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ARTICLE COMMENTARY

Treatment and diagnosis of severe KPC-producing *Klebsiella pneumoniae* infections: a perspective on what has changed over last decades

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ABSTRACT

Antimicrobial resistance is a global health threat. Among Gram-negative bacteria, resistance to carbapenems, a class of β-lactam antibiotics, is usually a proxy for difficult-to-treat resistance, since carbapenem-resistant organisms are often resistant to many classes of antibiotics. Carbapenem resistance in the Gram-negative pathogen *Klebsiella pneumoniae* is mostly due to the production of carbapenemases, enzymes able to hydrolyze carbapenems, and *K. pneumoniae* carbapenemase (KPC)-type enzymes are overall the most prevalent carbapenemases in *K. pneumoniae*. In the last decade, the management of severe infections due to KPC-producing *K. pneumoniae* (KPC-Kp) in humans has presented many peculiar challenges to clinicians worldwide. In this perspective, we discuss how the treatment of severe KPC-Kp infections has evolved over the last decades, guided by the accumulating evidence from clinical studies, and how recent advances in diagnostics have allowed to anticipate identification of KPC-Kp in infected patients.

KEY MESSAGES

- In the last decade, the management of severe infections due to KPC-Kp has presented many peculiar challenges to clinicians worldwide
- Following the introduction in clinical practice of novel β-lactam/β-lactamase inhibitor combinations and novel β-lactams active against KPC-producing bacteria, the management of severe KPC-Kp infections has witnessed a remarkable evolution
- Treatment of severe KPC-Kp infections is a highly dynamic process, in which the wise use of novel antimicrobials should be accompanied by a continuous refinement based on evolving clinical evidence and laboratory diagnostics

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Introduction

Antimicrobial resistance has been estimated to cause at least 700,000 people deaths worldwide each year, possibly rising to 10 million by 2050 [1]. Since early 1990s, resistance to carbapenems, a class of β-lactam antibiotics, has been reported with increasing frequency among Gram-negative bacteria [2], usually as a proxy for difficult-to-treat resistance. Indeed, carbapenem-resistant organisms are often resistant to many classes of antibiotics, thereby complicating the treatment of infections caused by these bacteria in humans [3].

Among major Gram-negative pathogens, *Klebsiella pneumoniae* is one of the most affected by carbapenem resistance. In this species, carbapenem resistance is mostly due to the production of β-lactamases able to hydrolyze carbapenems (i.e. carbapenemases), although combinations of other different mechanisms may also occur [4,5]. *K. pneumoniae* carbapenemase (KPC)-type enzymes are overall the most prevalent acquired carbapenemases in *K. pneumoniae* and became of major relevance after their emergence and global spread during the first decade of the 21st century [6]. KPC enzymes belong to molecular class A of β-lactamases (Ambler classification), whose active site

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contains a serine residue, are usually plasmid-encoded, and are able to hydrolyze a very broad spectrum of β-lactam substrates, including penicillins, cephalosporins, monobactams and carbapenems. They are weakly inhibited by traditional β-lactam inhibitors (i.e. clavulanic acid and tazobactam), but efficiently inhibited by the novel β-lactamase inhibitors (i.e. diazabicyclooctanes and boronates) [7,8].

In the last decade, following the introduction in clinical practice of novel β-lactam/β-lactamase inhibitor combinations and novel β-lactams active against KPC-producing bacteria, the management of severe infections due to KPC-producing *K. pneumoniae* (KPC-Kp) in humans has witnessed a remarkable evolution. In this perspective, we discuss how the treatment of severe KPC-Kp infections has evolved over the last decades, guided by the accumulating evidence from clinical studies, and how recent advances in diagnostics have allowed to anticipate identification of KPC-Kp in infected patients.

