JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Brief Original Article

The combined-disk boronic acid test as an accurate strategy for the detection of KPC carbapenemase in Central America

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Abstract

Introduction: Carbapenemase-producing *Klebsiella pneumoniae* (KPC) outbreaks may cause a huge economical burden on developing countries. Furthermore, KPC can be challenging to detect. We describe the laboratory strategy for the detection of KPC from 2011 to 2013 in a tertiary care hospital in Central America with approximately 1,000 beds.

Methodology: A retrospective analysis of a clinical laboratory database was done to determine the pragmatic application of the combined-disk boronic acid test during a KPC outbreak in Panama. A total of 1,026 *Klebsiella pneumoniae* isolates were found, of which 133 were positive for KPC. The strategy during two phases was described according to the test employed as a confirmatory test for KPC. After the *K. pneumoniae* isolates were detected by the VITEK 2 system, bla_{KPC} polymerase chain reaction (PCR) and the combined-disk boronic acid test were employed as a confirmatory test during phase one. The combined-disk boronic acid test was employed as a confirmatory test for KPC during phase two.

Results: The sensitivity, specificity, positive predictive value, and negative predictive value of the boronic acid test were 100%, 97%, 91%, and 100%, respectively, when bla_{KPC} PCR was employed as a confirmatory test during the start of the outbreak. Afterwards, modified VITEK 2 system parameters resulted in 116 suspicious KPC samples and the boronic acid test confirmed 102 isolates.

Conclusions: The use of an automated bacterial identification system and the boronic acid test for the detection of KPC was an effective and low-cost strategy for a clinical laboratory in Panama during an outbreak.

Key words: carbapenemase; disease outbreaks; drug resistance; polymerase chain reaction; public health.

J Infect Dev Ctries 2016; 10(3):298-303. doi:10.3855/jidc.7213

(Received 31 May 2015 – Accepted 21 August 2015)

Introduction

Antimicrobial resistance is an increasing public health issue worldwide [1]. Carbapenemase-producing *Klebsiella pneumoniae* (KPC) has sparked interest due to the high antibiotic resistance that causes increased mortality compared to other strains [2,3]. Multiple outbreaks have been reported worldwide that have led to the application of several detection and control strategies [4-6]. Colombia was the first country in Latin America to report KPC in 2008, which caused approximately 14 deaths [7].

The routine detection of KPC can be challenging and expensive [8,9]. The initial detection through disk diffusion agar remains difficult, and automated bacterial identification systems have low diagnostic accuracy [10,11]. Several tests have been employed to detect KPC, including the Hodge test, the modified Hodge test, and the boronic acid test [9,12]. The diagnostic accuracy of these tests varies and these tests are sometimes used as confirmatory tests. However, molecular tests have remained the gold standard for the identification and characterization of the resistance mechanism [6,13].

The objective of this report was to describe the laboratory strategy for the detection of KPC from 2011 to 2013 in a tertiary care hospital in Central America that has approximately 1,000 beds. We also aimed to determine the diagnostic accuracy, pragmatically and retrospectively, of the combined-disk boronic acid test during an outbreak of KPC.

Methodology

The clinical laboratory database of Complejo Hospitalario Dr. Arnulfo Arias Madrid was analyzed

retrospectively. This database contains the *K. pneumoniae* isolates from 2011–2013 that were determined using the VITEK 2 Compact system (bioMérieux, Marcy L'Etoile, France). All 1,026 isolates were checked, including suspicious and confirmed cases. Only the first positive isolate for *K. pneumoniae* was taken into account per subject. No clinical data, including infection or carrier status, was available. The institutional review board of the hospital approved this study.

