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A Cost-Effective Method for Identifying *Enterobacterales* with OXA-181

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ABSTRACT OXA-181 is the second most common global OXA-48-like carbapenemase and is endemic in the Indian subcontinent. Molecular studies have shown that *Enterobacterales* with OXA-181 are often introduced into regions of nonendemicity. Distinguishing OXA-181 from other OXA-48-like enzymes often requires sequencing, which is rather expensive and time-consuming. A specific PCR (i.e., OXA181PCR) for the detection of $bla_{OXA-181}$ was validated using a global collection ($n = 315$) of bacteria with well-characterized carbapenemases and showed 100% sensitivity and specificity (95% confidence interval [CI], 94.1 to 100 and 98.6 to 100, respectively) for detecting bacteria with OXA-181. The OXA181PCR subsequently gave positive results on 58/160 (36%) *Enterobacterales* with OXA-48-like carbapenemases from the 2015 INFORM surveillance program. The $bla_{OXA-181}$-positive *Enterobacterales* were present in 9 countries spanning 5 continents, illustrating the global distribution of OXA-181. This methodology can easily be incorporated into molecular surveillance programs to provide accurate information about the prevalence of OXA-181. A loop-mediated isothermal amplification (LAMP)-OXA48 assay overall performed well for detecting OXA-48-like enzymes but showed poor specificity due to false-positive results with non-OXA carbapenemases.

KEYWORDS detection, OXA-181, carbapenemases

Enterobacterales with OXA-48-like carbapenemases are important causes of carbapenem resistance, and genomic surveillance studies have shown them to be the most common carbapenemase in certain regions of the world (e.g., the Middle East, North Africa, and European countries such as Belgium and Spain) (1). Bacteria with these enzymes are introduced on a regular basis into regions of nonendemicity, where they have been responsible for nosocomial outbreaks. OXA-48, OXA-181, OXA-232, OXA-204, OXA-162, and OXA-244 are the most common global enzymes identified among the OXA-48-like carbapenemases (1).

OXA-181 is the second most common global OXA-48-like derivative and was first described in Indian hospitals in 2006 to 2007 (2). That was followed by reports from France (3), the Netherlands (4), Oman (5), and New Zealand (6) in 2011. Since 2014, the description of OXA-181 escalated on a global scale, with numerous reports from the Middle East, Asia, Africa, Europe, and North America (7). Currently, *Enterobacterales* with $bla_{OXA-181}$ are endemic on the Indian subcontinent (e.g., India [8, 9], Pakistan [10], Bangladesh [11], and Sri Lanka [12]). Nosocomial outbreaks have also been described in Angola (13), South Africa (14), São Tomé and Príncipe (15), and the United Arab

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TABLE 1 PCR primers and amplification conditions[a]

PCR Name and Primers	Sequence	Target[b]	Amplicon size (bp)
OXA181PCR			
OXA181ASP_336Tspe-F1	3′-GGAATCGTGACCATGACTTACTT-5′	bla_{OXA} nt	349
OXA181ASP_640Aspe-R1	3′-CAGCCAATCTTAGGTTCGATTAT-5′	nt 313–661	
OXA48LPCR			
OXA-48-L_722-F1	3′-TGCCCACATCGGATGGTT-5′	bla_{OXA} nt	60
OXA-48-L_781-R1	3′-CCTGTTTGAGCACTTCTTTTGTGA-5′	nt 722–781	
16SControl			
16S rRNA-F1	3′-TGGAGCATGTGGTTTAATTCGA-5′	nt 776–797	159
16S rRNA-R1	3′-TGCGGGACTTAACCCAACA-5′	nt 916–934	

[a]Each amplification was performed with 1× AmpliTaq Gold 360 master mix (Thermo Fisher, Mississauga, ON, Canada), 0.1 to 0.2 μM for each primer, and 3 μl of DNA in a total volume of 25 μl. Thermocycling was done using a Veriti thermal cycler (Applied Biosystems, Foster City, CA, USA), and the conditions were as follows: initial denaturation, 95°C for 3 min; 30 cycles of 95°C for 40 s, 52°C for 40 s, and 72°C for 40 s; and a final extension of 72°C for 7 min.
[b]nt, Nucleotides.

Emirates (UAE) (16). Previous travel to India (17), Nigeria (18), or the Middle East (19) was identified as a possible risk factor of infections with OXA-181-producing bacteria.