Evolution of treatment algorithms and clinical studies

The old era (2013–2018)

Back to the early 2010s, the treatment of severe infections due to KPC-Kp mostly relied on combinations of two or even three antibacterial agents, almost invariably including a polymyxin or an aminoglycoside, that belong to two classes of antibiotics potentially associated with significant nephrotoxicity [9,10]. To understand such a peculiar prescribing pattern, it should be first reminded that, besides being resistant to carbapenems, KPC-Kp are very often resistant to other classes of commonly used antibiotics, such as penicillins, third and fourth-generation cephalosporins, and fluoroquinolones (owing both to the broad spectrum of activity of KPC-type enzymes against β-lactams other than carbapenems and to the genetic linkage of bla_{KPC} to other resistance determinants located on the same plasmid, commonly including genes conferring resistance to aminoglycosides, quinolones, trimethoprim, sulphonamides, and tetracyclines) [11,12]. It was thus not uncommon for aminoglycosides or, more frequently, polymyxins, to remain the only class/classes of antibiotics showing *in vitro* activity against KPC-Kp isolates from infected patients, and polymyxins were thus frequently selected as first-line treatment.

The fact that polymyxins retained activity against KPC-Kp on most occasions was no surprise. Polymyxins, of which those currently available for use in humans are polymyxin B and polymyxin E (the latter also known as colistin), are bactericidal lipopeptides mainly exerting their antimicrobial activity through interaction with the lipopolysaccharide of Gram-negative bacteria and permeabilization of their outer membrane [13]. They became available in the 1950s, but their use in humans was soon abandoned, with few exceptions, because of concerns about their nephrotoxicity and the concomitant availability of other less toxic classes of antibiotics. The lack of widespread use of polymyxins for several decades is thought to have massively relieved the selective pressure for polymyxin resistance in human pathogens, thereby justifying the very high frequency of susceptibility even among KPC-Kp isolates when, at the beginning of the current century, polymyxins started to be used again and conspicuously, for the treatment of severe infections due carbapenem-resistant Gram-negative bacteria. However, crude mortality rates of severe KPC-Kp infections treated with polymyxins were generally higher than those registered in patients with severe infections due to carbapenem-susceptible Kp (CS-Kp) infections treated with carbapenems [14]. The reasons for such worse mortality rates are still not completely clear even today. The principal suspected culprits are a suboptimal efficacy of polymyxins and an increased frequency of inappropriate empirical therapy in KPC-Kp infections than in CS-Kp infections. Other reasons such as increased KPC-Kp virulence are overall far less likely, although still remaining to be definitely ruled out [14,15]. Suboptimal efficacy of polymyxins is a plausible argument. Certainly, polymyxins have been tremendously useful for treating severe KPC-Kp infections in the last 20 years (in the presence of scant alternatives), but several shortcomings could have played a role in unfavorably influencing their efficacy. The first is that also studies on the pharmacokinetic/pharmacodynamic (PK/PD) properties of polymyxins were largely abandoned starting from the middle of the past century, therefore, around twenty years ago, the available PK/PD data guiding administration of polymyxins were not updated according to current standards. For example, it is now known that intravenous polymyxins have a reduced lung penetration and that, for colistin, there could be an interpatient variability in the conversion of the prodrug (colistimethate) into the active moiety (colistin) [16]. In addition, some other factors likely and unfavorably influenced efficacy of polymyxins before the availability of updated PK/PD guidance (the international guidelines for optimizing the use of polymyxins in clinical practice, that represented the sum of all the crucial efforts of different research

groups worldwide, were released only at the beginning of 2019) [15]. In the early 2010s, colistin maintenance dosages were usually of 6 million international units (MIU), and without a loading dose, whereas it is now well recognized that maintenance doses in patients with normal kidney function should be of 9 MIU daily (and possibly even >9 MIU in critically ill patients with augmented renal clearance), after an initial loading dose of 9 MIU [17]. In addition, gradient tests were widely used for colistin susceptibility testing at the time, but it was lately recognized that false susceptibility results could arise in a non-negligible proportion of cases [18].