Tests for the detection of K. pneumoniae and KPC

A *K. pneumoniae* isolate was identified using the VITEK 2 system. A PCR test for the detection of the gene bla_{KPC} was employed to detect KPC. The primers used were the following: 5′-AACAAGGAATATCGTTGATG-3′ and reverse primer 5′-AGATGATTTTCAGAGCCTTA-3′ (Integrated DNA Technologies, Coralville, USA). The combined-disk boronic acid test, containing 300 µg of 3-aminophenyl-boronic acid (APBA), was used as a confirmatory test in phase two for the detection of KPC (catalogue code B1241627, Britania Laboratory, Buenos Aires, Argentina) [14], only after the diagnostic accuracy was determined from January 2011–May 2011 (phase one) by comparing it to the PCR test results in 131 samples. An imipenem disk (10 µg) with a distance of 20 mm was used for APBA inhibition. Two trained operators interpreted the test results. This policy in phase one to screen all positive *K. pneumoniae* samples by two methods was due to two positive KPC samples detected at the end of 2010.

An in-house method for rectal swab surveillance was employed to detect KPC. The method consisted of a medium of 5 mL tryptic soy broth containing a 10 µg meropenem disk, similar to a method described previously [15]. Precise data on the amount of rectal swabs collected were not available.

Phases

The results were divided into two phases. Phase one included results from January 2011 to May 2011. All *K. pneumoniae* isolates were sent to the Public Health Central Reference Laboratory to perform the combined-disk boronic acid test and a PCR for bla_{KPC}. In phase one, PCR for bla_{KPC} was performed in all samples (131) since the combined-disk boronic acid test required evaluation for diagnostic accuracy and a national algorithm was not available in this phase. The combined-disk boronic acid test was used according to the recommendations by the Carlos G. Malbran Laboratory [14].

The VITEK 2 system employs a software that relates the results of the drug susceptibility tests to potential resistance mechanisms. The ASTN087 (bioMérieux, Marcy L'Etoile, France) dilution card was employed in the system for meropenem and imipenem. Automated bacterial identification systems have a poor diagnostic accuracy for the detection of carbapenemase-resistant microorganisms, including the VITEK 2 system's default criteria (non-susceptibility result to at least one carbapenem according to Clinical and Laboratory Standards Institute breakpoints). Therefore, during phase 2, a minimum inhibitory concentration ≥ 2 µg/mL for imipenem and ≥ 1 µg/mL for meropenem was used as a suspicious isolate of KPC, which, according to previous recommendations, had 98% sensitivity and 94% specificity [16]. After the diagnostic accuracy of the combined-disk boronic acid test was determined in phase one, all KPC suspicious strains were later confirmed by this test.

Results

From 2011 to 2013, 1,026 *K. pneumoniae* isolates were detected by the VITEK 2 system, considering only the first bacterial isolate per subject. According to the VITEK 2 system, 17.6% of isolates were suspicious for KPC, of which 12.9% were then confirmed to be positive. These isolates were obtained from blood cultures (23%), wound secretions and ulcers (20%), urine (17%), endotracheal secretions (15%), abscesses (3%), and other type of samples (22%).

Phase one: Determining the diagnostic accuracy of the boronic acid test

In phase one, 131 *K. pneumoniae* samples were isolated, of which 65 were suspicious for KPC according to the VITEK 2 system's default criteria. Of the 65 suspicious KPC suspicious isolates, 31 were confirmed to be positive. By PCR, 61.2% (9/31) of KPC-positive samples were from male subjects. The median age of the patients was 54.8 years with a standard deviation of 4.23 years. KPC corresponded to 23.6% (31/131) of the total *K. pneumoniae* isolates during phase one. The confirmed KPC samples were isolated from subjects in the intensive care unit (64%), and the rest were from other medical wards. During this phase, 67% of the total confirmed KPC isolates were detected using rectal swabs.

All the *K. pneumoniae* isolates were evaluated by employing the boronic acid test and bla_{KPC} PCR. Three samples were positive in the boronic acid test and bla_{KPC} negative (boronic acid test false positive). The sensitivity and specificity of the boronic acid test were

100% and 97%, respectively. The positive predictive value (PPV) and the negative predictive value (NPV) were 91% and 100%, respectively (Table 1). APBA can inhibit other class A carbapenemases, and this could have resulted in the three samples that were APBA false positive.