The laboratory detection of OXA-48-like *Enterobacterales* with phenotypic methods has improved recently but remains challenging due to their weak carbapenemase activities (20). In-house and commercial PCR-based molecular confirmation methods have good sensitivities and specificities for detecting OXA-48-like enzymes (20). Distinguishing OXA-181 from other OXA-48-like enzymes often requires sequencing, which is rather expensive and time-consuming (20). This has precluded genomic surveillance studies to routinely identify OXA-181, and therefore, the true global prevalence of this enzyme is currently unknown.

A study was designed to identify OXA-181 among *Enterobacterales* with OXA-48-like carbapenemases using a simple, cost-effective PCR methodology. We also validated the performances of an in-house PCR and a previously published in-house loop-mediated isothermal amplification (LAMP) assay for the detection of OXA-48-like enzymes (21).

MATERIALS AND METHODS

We designed a $bla_{OXA-181}$-specific PCR (named OXA181PCR) that targeted a region between nucleotides 313 and 661 on the OXA gene for the identification of OXA-181. The amplification of a 349-bp DNA fragment was indicative of the presence of $bla_{OXA-181}$, while no amplification demonstrated a negative test. The PCR was combined with 16S primers (named 16SControl) to indicate the presence of bacterial DNA. We also validated an in-house PCR (named OXA48LPCR) that targeted a region between nucleotides 722 and 781 on the OXA gene for the identification of OXA-48-like enzymes. The sequences and PCR conditions for OXA181PCR, OXA48LPCR, and 16SControl are shown in Table 1.

The reason for including the LAMP assay (named LAMP-HNB_OXA48) was that this method does not require a thermocycler or specialized equipment for visualization and had been used for the detection of a wide variety of microorganisms, making it ideal to use for the identification of antimicrobial resistance determinants in low- and middle-income countries (LMICs). The original evaluation of the OXA-48-like LAMP assay only included isolates with bla_{OXA-48} ($n = 3$) and $bla_{OXA-181}$ ($n = 3$); therefore, the accuracy is unknown for the detection of different OXA-48-like enzymes (21).

OXA181PCR, OXA48LPCR, and LAMP-HNB_OXA48 were validated using a global collection of clinical Gram-negative bacteria ($n = 315$) with different carbapenemases (Table 2). These included the following OXA-48-like enzymes: OXA-48 ($n = 110$), OXA-181 ($n = 61$), OXA-244 ($n = 13$), OXA-163 ($n = 11$), OXA-232 ($n = 7$), OXA-162 ($n = 5$), OXA-370 ($n = 2$), and 1 each of OXA-204, -245, -252, -405, -439, and -484. The enzymes were previously characterized with whole-genome sequencing as part of several ongoing global molecular epidemiology studies using procedures described before (22). The microbes (e.g., *Acinetobacter* spp. [$n = 20$], *Citrobacter* spp. [$n = 5$], *Enterobacter* spp. [$n = 73$], *Escherichia coli* [$n = 27$], *Klebsiella pneumoniae* [$n = 181$], and others [$n = 9$]) were obtained from North America (i.e., Canada, United States), Latin America (i.e., Mexico, Guatemala, Colombia, Brazil, Argentina), Europe (i.e., Spain, France, Belgium, Italy, Greece, Romania, Serbia, Germany, Turkey, Russia), Africa (i.e., Egypt, Morocco, Tunisia, Kenya, Nigeria, South Africa), the Middle East (i.e., UAE, Saudi Arabia, Israel, Lebanon, Jordan), Asia (i.e., India, Vietnam, Thailand, Philippines, Japan), and Oceania (i.e., Australia). The *Acinetobacter* spp. were positive for OXA-23 ($n = 13$), OXA-40 ($n = 2$), OXA-58 ($n = 4$), and OXA-143 ($n = 1$).

