EXPERT REVIEWS

Screening and deciphering antibiotic resistance in *Acinetobacter baumannii*: a state of the art

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Acinetobacter baumannii, recognized as a serious threat in healthcare facilities, has the ability to develop resistance to antibiotics quite easily. This resistance is related to either gene acquisition (horizontal gene transfer) or mutations in the genome, leading to gene disruption, over- or down-expression of genes. The clinically relevant antibiotic resistances in *A. baumannii* include resistance to aminoglycosides, broad-spectrum cephalosporins, carbapenems, tigecycline and colistin, which are the last resort antibiotics. The intrinsic and acquired resistance mechanisms of *A. baumannii* are presented here, with special focus on β-lactam resistance. The most up-to-date techniques for identification, including phenotypical and molecular tests, and screening of those emerging resistance traits are also highlighted. The implementation of early detection and identification of multidrug-resistant *A. baumannii* is crucial to control their spread.

Keywords: β-lactamases • *Acinetobacter baumannii* • carbapenemase • detection • resistance

Acinetobacter baumannii and its related species can develop acquired resistance to antibiotics quite easily. In particular, the increased resistance to broad-spectrum antibiotics, such as broad-spectrum β-lactams, quinolones or aminoglycosides has now been widely identified.

This issue of acquired resistance in *Acinetobacter* spp. is reinforced by the fact that these species naturally exhibit resistance to many antibiotics (amoxicillin, narrow-spectrum cephalosporins, ertapenem, trimethoprim and chloramphenicol). In addition, they may easily acquire resistance determinants from other Gram-negative species (e.g., members of the *Enterobacteriaceae* family or *Pseudomonas aeruginosa*) with which they share common habitats. This article presents the diversity of intrinsic and acquired resistance mechanisms that may be identified in *A. baumannii*, with a focus on resistance to β-lactams and, in particular, to carbapenems. In addition, this article shows current international break points of susceptibility/resistance (Table 1) and presents the up-to-date techniques necessary for identification of resistant isolates and corresponding resistance mechanisms. Recent studies related to the cost of the identification procedures, the treatment options and the adequate infection control measures are also discussed.

Resistance to β-lactams in *A. baumannii*

Naturally occurring β-lactamases

A. baumannii produces an intrinsic AmpC-type cephalosporinase that is encoded by the bla_{ADC}-like genes. Most often, those genes are expressed at low levels and do not interfere with the efficacy of expanded-spectrum cephalosporins [1]. However, insertion of IS*Aba1* upstream of the bla_{ADC} gene has been shown to be responsible for the overexpression of this β-lactamase gene by providing strong promoter sequences, resulting in resistance to expanded-spectrum cephalosporins [2,3]. It is worth noting that extended-spectrum AmpC-type β-lactamases have been identified in *A. baumannii*. The enzymes are point mutants of narrow-spectrum AmpCs, in which several amino acid substitutions confer an increased activity toward expanded-spectrum cephalosporins and monobactams [4,5]. Another chromosomally encoded and intrinsic β-lactamase, OXA-51 (and its relatives), has been identified in *A. baumannii* [6]. These enzymes hydrolyse carbapenems at very low levels. Nonetheless, the corresponding genes may be overexpressed (again through the

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Table 1. MICs (µg/ml) break points for *Acinetobacter baumannii* (2012).

Antibiotics	CLSI			EUCAST			CA-SFM		
	S	*I*	*R*	*S*	*I*	*R*	*S*	*I*	*R*
β-lactams									
Ticarcillin	≤16	32–64	≥128	NA	NA	NA	≤16	ND	>64
Ticarcillin/clavulanate	≤16/2	32/2–64/2	≥128/2	NA	NA	NA	≤16/2	ND	>64/2
Piperacillin	≤16	32–64	≥128	NA	NA	NA	≤16	ND	>64
Piperacillin/tazobactam	≤16/4	32/4–64/4	≥128/4	NA	NA	NA	>64/4	ND	>64/4
Cefotaxime	≤8	16–32	≥64	NA	NA	NA	NA	NA	NA
Ceftazidime	≤8	16–32	≥32	NA	NA	NA	≤4	ND	>8
Cefepime	≤8	16–32	≥64	NA	NA	NA	≤4	ND	>8
Imipenem	≤4	8	≥16	≤2	ND	>8	≤2	ND	>8
Meropenem	≤4	8	≥16	≤2	ND	>8	≤2	ND	>8
Doripenem	NA	NA	NA	≤1	ND	>4	≤1	ND	>4
Non-β-lactams									
Gentamicin	≤4	8	≥16	≤4	ND	>4	≤4	ND	>4
Tobramycin	≤4	8	≥16	≤4	ND	>4	≤4	ND	>4
Amikacin	≤16	32	≥64	≤8	ND	>16	≤8	ND	>16
Netilmicin	≤8	16	≥32	≤4	ND	>4	≤4	ND	>4
Cirpofloxacin	≤1	2	≥4	≤1	ND	>1	ND	ND	ND
Levofloxacin	≤2	4	≥8	≤1	ND	>2	ND	ND	ND
Tetracyline	≤4	8	≥16	NA	NA	NA	≤4	ND	>8
Tigecycline	NA	NA	NA	NA	NA	NA	NA	NA	NA
Colistin	≤2	ND	≥4	≤2	ND	>2	≤2	ND	>2
Rifampicin	NA	NA	NA	NA	NA	NA	≤4	ND	>16

CA-SFM: Comité de l'Antibiogramme-Société Française de Microbiologie; CLSI: Clinical and Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; I: Intermediate susceptibility; NA: Not applicable; ND: No data available; R: Resistant; S: Susceptible.

occurrence of efficient promoters either brought about by IS*Aba1* or IS*Aba9*), leading to a more significant effect and, therefore, reducing susceptibility to carbapenems [7–9].

Acquired narrow- & extended-spectrum β-lactamases

Narrow-spectrum β-lactamases being inhibited by clavulanic acid (e.g., TEM-1 and TEM-2; CARB-4, CARB-5 and CARB-14; SHV-1, SHV-56 and SHV-71; and SCO-1) or being resistant to the action of clavulanic acid (e.g., OXA-21 and OXA-37) have been reported in *A. baumannii* [10,11]. These enzymes hydrolyze narrow-spectrum penicillins, but they usually do not include cephalosporins and carbapenems in their hydrolytic profile.