Phase two

In the second phase, 116 suspicious isolates were detected, of which 102 were confirmed positive for KPC by the boronic acid test. The samples were isolated from subjects in the intensive care unit (34%), and the rest were from other medical wards. The resistance percentage for imipenem for the rest of 2011 (June–December) was 26%, for 2012 was 12%, and for 2013 was 7.5%. Of the total KPC isolates during phase 2, 96% were resistant to imipenem and 97% were resistant to meropenem. During this phase, 55.9% of the confirmed KPC isolates were detected using swabs.

Of the total KPC isolates in both phases, 94% were resistant to imipenem and 97% to meropenem. Of the confirmed KPC isolates, 58.6% were also detected using rectal swabs (Table 2).

Discussion

The results of this report suggest an effective laboratory strategy to be employed in developing countries such as Panama, considering the measures applied during the two phases described. Several recommendations have been issued regarding the optimum techniques for the detection of KPC [17,18]. A report in Greece described a similar strategy to ours, employing an automated system and the boronic acid test as a confirmatory test for the detection of KPC [19]. Nonetheless, the capacity to implement the

Table 1. The first positive samples for KPC from January–May 2011.

Case	Year	Month	Sex (male/female)	Sample	MIC IPM	MIC MEM	Boronic acid test result	PCR test result
1	2011	1	M	Blood	8	> 16	Positive	Positive
2	2011	1	F	Blood	2	4	Positive	Positive
3	2011	3	M	Blood	4	> 8	Positive	Positive
4	2011	4	F	Blood	8	> 16	Positive	Positive
5	2011	4	F	Urine	< 2	< 1	Positive	Positive
6	2011	4	F	Blood	8	> 16	Positive	Positive
7	2011	4	F	Urine	> 16	> 16	Positive	Positive
8	2011	4	M	ETS	> 8	> 8	Positive	Positive
9	2011	4	F	Blood	2	4	Positive	Positive
10	2011	4	F	Urine	4	> 8	Positive	Positive
11	2011	3	M	ETS	4	4	Positive	Positive
12	2011	5	M	ETS	> 16	> 16	Positive	Positive
13	2011	5	M	Blood	> 4	> 8	Positive	Positive
14	2011	5	M	ETS	> 16	> 16	Positive	Positive
15	2011	5	F	Blood	> 16	> 16	Positive	Positive
16	2011	5	M	Blood	1	2	Positive	Positive
17	2011	5	F	Blood	2	4	Positive	Positive
18	2011	5	M	ETS	> 16	> 16	Positive	Positive
19	2011	5	M	ETS	> 16	> 8	Positive	Positive
20	2011	5	F	Cervix	> 8	> 8	Positive	Positive
21	2011	5	M	Abdominal fluid	> 16	> 8	Positive	Positive
22	2011	5	M	ETS	2	> 4	Positive	Positive
23	2011	5	M	ETS	2	> 4	Positive	Positive
24	2011	5	F	Biliar secretion	2	> 4	Positive	Positive
25	2011	5	F	Blood	1	2	Positive	Positive
26	2011	5	M	Urine	1	2	Positive	Positive
27	2011	5	M	ETS	4	8	Positive	Positive
28	2011	5	M	ETS	> 16	> 16	Positive	Positive
29	2011	5	M	ETS	> 4	8	Positive	Positive
30	2011	5	M	Blood	2	> 4	Positive	Positive
31	2011	5	M	Blood	> 16	> 16	Positive	Positive

MIC: minimum inhibitory concentration; IPM: imipenem; MEM: meropenem; PCR: polymerase chain reaction; ETS: endotracheal secretion.

Table 2. *Klebsiella pneumoniae* isolates, KPC isolates, imipenem and meropenem resistance percentages, colonized and infection cases, and KPC isolates also detected by employing rectal swab screening from 2011–2013.