The suspicion of reduced polymyxins efficacy eventually led clinicians to consider the addition of other agents to polymyxins, in turn leading to the administration of polymyxin-based combination regimens (see Figure 1 for a summary of the most frequently used companion agents and of their characteristics). Polymyxin-based combination regimens have been used for many years between 2010 and 2018. During the same period, many observational studies were conducted to compare clinical outcomes (mainly short-term mortality) between patients with severe KPC-Kp infections treated with polymyxin monotherapy and patients with severe KPC-Kp infections treated with polymyxin-based combinations. However, although some large Italian and Greek studies suggested an advantage of combinations over monotherapy in terms of mortality, this topic remained debated for several years, partly due to many inherent weaknesses of observational, nonrandomized studies in evaluating this topic [19,20]. In 2017, the results of the large, multinational INCREMENT study seemed to tip the balance more solidly in favor of combinations, although certainty of evidence still remained low due

Figure 1. Combination therapies frequently employed before availability of novel β-lactam/β-lactam inhibitor (BL/BLI) combinations and β-lactams (BL) for the treatment of severe infections caused by KPC-producing *Klebsiella pneumoniae*. CNS: central nervous system; KPC-Kp: *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae*; MIC: minimum inhibitory concentration; PK/PD: pharmacokinetic/pharmacodynamic. Information included in the table is from Refs. [9,15,19–34].

to the observational, retrospective nature of the study [35]. Furthermore, as shown in Figure 1, the employed combinations were highly heterogeneous (they were mostly colistin-based regimens, but with heterogeneous companion agent/s). Such a large heterogeneity in the type of selected companion agent/s possibly reflected the lack of high-level evidence (and, in turn, solid guidelines) dictating which combinations to use in the different clinical scenarios.

Overall, while certainly having been useful for improving our ability to treat severe KPC-Kp infections with the few available agents from 2010 to 2018, observational studies conducted in those years eventually led to sufficient consensus only after 2017. This is not a criticism, but rather a consideration reminding us about the importance of the following: (i) pursuing high certainty evidence from randomized controlled trials; (ii) improving national and multinational efforts to conduct large and well-designed observational studies (to provide lower but still acceptable evidence whenever randomized studies are unfeasible). Indeed, although we now have novel agents that have revolutionized the treatment of severe KPC-Kp infections (see next section), we cannot exclude that we will face again a similar situation in the future, since antimicrobial resistance has proved to be highly dynamic and disseminate very rapidly. We should not remain unprepared.

The era of novel β-lactam/β-lactamase inhibitor combinations and β-lactams active against KPC-Kp (2019–onward)

Novel β-lactam/β-lactamase inhibitor (BL/BLI) combinations showing *in vitro* activity against KPC-Kp and currently approved for use in humans are ceftazidime-avibactam, meropenem-vaborbactam, and imipenem-cilastatin-relebactam [8]. The first that became available is ceftazidime/avibactam (first approved by the Food and Drug Administration [FDA] and the European Medicines Agency [EMA] in 2015 and 2016 in the US and Europe, respectively), followed by meropenem/vaborbactam (first approved by the FDA and the EMA in 2017 and 2018, respectively) and imipenem/relebactam (first approved by the FDA and the EMA in 2019 and 2020, respectively). In addition, the recently approved siderophore cephalosporin cefiderocol (first approved by the FDA and the EMA in 2019 and 2020, respectively) also shows activity against KPC-Kp [36,37]. Two important considerations should be made regarding these novel agents. The first is that they made KPC-Kp treatable again with β-lactams, thereby removing the label of 'difficult-to-treat resistance' (i.e. resistance to all β-lactams and fluoroquinolones) [21]. The second is that, for some of these novel agents, besides classical indication-based randomized trials (e.g. patients with complicated urinary infection, nosocomial pneumonia), also pathogen-directed randomized trials (i.e. patients with carbapenem-resistant Gram-negative bacteria infections) were conducted [38,39]. Nonetheless, while these pathogen-directed studies are certainly a crucial innovation, it still remains difficult to enroll a high number of patients with infections due to resistant organisms (KPC-Kp included) in randomized trials, thus their populations are frequently small. This inherently reduces the potential to generalize their results, as well as possibly precluding adequate mitigation of both measured and unmeasured confounding. The solution to this issue is still debated. Indeed, it is also true that pragmatically widening the classically strict inclusion criteria of randomized trials (for allowing enrollment of larger samples of patients with infection due to resistant organisms) could confound results by leading to inclusion of several patients with other factors contributing to their prognosis (e.g. too severe acute conditions or high burden of baseline comorbidities). Novel designs and methods for randomized trials are in development or under evaluation for improving our ability to deal with this issue [40]. In the meantime, high certainty evidence guiding the use of novel agents for KPC-Kp infections remains mostly indirect, drawn from large, randomized trials conducted predominantly in patients with infection due to carbapenem-susceptible bacteria, and thus with some inherent uncertainty about the legitimacy of extrapolating results also to KPC-Kp infections.