After the initial validation process, the OXA181PCRs were subsequently used on a collection of *Enterobacterales* with carbapenemases ($n = 437$) obtained in 2015 from the INFORM global surveillance

TABLE 2 Performance and validation of OXA48LPCR, LAMP-HNB_OXA48, and OXA181PCR

Enzymes	No. of OXA48LPCR-positive enzymes	No. of LAMP-HNB_OXA48-postive enzymes	No. of OXA181PCR-positive enzymes
OXA-48-like ($n = 215$)	213	211	61
OXA-48 ($n = 110$)	110	110	0
OXA-181 ($n = 61$)	60	61	61
OXA-244 ($n = 13$)	13	12	0
OXA-163 ($n = 11$)	11	10	0
OXA-232 ($n = 7$)	6	7	0
OXA-162 ($n = 5$)	5	4	0
OXA-370 ($n = 2$)	2	1	0
Others ($n = 6$; 1 each of OXA-204, -252, -405, -439, -245, -484)	6	6	0
Non-48 OXAs ($n = 20$)	0	0	0
OXA-23 ($n = 13$)	0	0	0
OXA-40 ($n = 2$)	0	0	0
OXA-58 ($n = 4$)	0	0	0
OXA-143 ($n = 1$)	0	0	0
Non-OXAs ($n = 80$)	0	14	0
NDMs ($n = 20$)	0	2	0
VIMs ($n = 20$)	0	3	0
IMPs ($n = 20$)	0	5	0
KPCs ($n = 20$)	0	4	0
INFORM isolates ($n = 437$)	158	192	58
IMPs ($n = 2$)	0	1	0
KPCs ($n = 200$)	0	29	0
KPCs + VIMs ($n = 2$)	0	0	0
NDMs ($n = 50$)	0	2	0
NDMs + OXA-48-like ($n = 18$)	17	18	1
OXA-48-like ($n = 137$)	136	134	57
VIMs ($n = 23$)	0	3	0
VIMs + OXA-48-like ($n = 5$)	5	5	0

program to identify OXA-181 among *Enterobacterales* with OXA-48-like carbapenemases (23) (Table 2). Agreement between results obtained with the OXA48LPCR and LAMP-HNB_OXA48 methodologies were compared with the 437 INFORM isolates using Cohen's kappa coefficient (23) (Table 2). The INFORM surveillance program collected clinically relevant nonrepeat Gram-negative bacteria from intra-abdominal, urinary tract, blood, skin and soft tissue, and lower respiratory tract infection specimens from 42 countries in North America, Latin America, Europe, the Middle East, Asia, Africa, and Oceania. INFORM screened for the presence of carbapenemase genes using a multiplex PCR as described previously (23). The 2015 INFORM collection included the following carbapenemases (Table 2): IMPs ($n = 2$), KPCs ($n = 200$), KPCs + VIMs ($n = 2$), NDMs ($n = 50$), NDMs + OXA-48-like ($n = 18$), OXA-48-like ($n = 137$), VIMs ($n = 23$), and VIMs + OXA-48-like ($n = 5$).

All analyses were done in R (24). Using the initial set of isolates, sensitivities, specificities, and respective 95% confidence intervals (95% CI) were estimated for each test relative to whole-genome sequencing, assumed to be a perfect test. Agreement between tests was measured using Cohen's kappa coefficient.

For frequency estimation of OXA-181 in the carbapenemase-producing INFORM global isolates, we used a Bayesian framework where our OXA181PCR was assumed to be an imperfect diagnostic test. Generalized linear mixed models were used where logit of OXA-181 carbapenemases in INFORM isolates was considered the outcome. Estimated logit values were converted to population-averaged values (25) for presentation. Country-random effects, assumed to be normally distributed, were used to deal with the lack of independence of isolates obtained from the same countries. Sensitivity and specificity prior distributions were generated using results from the initial validation, where the 95% CIs initially estimated were used as quantiles for determining optimal beta distributions. For all other parameters (e.g., probability of OXA-181-like carbapenemases, country-random effects), noninformative priors were used. A sensitivity analysis was implemented for estimating the impact of prior distributions in our estimates, where different parameters for beta distributions were tested and posterior distributions were compared visually. A Markov chain Monte Carlo (MCMC) approach using Gibbs sampling was performed with 6 chains in parallel with a total of 200,000 iterations in R using the runjags package (26). Visual inspection of chains, autocorrelation plots, and effective sample sizes (ESS) were used as measures of efficacy, where an ESS of at least 20,000 was deemed appropriate. Posterior distribution plots were visualized, and summary statistics as well as 95% credible intervals (CR) were estimated.