The first extended-spectrum β-lactamase (ESBL) identified in *A. baumannii* was PER-1 [12]. The bla_{PER-1} gene is part of a composite transposon, namely Tn*1213*, bracketed by two unrelated insertion sequences, namely IS*Pa12* and IS*Pa13*. Several variants of PER-1 have been identified in *A. baumannii*. Among them are the bla_{PER-2} and the bla_{PER-7} genes. The PER-7 ESBL exhibited increased resistance to broad-generation cephalosporins and monobactams as compared with PER-1 [13,14]. PER-2, which is distantly related to PER-1, has been found exclusively among South American *A. baumannii* isolates, associated with a single copy of IS*Pa12* and possessing the promoter sequences leading to expression of the corresponding gene [14]. Another ESBL identified in *A. baumannii* is VEB-1, initially identified in France in a series of nosocomial isolates that had disseminated at a nationwide level. The bla_{VEB-1} gene was identified as a gene cassette inserted into class 1 integrons varying in size and structure [15–17]. *A. baumannii* isolates harboring the bla_{VEB-1} gene have also been identified in Belgium, Taiwan and South America [14,15,18,19]. Another group of ESBLs encountered in *A. baumannii* corresponds to the Guiana extended-spectrum β-lactamase (GES) enzymes [20]. Some of the GES variants possess a significant carbapenemase activity (**Figure 1**) [21,22]. Several CTX-M-producing *A. baumannii* isolates have been identified, with a CTX-M-2 producer in Japan, CTX-M-2/-43 producers in Bolivia and USA and also recently CTX-M-15 producers in Haiti [23–26]. However, CTX-M enzymes, which are by far the most common ESBLs identified in *Enterobacteriaceae*, are still very scarce in *Acinetobacter* spp. Rare identification of bla_{SHV}-type (bla_{SHV-5}, bla_{SHV-12} and bla_{SHV-18}) and bla_{TEM}-type (bla_{TEM-92}, $bla_{TEM-116}$, $bla_{TEM-128}$ and $bla_{TEM-150}$) ESBL genes have been reported in *A. baumannii*, which have been identified either on the chromosome or on plasmids [11]. Production of ESBLs in *A. baumannii* leads to resistance against expanded-spectrum cephalosporins. In addition, those ESBL-encoding genes are mostly associated with a series of other antibiotic resistance genes, including those conferring resistance to aminoglycosides. However, exchanges of ESBL-encoding genes from *Enterobacteriaceae* to *A. baumannii* remain rare.

Acquired carbapenem-hydrolysing β-lactamases

Many acquired β-lactamases have been identified as a source of carbapenem resistance in *A. baumannii*. They are either Ambler class A (clavulanic acid-inhibited ESBLs), class B (metallo-β-lactamases [MBLs]) or class D (oxacillinases) β-lactamases; however, the latter (also termed carbapenem-hydrolyzing class D β-lactamases [CHDLs]) are the most commonly identified carbapenemases in *A. baumannii*. These enzymes possess a weaker

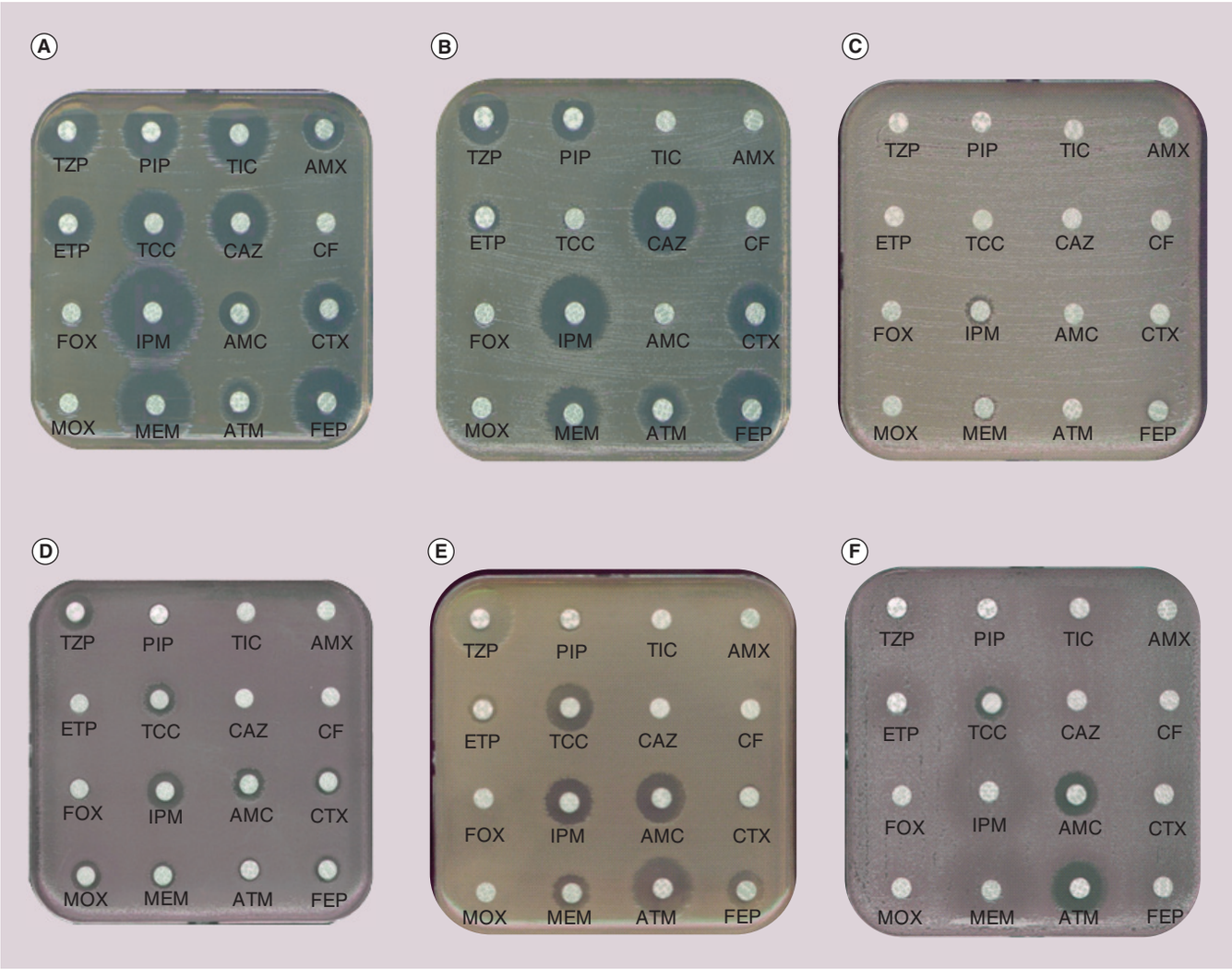

Figure 1. Susceptibility patterns of *Acinetobacter baumannii*. (A) *A. baumannii* wild-type strain; **(B)** *A. baumannii* wild-type strain producing OXA-23; **(C)** *A. baumannii* clinical isolate producing OXA-23 and overproducing natural cephalosporinase; **(D)** *A. baumannii* clinical isolate producing GES-14; **(E)** *A. baumannii* clinical isolate producing IMP-4 and **(F)** *A. baumannii* clinical isolate producing NDM-1. AMC: Amoxicillin/clavulanate; AMX: Amoxicillin; ATM: Aztreonam; CAZ: Ceftazidime; CF: Cephalothin; CTX: Cefotaxime; ETP: Ertapenem; FEP: Cefepime; FOX: Cefoxitin; IPM: Imipenem; MEM: Meropenem; MOX: Moxalactam; PIP: Piperacillin; TCC: Ticarcillin/clavulanate; TIC: Ticarcillin; TZP: Piperacillin/tazobactam.

carbapenemase activity compared with MBLs. Furthermore, they do not possess a significant activity toward expanded-spectrum cephalosporins, but their frequent association with other resistance mechanisms (including efflux pump systems and impermeability) often leads to high levels of resistance to carbapenems in *A. baumannii*. Their contribution to the carbapenem-resistant phenotype in clinical isolates has been experimentally demonstrated by gene knockout [27]. The inactivation of the gene, and consequently of the corresponding enzymes, in clinical isolates has been shown to restore the efficacy of carbapenems, demonstrating the crucial role of those enzymes in the resistance phenotype [27].