Nonetheless, two important considerations can be firmly made: (i) differently from efficacy, safety can be more directly extrapolated to patients with KPC-Kp infections, with novel BL and BL/BLI combinations being less nephrotoxic than previously polymyxin-based or aminoglycoside-based regimens; (ii) initial observational evidence on the use of novel agents for treating severe KPC-Kp infections is suggesting lower mortality than previously registered with the use of previous standard of care [41,42]. Although with lower certainty of evidence than randomized trials, this observational evidence has guided the development of two much awaited guidance document/guidelines for the treatment of resistant organisms of concerns, including also KPC-Kp, from the infectious Diseases Society of America (IDSA) and the European Society of Clinical Microbiology and Infectious Diseases (ESCMID)

[36,37]. A summary of current recommendations for the treatment of KPC-Kp infections, as well as of the evidence underlying their development, is available in Table 1. Certainly, there are still some important unanswered questions that will need to be addressed in the forthcoming years (e.g. to optimize the use of novel agents in patients with impaired renal function, renal replacement therapy, and/or pneumonia [43–46]), but it cannot be denied that we are entering a completely new era in the treatment of severe KPC-Kp infections. This also considering that other BL or BL/BLI showing *in vitro* activity against KPC-Kp such as ARX-1796, aztreonam/avibactam, cefepime/taniborbactam, cefepime/zidebactam, ceftaroline/avibactam, meropenem/nacubactam, ETX1317, QPX7728, and VNRX-7145 are under clinical development and may become available in the future [47].

Table 1. Current IDSA and ESCMID recommendations for the treatment of severe infections caused by KPC-producing *Klebsiella pneumoniae*.

Guidelines/Guidance document	Recommended treatment for severe KPC-Kp infections	Comments
ESCMID guidelines [36]	• For patients with severe infections due to CRE, including KPC-Kp, the guidelines suggest meropenem/vaborbactam or ceftazidime/avibactam if active *in vitro* *(Conditional recommendation with low/ moderate level of evidence)* • In patients with CRE infections resistant to meropenem/vaborbactam and ceftazidime/ avibactam, the guidelines conditionally recommend cefiderocol *(Conditional recommendation with low level of evidence)* • The guidelines state that there is currently no evidence to recommend for or against the use of imipenem/relebactam for severe CRE infections *(No recommendation)* • For patients with CRE infections susceptible to and treated with ceftazidime-avibactam, meropenem-vaborbactam, or cefiderocol, the guidelines do not recommend combination therapy *(Strong recommendation with low level of evidence)* • For the targeted treatment of severe infections caused by CRE resistant to novel agents and susceptible *in vitro* only to polymyxins, aminoglycosides, tigecycline and/or fosfomycin, the guidelines suggest treatment with more than one drug active *in vitro*, with no recommendation for or against specific combinations *(Conditional recommendation with moderate level of evidence)*	• The ESCMID recommendations refer to targeted treatment (i.e. after susceptibility test results, whereas the optimal place in therapy of novel agents for the empirical treatment (i.e. before the identification of the causative agent and susceptibility test) of severe infections in patients at risk of KPC-Kp etiology remains to be provided in official documents of major infectious diseases scientific societies • Recommendations of ESCMID are mostly similar to those of IDSA, although a difference, likely relying on the different development methods, is the lack of recommendations on the role of imipenem/ relebactam in the ESCMID guidelines, while this agent is among first-line choices recommended by IDSA. This possibly relied on the fact that the in IDSA guidance document (the first version was released before the ESCMID guidelines) was conceived to delineate the way to correctly use novel agents that had become available and were (are) less toxic than previously used approaches. In turn, this prioritized urgency toward extensive and comprehensive methodological adherence to GRADE methodology and guidelines development. On the other hand, ESCMID guidelines are methodologically in line with standard requirements for guidelines development. This nonetheless does not allow to provide recommendations for those areas (e.g. use of imipenem/relebactam) for which clinical evidence for the treatment of CRE infections is still preliminary or absent despite well supported by preclinical evidence. In this regard, it has been suggested that ESCMID and IDSA approaches should be considered as complementary, with a joint approach being required for future refinement of current recommendations with the availability of novel evidence [48]
IDSA guidance document [37] (No strength of recommendation and level of evidence provided)	• Ceftazidime-avibactam, meropenem-vaborbactam, and imipenem/relebactam are preferred treatment options for severe infections caused by KPC-Kp resistant to both ertapenem and meropenem. Cefiderocol is a further alternative in the case of pyelonephritis and complicated urinary tract infections • Polymyxin B and colistin should be avoided for the treatment of severe infections caused by CRE • Combination of a novel β-lactam agent with an aminoglycoside, fluoroquinolone, or polymyxin is not routinely recommended for the treatment of infections caused by CRE	• The IDSA recommendations refer to targeted treatment (i.e. after susceptibility test results, whereas the optimal place in therapy of novel agents for the empirical treatment (i.e. before the identification of the causative agent and susceptibility test) of severe infections in patients at risk of CRE etiology remains to be provided in official documents of major infectious diseases scientific societies