RESULTS

Validation of OXA181PCR, OXA48LPCR, and LAMP-HNB_OXA48 using isolates with sequenced carbapenemases. The OXA48LPCR had a sensitivity of 99% (95% CI, 96.7 to 99.9) and a specificity of 100% (95% CI, 96.3 to 100), and the LAMP-HNB_OXA48 had a sensitivity of 98% (95% CI, 98.1 to 99.5) and a specificity of 86% (95% CI, 77.6 to 92.1) for detecting *Enterobacterales* with OXA-48-like enzymes (Table 2). The OXA181PCR had a sensitivity and specificity of 100% (95% CI, 94.1 to 100 and 98.6 to 100, respectively) for detecting *Enterobacterales* with $bla_{\text{OXA-181}}$. When using OXA181PCR and OXA48LPCR in a multiplex PCR approach, the specificity of OXA181PCR dropped to 96% (due to OXA-232 [$n = 7$] that gave false-positive results). Repeat testing with LAMP-HNB_OXA48 resolved 8/14 of the non-OXA false positives (i.e., 3/5 of the IMPs, 2/4 of the KPCs, 2/3 of the VIMs, and 1/2 of the NDMs that initially were positive tested negative on repeated testing).

Performances of OXA181PCR, OXA48LPCR, and LAMP-HNB_OXA48 using isolates from the 2015 INFORM surveillance program. The OXA48LPCR showed excellent agreement ($\kappa = 0.99$, $P < 0.001$) with the multiplex PCR results to detect OXA-48 like enzymes; none of the non-OXA-48-like and 158/160 (99%) of the OXA-48-like enzymes tested positive. The LAMP-HNB_OXA48 showed very good agreement ($\kappa = 0.82$, $P < 0.001$) with the multiplex PCR results; 35/277 (13%) of the non-OXA-48-like and 157/160 (98%) of the OXA-48-like enzymes tested positive (Table 2). The OXA181PCR gave positive results on 58/160 (36%) of the OXA-48-like enzymes, with an estimated frequency of 13.4% (95% Bayesian credible interval, 5.5 to 26.7) of OXA-181 among the carbapenemase-producing isolates obtained from the INFORM surveillance program. These bacteria were obtained from South Africa, South Korea, Mexico, Australia, Thailand, Taiwan, Kuwait, Turkey, and Belgium, illustrating the global distribution of *Enterobacterales* with OXA-181.

DISCUSSION

In 2014, a report from the World Health Organization demonstrated that when antimicrobial resistance (AMR) surveillance data are available, they can be beneficial for selecting treatment choices, understanding AMR trends, identifying priority areas for interventions, and monitoring the impact of such interventions (27). The report revealed the lack of adequate surveillance programs in many parts of the world and identified certain bacteria, including carbapenem-resistant *E. coli* and *K. pneumoniae*, on which global surveillance data are urgently required.

Surveillance studies that use molecular methodologies to identify carbapenemases have shown that OXA-48-like β-lactamases are the second or third most common carbapenemase among global *Enterobacterales* (28, 29). Data from the SMART (2008 to 2014) and INFORM (2012 to 2014) global surveillance programs show that 27% of carbapenemase-producing *Enterobacterales* ($n = 1{,}615$) were positive for OXA-48-like carbapenemases (compared to 55% for KPCs and 26% for NDMs). In certain areas (e.g., the Middle East, North Africa, and European countries such as Belgium and Spain), OXA-48-like enzymes were the most common carbapenemase among *Enterobacterales*. Unfortunately, the identification of OXA-181 among OXA-48-like enzymes requires sequencing that is not often routinely performed in global surveillance programs or clinical laboratories that use genomic methodologies to identify carbapenemases. Previous studies reported the frequency of OXA-181 among *Enterobacterales* with carbapenemases that ranged from 1.8 to 24.5%, although they were not uniformly designed in terms of population and methodologies (30, 31). The global frequency of OXA-181 in this study was estimated to be around 13%, using the INFORM global collection of *Enterobacterales* with carbapenemases. This value could potentially be used to infer the prevalence of OXA-181-producing isolates among global *Enterobacterales* with carbapenemases from human patients.

LMICs bear a considerable burden from the widespread prevalence of AMR bacteria but unfortunately lack the adequate diagnostic tools and surveillance systems to resolve and curb the spread of such bacteria (32). LMICs urgently need on-site rapid,

accurate, and cost-effective genomic technologies to identify country-specific AMR determinants. The genomic methodologies should be simple, user-friendly, and accordant with local economic and social constraints (32).