The CHDL OXA-23, together with its point mutant derivative OXA-27, constitute the major subgroup of CHDLs in term of geographical distribution [6]. Indeed, OXA-23 is the most widespread CHDL in *A. baumannii* worldwide [10,28,29]. The $bla_{\text{OXA-23}}$ gene can be found in various transposon structures, namely Tn*2006*, Tn*2007* and Tn*2008* [28,30,31]. Interestingly, the progenitor of $bla_{\text{OXA-23}}$ has been identified, being *Acinetobacter radioresistens*, a nonpathogenic and environmental species [32]. Another CHDL subgroup is made of OXA-40 (also named OXA-24), OXA-25, OXA-26 and OXA-72. OXA-24/40 producers are particularly prevalent in the USA, Spain and Portugal [6]. The third CHDL subgroup consists of OXA-58 and its variants OXA-96, OXA-97 and OXA-164, with the $bla_{\text{OXA-58}}$ gene being predominant and identified worldwide [6,33]. These genes are mostly plasmid-encoded and associated with insertion sequences that play a role in their expression but not acquisition. It is worth noting that the $bla_{\text{OXA-58}}$ gene has been identified in different *Acinetobacter* species, such as in *Acinetobacter junii*, *Acinetobacter pittii*

(formerly *Acinetobacter* genomic species 3) and *Acinetobacter nosocomialis* (formerly *Acinetobacter* genomic species 13TU) [6,34]. Finally, OXA-143 constitutes the last CHDL subgroup, which has been recently identified in South American isolates [35,36].

MBLs are important carbapenemases [37]. Although reported mostly from *P. aeruginosa* and *Enterobacteriaceae*, four groups of MBLs have also been described in *A. baumannii*, namely active on imipenem β-lactamase (IMP)-type, Verona integron-encoded metallo-β-lactamase (VIM)-type, Seoul imipenemase (SIM)-type and New Delhi metallo-β-lactamase (NDM)-type enzymes [37–39]. NDM-type enzymes are considered as emerging in *A. baumannii* [38,39]. Analysis of the region surrounding the bla_{NDM-1} gene revealed that the promoter sequences are present in a remnant of an insertion sequence found in the *Acinetobacter* genus, namely IS*Aba125* [40]. In *A. baumannii*, the bla_{NDM-1} gene is part of a composite transposon made of two copies of IS*Aba125* [41]. Downstream of bla_{NDM-1}/bla_{NDM-2}, eight open reading frames have been identified. The first one corresponded to the ble_{MBL} gene, which encodes a protein that confers resistance to bleomycin, known as an anticancer drug [41]. Following the ble_{MBL} gene, several gene-encoding putative proteins sharing similarities with genes identified from the *Brevundimonas* and *Xanthomonas* genus have been identified, probably indicating an environmental source of the bla_{NDM-1} gene [41].

The corresponding genes have been reported mostly in *Enterobacteriaceae*, but recent reports from India, China and Europe indicate that their occurrence in *A. baumannii* has very likely been underestimated [38,42,43]. These recent reports also indicated that a different reservoir of NDM-producing *A. baumannii* may be identified in China and India in particular. The NDM-1-producing isolates identified showed high-level resistance to all β-lactams, including carbapenems (imipenem and meropenem). Overall, MBLs are responsible for high levels of resistance to carbapenems in *A. baumannii*.

Finally, several class A carbapenemases have been identified in *A. baumannii*. *Klebsiella pneumoniae* carbapenemase (KPC)-positive *Acinetobacter* spp. isolates belonging to the *Acinetobacter calcoaceticus–A. baumannii* complex have been identified in Puerto Rico [44]. GES enzymes possessing some carbapenemase activity are increasingly identified in *A. baumannii*, with several variants being described worldwide [20–22,45]. These GES variants possessing significant carbapenemase activity identified in *A. baumannii* are GES-11 and GES-14 [21,22,45,46], with the bla_{GES-14} and bla_{GES-11} genes located on conjugative plasmids [45].

Nonenzymatic β-lactam resistance

Resistance to β-lactams is mainly due to production of β-lactamases in *Acinetobacter* spp. However, the involvement of efflux pump systems, porin modifications or loss and the modification of penicillin-binding proteins have also been reported. Regarding the resistance through modification of penicillin-binding proteins, very few studies are available and the role of these modifications in carbapenem resistance in *A. baumannii* cannot be clearly assessed [47]. Several reports indicated that changes of porin nature or production level may be linked to resistance or decreased susceptibility to carbapenems [10]. The AdeABC (*Acinetobacter* drug efflux) pump belonging to the resistance–nodulation–cell division family has been shown to play a role in β-lactam resistance [48]. Knockout experiments and overexpression of naturally occurring efflux pumps showed that this system mainly affects the efficacy of cefepime, cefpirome and cefotaxime [48]. In association with production of CHDLs, it may confer an additional level of resistance to carbapenems [27].

Identification of the β-lactam resistance mechanisms

As aforementioned, many broad-spectrum β-lactamases have been described in *A. baumannii* and many of them are capable of carbapenem hydrolysis. Therefore, identifying the resistance mechanism is important in order to evaluate whether it is enzymatic or not and, therefore, whether it may be transferable or not.