CRE: carbapenem-resistant *Enterobacterales*; ESCMID: European Society of Clinical Microbiology and Infectious Diseases; GRADE: Grading of Recommendations Assessment, Development, and Evaluation; IDSA: Infectious Diseases Society of America; KPC-Kp: *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae*.

The availability of novel agents is a true revolution and may help reducing the high mortality of KPC-Kp infections we frequently witnessed in the past decade. However, we should not let our guard down and allow indiscriminate use of these novel agents, in line with antimicrobial stewardship principles. Notably, this does not mean that we should not use novel agents, but that they will need to be used wisely and appropriately. Indeed, reports of resistance to novel agents are increasing worldwide. More in detail, resistance to ceftazidime/avibactam has been increasingly documented among KPC-Kp over the last few years, mostly owing to the emergence of mutated KPC variants [49], although other mechanisms (e.g. increased production of KPC, permeability defects, overexpression of efflux pumps, and production of other transferable – mutated – class A or class C and class D β-lactamases) may also contribute [49–53]. In addition, cases of KPC-Kp resistant also to meropenem/vaborbactam have been recently reported. To this regard, it should be noted that a marked overproduction of KPC associated with impairment of major porins may led to development of cross-resistance to ceftazidime/avibactam, meropenem/vaborbactam, and imipenem/relebactam in KPC-Kp [53–58]. Finally, some KPC variants have also been associated with reduced susceptibility to cefiderocol [59,60]. Concerted efforts aimed at slowing down the development of resistance to these novel agents are therefore needed to minimize this problem and its potential unfavorable impact on patients' outcome in the forthcoming future.

The key role of the laboratory for the rapid diagnosis of KPC-Kp infections: what has changed over the years?

Until the first decade of the new century, from the diagnostic laboratory perspective, the principal strategies for the diagnosis of KPC-Kp infections were based on phenotypic screening followed by confirmatory tests. The screening relied upon the detection of a reduced susceptibility to carbapenems, since carbapenemase production does not necessarily confer resistance to carbapenems and may cause an increase in minimum inhibitory concentration (MIC) that remains below the clinical breakpoints for resistance. Nonsusceptibility to ertapenem was considered by the Clinical and Laboratory Standards Institute (CLSI) as the most sensitive indicator of carbapenemase production [61], while a meropenem MIC $\geq$ 0.125 mg/L (i.e. above the epidemiological cut-off value [ECOFF]) was considered the best compromise of sensitivity and specificity for screening of carbapenemase producers by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [62]. The phenotypic screening could be performed with standard antimicrobial susceptibility testing (AST), provided that the adopted system measured MIC values low enough to intercept the screening breakpoints, or disk diffusion was used.

