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Acquired carbapenemases in Gram-negative bacterial pathogens: detection and surveillance issues

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Abstract

Acquired carbapenemases are emerging resistance determinants in Gram-negative pathogens, including *Enterobacteriaceae*, *Pseudomonas aeruginosa* and other Gram-negative non-fermenters. A consistent number of acquired carbapenemases have been identified during the past few years, belonging to either molecular class B (metallo-β-lactamases) or molecular classes A and D (serine carbapenemases), and genes encoding these enzymes are associated with mobile genetic elements that allow their rapid dissemination in the clinical setting. Therefore, detection and surveillance of carbapenemase-producing organisms have become matters of major importance for the selection of appropriate therapeutic schemes and the implementation of infection control measures. As carbapenemase production cannot be simply inferred from the resistance profile, criteria must be established for which isolates should be suspected and screened for carbapenemase production, and for which tests (phenotypic and/or genotypic) should be adopted for confirmation of the resistance mechanism. Moreover, strategies should be devised for surveillance of carbapenemase producers in order to enable the implementation of effective surveillance programmes. The above issues are addressed in this article, as a follow-up to an expert meeting on acquired carbapenemases that was recently organized by the ESCMID Study Group for Antibiotic Resistance Surveillance.

Keywords: β-Lactamases, carbapenemases, Gram-negative bacteria, laboratory detection, review, surveillance

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Introduction

The vast majority of acquired carbapenemases belong to three of the four known classes of β-lactamases, namely Ambler class B (metallo-β-lactamases (MBLs)), Ambler class A, and Ambler class D (oxacillinases (OXAs)). The bacterial host ranges of these three distinct classes of enzyme, which confer clinically significant resistance to carbapenems, varies (Table 1). MBLs, mainly of the VIM and IMP types, have spread mostly in *Pseudomonas aeruginosa*, and, at least in some regions, in *Acinetobacter baumannii* and *Enterobacteriaceae*, especially *Klebsiella pneumoniae* [1,2]. Also, the latter species has been the main producer of the KPC-type class A carbapenemases so far [1,3], although other species in which these enzymes have been identified include

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TABLE 1. Species distribution of clinically relevant acquired carbapenemases

Organism	MBLs (class B)	Class A KPC (GES)	OXA (class D)
Pseudomonads			
Pseudomonas aeruginosa	**++**	+	+
Pseudomonas putida	+	+	
Acinetobacter baumannii	**+**[a]		**++**
Acinetobacter spp.	+		+
Enterobacteria			
Klebsiella pneumoniae	**+**[a]	**++**	+
Escherichia coli	+	+	+
Proteus mirabilis	+		+
Providencia spp.	+		
Klebsiella oxytoca	+	+	
Serratia marcescens	**+**[a]	+	
Enterobacter spp.	**+**[a]	+	
Citrobacter freundii	+	+	
Morganella morganii	+		
Salmonella enterica		+	
Raoultella spp.		+	

MBL, metallo-β-lactamase.
++, prevalent species–enzyme type combinations; +, occasionally reported species–enzyme type combinations.
[a]Endemic in certain regions.
Crosses in bold denote higher prevalence in the respective species.

Klebsiella oxytoca, *Salmonella enterica*, *Escherichia coli*, *Enterobacter* spp., and *Pseudomonas* spp. [1,4–7]. In addition, rarer class A enzymes of the GES type, which are typical extended-spectrum β-lactamases (ESBLs) with the activity spectrum expanded towards carbapenems, have been identified in *P. aeruginosa*, *K. pneumoniae* and *E. coli* [8,9]. Acquired OXA-type carbapenem-hydrolyzing class D β-lactamases (CHDLs) of the OXA-23, OXA-24/OXA-40 and OXA-58 subfamilies are common among *A. baumannii* isolates [1,10], and OXA-48 has been detected in *K. pneumoniae* and *E. coli* isolates [11,12].

The potential for further evolution of this scenario should not be ignored. Rapid changes in the carbapenemase host range may occur, because these genes are associated with mobile genetic elements, and the emergence of novel enzyme types and variants can be expected in the future.

Laboratory Detection of Carbapenemase-producing Organisms

Detection of carbapenemase-producing (CP) organisms in the clinical microbiology laboratory is a matter of major importance for the choice of appropriate therapeutic schemes and the implementation of infection control measures. Detection of carbapenemase producers, however, poses a number of difficulties, as it cannot be based simply on the resistance profile, and as the relevant methodology based on specific tests has not yet been well standardized.