Many multidrug-resistant *A. baumannii* clinical isolates share a resistance to all β-lactams, giving rise to a resistance phenotype that is difficult to interpret (Figure 1). Several phenotypic tests have been developed to decipher the β-lactam resistance mechanisms and to facilitate the interpretation of the antimicrobial susceptibility results (Table 2). These methods are summarized in Table 2 and in Figure 2. The use of media supplemented with cloxacillin (250 mg/l) is the main technique advocated to inhibit the naturally occurring *Acinetobacter*-derived cephalosporinase and thus evaluate whether this chromosomally encoded β-lactamase is responsible *per se* for the β-lactam resistance pattern observed. Two Mueller–Hinton plates are used, with and without cloxacillin, both being inoculated with a 0.5 McFarland culture of *A. baumannii*. Discs of ceftazidime and cefepime are placed at three different distances (0.75, 1 and 1.5 cm) to a disc supplemented with clavulanate. The entire restoration of activity of ceftazidime and cefepime on plates supplemented with cloxacillin may rule out the production of MBL or ESBL – activities that are not inhibited by cloxacillin. On the other hand, the synergistic images that may appear between cephalosporins and clavulanate strongly suggest the production of an ESBL. However, these phenotypic tests may be difficult to interpret. Naas *et al*. suggested that incubation of the plates at room temperature may facilitate this interpretation by reinforcing the synergy images with clavulanic acid [16].

The Etest® MBL strip (bioMérieux, Marcy l'Étoile, France) is one of the techniques advocated for detection of MBL based on inhibition of MBL activity by ethylenediaminetetraacetic acid (EDTA) [49]. Etest strips have a good sensitivity for detection of MBL producers [50], except for those isolates exhibiting low MIC for carbapenems (e.g., MIC value of imipenem at 4 mg/l for an IMP-4 producer) [50] giving rise to noninterpretable results. Susceptibility to imipenem is usually restored in the presence of EDTA, thus highlighting the significant contribution of MBLs (NDM, VIM, SIM and IMP) for carbapenem resistance. However, several strains producing OXA-23 or OXA-40 that are MBL-negative may give false-positive results [50]. This showed that the intrinsic effect of EDTA on the growth of *A. baumannii* may interfer with the result of this test, generating false-positive results. Other techniques can be used to detect MBL production using the same EDTA inhibition principle [51]; the combined-disc test uses

two imipenem discs (10 μg) with one supplemented with 292-μg EDTA. An increase in the inhibition zone diameter of >5 mm around the disc with EDTA is considered as a positive test. The double-disc synergy test is performed by using an imipenem disc (10 μg) placed at 20 mm (center to center) from a blank filter disk containing 292 μg EDTA. If the test is positive, a synergistic image between EDTA and imipenem is observed. The aztreonam disc (30 μg) can also be used as a marker of suspicion for MBL production when the diameter of inihibition is higher than that observed for imipenem or meropenem (even though aztreonam is known to be naturally weakly active on *A. baumannii*).

The modified Hodge test has been widely used for screening of carbapenemase production. This test may be performed using *Escherichia coli* ATCC25922 as the indicator organism, as it is susceptible to all β-lactams at a turbidity of 0.5 McFarland, with a carbapenemase producer as the positive control and a carbapenem-resistant but noncarbapenemase producer as the negative control [52]. While VIM and IMP producers can be detected quite easily, NDM producers are not accurately detected using this technique, which is also time consuming (takes 48 h). CHDL producers are also usually difficult to detect using this technique. Overall, this test is neither sensitive nor specific for detecting carbapenemase activity from carbapenemase-positive *A. baumannii* and shall be discarded.

Table 2. Laboratory methods for the detection of β-lactam resistance in *Acinetobacter baumannii*.

Methods	Principle	Target	Efficacy	Cost	Ref.
PCR	Molecular test	Resistance genes	+++	$$$	[45,50,55]
Sequencing	Molecular test	Resistance genes	+++	$$$	[45,50,55]
Real-time PCR	Molecular test	Resistance genes	+++	$$$	[56]
DNA hybridization	Molecular test	Resistance genes	++	$$$	
DNA microarray	Molecular test	Resistance genes	+++	$$$	[57]
UV spectrometry	Biochemical test	Carbapenem hydrolysis	+	$	[50,96]
MALDI–TOF	Biochemical test	Carbapenem hydrolysis	+++	$$$	[53]
CarbaNP test†	Biochemical test	Carbapenem hydrolysis	+++	$	[58,59]
Cloxacillin test	Phenotypical test	Cephalosporinase overproduction	+++	$$	[22]
Etest® MBL	Phenotypical test	MBL production	++	$$$	[50]
IPM + EDTA on disks	Phenotypical test	MBL production	++	$	[50,51]
Modified Hodge test	Phenotypical test	Carbapenemase production	+/-	$$	[50,51]
Disk combination methods	Phenotypical test	ESBL production, carbapenemase production	++	$	[50,51]
Vitek cards	Phenotypical test	β-Lactam resistance	++	$$	[100]

†Optimization of the CarbaNP technique for *Acinetobacter baumannii* (CarbaAcineto NP test) has been obtained very recently [Nordmann P, Unpublished Data].
+: Weak efficiency; ++: Reliable efficiency; +++: High efficiency; +/-: Often uninterpretable; $: Low cost; $$: Intermediate cost; $$$: High cost; CarbaNP: Carbapenemase Nordmann–Poirel test; EDTA: Ethylenediaminetetraacetic acid; ESBL: Extended-spectrum β-lactamase; IPM: Imipenem; MALDI–TOF: MALDI–time of flight; MBL: Metallo-β-lactamases.

Biochemical detection of carbapenemase activity using UV spectrophotometry may be also an alternative detection technique. A 10 ml sample of *A. baumannii* overnight broth cultured isolates are centrifuged and then sonicated, giving rise to an enzymatic crude extract. Specific activities for carbapenems can be measured using a UV spectrophotometer at a wavelength value of 297 nm for imipenem. Again, while good results may be obtained with VIM, SIM and IMP producers, detection of NDM producers remains very difficult, with many isolates giving false-negative results [50]. The standard mean of specific activities obtained for the NDM producers was evaluated at 5.7 mU/mg of proteins [50].

Another biochemical detection method is based on MALDI–time of flight (MALDI–TOF), which has recently been developed. This method is based on the detection of native imipenem and its natural degradation product, both molecules being detected according to their different masses (300 m/z for imipenem and 254 m/z for imipenemoic acid). The hydrolysis of imipenem is revealed by the observation of a peak at 254 m/z. In a recent study, 106 *A. baumannii* strains including 63 well-characterized carbapenemase-producing (mainly OXA-23 producers) and 79 carbapenem-susceptible (and, therefore, probably carbapenemase-negative) strains were studied [53]. The mixture of *A. baumannii* and imipenem was centrifuged and the supernatant analyzed by MALDI–TOF. The result was interpreted as positive for carbapenemase production if the specific peak for imipenem at 300 m/z disappeared during the incubation time and if the peak of the natural metabolite at 254 m/z increased, with the ratio between the peak for imipenem and its metabolite being 0.5 [53]. This assay showed excellent sensitivity and specificity. This constitutes, therefore, a rapid method for those laboratories possessing MALDI–TOF technology. However, this technique requires expensive equipment along with trained microbiologists (Table 2).

