-lactamase involved in resistance to carbapenems in *Acinetobacter baumannii*. Antimicrob Agents Chemother 2005;49:202–8.
Kuo SC, Lee YT, Yang Lauderdale TL, Huang WC, Chuang MF, Chen CP, et al. Contribution of *Acinetobacter*-derived cephalosporinase-30 to sulbactam resistance in *Acinetobacter baumannii*. Front Microbiol 2015;6:231.
Rodriguez-Martinez JM, Nordmann P, Ronco E, Poirel L. Extended-spectrum cephalosporinase in *Acinetobacter baumannii*. Antimicrob Agents Chemother 2010;54:3484–8.
Mack AR, Barnes MD, Taracila MA, Hujer AM, Hujer KM, Cabot G, et al. A standard numbering scheme for class C beta-lactamases. Antimicrob Agents Chemother 2020;64:e01841-19.
Adams MD, Wright MS, Karichu JK, Venepally P, Fouts DE, Chan AP, et al. Rapid Replacement of *Acinetobacter baumannii* Strains Accompanied by Changes in Lipooligosaccharide Loci and Resistance Gene Repertoire. mBio 2019;10: e00963-13.
Wright MS, Haft DH, Harkins DM, Perez F, Hujer KM, Bajaksouzian S, et al. New insights into dissemination and variation of the health care-associated pathogen *Acinetobacter baumannii* from genomic analysis. mBio 2014;5: e00963-13.
Wright MS, Iovleva A, Jacobs MR, Bonomo RA, Adams MD. Genome dynamics of multidrug-resistant *Acinetobacter baumannii* during infection and treatment. Genome Med 2016;8:26.
Kaitany KC, Klinger NV, June CM, Ramey ME, Bonomo RA, Powers RA, et al. Structures of the class D Carbapenemases OXA-23 and OXA-146: mechanistic basis of activity against carbapenems, extended-spectrum cephalosporins, and aztreonam. Antimicrob Agents Chemother 2013;57:4848–55.
Santillana E, Beceiro A, Bou G, Romero A. Crystal structure of the carbapenemase OXA-24 reveals insights into the mechanism of carbapenem hydrolysis. Proc Natl Acad Sci U S A 2007;104:5354–9.
Schneider KD, Ortega CJ, Renck NA, Bonomo RA, Powers RA, Leonard DA. Structures of the class D carbapenemase OXA-24 from *Acinetobacter baumannii* in complex with doripenem. J Mol Biol 2011;406:583–94.
Smith CA, Antunes NT, Stewart NK, Toth M, Kumarasiri M, Chang M, et al. Structural basis for carbapenemase activity of the OXA-23 beta-lactamase from *Acinetobacter baumannii*. Chem Biol 2013;20:1107–15.
Stewart NK, Smith CA, Antunes NT, Toth M, Vakulenko SB. Role of the hydrophobic bridge in the carbapenemase activity of class D beta-Lactamases. Antimicrob Agents Chemother 2019;63: e02191-18.
Grosso F, Quinteira S, Poirel L, Novais A, Peixe L. Role of common blaOXA-24/OXA-40-carrying platforms and plasmids in the spread of OXA-24/OXA-40 among *Acinetobacter* species clinical isolates. Antimicrob Agents Chemother 2012;56:3969–72.
Zander E, Nemec A, Seifert H, Higgins PG. Association between beta-lactamase-encoding *bla*(OXA-51) variants and DiversiLab rep-PCR-based typing of *Acinetobacter baumannii* isolates. J Clin Microbiol 2012;50:1900–4.
Barnaud G, Zihoune N, Ricard JD, Hippeaux MC, Eveillard M, Dreyfuss D, et al. Two sequential outbreaks caused by multidrug-resistant *Acinetobacter baumannii* isolates producing OXA-58 or OXA-72 oxacillinase in an intensive care unit in France. J Hosp Infect 2010;76:358–60.
Franolic-Kukina I, Bedenic B, Budimir A, Herljevic Z, Vranes J, Higgins PG. Clonal spread of carbapenem-resistant OXA-72-positive *Acinetobacter baumannii* in a Croatian university hospital. Int J Infect Dis 2011;15:e706–9.
Lee K, Kim MN, Choi TY, Cho SE, Lee S, Whang DH, et al. Wide dissemination of OXA-type carbapenemases in clinical *Acinetobacter* spp. isolates from South Korea. Int J Antimicrob Agents 2009;33:520–4.
Lu PL, Doumith M, Livermore DM, Chen TP, Woodford N. Diversity of carbapenem resistance mechanisms in *Acinetobacter baumannii* from a Taiwan hospital: spread of plasmid-borne OXA-72 carbapenemase. J Antimicrob Chemother 2009;63:641–7.
Tian GB, Adams-Haduch JM, Taracila M, Bonomo RA, Wang HN, Doi Y. Extended-spectrum AmpC cephalosporinase in *Acinetobacter baumannii*: ADC-56 confers resistance to cefepime. Antimicrob Agents Chemother 2011;55:4922–5.
Jeon JH, Hong MK, Lee JH, Lee JJ, Park KS, Karim AM, et al. Structure of ADC-68, a novel carbapenem-hydrolyzing class C extended-spectrum beta-lactamase isolated from *Acinetobacter baumannii*. Acta Crystallogr D Biol Crystallogr 2014;70:2924–36.
De Luca F, Benvenuti M, Carboni F, Pozzi C, Rossolini GM, Mangani S, et al. Evolution to carbapenem-hydrolyzing activity in noncarbapenemase class D beta-lactamase OXA-10 by rational protein design. Proc Natl Acad Sci U S A 2011;108:18424–9.