Which isolates should be tested for carbapenemase production?

The production of a given carbapenemase may confer a particular β-lactam resistance phenotype, depending on the bacterial species, the expression level, the enzyme type or variant, and the presence of additional resistance mechanisms such as permeability reduction and/or efflux and/or activity of other β-lactamases [1,13]. A significantly elevated MIC (or a decreased inhibition zone with disk diffusion testing) of a carbapenem should make a clinical isolate eligible for further testing for carbapenemase production by means of specific methods (see below). However, carbapenem MICs for carbapenemase producers may vary within a broad range of values, and even lay within the susceptibility range, as defined by either the current CLSI or the EUCAST clinical breakpoints (Table 2). Indeed, such low levels of resistance to carbapenems have often been observed in *Enterobacteriaceae* producing carbapenemases of different types [14–16], *Acinetobacter* isolates producing MBLs [17] and CHDLs, [18] and, although rarely, among MBL-producing *P. aeruginosa* isolates [19]. In order to propose selection criteria for clinical isolates undergoing specific testing for carbapenemase detection (screening), one should take into account the carbapenem MIC ranges reported so far for CP isolates (Table 3), the distribution of carbapenem MICs in wild-type microorganisms (Table 2; see also MIC distributions of wild-type microorganisms at http://www.eucast.org), and certain characteristics of resistance phenotypes conferred by various mechanisms.

In a previous discussion paper on MBLs, it was proposed that MBL production in *P. aeruginosa*, other *Pseudomonas* spp.

TABLE 2. CLSI and EUCAST carbapenem clinical breakpoints and epidemiological cut-off values (ECOFFs) (MIC values, mg/L)

Organisms	CLSI		EUCAST		
	S (≤)	R (≥)	S (≤)	R (>)	ECOFF (>)
Enterobacteriaceae					
Imipenem	4	8	2	8	1–4
Meropenem	4	8	2	8	0.125–0.25
Ertapenem	2	4	0.5	1	0.064
Doripenem	ND	ND	1	4	0.064
Pseudomonas aeruginosa					
Imipenem	4	16	4	8	4
Meropenem	4	16	2	8	2
Doripenem	ND	ND	1	4	1
Acinetobacter spp.					
Imipenem	4	16	2	8	1
Meropenem	4	16	2	8	2
Doripenem	ND	ND	1	4	1

ND, not defined.

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TABLE 3. Ranges of carbapenem MICs observed in clinical isolates producing acquired carbapenemases

Organism/enzyme type	MICs (mg/L)		
	Imipenem	Meropenem	Ertapenem
Pseudomonas aeruginosa/MBL	2 to >64	2 to >64	–
P. aeruginosa/KPC	>64	>32	–
Acinetobacter baumannii/MBL	2 to >64	2 to >64	–
A. baumannii/OXA	1 to >64	1 to >64	–
Enterobacteriaceae AmpC(–)/MBL	0.5 to >64	0.25 to >64	0.5–4(?) Not enough data[a]
Enterobacteriaceae AmpC(+)/MBL	1 to >32	1 to >32	Not enough data[a]
Enterobacteriaceae AmpC(–)/KPC	0.5 to >64	1 to >32	0.5 to >64
Enterobacteriaceae AmpC(+)/KPC	8 to >64	4–64	8 to >64
Enterobacteriaceae AmpC(–)/OXA (OXA-48)	1 to >64	0.5–64	4 to >64

MBL, metallo-β-lactamase.
[a]Most of the papers do not include ertapenem MIC values.
(?)Upper limit uncertain.

and *Acinetobacter* spp. should be further investigated in isolates that are non-susceptible to carbapenems (imipenem and/or meropenem) and resistant either to ticarcillin, ticarcillin–clavulanate or ceftazidime [20]. In general, this proposal seems still to be appropriate for MBLs and could also be extended to other carbapenemases, even though there are some differences in the breakpoints recommended by different institutions. For example, the EUCAST and CLSI guidelines define as imipenem-non-susceptible those strains of *A. baumannii* with MICs of ≥4 mg/L or ≥8 mg/L, respectively, and as meropenem-non-susceptible those strains of *A. baumannii* and *P. aeruginosa* with MICs of ≥4 mg/L or ≥8 mg/L, respectively (Table 2).