Phase	Year	*Klebsiella pneumoniae* isolates[a]	KPC suspect isolates[a]	Confirmed KPC isolates[a]	KPC isolates IPM resistance	KPC isolates MEM resistance	Detection also by rectal swab screening
1	January 2011–May 2011	131	65/131 (49.6%)[b]	31/65 (47.7%)	29/31 (94.0%)	30/31 (96.8%)	21/31 (67.7%)
2	June 2011–December 2011	143	43/143 (30.1%)[c]	38/43 (88.4%)	36/38 (94.7%)	36/38 (94.7%)	18/38 (47.3%)
2	2012	380	45/380 (11.8%)[c]	41/45 (91.1%)	39/41 (95.1%)	41/41 (100%)	23/41 (56.1%)
2	2013	372	28/372 (7.5%)	23/28 (82.1%)	21/23 (91.3%)	22/23 (95.7%)	16/23 (69.5%)
Total		**1,026**	**181/1,026 (17.6%)**	**133/181 (73.5%)**	**125/133 (94.0%)**	**129/133 (97%)**	**78/133 (58.6%)**

Percent KPC carbapenemase positive was determined by dividing the confirmed KPC isolates by the total Klebsiella pneumoniae isolates. In phase one, the 131 K. pneumoniae isolates (carriers and infected) were sent to the public health central reference laboratory. The KPC isolates imipenem and meropenem resistance percentages were calculated by dividing the quantity of the total confirmed KPC isolates with resistance to imipenem or meropenem by the confirmed KPC isolates in each period. Detection by rectal swab screening corresponds to KPC isolates that were detected in one sample type and also by rectal swab screening; *IMP: imipenem; MEM: meropenem; [a] Carriers and infected; [b] Suspect isolate in phase one according to the non-susceptibility result to at least one carbapenem according to CLSI breakpoints; [c] Suspect isolate in phase two according to the modified VITEK 2 system criteria.*

recommendations will depend on the health system of each country and the institutions where any outbreak occurs [20].

In Panama, the VITEK 2 system was introduced for routine employment in microbiology departments in 2010. Therefore, Panama integrated the VITEK 2 system for KPC suspect sample detection strategy and then looked for an alternative to the PCR test for the confirmation of KPC. Previously, a flowchart for the detection of KPC, using the VITEK 2 system, determined that a minimum inhibitory concentration for imipenem ≥ 2 and meropenem ≥ 1 provided a sensitivity of 98%, a specificity of 94%, and a PPV of 95% to detect KPC [16]. Several studies have shown that the boronic acid test has a sensitivity of 89%–100%, a specificity of 100%, a PPV of 100%, and a NPV of 100% for the detection of KPC [14,21]. However, very few reports have described the pragmatic application of the boronic acid test during outbreaks of KPC. This is reflected in the high PPV and NPV of the boronic acid test in our report, and also in the PPV lower than 100%. The high PPV and NPV confirms the usefulness of this test in situations were the main objective is to ensure the identification of KPC-suspicious samples.

The utility of the boronic acid compounds have recently been established, standing out as efficient inhibitors that can be used in disk potentiation tests, to differentiate KPC producers from those producing extended-spectrum beta lactamase [10,22]. However, it is important to note that there are different boronic acid compounds and different concentrations that are used. A recent study showed that phenyl boronic acid had a superior diagnostic accuracy compared to APBA [22].

The boronic acid test presents some advantages when compared to other phenotypic tests, namely the Hodge test, including its lower cost, faster turnaround time, higher specificity, and the relative simplicity of the test [9,23]. In Panama, each PCR test had a cost of US $50, while the cost of each boronic acid test was US $5 only. Mean time to report a PCR test result was approximately three days, while the boronic acid test result was reported within 24 hours. Also, PCR tests are not routinely available in hospitals of many low- and middle-income countries.

Several reports have shown the efficacy of the early adoption of strict measures to contain an outbreak. These measures include informing the healthcare personnel about the outbreak, the transmission mechanism, and the need for strict adherence to hygiene protocols [6]. Also, contact precautions were undertaken, as well as rectal swab screening of inpatients with a history of hospitalization, and a protocol about control measures for the country was elaborated [24]. Although rectal swab tests may have low sensitivity to detect colonization of Enterobacteriaceae-producing KPC, their low cost could make it useful in outbreaks [25]. Nonetheless, a more recent study showed a high diagnostic accuracy of direct rectal swab screening by combined-disk boronic acid test for active surveillance purposes of KPC [26]. The decrease in the number of cases seen by 2013 likely reflects the effectiveness of these measures.