Since a reduced carbapenem susceptibility could also be due to different mechanisms (e.g. outer membrane permeability defects coupled with production of extended-spectrum β-lactamases [ESBL]) [63], and since several different carbapenemases can be found in *K. pneumoniae* in addition to KPC (e.g. class B [IMP, VIM, NDM] or class D [OXA-48-like] β-lactamases), confirmatory tests were needed to confirm\rule out a carbapenemase activity and to identify the carbapenemase type [64]. The modified Hodge test (MHT) was initially recommended by CLSI to confirm carbapenemase production in isolates positive to the phenotypic screening [61]. However, this test proved difficult to interpret in some cases and suffered from several limitations (Table 2), which led the CLSI to abandon its endorsement in 2018. Easier and faster phenotypic tests for the detection of carbapenemase production were developed, such as the modified carbapenem inactivation method (mCIM) and the carba NP test. However, also these tests (like MHT) did not inform about the carbapenemase type [65], except for subsequent updates of carba NP which allow discrimination between the different classes of carbapenemases (i.e. classes A, B, and D) and identification of KPC producers [66]. Other phenotypic assays were developed based on the inhibitory properties that several molecules retain against KPC enzymes, such as boronic acid and its derivatives (i.e. phenylboronic [PBA] and 3-aminophenylboronic acid [APBA]), a feature exploited by combined-disk tests (CDTs), also known as disc-inhibitors synergy tests. CDTs, which are performed by comparing the diameter of the growth-inhibitory zone around an indicator β-lactam disk (containing cefepime or imipenem, meropenem and/or ertapenem) plus the inhibitor to that around the corresponding β-lactam disk alone, exhibit high sensitivities and specificities for the detection of KPC, and can also provide accurate information about isolates expressing KPC plus ESBL or class B carbapenemases (i.e. metallo-β-lactamases [MBL]) when coupled with inhibitors of these enzyme types (i.e. clavulanic acid for ESBL, and ethylenediaminetetraacetic acid [EDTA] for MBL) [65]. Following a similar approach, addition of PBA to gradient MIC strip susceptibility tests (e.g. E-

Table 2. Overview of established laboratory methods for detection of KPC-type carbapenemases in patients with severe infections caused by KPC-producing *Klebsiella pneumoniae*.

		Time to results	Specimen	Information provided	Limitation(s)	Note	Commercial assay(s)	Ref.
Phenotypic tests	Modified Hodge test (MHT)	18–24 h	Pure culture	Carbapenemase activity	• False-positive results for high-level AmpC producers or CTX-M-type ESBL producers • Weak detection of NDM producers	Early CLSI-endorsed method	–	[64,65,69]
	Modified carbapenemase inactivation method (mCIM)	18–24 h	Pure culture	Carbapenemase activity	–	Laboratory developed test	–	[64,65,68]
	Gradient MIC strip	18–24 h	Pure culture	Production of KPC or MBL	• Poor specificity with AmpC	–	ETEST® KPC	[66–68]
	(Multidisc) combined-disk tests (CDT)	18–24 h	Pure culture	Production of KPC, MBL or OXA-48	• Identification of carbapenemase Producers among AmpC-positive species (e.g. *Enterobacter* spp.)	Laboratory developed test	–	[64,65,68]
	Carba NP test II	30 min–2 h	Pure culture	Production of class A, B or D β-lactamases	• Weak detection of OXA-48 producers	CLSI-endorsed method	RAPIDEC® CARBA NP	[64,68,69]
Genotypic tests	MALDI-TOF MS	30 min	Pure culture	Identification of a gene product (often) accompanying KPC	• Alternate MALDI-TOF instrument Settings (on the usual instrument) • Identification limited by the presence of bla_{KPC} aboard of pKpQIL-like plasmids	–	–	[68,70]
	Lateral flow immunoassay (LFIA)	20 min	Pure culture, positive blood culture, rectal swab	Production of KPC, MBL (VIM, IMP, NDM), OXA-48-like	• Possible false-negative results with new enzyme variants • Non high throughput • False-negative results may occur with some KPC variants (e.g. KPC-31) • Unable to detect novel carbapenemases	–	NG Test Carba 5® RESIST-4 O.K.N.V.® KPC K-SeT	[68,71]
	PCR, real time-PCR	50 min–4 h	Pure culture rectal swab	Detection of specific carbapenemase genes (e.g. bla_{KPC}, bla_{NDM}, bla_{IMP}, bla_{VIM}, $bla_{OXA\ 48\text{-}like}$)	• Unable to detect novel carbapenemases	Include laboratory developed test	Hyperplex SuperBug ID Check-Direct CPE assay CRE ELITe MGB® kit Revogene® Carba C Novodiag® CarbaR+ AllPlex™ Entero-DR assay Xpert Carba-R test CARBAPLEX®	[72,73]

(*continued*)

Table 2. Continued.