In the case of *Enterobacteriaceae*, it is now suggested that carbapenemase-detecting phenotypic tests should be performed in isolates exhibiting even a small reduction of susceptibility to carbapenems, including ertapenem, which was recently found to be a sensitive marker of KPC production [21]. The CLSI recommends MIC values of ≥2 mg/L for ertapenem, imipenem or meropenem, and resistance to at least one cephalosporin of subclass III (cefotaxime, ceftriaxone or ceftazidime) as indicators of possible carbapenemase production in *Enterobacteriaceae* [22]. However, the CLSI experience is mostly based on KPC producers. Even less stringent criteria have been proposed, which recommend ertapenem or meropenem MICs of ≥0.5 mg/L or imipenem MICs of ≥1 mg/L as cut-off values for suspicion of carbapenemase production [23]. Similarly, for the disk diffusion methodology, the CLSI proposes zone diameter cut-off values of ≤21 mm for ertapenem or meropenem [22], whereas other authors have recently recommended ≤21 mm for ertapenem, ≤20 mm for imipenem or even ≤27 mm for meropenem [23]. An adequately high sensitivity is expected with application of the less stringent criteria, although a number of non-specific, false-positive results may be obtained, especially with ertapenem and with isolates producing either higher amounts of AmpC-type cephalosporinases or CTX-M ESBLs and with porin alterations [23,24]. Nevertheless, further adjustment of the cut-offs may be necessary following the growing experience with CP clinical isolates.

A concomitant examination of additional phenotypic traits could assist in detection of CP organisms. Irrespective of the actual levels of resistance to carbapenems, acquisition of a carbapenemase affects the susceptibility to a wide variety of β-lactam antibiotics. In Table 4, the expected 'baseline' resistance profiles conferred by carbapenemase production in

TABLE 4. Expected phenotypes of carbapenemase producers for selected β-lactams

Organism	Enzyme	AMP	TIC	TZP	CAZ	ATM	IMP	ETP
Pseudomonas aeruginosa	MBL	–	R	R	R	S	R	–
	KPC	–	R	R	R	R	R	–
Acinetobacter baumannii	MBL	R	R	R	R	S	I/R	–
	OXA	R	R	I	S	S	I/R	–
Enterobacteriaceae AmpC(–)	MBL	R	R	S/I	R	S	S/I/R	I/R
Enterobacteriaceae AmpC(+)	MBL	–	R	S/I	R	S	S/I/R	I/R
Enterobacteriaceae AmpC(–)	KPC/GES	R	R	R	I/R	R	S/I/R	I/R
Enterobacteriaceae AmpC(+)	KPC/GES	–	R	R	R	R	I/R	I/R
Enterobacteriaceae AmpC(–)	OXA-48	R	R	R	I/S	S	S/I	S/I

MBL, metallo-β-lactamase; AMP, ampicillin; TIC, ticarcillin; TZP, piperacillin/tazobactam; CAZ, ceftazidime; ATM, aztreonam; IMP, imipenem; ETP, ertapenem.
Phenotypes may vary for many organism–compound combinations, depending on enzyme variants, expression level or additional mechanisms.

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Gram-negative bacteria are indicated. A CP isolate is expected to exhibit resistance at least to penicillins and narrow-spectrum cephalosporins. Also, production of either a KPC-like or GES-like class A carbapenemase or of an MBL commonly mediates resistance to expanded-spectrum cephalosporins, such as ceftazidime, cefotaxime and ceftriaxone [1]. Nevertheless, activity of the latter drugs against CHDL producers is not necessarily compromised [10], and activity against organisms with other, rare class A carbapenemases (e.g. SME, IMI and NMC-A) is usually not compromised at all [1].

Finally, it should be underscored that determination of carbapenem MICs in organisms producing either a KPC or an MBL can be problematic. There have been studies reporting relatively low reproducibility for most of the conventional methods used, as well as discrepant results among the methods [25–27]. At least in part, these problems could be due to a strong inoculum effect and to the often low carbapenem MICs mediated by these β-lactamases in *Enterobacteriaceae*. Therefore, special care should be applied in preparing bacterial inocula, mostly when automated systems are used [25,27]. Under-inoculation has been suspected as the main cause of false-negative results in the detection of KPC producers [26,27]. Inconsistencies have also been reported for the gradient diffusion methodologies, such as the Etest [27–29]. Moreover, the latter methods are not considered to be appropriate for the KPC-producing organisms, owing to their heterogeneous growth, which makes the interpretation very difficult [27]. The broth microdilution and disk diffusion methods are considered to be more reliable for the detection of all types of carbapenemase-mediated resistance.