Conclusions

The results of this report suggest an effective laboratory strategy to be employed in countries such as Panama, considering the measures applied during the two phases described. Nonetheless, the use of similar strategies requires a previous assessment in each country, according to its healthcare system, for the long-term use of a phenotypic test as a confirmatory test for the detection of KPC. Finally, we believe that the combination of an automated system and the boronic acid test, especially in low- and middle-income countries, could be useful in clinical laboratories for the detection of KPC.

Acknowledgements

We would like to thank Boris Castillo from the Infectious Diseases Department and the Microbiology Department of Complejo Hospitalario Dr. Arnulfo Arias Madrid. LCRSP Microbiology Group includes Raquel de Bolaños, Rubén Ramis, José Moreno.

References

1. World Health Organization (2014) Antimicrobial resistance: global report on surveillance 2014. Available: http://www.who.int/drugresistance/documents/surveillancereport/en/. Accessed May 6, 2015.
2. Tängdén T, Giske CG (2014) Global dissemination of extensively drug-resistant carbapenemase-producing Enterobacteriaceae: clinical perspectives on detection, treatment and infection control. J Intern Med 277: 501-512.
3. Harris PNA, Tambyah PA, Paterson DL (2015) β-lactam and β-lactamase inhibitor combinations in the treatment of extended-spectrum β-lactamase producing Enterobacteriaceae: time for a reappraisal in the era of few antibiotic options? Lancet Infect Dis 15: 475-485.
4. Nordmann P (2014) Carbapenemase-producing Enterobacteriaceae: overview of a major public health challenge. Med Mal Infect 44: 51-56.
5. Nordmann P, Naas T, Poirel L (2011) Global spread of Carbapenemase-producing Enterobacteriaceae. Emerging Infect Dis 17: 1791-1798.
6. Munoz-Price LS, Poirel L, Bonomo RA, Schwaber MJ, Daikos GL, Cormican M, Cornaglia G, Garau J, Gniadkowski M, Hayden MK, Kumarasamy K, Livermore DM, Maya JJ, Nordmann P, Patel JB, Paterson DL, Pitout J, Villegas MV, Wang H, Woodford N, Quinn JP (2013) Clinical epidemiology of the global expansion of Klebsiella pneumoniae carbapenemases. Lancet Infect Dis 13: 785-796.
7. Lopez JA, Correa A, Navon-Venezia S, Correa AL, Torres JA, Briceño DF, Montealegre MC, Quinn JP, Carmeli Y, Villegasz MV (2011) Intercontinental spread from Israel to Colombia of a KPC-3-producing Klebsiella pneumoniae strain. Clin Microbiol Infect 17: 52-56.
8. Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL (2012) Carbapenemases in Klebsiella pneumoniae and other Enterobacteriaceae: an evolving crisis of global dimensions. Clin Microbiol Rev 25: 682-707.
9. Nordmann P, Gniadkowski M, Giske CG, Poirel L, Woodford N, Miriagou V (2012) Identification and screening of carbapenemase-producing Enterobacteriaceae. Clin Microbiol Infect 18: 432-438.
10. Nordmann P, Cuzon G, Naas T (2009) The real threat of Klebsiella pneumoniae carbapenemase-producing bacteria. Lancet Infect Dis 9: 228-236.
11. Doern CD, Dunne WM, Burnham AD (2011) Detection of Klebsiella pneumoniae carbapenemase (KPC) production in non-Klebsiella pneumoniae Enterobacteriaceae isolates by use of the Phoenix, Vitek 2, and disk diffusion methods. J Clin Microbiol 49: 1143-1147.
12. Hirsch EB, Tam VH (2010) Detection and treatment options for Klebsiella pneumoniae carbapenemases (KPCs): an emerging cause of multidrug-resistant infection. J Antimicrob Chemother 65: 1119-1125.