We described such an approach that is accurate, rapid, simple, and cost-effective to screen for *Enterobacterales* with OXA-48-like enzymes (using OXA48LPCR or LAMP-HNB_OXA48) and to identify OXA-181 among them (using OXA181PCR). Unfortunately, combining OXA181PCR and OXA48LPCR in a multiplex PCR approach led to a decrease in the specificity of OXA181PCR (i.e., *Enterobacterales* with OXA-232, a close relative of OXA-181, gave false-positive results in the multiplex PCR). The OXA181PCR is cost-effective (i.e., $3.75 versus $19 for sequencing) and very easy to interpret compared to sequencing (i.e., amplification of a 349-bp fragment is indicative of OXA-181). Such methodologies can easily be incorporated in global AMR molecular surveillance programs and are especially suited for LMIC surveillance networks. This will enable surveillance networks to determine the prevalence/frequency of OXA-181 among *Enterobacterales* with OXA-48-like enzymes and be helpful from an epidemiological perspective, especially in an outbreak setting. There is also a potential infection prevention and control benefit in regions of nonendemicity to determine if a patient is infected/colonized with OXA-181-producing *Enterobacterales*. This will enable practitioners to establish an epidemiological link with previous travel to a region of endemicity.

The workflow will initially screen for OXA-48-like enzymes among carbapenem-resistant *Enterobacterales* using either OXA48LPCR, LAMP-HNB_OXA48, or in-house or commercial systems and then to confirm OXA-181 using OXA181PCR. Such an approach will provide accurate information about the prevalence of OXA-181. Our results with the 2015 INFORM surveillance program show that *Enterobacterales* with $bla_{\text{OXA-181}}$ were present in 9 countries (e.g., South Africa, South Korea, Mexico, Australia, Thailand, Taiwan, Kuwait, Turkey, and Belgium), illustrating the global distribution of OXA-181.

In summary, OXA48LPCR showed excellent sensitivities and specificities in screening for OXA-48-like enzymes, while OXA181PCRs were able to reliably distinguish OXA-181 from other OXA-48-like enzymes. In regions of endemicity with a high prevalence of *Enterobacterales* with OXA-181, the OXA181PCR will be a good option as a "first-line" molecular confirmation test. The LAMP-HNB_OXA48 overall performed reasonably but showed poor specificity due to false-positive results with non-OXA carbapenemases (Table 2). We are currently working on a similar simple PCR approach to identify OXA-48 among OXA-48-like enzymes.

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REFERENCES

1. Pitout JDD, Kock MM, Strydom K-A, Matsumura Y. 2019. The global ascendency of OXA-48-type carbapenemases. Clin Microbiol Rev 32:e00102-19. https://doi.org/10.1128/CMR.00102-19.
2. Castanheira M, Deshpande LM, Mathai D, Bell JM, Jones RN, Mendes RE. 2011. Early dissemination of NDM-1- and OXA-181-producing *Enterobacteriaceae* in Indian hospitals: report from the SENTRY Antimicrobial Surveillance Program, 2006–2007. Antimicrob Agents Chemother 55:1274–1278. https://doi.org/10.1128/AAC.01497-10.
3. Poirel L, Ros A, Carricajo A, Berthelot P, Pozzetto B, Bernabeu S, Nordmann P. 2011. Extremely drug-resistant *Citrobacter freundii* isolate producing NDM-1 and other carbapenemases identified in a patient returning from India. Antimicrob Agents Chemother 55:447–448. https://doi.org/10.1128/AAC.01305-10.
4. Kalpoe JS, Al Naiemi N, Poirel L, Nordmann P. 2011. Detection of an Ambler class D OXA-48-type beta-lactamase in a *Klebsiella pneumoniae* strain in the Netherlands. J Med Microbiol 60:677–678. https://doi.org/10.1099/jmm.0.028308-0.
5. Potron A, Nordmann P, Lafeuille E, Al Maskari Z, Al Rashdi F, Poirel L. 2011. Characterization of OXA-181, a carbapenem-hydrolyzing class D beta-lactamase from *Klebsiella pneumoniae*. Antimicrob Agents Chemother 55:4896–4899. https://doi.org/10.1128/AAC.00481-11.
6. Williamson DA, Heffernan H, Sidjabat H, Roberts SA, Paterson DL, Smith M, Freeman JT. 2011. Intercontinental transfer of OXA-181-producing *Klebsiella pneumoniae* into New Zealand. J Antimicrob Chemother 66:2888–2890. https://doi.org/10.1093/jac/dkr396.
7. Mairi A, Pantel A, Sotto A, Lavigne JP, Touati A. 2018. OXA-48-like carbapenemases producing *Enterobacteriaceae* in different niches. Eur J Clin Microbiol Infect Dis 37:587–604. https://doi.org/10.1007/s10096-017-3112-7.
8. Mahalingam N, Manivannan B, Khamari B, Siddaramappa S, Adak S, Bulagonda EP. 2018. Detection of antibiotic resistance determinants and their transmissibility among clinically isolated carbapenem-resistant *Escherichia coli* from South India. Med Princ Pract 27:428–435. https://doi.org/10.1159/000489885.
9. Mohanty S, Gajanand M, Gaind R. 2017. Identification of carbapenemase-mediated resistance among *Enterobacteriaceae* bloodstream isolates: a