Phenotypic tests for detection of carbapenemase production

A number of simple phenotypic tests, most of them in the disk diffusion format, have been described and evaluated as methodologies for the specific detection of CP organisms.

The clover leaf method (or modified Hodge test (MHT)) has been extensively used as a general phenotypic method for the detection of carbapenemase activity [22,30], and it has been the only method of carbapenemase detection so far recommended by the CLSI [22]. The test is based on the inactivation of a carbapenem by either whole cells or cell extracts of the CP organisms, which enables a carbapenem-susceptible indicator strain to extend growth towards a carbapenem disk, along the streak of inoculum of the test strain or extract thereof. The assay is, overall, sensitive for the detection of a carbapenemase-mediated mechanism of resistance to carbapenems but does not provide information regarding the type of carbapenemase involved. Moreover, there have been reports of false-positive results, mostly generated by CTX-M-producing strains with reduced outer membrane permeability, and some investigators have raised the problem of difficulties in the interpretation of the clover leaf test for weak carbapenemase producers, particularly for MBLs in *Enterobacteriaceae* [23]. In the case of MBL producers, it has been suggested that addition of zinc sulphate may improve the MHT performance [31]. In settings where KPC-producing *K. pneumoniae* isolates are endemic, the accurate determination of levels of susceptibility to ertapenem and the MHT have been proposed as methods that are sensitive enough for detection of those isolates, although with insufficient specificity [21].

Several inhibitor-based tests have been developed for the specific detection of MBL producers. These are based on the synergy between MBL inhibitors—such as EDTA [30,32], EDTA plus 1,10-phenanthroline [33], thiol compounds (2-mercaptopropionic acid or sodium mercaptoacetic acid) [31,34] and dipicolinic acid [35]—and a carbapenem (imipenem and/or meropenem) and/or an oxyimino-cephalosporin (ceftazidime) as indicator β-lactam compounds. These tests take advantage of the metalloenzyme dependence on zinc ions, and use the chelating agents to inhibit β-lactam hydrolysis. Various formats (disk diffusion or broth dilution) of EDTA-based synergy tests have been the most commonly used and evaluated [30–33]. The double-disk synergy test (DDST) and the combined disk test, using different amounts of EDTA and, in the case of DDST, different distances between the disks, exhibit high sensitivity even with isolates with low carbapenem resistance levels. It has been suggested that zinc supplementation of the culture medium may increase the sensitivity of the method [36], but this modification has not been thoroughly evaluated. The Etest MBL strip is also based on synergy between EDTA and imipenem, and has been credited with good sensitivity and specificity for detection of MBL-producing *P. aeruginosa* [37], although it has been repeatedly pointed out that its specificity might be impaired by, among other factors, the possible intrinsic activity of EDTA [28,38,39].

There have been studies reporting failures of EDTA-synergy tests to detect MBL production among *A. baumannii* isolates [17,40,41], and a better performance of thiol-based tests has been indicated [31]. Also, false-positive results in *A. baumannii* due to the presence of CHDLs have been reported [42]. The Etest MBL has proved inappropriate for detection of MBL-producing *Enterobacteriaceae* with low imipenem MICs (≤4 mg/L), but new Etest strips have recently been developed for the detection of MBLs in *Enterobacteriaceae*, with promising preliminary results [43].

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Despite the good performance of inhibitor tests in the detection of MBLs in general, it should be remembered that MBL inhibitors act non-specifically and affect other structures and processes (e.g. outer membrane permeabilization in *P. aeruginosa*) [38]. The risk of obtaining false or ambiguous results is certainly higher than in the case of ESBL detection, with the use of mechanism-based β-lactam inhibitors. Therefore, the results should be interpreted cautiously, and it is strongly recommended to have them confirmed with reference methodology (spectrophotometric assays—see below). All inhibitor-based synergy tests should include a control for the intrinsic activity of the inhibitor.