Molecular-based techniques, using specific primers, as summarized in Table 3, permit the identification of carbapenemase producers by targeting the corresponding genes. PCR allows the identification of known carbapenemases with a very high specificity and sensitivity. However, one of the caveats of these molecular techniques is a lack of detection of not-yet-identified carbapenemase genes. Multiplex PCR for detecting several carbapenemase genes should be adapted to *A. baumannii*, since recently developed multiplex PCR schemes were mainly aimed at identifying carbapenemases found in *Enterobacteriaceae*, but did not include CHDLs that are extremely prevalent among carbapenem-resistant *A. baumannii* [54,55]. Some real-time PCR schemes have been developed for the detection of carbapenemases [56]. The advantage of these techniques is that a result can be obtained within 3 h,

Table 3. Primers used for the detection of main resistance mechanisms.

Primers	Sequences	Target gene	Ref.
Pre-TEM-A	5′-GTA TCC GCT CAT GAG ACA ATA-3′	*bla*$_{TEM}$	[97]
Pre-TEM-B	5′-TCT AAA GTA TAT ATG AGT AAA CTT GGT CTG-3′	*bla*$_{TEM}$	[97]
SHV-A	5′-ATG CGT TAT WTT CGC CTG TGT-3′	*bla*$_{SHV}$	[97]
SHV-B	5′-TTA GCG TTG CCA GTG CTC G-3′	*bla*$_{SHV}$	[97]
CTX-M-A1	5′-SCS ATG TCG AGY ACC AGT AA-3′	*bla*$_{CTX-M}$	[97]
CTX-M-A2	5′-CCG CRA TAT GRT TGG TGG TG-3′	*bla*$_{CTX-M}$	[97]
GES-A	5′-ATG CGC TTC ATT CAC GCA C-3′	*bla*$_{GES}$	[45]
GES-B	5′-CTA TTT GTC CGT GCT CAG G-3′	*bla*$_{GES}$	[45]
PER-A	5′-ATG AAT GTC ATT ATA AAA GC-3′	*bla*$_{PER}$	[45]
PER-B	5′-AAT TTG GGC TTA GGG CAG AA-3′	*bla*$_{PER}$	[45]
VEB-A	5′-CGA CTT CCA TTT CCC GAT GC-3′	*bla*$_{VEB}$	[45]
VEB-B	5′-GGA CTC TGC AAC AAA TAC GC-3′	*bla*$_{VEB}$	[45]
VIM2004A	5′-GTT TGG TCG CAT ATC GCA AC-3′	*bla*$_{VIM}$	[98]
VIM2004B	5′-AAT GCG CAG CAC CAG GAT AG-3′	*bla*$_{VIM}$	[98]
IMP2004A	5′-ACA YGG YTT GGT DGT TCT TG-3′	*bla*$_{IMP}$	[98]
IMP2004B	5′-GGT TTA AYA AAA CAA CCA CC-3′	*bla*$_{IMP}$	[98]
NDM-1A	5′-GGT TTG GCG ATC TGG TTT TC-3′	*bla*$_{NDM}$	[50]
NDM-1B	5′-CGG AAT GGC TCA TCA CGA TC-3′	*bla*$_{NDM}$	[50]
SIM-1A	5′-TAC AAG GGA TTC GGC ATC G-3′	*bla*$_{SIM}$	†
SIM-1B	5′-TAA TGG CCT GTT CCC ATG TG-3′	*bla*$_{SIM}$	†
OXA-23A	5′-GAT GTG TCA TAG TAT TCG TCG-3′	*bla*$_{OXA-23}$	[45]
OXA-23B	5′-TCA CAA CAA CTA AAA GCA CTG-3′	*bla*$_{OXA-23}$	[45]
OXA-40A	5′-GTA CTA ATC AAA GTT GTG AA-3′	*bla*$_{OXA-40}$	[45]
OXA-40B	5′-TTC CCC TAA CAT GAA TTT GT-3′	*bla*$_{OXA-40}$	[45]
OXA-51A	5′-CTA ATA ATT GAT CTA CTC AAG-3′	*bla*$_{OXA-51}$ and derivatives	[45]
OXA-51B	5′-CCA GTG GAT GGA TGG ATA GAT TAT C-3′	*bla*$_{OXA-51}$ and derivatives	[45]
OXA-58A	5′-CGA TCA GAA TGT TCA AGC GC-3′	*bla*$_{OXA-58}$	[45]
OXA-58B	5′-ACG ATT CTC CCC TCT GCG C-3′	*bla*$_{OXA-58}$	[45]
OXA-143A	5-AGT TAA CTT TCA ATA ATT G-3′	*bla*$_{OXA-143}$	[45]
OXA-143B	5′-TTG GAA AAT TAT ATA ATC CC-3′	*bla*$_{OXA-143}$	[45]
OXA-CHDL A	5′-CCH GCH TCD ACH TTY AAR AT-3′	All carbapenem-hydrolyzing class D β-lactamases	[99]
OXA-CHDL B	5′-KYH AYA BCC MWK SCC CAD CC-3′	All carbapenem-hydrolyzing class D β-lactamases	[99]
ISAba1-B	5′-CAT GTA AAC CAA TGC TCA CC-3′	IS*Aba1*	[3]
5′CS	5′-TCT CGG GTA ACA TCA AGG-3′	5′CS of class 1 integrons	[22]
3′CS	5′-AA GCA GAC TTG ACC TGA-3′	5′CS of class 1 integrons	[22]
armA-F	5′-ATT TTA GAT TTT GGT TGT GGC-3′	*armA*	[98]
armA-R	5′-ATC TCA GCT CTA TCA ATA TCG-3′	*armA*	[98]
aac(6′)-Ib-for	5′-TTG CAA TGC TGA ATG GAG AG-3′	*aac(6′)-Ib*	[97]
aac(6′)-Ib-rev	5′-CGT TTG GAT CTT GGT GAC CT-3′	*aac(6′)-Ib*	[97]
gyrA-for	5′-AAA TCT GCC CGT GTC GTT GGT-3′	*gyrA*	[23]
gyrA-rev	5′-GCC ATA CCT ACG GCG ATA CC-3′	*gyrA*	[23]
parC-for	5′-AAA CCT GTT CAG CGC CGC ATT-3′	*parC*	[23]
parC-rev	5′-AAA GTT GTC TTG CCA TTC ACT-3′	*parC*	[23]
arr-2-for	5′-GAT AAT TAC AAG CAG GTG CAA GG-3′	*arr-2*	†
arr-2-rev	5′-TCT AAC GAA TCC AAC ATT CCC-3′	*arr-2*	†

†Not published.

which is significantly faster than classic PCR. This assay can detect the presence of six different carbapenemase gene types in a single 3-h-long PCR with high sensitivity and specificity [56].