	Time to results	Specimen	Information provided	Limitation(s)	Note	Commercial assay(s)	Ref.
Loop-mediated isothermal amplification (LAMP)	25 min	Pure culture	Detection of specific carbapenemase genes (e.g. bla_{KPC}, bla_{NDM}, bla_{IMP}, bla_{VIM}, $bla_{OXA\ 48\text{-}like}$)	• Unable to detect novel carbapenemases	–	eazyplex® superBug	[72]
Syndromic assay	40 min–6 h	Positive blood culture, whole blood, respiratory sample, synovial fluid	Detection of specific carbapenemase genes (e.g. bla_{KPC}, bla_{NDM}, bla_{IMP}, bla_{VIM}, $bla_{OXA\ 48\text{-}like}$) and pathogens	• Limited spectrum of target carbapenemases • Unable to detect novel carbapenemases • Multiple positive results or targets may complicate test interpretation • Higher cost than confirmatory molecular tests based on PCR\RT-PCR • False-positive results may occur • No detection of off-target pathogens	Minimal hands-on time, highly automated	Biofire® FilmArray panels (Blood Culture Identification 2, Pneumonia Panel plus, Bone and Joint Infection) Unyvero® ePlex® BCID-GN panel Verigene® (BC-GN) T2Resistance Panel \ T2Bacteria Panel	[74,75]
Whole-genome sequencing (WGS)	2 h–3 days	Pure culture	Detection of all resistance genes	• Need of trained microbiologists • Need of dedicated instrumentation, infrastructures • Longer time to results than other molecular assays	Not yet FDA- / CE- IVD cleared	–	[76]

CE: Conformite Europeenne; CLSI: Clinical and Laboratory Standards Institute; CTX-M: active on cefotaxime, first isolated in Munich; ESBL: extended-spectrum β-lactamases; FDA: Food and Drug Administration; IMP: active on imipenem; IVD: *in vitro* diagnostic; KPC: *Klebsiella pneumoniae* carbapenemase; MALDI-TOF: matrix assisted laser desorption/ionization - time of flight; MBL: metallo-β-lactamases; MIC: minimum inhibitory concentration; NDM: New Delhi MBL; OXA: oxacillinase; PCR: polymerase chain reaction; TAT: turnaround time from pure culture of isolate; VIM: Verona integron-encoded.

test) has also been used as confirmation assay for recognition of KPC producers (or MBL using EDTA) [66,67]. Although these tests are simple, unexpensive, and relatively efficient in detecting specific carbapenemases, all require prior bacterial culture from the clinical specimen, so that the long time to results represents the main drawback for most of them (Table 2). Moreover, as with all phenotypic tests, potential false negative results can be achieved in the case of unexpressed or minimally expressed carbapenemase genes [68].

The recent development of rapid multiplex lateral-flow immunochromatographic assays (LFIAs), based on immunological detection of epitopes of carbapenemase enzymes, has partially overcome the above limitations. LFIAs represent easy, rapid, and reliable confirmatory tests for the detection of the most widespread and clinically important carbapenemases found in *Enterobacterales* (i.e. NDM-, KPC-, IMP-, VIM-and OXA-48-like enzymes) from bacterial cultures on solid media and were also proven useful for the detection of carbapenemases directly from positive blood cultures with high sensitivities and specificities (>96%) [71,77]. It should be noted, however, that some recently emerged KPC variants showing reduced susceptibility to ceftazidime-avibactam (e.g. KPC-31) can be associated with relevant detection issues with LFIAs [78]. The possible use of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for the detection of a gene product encoded by the pKpQIL plasmid, the most successful genetic support driving the global spread of bla_{KPC}, has also been investigated. This approach relies on the detection of *a* ~11,109-Da mass peak corresponding to the cleavage product of a hypothetical protein (designated p019 or pKpQIL_019) that is fairly closely linked to pKpQIL-like plasmids, and could be of help for the rapid and unexpensive tracking of KPC-producing strains [70,79], although suffering from some limitations (Table 2).