Specific phenotypic assays for the identification of KPC-producing strains (and of those producing other class A carbapenemases) are based on the inhibitory effect of boronic acids, usually 3-aminophenylboronic acid (APB), although the mechanism of inhibition is not known. Several versions of such tests have been recently developed, differing in performance [23,44,45]. Although, in one of these studies, the DDST approach was found to work well [23], the combined disk test variant has been applied more often and, so far, evaluated as being better. Of several indicator β-lactams tested, either meropenem [45] or imipenem [23] were pointed out as the preferable compounds. Also different cut-off values of zone diameter differences between disks with a carbapenem plus APB and the carbapenem alone were proposed as being indicative of production of KPC (or another class A carbapenemase) (≥4 to ≥7 mm). With the use of meropenem disks, with or without 400 μg of APB, the specificity in diagnosing KPC-producing *K. pneumoniae* isolates and differentiating them from plasmidic AmpC-producing *K. pneumoniae* and *E. coli* proved excellent [45]. Apart from the disk diffusion approaches, a method has been developed in which MICs of carbapenems are evaluated both in the absence and in the presence of APB (0.3 g/L) by agar dilution [23]. A three-fold or greater reduction of carbapenem MIC in the presence of APB has been proposed as the cut-off value for positive isolates. In general, it seems that the boronic acid-based methods exhibit high sensitivity in the detection of KPC producers, which makes these methods very promising. However, their specificity needs further evaluation. Preliminary—and often still unpublished—observations indicate a tendency for false-positive results to be generated, mostly as a result of organisms with reduced susceptibility to carbapenems because of high-level expression of AmpC-type cephalosporinases and porin alterations [23]. This is not surprising when one considers the significant inhibition of AmpCs by boronic acids [46]. It is noteworthy that the APB-based assays failed to detect the KPC-producing *Klebsiella* isolates in the case of co-production of VIM enzyme [47].

The enzymatic properties of CHDLs have prevented the development of specific phenotypic tests for their detection. Even the utility of the MHT for CHDL producers has not been systematically studied. Thus, the definitive identification of such organisms requires molecular techniques, e.g. PCR assays specific for the respective genes.

A group of experts from EUCAST and the ESCMID Study Group for Antibiotic Resistance Suveillance (EARSS) has recommended the following procedures for the detection of class A and B enzymes in *Enterobacteriaceae*. For class A enzymes (KPC or other enzymes), production is suspected when a difference of ≥4 mm in the zone diameter is observed between meropenem (10 μg) and meropenem plus boronic acid (600 μg). As boronic acid can also inhibit class C enzymes, comparison between the zone diameters of meropenem and meropenem plus cloxacillin (750 μg) disks suggests the presence of a strain hyperproducing the chromosomal AmpC or producing a plasmid-encoded AmpC when the latter diameter is increased by ≥5 mm. The detection of class B enzymes is based on a disk combination test using meropenem and meropenem plus EDTA (0.25 M). The test is considered to be positive when there is an increase in the zone diameter of ≥5 mm. This approach is, overall, similar to that proposed by different investigators who have recommended the use of different disks containing a carbapenem disk with EDTA and boronic acid but with different concentrations [44,45], with the exception that these protocols cannot differentiate class A carbapenemases from the combination of class C β-lactamases or ESBLs and porin loss. However, when meropenem disks with 400 μg of boronate were used, it was possible to accurately discriminate KPC producers from KPC-negative *K. pneumoniae* showing reduced carbapenem susceptibility due to permeability defects, using suitable breakpoints [45]. The authors claim that, with these disk tests, carbapenemases can easily be detected from the first isolation day with no need for estimation of Etest carbapenem MICs and of carbapenemase production on the next day using the clover leaf test.

Carbapenemase detection by spectrophotometric assays

Spectrophotometric measurement of carbapenem hydrolysis is considered to be the reference standard method for detection of carbapenemase production in a suspected CP organism. Hydrolysis of carbapenems in the presence or absence of inhibitors (i.e. EDTA for MBLs, tazobactam or clavulanic acid for KPCs, NaCl for most CHDLs), performed with crude cell extracts or partially purified enzymes, could provide additional information concerning the enzyme type.

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These laborious and technically demanding assays should be performed in reference laboratories.

Detection of carbapenemase genes by molecular methods

Molecular methods such as simplex and multiplex PCRs, real-time PCR, DNA hybridization and sequencing have been commonly used for the identification of carbapenemase genes in research laboratories and reference centres. Nowadays, some of these methods, mostly PCR, are routinely performed in some clinical laboratories in order to circumvent the problems of the phenotypic detection of CP organisms. Apart from the 'in-house' assays, there are also commercially available kits based on PCR and hybridization, examples being Hyplex MBL ID and Hyplex CarbOxa ID (BAG Health Care, Lich, Germany) for the detection of bla_{VIM}(1–13) and bla_{IMP}(1–22) genes and of bla_{OXA} carbapenemase genes, respectively. This methodology allows detection of the carbapenemase-encoding genes directly from clinical samples [48,49].