New detection techniques have been developed to identify broad-spectrum β-lactamases in *A. baumannii*. One of the latest techniques is the DNA microarray, a molecular-based technology that has the potential to detect a large number of genes within a single reaction. A commercial DNA microarray test, the Check-MDR CT102 microarray (Check-Points BV, Wageningen, The Netherlands), has been evaluated, being a further refinement of an ESBL-detection microarray, which, in addition to the previously detected genes, includes clinically relevant carbapenemase-encoding genes, such as *OXA-48*, *VIM*, *IMP* and *NDM-1* [57]. Excellent sensitivity and specificity have been found for the tested genes, showing accurate identification of common ESBLs and carbapenemase producers from bacterial cultures. Nonetheless, this technique has not been yet validated for *A. baumannii* [57].

The most promising technique for rapid and accurate identification of any carbapenemase producer is the carbapenemase Nordmann–Poirel test [58]. This test is based on biochemical detection of the hydrolysis of a β-lactam ring of a carbapenem by any carbapenemase. It is rapid (30 min), costless, easy to handle and highly specific and sensitive; it may be implemented worldwide. This test has now been extensively validated worldwide for detection of carbapenemase activities in *Enterobacteriaceae* and *P. aeruginosa* [58,59]. The authors' recent unpublished and personal data using a modified version of the carbapenemase Nordmann–Poirel test, named CarbaAcineto Nordmann–Poirel test, showed that it may work for detection of carbapenemase activity in *Acinetobacter* spp. as well [Nordmann P, Unpublished Data].

Clinically relevant non-β-lactam resistance mechanisms

Resistance to quinolones & fluoroquinolones

Quinolones and fluoroquinolones inhibit bacterial DNA replication by targeting the DNA gyrase (encoded by *gyrA* and *gyrB* genes) and DNA topoisomerase IV (encoded by *parA* and *parC* genes) enzymes [60]. Several specific mutations occurring in the quinolone-resistance-determining region of those proteins may have a significant impact on the susceptibility to quinolones and fluoroquinolones. These mutations lead to a lower affinity and reduce the binding of the quinolones to the enzyme–DNA complex. These types of mutations, as observed in many other bacterial species, have been extensively reported in *A. baumannii*. The Ser-86-Leu substitution in *gyrA*, together with the Ser-80-Leu substitution in *parC*, are commonly identified, significantly increasing the MICs of ciprofloxacin [61]. Efflux-mediated resistance to quinolones has also been described to involve efflux pumps that are intrinsic in *A. aumannii* (namely AdeABC, AdeIJK and AdeFGH) [48]. These systems are able to pump out quinolones and therefore contribute to high-level resistance to these compounds in synergy with mutation(s) in the gyrase/topoisomerase(s). Resistance to quinolones is widely distributed throughout the world [29]. So far, no validated plasmid-mediated mechanism of resistance has been identified in *A. baumannii*. Quinolone resistance can be easily identified through antimicrobial susceptibility testing.

Resistance to aminoglycosides

Aminoglycosides belong to antibiotic families that target bacterial translation [62]. Resistance to aminoglycosides is mainly due to the aminoglycoside-modifying enzyme (AME) [63]. Multiple AMEs, including phosphotransferases, acetyltransferases (in particular AAC[6′]-Ib) and adenyltransferases, have been reported in *A. baumannii*. Most of the time, aminoglycoside resistance in *Acinetobacter* spp. involves production of AMEs, and all three classes have been identified in *A. baumannii* [29]. The main AMEs found in *A. baumannii* are AAC(3′)-I-modifying gentamicin and fortimicin, APH(3′)-VI-modifying amikacin, kanamycin and neomycin, and AAC(6′)-Ib-modifying tobramycin, netilmicin and amikacin. The association of several AMEs can lead to a pan-resistance against all aminoglycosides.

The second and most recent mechanism of aminoglycoside resistance involves a target modification operated by a 16S rDNA methylase. The ArmA enzyme methylates the active site of the ribosome, giving rise to a cross-resistance to all aminoglycosides [62,63]. This resistance trait is currently emerging and has been described in different parts of the world. Phenotypic and molecular techniques are needed to detect this mechanism. No specific phenotypic method exists for detecting 16S rRNA methylases, except for molecular methods based on the detection of the corresponding genes. However, their production can be suspected by observing a lack of inhibition zone for gentamicin and amikacin discs (two different 4,6-disubstituted deoxystreptamine) that are generally not modified by a single enzyme. A double zone of inhibition is often observed around the amikacin disc for most ArmA producers. In this case, PCR has to be used to accurately detect the corresponding genes [64,65].

Resistance to rifampicin, cyclines & colistin

Rifampicin binds to conserved amino acids in the active site of the bacterial RNA polymerase, consequently blocking transcription initiation. Resistance to rifampicin results mostly from chromosomal mutations leading to amino acid changes in the active site of the RNA polymerase [66]. A recent study in Italy focusing on rifampicin resistance in *A. baumannii* showed that substitutions in the RNA polymerase were mainly focused not only in His535 and Leu542, but also in Asp525 and Pro544, which were substituted by different amino acids [67]. Interestingly, the membrane permeability and efflux pump systems also seem to play a role in isolates that exhibited reduced susceptibility to rifampicin [67]. This finding has been evidenced by the restoration of susceptibility to rifampicin in the presence of phenyl-arginine-β-naphthylamide or 1-(1-naphthylmethyl)-piperazine, which are efflux pump inhibitors. In addition, resistance to rifampicin has sometimes been found to be transferable in *A. baumannii* when involving the *arr-2* gene, which encodes a rifampicin ADP-ribosylating transferase that inactivates rifampicin by ribosylation [68]. However, the *arr-2* gene seems to not be widespread in *A. baumannii*, and rifampicin therefore remains active against most multidrug-resistant isolates, thus remaining an interesting therapeutic option [16,68].

Tetracyclines have a bacteriostatic activity by reversibly binding to the 30S ribosomal subunit, therefore inhibiting protein

