bla_{VIM} and bla_{OXA}-mediated carbapenem resistance among Acinetobacter baumannii and Pseudomonas aeruginosa isolates from the Mulago hospital intensive care unit in Kampala, Uganda

Dickson Aruhomukama¹, Christine F. Najjuka¹, Henry Kajumbula¹, Moses Okee¹, Gerald Mboowa¹,², Ivan Sserwadda¹, Richard Mayanja², Moses L. Joloba¹,² and David P. Kateete¹,²*

Abstract

Background: Between January 2015 and July 2017, we investigated the frequency of carbapenem resistant Acinetobacter baumannii (CRAB) and carbapenem resistant Pseudomonas aeruginosa (CRPA) at the Mulago Hospital intensive care unit (ICU) in Kampala, Uganda. Carbapenemase production and carbapenemase gene carriage among CRAB and CRPA were determined; mobility potential of carbapenemase genes via horizontal gene transfer processes was also studied.

Methods: Clinical specimens from 9269 patients were processed for isolation of CRAB and CRPA. Drug susceptibility testing was performed with the disk diffusion method. Carriage of carbapenemase genes and class 1 integrons was determined by PCR. Conjugation experiments that involved bla\textsubscript{VIM} positive CRAB/CRPA (donors) and sodium azide resistant Escherichia coli J53 (recipient) were performed.

Results: The 9269 specimens processed yielded 1077 and 488 isolates of Acinetobacter baumannii and Pseudomonas aeruginosa, respectively. Of these, 2.7% (29/1077) and 7.4% (36/488) were confirmed to be CRAB and CRPA respectively, but 46 were available for analysis (21 CRAB and 25 CRPA). Majority of specimens yielding CRAB and CRPA were from the ICU (78%) while 20 and 2% were from the ENT (Ear Nose & Throat) Department and the Burns Unit, respectively. Carbapenemase assays performed with the MHT assay showed that 40 and 33% of CRPA and CRAB isolates respectively, were carbapenemase producers. Also, 72 and 48% of CRPA and CRAB isolates respectively, were metallo-beta-lactamase producers. All the carbapenemase producing isolates were multidrug resistant but susceptible to colistin. bla\textsubscript{VIM} was the most prevalent carbapenemase gene, and it was detected in all CRAB and CRPA isolates while bla\textsubscript{OXA-23} and bla\textsubscript{OXA-24} were detected in 29 and 24% of CRAB isolates, respectively. Co-carriage of bla\textsubscript{OXA-23} and bla\textsubscript{OXA-24} occurred in 14% of CRAB isolates. Moreover, 63% of the study isolates carried class 1 integrons; of these 31% successfully transferred bla\textsubscript{VIM} to E. coli J53.

(Continued on next page)
Conclusions: CRAB and CRPA prevalence at the Mulago Hospital ICU is relatively low but carbapenemase genes especially bla<sub>IM</sub> and bla<sub>OXA-23</sub> are prevalent among them. This requires strengthening of infection control practices to curb selection and transmission of these strains in the hospital.

Keywords: Carbapenem resistant Acinetobacter, Carbapenem resistant Pseudomonas aeruginosa, Carbapenemase genes, Class 1 integrons, Conjugation, Intensive care unit, ICU, Mulago hospital, Uganda

Background
Carbapenemases are a highly potent class of antibiotics used to treat infections caused by multidrug resistant (MDR) Gram negative bacteria. However, the effectiveness of these antibiotics is currently being threatened by the emergence of carbapenem resistant bacteria [1, 2]. Carbapenemases are the main mechanism by which resistance to carbapenems occurs [1, 3–5], and they belong to three of the four β-lactamase classes A, B and D [1–5]. Class D carbapenemases are the OXA-β-lactamases [4], further subdivided into various sub-groups mainly bla<sub>OXA-23</sub>, bla<sub>OXA-24/40</sub>, bla<sub>OXA-58</sub>, bla<sub>OXA-48</sub>, bla<sub>OXA-51</sub> and bla<sub>OXA-143</sub> [1, 4]. These OXA-type β-lactamases occur widely in Acinetobacter with the most abundant being bla<sub>OXA-51</sub>, which is chromosomally encoded hence intrinsic to these species but it may confer resistance to carbapenems when its expression is up-regulated by genetic re-organization [1, 4, 6]. Class B carbapenemases are also known as the metallo-β-lactamases (MBLs) [1, 3]; they are mostly encoded by integron-borne mobile gene cassettes and hence, they are transferable amongst various bacteria via horizontal gene transfer mechanisms notably conjugation [3]. Class A carbapenemases include the Klebsiella pneumoniae carbapenemase (KPC) family that can be plasmid encoded or chromosomal [2, 5].

The hospital environment, mostly the intensive care units (ICUs), is a hotspot of antimicrobial resistance and source of MDR bacterial infections [7], particularly infections involving Acinetobacter species and Pseudomonas aeruginosa. The aim of this study was to determine the prevalence of carbapenem resistant Acinetobacter baumannii (CRAB) and carbapenem resistant Pseudomonas aeruginosa (CRPA) in clinical specimens from the Mulago Hospital ICU. Also, carriage of carbapenemase encoding genes and class 1 integrons was investigated, as well as mobility potential of the genetic elements among CRPA and CRAB isolates.