Commercial kits of this type seem to be promising, and their thorough evaluation in multicentre studies must be considered.

Surveillance of Carbapenemase-producing Microorganisms

Importance of surveillance and the current situation

Surveillance of the occurrence and spread of resistant organisms is a crucial step for containment of antimicrobial resistance [50]. ESBL-producing organisms have drawn the attention of national and international surveillance systems, probably because ESBLs have become a public health problem in most countries, and there are well-standardized protocols and tools for phenotypic detection of these enzymes [22]. Unlike this situation, acquired carbapenemases have been reported in fewer geographical areas, and endemicity seems to be still limited to certain countries, such as Greece, Italy, Israel, Japan, Brazil and the USA [1–3,51]. Moreover, difficulties in the phenotypic detection of these isolates and the lack of appropriate standardized methods (see above) complicate surveillance studies and could lead to an underestimation of the actual epidemiological impact of CP organisms. The dearth of surveillance data on bacteria producing acquired carbapenemases could also reflect the fact that different resistance mechanisms can affect carbapenems, whereas the presence of multiple mechanisms is often required for an isolate to be resistant to this antimicrobial class [52]. In fact, expression of acquired carbapenemases alone, especially in *Enterobacteriaceae*, often results in carbapenem MIC values that remain lower than the current breakpoints (see above).

For these reasons, in surveillance data, only carbapenem resistance is normally recorded, but not the resistance mechanisms involved. This can be further complicated by the fact that the antimicrobial susceptibility testing committees, the CLSI and EUCAST, have defined different clinical breakpoints for carbapenems (Table 2). Although EUCAST breakpoints are, overall, lower than those defined by the CLSI, they cannot be used accurately to define or to discriminate which isolates are putative carbapenemase producers, as the carbapenem MIC values for these isolates may remain lower than the breakpoints (see above). EUCAST has also established the epidemiological cut-off values (ECOFFs) that discriminate wild-type isolates, lacking a resistance mechanism, from those possessing any resistance mechanisms affecting the considered antibiotic (Table 2). Although the use of ECOFFs could clearly favour carbapenemase surveillance systems, some investigators have raised the possibility of the isolation of *Enterobacteriaceae* for which carbapenem MICs are below ECOFFs [16,53]. If these isolates become prevalent, and further studies demonstrate their clinical relevance, additional drawbacks should be resolved in surveillance studies and also in the process of setting breakpoints. The use of the most affected carbapenem as an indicator of the presence of a resistance mechanism, or several carbapenems at the same time, might help to partially solve this problem. Finally, it should also be noted that carbapenem breakpoints are currently under discussion both at the CLSI and EUCAST, and some revisions are expected in the near future.

Another approach to the surveillance of acquired carbapenemases is to establish criteria based on phenotypes in order to indicate the potential presence of these enzymes. This approach should be combined with confirmatory tests that resolve suspected isolates. This was partially addressed in a previous document, where phenotypes endowed with the production of a carbapenemase were considered and a low-stringency consensus was established [20]. This consensus still retains its validity (see above). In the particular case of MBLs, resistance to ceftazidime and susceptibility to aztreonam might also indicate the presence of these enzymes. Nevertheless, this phenotypic rule should be used with caution, as it is not infrequent for MBL-producing isolates, particularly *Enterobacteriaceae*, to also have other resistance mechanisms affecting monobactams, such as ESBLs, plasmid-mediated AmpCs, or hyperproduction of chromosomal β-lactamases [16,54,55].

As one of the main objectives of antimicrobial surveillance systems is to detect and warn of the emergence and spread of new resistance mechanisms, carbapenemase surveillance

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systems are urgently needed. Early warning systems at a regional or national level should ensure the detection of the emergence of CP organisms in areas where such isolates have not previously been reported. Moreover, continuous surveillance efforts in countries with high prevalence should provide data for the monitoring of both resistance trends and the impact of control strategies. A common database at the international or regional level is also desirable. The EARSS system is an example of a successful effort funded by public health authorities (http://www.rivm.nl/earss/). In addition, private surveillance systems, generally funded by pharmaceutical companies, such as the SENTRY, TEST, SMART or MYSTIC programmes, should also contribute to the knowledge of carbapenemase-producing organisms.