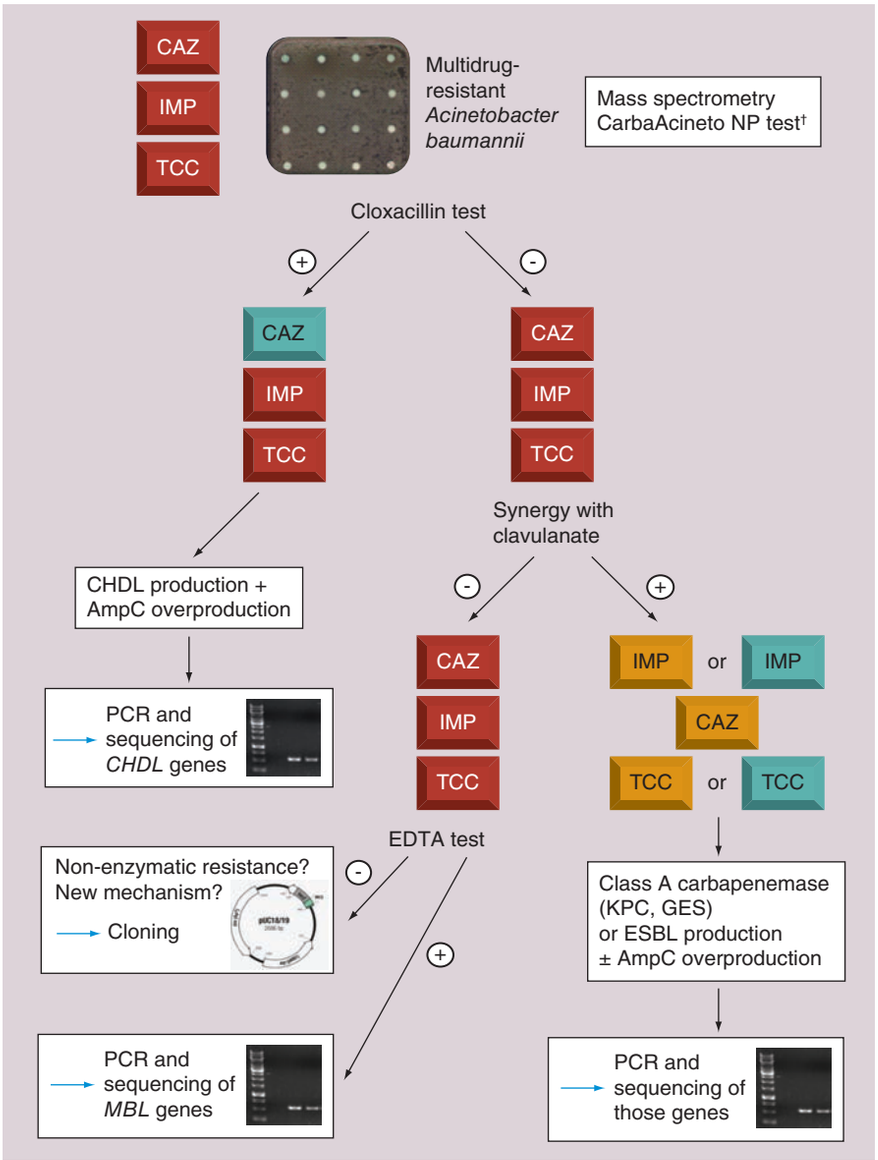

Figure 2. Identifying β-lactam resistance. Red indicates high-level resistance, orange indicates moderate resistance and green indicates susceptibility.
†Extensive validation of the CarbaAcineto NP test in the near future and use of mass spectrometry may change this decision chart.
+: Positive test result; -: Negative test result; CarbaAcineto NP test: Carbapenemase Nordmann–Poirel test optimized for *Acinetobacter baumannii*; CAZ: Ceftazidime; CHDL: Carbapenem-hydrolyzing class D β-lactamase; ESBL: Extended-spectrum β-lactamase; GES: Guiana extended-spectrum; IPM: Imipenem; KPC: *Klebsiella pneumoniae* carbapenemase; MBL: Metallo-β-lactamase; TCC: Ticarcillin/clavulanate.

translation [69]. Resistances to tetracyclines in *A. baumannii* have often been reported [29,70]. The TetA and TetB proteins are constituents of efflux pumps that extrude tetracyclines from the bacterial cell. In addition, protection of the ribosome may be mediated by the widely distributed TetM determinant [29,70]. These resistance determinants confer high-level resistance to tetracycline but do not modify the efficacy of tigecycline (a new member of the cycline family). Resistance to tigecycline is mediated by the overexpression of an intrinsic efflux pump (Ade-derivate systems) [48]. The MICs to tigecycline may be variable and no official breakpoints for *Acinetobacter* spp. are currently available for this drug (Table 1), even though Jones *et al.* have published guidelines for interpreting tigecycline MICs (susceptible, MIC: ≤2 µg/ml; resistant, MIC: ≥8 µg/ml) [71].

Colistin and polymyxins are old antibiotics that are becoming a last resort against extremely drug-resistant bacteria. These compounds bind to and disrupt the negatively charged outer membrane of Gram-negative bacteria [72]. So far, the main mechanisms of resistance identified correspond to the modification of lipid A, an essential component of the bacterial lipopolysaccharide, due to a mutation in *pmrA* or *pmrB* genes, leading to a reduction of the net negative charge of the outer-membrane protein [73]; the proteolytic cleavage of the antibiotic followed by exclusion of the peptides by efflux and complete loss of lipopolysaccharide production, either by inactivation of *lpxA*, *lpxC* and *lpxD* from either mutation or insertion of insertion sequence IS*Aba11* [10,74–76]. Although this phenotype remains rare, colistin-resistant *A. baumannii* have been reported [29,70]. A phenomenon of heteroresistance to colistin has been observed in *Acinetobacter* spp. It is defined as a resistant subpopulation among a population of colistin-susceptible *A. baumannii* (with MIC of colistin ≤2 mg/l) and may be due to potential suboptimal recommended dosage regimens [77]. The heteroresistance to colistin is difficult to assess and its definition varies among different reports [74]. The weak agar diffusion of colistin limits the predictive accuracy of the disc-diffusion technique and, consequently, MIC values have to be determined by Etest or broth dilution techniques.

Cost of detection methods of antibiotic resistance in A. baumannii

The cost of many screening methods imposes a limit for the identification of the resistance mechanisms and consequently for better control of the spread of multidrug-resistant *A. baumannii*. The precise identification of resistance mechanisms may indeed give precious epidemiological information. The threat of a plasmid-mediated diffusion is higher than chromosomal resistance. However, screening methods for patients carrying *A. baumannii* should be performed upon hospital admission using either swabs or sponges, as recently described by Doi *et al.* [78]. The authors showed that screening using a sponge or a

swab was sensitive enough to detect *A. baumannii*. However, this method is very expensive and should preferably be used when the prevalence of *A. baumannii* is suspected to be >1% [78].

Most multidrug-resistant *A. baumannii* (including those resistant to carbapenems) are resistant to broad-spectrum cephalosporins. Therefore, screening media designed for detection of ESBL producers (e.g., chromID® ESBL-containing cefpodoxime [bioMérieux, Marcy l'Etoile, France]) may be used. Several media containing a carbapenem (e.g., Brilliance™ CRE [Oxoid, Cambridge, UK] or CHROMagar™ KPC [CHROMagar, Paris, France]) may also be used. These have a claimed sensitivity, since high MICs values of carbapenems are usually high for carbapenemase-producing *A. baumannii* [79]; however, there is currently no extensive clinical validation available.

The cost of PCR/sequencing is rather high as for all molecular techniques including checkpoint CT012. Biochemical tests based on imipenem hydrolysis detected by UV spectrophotometry or mass spectrometry are less expensive for each isolate (once the appropriate equipment has been acquired) but can only be performed in reference laboratories. For the detection of MBLs, the use of Etest MBL is advocated but the use of a double-disc synergy test (supplemented with imipenem and imipenem/EDTA on each extremity, respectively) is cost saving, since Etest MBL is expensive and both techniques give similar results [50]. The use of the CarbaAcineto NP test offers not only a rapid result, but it is also the cheapest solution for the detection of any kind of carbapenemase activity in *A. baumannii* (less than €1–2).