Methods
Study design and setting
This was a cross sectional study conducted at Mulago National Referral Hospital in Kampala, Uganda. Culturing and other laboratory procedures were performed at the laboratories of the Departments of Medical Microbiology and Immunology & Molecular Biology, Makerere University College of Health Sciences.

Samples
The bacteria investigated were isolated from clinical specimens referred by physicians for diagnostic testing (mainly culturing and identification of bacterial pathogens) in the period between 2015 and 2017. During this period, the Clinical Microbiology Laboratory processed clinical specimens from a total of 9269 patients for isolation of CRAB and CRPA. The specimens included blood, tracheal aspirates, ear swabs, catheter tips, sputum, pus swabs, throat swabs etc. Majority (78%) of the specimens yielding CRAB and CRPA isolates were from the ICU while 20% were from the Ear Nose & Throat (ENT) department. The Burns unit contributed the remaining 2% of the isolates.

Culturing, identification and drug susceptibility testing
Culturing and identification of isolates to species level was performed as described previously [1]. Briefly, presumptive identification of the isolates was based on colony morphology, Gram staining characteristics and biochemical properties. Biochemical tests included triple sugar iron (TSI), Sulphur indole motility test (SIM), as well as citrate, urease and oxidase tests. Pseudomonas aeruginosa colonies were identified by their spreading pattern, serrated edges, fruity sweet-grape smell and bright green color. In addition, a negative TSI, positive catalase and oxidase tests and growth at 42°C were also used to identify Pseudomonas aeruginosa. Acinetobacter isolates were identified based on negative oxidase and glucose fermentation tests, negative catalase and motility tests and their inability to grow under anaerobic conditions. Further, to identify Acinetobacter baumannii, PCR-detection of the bla<sub>OXA-51</sub> gene intrinsic to this species was performed [1, 6, 8].

Following isolate identification, drug susceptibility testing was performed using the Kirby Bauer disk diffusion method on Mueller Hinton agar (MHA) (BiolabZrt Budapest, Hungary), as recommended by the Clinical Laboratory Standards Institute (CLSI, 2014) and elsewhere [1, 9, 10]. Briefly, three colonies of the test isolate
were emulsified into sterile saline and the resulting suspension adjusted to turbidity of 0.5 McFarland. Antibiotic discs (BiolabZrt Budapest, Hungary) screened against the isolates were imipenem (10 μg), meropenem (10 μg), piperacillin/tazobactam (100 μg /10 μg), colistin (10 μg), gentamicin (10 μg), amikacin (30 μg), ciprofloxacin (5 μg), ceftazidime (30 μg), and ceftazidime (30 μg). Interpretation of susceptibility testing results was according to CLSI guidelines (2014). Quality control procedures were done using Pseudomonas aeruginosa ATCC 27853 and Escherichia coli ATCC 35218/25922 [1, 9, 10].

**Carbapenemase assays**

Phenotypic screening for carbapenemase activity was done using the Modified Hodge Test (MHT). Additionally, combination disc tests, imipenem-EDTA and boronic acid inhibition tests, were used to screen for MBL and KPC production respectively, as described previously [1, 9, 11]. In the MHT assay, a 1:10 dilution of the indicator strain (Klebsiella pneumoniae ATCC 700603 or E. coli ATCC 25922) was made by diluting 0.5 ml of culture (0.5 McFarland) to 5 ml with sterile normal saline. Then, a lawn culture (1:10 dilution) was streaked onto a MHA plate using a sterile swab. A 10 μg meropenem disk was placed in the center of the test area on the MHA plate, and test isolates streaked in a straight line. Then, a lawn culture (1:10 dilution) was streaked onto the MHA plate using a sterile swab. A 10 μg meropenem disk was placed in the center of the test area on the MHA plate, and test isolates streaked in a straight line from the edge of the disk to the edge of the plate. Klebsiella pneumoniae ATCC BAA-1705 and Klebsiella pneumoniae ATCC BAA-1706 were used as positive and negative controls, respectively [1, 12–14]. Furthermore, isolates that were found to be non-susceptible to imipenem or meropenem were also screened for MBL activity. In the MBL assay, overnight liquid cultures of the test isolates (adjusted to 0.5 McFarland) were streaked on a MHA plate. Two discs of imipenem (10 μg) were placed 15 mm apart (center-to-center); of the two discs, one was impregnated with 10 μl of 0.5 M EDTA to achieve a disc content of 1.5 mg EDTA. Following this, incubation was done at 37 °C, overnight. An increase in inhibition zone diameter by ≥5 mm in the EDTA-supplemented disc was interpreted as positive for MBL production [1]. Isolates that were positive were also screened for KPC production. In this test, a 10 μg meropenem disc and another 10 μg meropenem disc containing 3- AminoPhenyl boronic Acid (3-APBA) (300 μg/ml) (Tokyo Chemical Co. Ltd., Japan) were placed 20 mm apart, center-to-center. An increase in inhibition zone diameter by ≥5 mm around the combination disc compared to the meropenem disc alone was considered positive for KPC production [11].