During the past two decades, following the technological advances in diagnostics and the need to increase rapidity of microbiologic diagnosis, several molecular tools based on different nucleic acid amplification tests (NAATs) (e.g. real-time PCR and loop-mediated isothermal amplification), possibly in combination with microarrays, have rapidly taken a prominent place in the clinical laboratory [72,73]. Overall, NAATs are advantageous over phenotypic methods due to faster turnaround times, higher sensitivity, possible use directly with clinical samples, and ability to give direct information about the nature of the carbapenemase genes (Table 2), that nowadays has relevant therapeutic implications. In fact, while knowledge of the carbapenemase type was initially relevant only for epidemiological and infection control purposes, the exact and rapid identification of carbapenemase genes has become of the utmost importance following the advent of novel BL and BL/BLI combinations, which differentially cover class A, B and D carbapenemases [7,80]. Although in early stages NAATs mainly consisted of laboratory-developed assays, typically employed by diagnostic laboratories with advanced technical expertise, at present, several FDA-cleared and CE-cleared *in vitro* diagnostic (IVD) commercial assays are available in automated formats, of which some are also suitable for point-of-care testing. These assays allow for confirmation of bla_{KPC}, as well as of other carbapenemase-encoding genes (i.e. bla_{NDM}, bla_{IMP}, bla_{VIM} and $bla_{OXA\ 48\text{-}like}$) from bacterial isolates exhibiting a reduced susceptibility to carbapenems or even directly from clinical specimens, with a short time to results (Table 2).

In the last decade, molecular testing of carbapenemase genes has also been incorporated into syndromic panels, providing a marked reduction of the time to diagnosis and significant benefits for antimicrobial stewardship for bloodstream infections, starting from positive blood cultures, for lower respiratory tract infections, starting from bronchoalveolar lavage or bronchial aspirate specimens, and for bone and joint infections, starting from synovial fluid [74] (Table 2); similar platforms can provide identification of the most common pathogens, and of the most clinically-relevant associated resistance genes, in a timeframe of 1–5 h directly from blood or deep respiratory samples [75,81], and invariably include the bla_{KPC} target. However, although syndromic panels are powerful tools that may assist in a timely manner diagnosis of infections, it should also be noted that these assays only detect a predefined range of carbapenemases and/or pathogens, proving of major value only when they render a positive result. Moreover, in some cases, potential interpretation issues may occur upon detection of the KPC-encoding gene, since this does not always correlate with the susceptibility phenotype against certain β-lactamase inhibitors (i.e. the inhibitory activity of avibactam and vaborbactam can be hindered by the presence of overexpressed enzymes or mutated KPC variants) [82–84].

In recent years, identification of KPC-producing organisms has been increasingly centered on molecular testing, also including whole genome sequencing (WGS). Although WGS can potentially provide

untapped information regarding novel KPC enzymes as well as the whole resistance genes' content, not suffering from the major limitations of other molecular assays (i.e. off-target pathogens and/or carbapenemases), the long time to results, costs and infrastructure limitations actually make its implementation in the routine laboratory workflow a big challenge [76], so that its use is primarily demanded for epidemiological purposes.

Conclusion

As with other β-lactamases, a notable diversification has been observed with KPC enzymes, following their emergence in the clinical setting. Currently, at least 136 different allelic variants have been assigned (https://www.ncbi.nlm.nih.gov/pathogens/refgene/#KPC; last access on 30 November 2022), of which some already exhibit modification of the functional properties that may provide resistance also to the novel agents [85]. This should further remind us that the treatment of severe KPC-Kp infections is a highly dynamic process, in which the wise use of novel antimicrobials should be accompanied by a continuous refinement based on evolving clinical evidence and laboratory diagnostics. We should not waste all the tremendous steps forward made in the last decade.

Author contributions

DRG: conceptualization, supervision, writing of original draft, review, and editing; VDP, TG, and IK: writing of original draft, review and editing; AM, GMR, and MB: review and editing, supervision.

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