molecular study from India. Indian J Med Microbiol 35:421–425. https://doi.org/10.4103/ijmm.IJMM_16_386.
10. Nahid F, Zahra R, Sandegren L. 2017. A blaOXA-181-harbouring multiresistant ST147 *Klebsiella pneumoniae* isolate from Pakistan that represent an intermediate stage towards pan-drug resistance. PLoS One 12:e0189438. https://doi.org/10.1371/journal.pone.0189438.
11. Begum N, Shamsuzzaman SM. 2016. Emergence of carbapenemase-producing urinary isolates at a tertiary care hospital in Dhaka, Bangladesh. Ci Ji Yi Xue Za Zhi 28:94–98.
12. Hall JM, Corea E, Sanjeewani HD, Inglis TJ. 2014. Molecular mechanisms of beta-lactam resistance in carbapenemase-producing *Klebsiella pneumoniae* from Sri Lanka. J Med Microbiol 63:1087–1092. https://doi.org/10.1099/jmm.0.076760-0.
13. Kieffer N, Nordmann P, Aires-de-Sousa M, Poirel L. 2016. High prevalence of carbapenemase-producing *Enterobacteriaceae* among hospitalized children in Luanda, Angola. Antimicrob Agents Chemother 60:6189–6192. https://doi.org/10.1128/AAC.01201-16.
14. Lowe M, Kock MM, Coetzee J, Hoosien E, Peirano G, Strydom KA, Ehlers MM, Mbelle NM, Shashkina E, Haslam DB, Dhawan P, Donnelly RJ, Chen L, Kreiswirth BN, Pitout JDD. 2019. *Klebsiella pneumoniae* ST307 with blaOXA-181, South Africa, 2014–2016. Emerg Infect Dis 25:739–747. https://doi.org/10.3201/eid2504.181482.
15. Poirel L, Aires-de-Sousa M, Kudyba P, Kieffer N, Nordmann P. 2018. Screening and characterization of multidrug-resistant gram-negative bacteria from a remote African area, Sao Tome and Principe. Antimicrob Agents Chemother 62:e01021-18. https://doi.org/10.1128/AAC.01021-18.
16. Sonnevend A, Ghazawi A, Hashmey R, Haidermota A, Girgis S, Alfaresi M, Omar M, Paterson DL, Zowawi HM, Pal T. 2017. Multihospital occurrence of pan-resistant *Klebsiella pneumoniae* sequence type 147 with an ISEcp1-directed blaOXA-181 insertion in the *mgrB* gene in the United Arab Emirates. Antimicrob Agents Chemother 61:e00418-17. https://doi.org/10.1128/AAC.00418-17.
17. Peirano G, Ahmed-Bentley J, Fuller J, Rubin JE, Pitout JD. 2014. Travel-related carbapenemase-producing Gram-negative bacteria in Alberta, Canada: the first 3 years. J Clin Microbiol 52:1575–1581. https://doi.org/10.1128/JCM.00162-14.
18. Walkty A, Gilmour M, Simner P, Embil JM, Boyd D, Mulvey M, Karlowsky J. 2015. Isolation of multiple carbapenemase-producing Gram-negative bacilli from a patient recently hospitalized in Nigeria. Diagn Microbiol Infect Dis 81:296–298. https://doi.org/10.1016/j.diagmicrobio.2015.01.005.
19. Cho SY, Huh HJ, Baek JY, Chung NY, Ryu JG, Ki CS, Chung DR, Lee NY, Song JH. 2015. *Klebsiella pneumoniae* co-producing NDM-5 and OXA-181 carbapenemases, South Korea. Emerg Infect Dis 21:1088–1089. https://doi.org/10.3201/eid2106.150048.
20. Matsumura Y, Pitout JD. 2016. Recent advances in the laboratory detection of carbapenemase-producing *Enterobacteriaceae*. Expert Rev Mol Diagn 16:783–794. https://doi.org/10.1586/14737159.2016.1172964.