The importance of surveillance is underscored by the fact that the appearance of acquired carbapenemases in different countries has been associated with imported cases, mainly due to the transfer of patients from geographical areas where this problem is widely established. As an example, KPC-type enzymes in *K. pneumoniae* isolates were first reported in 2001 in North Carolina and, until 2005, the geographical distribution of these enzymes in *K. pneumoniae* was limited to the eastern USA [3]. In the New York area, KPC-producing strains became frequently encountered nosocomial pathogens, but were also detected in long-term-care facilities [4,56]. The emergence of KPC-producing *Enterobacteriaceae* in France was associated with travel to New York City [3], and international or intercontinental spread of KPC-producing *K. pneumoniae* isolates belonging to sequence type (ST) 258 has been reported from Israel [57] and several European countries, including Greece [58], the UK [59], Poland [60], Norway and Sweden [61], and Italy [62]. The emergence of these enzymes in the UK, Norway and Sweden was, in part, linked with previous patient hospitalization in Israel and Greece [59,61]. This was also the case for outbreaks due to VIM-1-producing *Enterobacteriaceae* reported in French hospitals [54,63].

TABLE 5. Target objectives of acquired carbapenemase surveillance systems

Target objectives	Specific indicators
Carbapenem resistance	Imipenem, meropenem, ertapenem, doripenem
Acquired carbapenemase-producing isolates	*Pseudomonas aeruginosa*
	Acinetobacter spp.
	Enterobacteriaceae (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* spp., *Proteus mirabilis*, others)
Types of enzyme	Class A (KPCs)
	Class B (mainly VIM and IMP)
	Class D (OXA variants affecting carbapenems)
Patients/individuals	Clinical (infections) and colonization (carriers) isolates
	Hospitalized and non-hospitalized patients
	Healthy population
Non-hospital compartments	Food-chain animals
	Environment

Case definition, denominators and epidemiological data

Like other surveillance systems, a system for surveying bacteria producing acquired carbapenemases should be able to record denominators, and to accurately establish incidence, prevalence and trends. Moreover, in light of the lessons learned with ESBLs, specific efforts should be made to clearly identify colonized and infected patients. Unlike the situation with ESBLs, animals in the food chain have not yet been associated with this problem, probably because the use of carbapenems in animals is prohibited. Nevertheless, contamination of aquatic settings with relevant carbapenemase producers [64,65] can favour and accelerate their spread to other settings; thus, surveillance studies targeting food-chain animals and environmental sources might contribute to tracing the potential pathways of carbapenemase dissemination and the possible involvement of non-human compartments.

Table 5 contains different target objectives for the surveillance of acquired carbapenemases. Relevant information should be recorded in each case, with a clear definition of cases and denominators. At patient or carrier levels, this information should include, among data, demographics and risk factors and/or clinical features, including previous hospitalizations, travel to countries with a high incidence of CP organisms, relationship with long-term-care facilities, previous underlying disease, and antibiotic exposure.

Generally speaking, it is recognized that a case definition is needed for an acquired carbapenemase surveillance programme. This should be implemented at different levels, including patients, isolates and clones. Even different enzymes and the corresponding genes should be considered. Despite the lack of high levels of expression of carbapenemases, case definitions are easier for *Enterobacteriaceae* than for *P. aeruginosa* and *Acinetobacter* spp., which can have different resistance mechanisms affecting carbapenems [52].

Surveillance of high-risk clones of carbapenemase-producing organisms

Surveillance systems should also be designed to investigate the population structure of CP organisms and to identify the so-called high-risk clones, as occurs with ESBLs [66]. Different typing systems can be used to broaden this epidemiological information.

Typing should be performed not only during outbreaks but also with sporadic isolates for comparison with 'epidemic' strains. In general, pulsed-field gel electrophoresis should not be used as the only reference typing system, especially in geographical areas with long-term persistence of

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acquired CP isolates. In this case, it is more useful to perform multilocus sequence typing to track clones and clonal complexes and to exchange information among different geographical areas, particularly to identify the emergence of highly epidemic clones in areas with low prevalence. This approach can also be useful for the identification of highly epidemic clones associated with specific carbapenemases, such as *P. aeruginosa* ST235, which mainly produces the VIM-1 enzyme but that was previously recognized to produce the PER-1 ESBL [67–69]. *K. pneumoniae* ST258 has been identified as a plasmid-mediated KPC carbapenemase producer in several countries [3,54,57–59,70]. The implementation of commercial easy-to-perform typing systems could also be useful, as recently shown with ESBL-producing isolates [71]. It is recommended that reference centres in different countries centralize data.