Treatment aspects of infections caused by A. baumannii

A. baumannii may cause pneumonia, wound infections, bacteremia, urinary tract infections and meningitis [80,81]. Among the identified risk factors leading to colonization or infection with *A. baumannii* (sometimes difficult to distinguish), prolonged hospitalization, intensive care unit admission, recent surgical procedures, antimicrobial agent exposure, central venous catheter use, prior hospitalization, nursing home residence and local colonization pressure on susceptible patients are well known [82–84]. Those infections can be treated with a combination of a β-lactam and an aminoglycoside. The combination of a β-lactam together with an aminoglycoside appears at least synergistic *in vitro* and allows a rapid bactericidal effect [85]. Fluoroquinolones also exhibited a rapid bactericidal effect against susceptible *A. baumannii* and therefore can be used in combination with a β-lactam [85]. The increasing resistance trend observed for fluoroquinolones, aminoglycosides and broad-spectrum β-lactams has consequently lead to the use of carbapenems alone or in combination with non-classical molecules, such as polymyxin, rifampin and sulbactam [82,85,86]. Tigecyline is often active against multidrug-resistant *A. baumannii*; however, recent reports described the emergence of tigecycline resistance [10,29]. Nevertheless, the current main problem in terms of resistance in *A. baumannii* is that carbapenems are often associated with multidrug or even pandrug resistance.

Since antibiotic-based therapies may become more and more limited when dealing with *A. baumannii*, alternative therapies are being explored. These experimental therapies include bacteriophage-based therapy or antibacterial peptides [87,88]. The main problem with these therapies is that their efficicacy has been evaluated only *in vitro*. The pharmacokinetic/pharmacodynamic profiles of these compounds, including half-life, diffusion in the host organism and potential degradation by human body fluids, limit their clinical efficacy. For an informative review of these new therapies, please refer to García-Quintanilla *et al.* [89].

The use of bacteriophage therapy is, from the authors' point of view, quite hazardous; the authors do not have enough data regarding the *in vivo* activity of such compounds. Moreover, the control of the virus after treatment seems to be impossible. In addition, it is likely that the emergence of bacteriophage-resistant strains under therapy will rapidly occur (modification of their membrane target site). However, the authors believe that antibiotic-use policies and control of antibiotic resistance are crucial for controlling the emergence and spread of antibiotic resistance in *A. baumannii*.

Control of antimicrobial-resistant bacteria

The control of multidrug resistance in *A. baumannii* will be one of the big challenges in clinical microbiology in the near future. It is indeed very likely that the now widely distributed bla_{NDM} carbapenemase genes, increasingly reported in *Enterobacteriaceae*, first spread among *Acinetobacter* spp. before disseminating into *Enterobacteriaceae* [41]. *A. baumannii* exhibits different factors potentially involved in the persistence of antimicrobial resistance in healthcare institutes (either antibiotics or antiseptics) and also exhibits a robust metabolism that is possibly responsible for higher survival on inorganic surfaces compared with most enterobacterial species [29,90]. As mentioned above, carbapenem-resistant *A. baumannii* are being increasingly reported and are often behind the occurrence of outbreaks [29,70]. Using molecular techniques, the clonal relationship between *A. baumannii* clinical isolates during outbreaks has been extensively studied. It has been demonstrated that most nosocomial outbreaks are caused by a single or a few *A. baumannii* clones [91]. The importance of hygiene measures has been clearly demonstrated for the control of hospital outbreaks caused by *A. baumannii*. Although no common strategy to eradicate colonization by *Acinetobacter* spp. in intensive care units is known, prevention of patient colonisation seems to be of primarily importance. The prevention of *A. baumannii* outbreaks should be performed in several steps: the detection of any multidrug-resistant *A. baumannii* should be performed at hospital admission; detection of *A. baumannii* carriage in the close environment of the index patient, at least in intensive care units; regular screening of the patient using swabs or sponges as described by Doi *et al.* [78]; and an enhanced isolation of carbapenem-resistant *A. baumannii*-infected patients or healthy carriers (cohorting) [29,92]. In reality, most of the containment measures for outbreak prevention involving multidrug-resistant *A. baumannii* are similar to those for preventing spread of any multidrug-resistant Gram-negative rods.

Expert commentary & five-year view

A. baumannii possesses an extraordinary capacity for acquiring antibiotic resistance determinants [81,93]. A high diversity of resistance determinants have been identified in these species [10]. The

bacteria may not only harbor the same carbapenemase genes as those identified in *Enterobacteriaceae* and *P. aeruginosa* (*VIM*, *IMP*, *KPC*, *NDM,* and so on) but may also acquire additional carbapenemase genes, such as the CHDL-encoding genes. Identification of carbapenem-resistant *A. baumannii* is extremely worrisome since carbapenems often remain the last resort antibiotics to treat multidrug-resistant *A. baumannii*-related infections. Unlike carbapenemase-producing *Enterobacteriaceae* that may exhibit a reduced susceptibility to carbapenems – in particular for OXA-48-producing *Enterobacteriaceae* [94] – most carbapenemase-producing *A. baumannii* often exhibit high MICs to carbapenems. This is probably a consequence of the intrinsic poor membrane permeability of *A. baumannii* (1000-fold less permeable than *E. coli*). However, OXA-58-producing but non-carbapenem-resistant *A. baumannii* have recently been identified [95], showing that some carbapenemase producers may remain susceptible to carbapenems.

Since reversal of antibiotic resistance (particularly to carbapenems) is rare in *A. baumannii*, it is unlikely that the increasing trends currently observed worldwide will be reversed. In addition, the likelihood of seeing another new drug that is effective against multidrug-resistant *A. baumannii* in the near future is limited. Consequently, strict infection-control measures will be crucial to counteract the impact of carbapenem-resistant *A. baumannii* on a large scale.

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Key issues

- *Acinetobacter baumannii* intrinsically possesses a reduced susceptibility to antibiotics, which is related to the intrinsic occurrence of two β-lactamase genes (one for AmpC and another for class D β-lactamases) whose expression can be modulated, together with efflux systems and permeability defects.
- The main mechanisms of carbapenem resistance in *A. baumannii* correspond to the Ambler class D carbapenem-hydrolyzing β-lactamases OXA-23, OXA-40 and OXA-58.
- Deciphering the antibiotic resistance mechanisms in *A. baumannii* remains very challenging considering the very high-resistance pattern that is usually observed.
- Molecular techniques, although difficult to implement worldwide, currently represent a gold standard for detection of resistance mechanisms in the species.
- Colistin, tigecycline and rifampicin often remain active against multidrug-resistant *A. baumannii*.

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