21. Srisrattakarn A, Lulitanond A, Wilailuckana C, Charoensri N, Wonglakorn L, Saenjamla P, Chaimanee P, Daduang J, Chanawong A. 2017. Rapid and simple identification of carbapenemase genes, *bla* $_{NDM}$, *bla* $_{OXA-48}$, *bla* $_{VIM}$, *bla* $_{IMP-14}$ and *bla* $_{KPC}$ groups, in Gram-negative bacilli by in-house loop-mediated isothermal amplification with hydroxynaphthol blue dye. World J Microbiol Biotechnol 33:130. https://doi.org/10.1007/s11274-017-2295-5.
22. Peirano G, Matsumura Y, Adams MD, Bradford P, Motyl M, Chen L, Kreiswirth BN, Pitout JDD. 2018. Genomic epidemiology of global carbapenemase-producing *Enterobacter* spp., 2008-2014. Emerg Infect Dis 24:1010–1019. https://doi.org/10.3201/eid2406.171648.
23. Kazmierczak KM, Bradford PA, Stone GG, de Jonge BLM, Sahm DF. 2018. *In vitro* activity of ceftazidime-avibactam and aztreonam-avibactam against OXA-48-carrying *Enterobacteriaceae* isolated as part of the International Network for Optimal Resistance Monitoring (INFORM) global surveillance program from 2012 to 2015. Antimicrob Agents Chemother 62:e00592-18. https://doi.org/10.1128/AAC.00592-18.
24. R Core Team. 2017. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
25. Dohoo IR, Martin W, Stryhn H. 2008. Veterinary epidemiologic research, 2nd ed, vol 1. VER Inc., Charlottetown, Prince Edward Island, Canada.
26. Denwood MJ. 2016. runjags: an R package providing interface utilities, model templates, parallel computing methods and additional distributions for MCMC models in JAGS. J Statistical Software 71:25.
27. World Heath Organization. 2014. Antimicrobial resistance: global report on surveillance 2014. http://www.who.int/drugresistance/documents/surveillancereport/en/.
28. de Jonge BL, Karlowsky JA, Kazmierczak KM, Biedenbach DJ, Sahm DF, Nichols WW. 2016. *In vitro* susceptibility to ceftazidime-avibactam of carbapenem-nonsusceptible *Enterobacteriaceae* isolates collected during the INFORM global surveillance study (2012 to 2014). Antimicrob Agents Chemother 60:3163–3169. https://doi.org/10.1128/AAC.03042-15.
29. Karlowsky JA, Lob SH, Kazmierczak KM, Badal RE, Young K, Motyl MR, Sahm DF. 2017. *In vitro* activity of imipenem against carbapenemase-positive *Enterobacteriaceae* isolates collected by the SMART global surveillance program from 2008 to 2014. J Clin Microbiol 55:1638–1649. https://doi.org/10.1128/JCM.02316-16.
30. Balm MND, Ngan G, Jureen R, Lin RTP, Teo JWP. 2013. OXA-181-producing *Klebsiella pneumoniae* establishing in Singapore. BMC Infect Dis 13:58. https://doi.org/10.1186/1471-2334-13-58.
31. Shanthi M, Sekar U, Arungiri K, Bramhne HG. 2013. OXA-181 beta lactamase is not a major mediator of carbapenem resistance in *Enterobacteriaceae*. J Clin Diagn Res 7:1986–1988. https://doi.org/10.7860/JCDR/2013/5884.3379.
32. Seale AC, Gordon NC, Islam J, Peacock SJ, Scott JAG. 2017. AMR surveillance in low and middle-income settings: a roadmap for participation in the Global Antimicrobial Surveillance System (GLASS). Wellcome Open Res 2:92. https://doi.org/10.12688/wellcomeopenres.12527.1.