In these STs and clonal complexes, virulence and pathogenicity should also be investigated. It is recommended that these studies should be restricted to reference laboratories, particularly in countries with low-level incidence.

Surveillance of carbapenemase genes and of cognate genetic platforms and plasmids

In a second step, specific surveillance studies should be designed to address the epidemiology of carbapenemase genes and the cognate genetic platforms participating in their expression, maintenance and mobilization. This should be organized in different geographical areas, at both national and international levels. Moreover, a catalogue of plasmids carrying carbapenemase genes should be established to contribute to the understanding of the spread of these enzymes.

In surveillance systems for acquired carbapenemases, co-resistances must also be monitored, as well as the association of carbapenemase genes with those affecting non-β-lactam antibiotics. Most of them have been demonstrated to be transferable, including those recently described as affecting fluoroquinolones (e.g. *qnr*, *aac(6′)-Ibcr* and *qepA*) or aminoglycosides (e.g. *arm* and *rtm*). Moreover, as some carbapenemase determinants are associated with integrons, other resistance cassettes, such as those involving aminoglycoside or trimethoprim, and sulphonamide (*sul*) resistance genes, should also be considered. In addition, it is interesting to monitor the simultaneous presence of other β-lactamase genes such as those encoding ESBLs or AmpC-type enzymes. This association is no longer a rarity [1,14,72,73].

Surveillance of carriers

Colonization with CP *Enterobacteriaceae*, particularly with CP *K. pneumoniae*, has been associated with several healthcare-associated factors, and higher mortality rates have been observed among patients infected with CP isolates than among those infected with non-CP isolates [74–76]. Moreover, patients with asymptomatic colonization are at risk of invasive infection [77]. In outbreaks or in settings where CP microorganisms are endemic, screening for asymptomatic carriers must be considered, as this procedure has been shown to be helpful in reducing the incidence of CP organisms [78]. The appropriate source/biological material for this procedure has not been specifically determined for CP *Enterobacteriaceae* but depends on the prevailing organism. Moreover, general guidelines for multidrug-resistant organisms should be followed (CDC, Healthcare Infection Control Practices Advisory Committee. Management of multidrug-resistant organisms in healthcare settings, 2006. Atlanta, GA: US Department of Health and Human Services, CDC, Healthcare Infection Control Practices Advisory Committee, 2007. Available at http://www.cdc.gov/ncidod/dhqp/pdf/ar/mdroguideline2006.pdf.). Suitable materials for the screening of CP strains are: (i) rectal swabs or stools for isolation of *Enterobacteriaceae*; (ii) respiratory specimens and/or stools for pseudomonads; and (iii) nasal swabs, axilla swabs and/or stools for *A. baumannii* [79].

Agar or broth media supplemented with a low-concentration carbapenem may be used. MacConkey agar containing imipenem at a concentration of 1 mg/L, or a 5-mL aliquot of tryptic soy broth containing a 10-μg disk of imipenem (resulting in a final concentration of imipenem of 2 mg/L), are suitable [80]. In addition, the use of MacConkey agar onto which a 10-μg disk of imipenem or ertapenem is placed has also been reported [77,78,80].

Regarding the KPC-producing isolates, screening with the new chromogenic medium (CHROMagar KPC; CHROMagar Company, Paris, France), which is supplemented with agents that inhibit the growth of carbapenem-susceptible organisms, has been proposed [81]. The method has been validated only in a small number of samples containing KPC-producing *K. pneumoniae* isolates. After the initial screening, the carbapenem-resistant isolates are investigated by phenotypic and molecular assays. This CHROMagar KPC medium is not selective for screening KPC-producing isolates, as it may also be used in screening for producers of other types of carbapenemases. Conversely, it should be noted that selective plates for ESBL-producing organisms (e.g. MacConkey agar containing ceftazidime or cefotaxime, or selective chromogenic agars) can be useful, although such a method is not yet validated. Bacteria growing on these plates must be checked for carbapenemases.

Molecular techniques such as PCR or real-time PCR have the potential to be used directly on biological material without prior cultivation. The approach could be useful,

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especially in outbreak settings or in areas with a high prevalence of CP microorganisms, where the timely cohorting of patients is extremely important for preventing the further spread of CP organisms.

In outbreak settings, after the initial screening, the putative carbapenemase producers should be forwarded to a reference laboratory for further confirmation of carbapenemase activity by spectrophotometric assays and for identification of different carbapenemases.

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Transparency Declaration

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