13. Krishna NK, Cunnion KM (2012) Role of molecular diagnostics in the management of infectious disease emergencies. Med Clin North Am 96: 1067-1078.
14. Pasteran F, Mendez T, Guerriero L, Rapoport M, Corso A (2009) Sensitive screening tests for suspected class A carbapenemase production in species of Enterobacteriaceae. J Clin Microbiol 47: 1631-1639.
15. Landman D, Salvani JK, Bratu S, Quale J (2005) Evaluation of techniques for detection of carbapenem-resistant Klebsiella pneumoniae in stool surveillance cultures. J Clin Microbiol 43: 5639-5641.
16. Pasteran F, Lucero C, Soloaga R, Rapoport M, Corso A (2011) Can we use imipenem and meropenem Vitek 2 MICs for detection of suspected KPC and other-carbapenemase producers among species of Enterobacteriaceae? J Clin Microbiol 49: 697-701.
17. Center for Disease Control and Prevention (2012) Guidance for Control of Carbapenem-Resistent Enterobacteriaceae, 2012 CRE Toolkit. Available: http://www.cdc.gov/hai/pdfs/cre/CRE-guidance-508.pdf. Accessed May 8, 2015.
18. Clinical and Laboratory Standard Institute (2012) Performance Standard for Antimicrobial Susceptibility testing: twenty second informational supplement. Available: http://clsi.org/standards/micro/. Accessed May 15, 2015.
19. Tsakris A, Kristo I, Poulou A, Markou F, Ikonomidis A, Pournaras S (2008) First occurrence of KPC-2-possessing Klebsiella pneumoniae in a Greek hospital and recommendation for detection with boronic acid disc tests. J Antimicrob Chemother 62: 1257-1260.
20. Bebell LM, Muiru AN (2014) Antibiotic use and emerging resistance: how can resource-limited countries turn the tide? Glob Heart 9: 347-358.
21. Tsakris A, Kristo I, Poulou A, Themeli-Digalaki K, Ikonomidis A, Petropoulou D, Pournaras S, Sofianou D (2009) Evaluation of boronic acid disk tests for differentiating KPC-possessing Klebsiella pneumoniae isolates in the clinical laboratory. J Clin Microbiol 47: 362-367.
22. Tsakris A, Themeli-Digalaki K, Poulou A, Vrioni G, Voulgari E, Koumaki V, Agodi A, Pournaras S, Sofianou D (2011) Comparative evaluation of combined-disk tests using different boronic acid compounds for detection of Klebsiella pneumoniae carbapenemase-producing enterobacteriaceae clinical isolates. J Clin Microbiol 49: 2804-2809.
23. Pournaras S, Poulou A, Tsakris A (2010) Inhibitor-based methods for the detection of KPC carbapenemase-producing

Enterobacteriaceae in clinical practice by using boronic acid compounds. J Antimicrob Chemother 65: 1319-1321.
24. Ministry of Health of Panama (2011). Carbapenemase producing Enterobacteriaceas controls measures. Available: http://190.34.154.88/Observatorio/KPC/A.%20DOCUMENTO%20-MEDIDAS%20DE%20CONTROL%20PARA%20BROTE%20KPC%20DEF.pdf. Accessed May 11, 2015.
25. Giani T, Tascini C, Arena F, Ciullo I, Conte V, Leonildi A, Menichetti F, Rossolini GM (2012) Rapid detection of intestinal carriage of Klebsiella pneumoniae producing KPC carbapenemase during an outbreak. J Hosp Infect 81: 119-122.
26. Pournaras S, Zarkotou O, Poulou A, Kristo I, Vrioni G, Themeli-Digalaki K, Tsakris A (2013) A combined disk test for direct differentiation of carbapenemase-producing enterobacteriaceae in surveillance rectal swabs. J Clin Microbiol 51: 2986-2990.

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Conflict of interests: No conflict of interests is declared.