**Detection of carbapenemase genes and class 1 integrons**

Molecular assays to determine carriage of carbapenemase encoding genes among CRAB and CRPA were performed as described previously [1, 15, 16]. The carbapenemase genes screened for included MBL encoding genes (blaIMP, blaVIM, blaSPM, blaNDM & blaKPC) and OXA-type genes (blaOXA-23, blaOXA-24 & blaOXA-58). Primers used in PCRs and amplicon analysis were described previously [1, 17].

**Conjugation and identification of transconjugants**

Conjugation experiments were performed as described previously [18, 19] using CRAB/CRPA as donors and E. coli J53 (F– met pro Azi+) which is resistant to sodium azide, as a recipient. Additional donor characteristics included co-carriage of class 1 integrons and blaVIM gene. CRBA/CRPA (donor) and E. coli J53 (recipient) were mixed in a ratio of 10:1 (donor:recipient) in Luria Bertani broth and the mixture incubated at 37 °C overnight. To select for transconjugants, serial dilutions of the cultures were plated on MacConkey agar containing ceftazidime (4 μg/ml) and sodium azide (100 μg/ml), which medium is toxic to donor cells. Confirmation of transfer was determined by PCR as described above to detect blaVIM among transconjugants.

**Quality control**

Well-characterized carbapenemase producing clinical strains of Klebsiella pneumoniae (Nr.8) and Pseudomonas aeruginosa from our collection [1, 9] were used as positive controls in molecular assays. For each gene that was PCR- amplified, PCR products were randomly selected and sequenced, and sequences confirmed through BLAST-searching at the National Centre for Biotechnology Information (https://blast.ncbi.nlm.nih.gov/Blast.cgi) as previously described [1]. ‘No template’ PCR controls and negative control PCRs with template DNA extracted from carbapenemase gene negative strains (K. pneumoniae DSMZ 9377, E. coli ATCC 25922 and P. aeruginosa ATCC 27853) were also included [1].

**Results**

A total of 9269 specimens from various wards at Mulago Hospital yielded 1077 and 488 isolates of Acinetobacter baumannii and Pseudomonas aeruginosa, respectively. Altogether, 2.7% (29/1077) and 7.4% (36/488) isolates were confirmed to be CRAB and CRPA respectively, Table 1. Acinetobacter species were detected in virtually all the tracheal aspirate samples and most of these samples yielded poly-microbial isolates that included Klebsiella pneumoniae and Escherichia coli.

CRAB and CRPA isolates recovered per year and hospital ward

The CRPA isolates recovered in 2015, 2016 and 2017 were 8/21 (38%), 4/21 (19%) and 9/21 (43%), respectively. The
yield per ward (ICU & ENT) was five & three isolates (2015); three & one isolate (2016); eight & one isolate (2017), respectively. Furthermore, the CRAB isolates recovered in 2015, 2016 & 2017 were 6/25 (24%), 3/25 (12%) and 16/25 (64%), respectively: The yield per ward was four & two in 2015 from ICU & ENT, respectively; three in 2016 from ICU; 13, two & one in 2017 from ICU, ENT & Burns Unit, respectively.

Carbapenemase activity among CRAB and CRPA isolates
Carbapenemase assays performed with the MHT assay showed that 40% (10/46) and 33% (15/46) of CRPA and CRAB isolates respectively, were positive for carbapenemase production. Furthermore, 72% (18/46) and 48% (10/46) of CRPA and CRAB isolates respectively, were MBL producers and none was positive for KPC, Table 2.

Prevalence of carbapenemase genes and class 1 integrons
bla
VIM was the most prevalent carbapenemase gene and it was detected in all CRPA and CRAB isolates, Table 3. Of note, the other MBL encoding genes i.e. bla
IMP, bla
SPM, and bla
NDM, were not detected; as well, carriage of the OXA-type carbapenemase genes was not detected in CRPA. Also, in agreement with activity assays, none of our isolates was positive for bla
KPC carriage. We however found the OXA-type carbapenemase genes to be prevalent among CRAB i.e. 29% & 24% for bla
OXA-23 & bla
OXA-24, respectively. Co-carriage of bla
OXA-23 and bla
OXA-24 was seen in 14% of the CRAB isolates. All CRAB isolates carried the bla
OXA-51 gene while none carried bla
OXA-58. Class 1 integrons of sizes 500 bp to 1000 bp were detected in 63% (29/46) of the isolates: 64% (16/25) & 62% (13/21) CRPA & CRAB respectively, Table 3. Furthermore, the classification of Acinetobacter baumanii based on bla
OXA-51 gene sequences showed that 12, eight and one CRAB isolate in this study belonged to pan-European International Clones II, I & III, respectively.

Drug susceptibility profiles of CRAB and CRPA isolates
bla
VIM-plus-bla
OXA-23 positive CRAB exhibited high levels of resistance to commonly used antibiotics especially ceftazidime (100%), gentamicin (88%), ciprofloxacin

| Specimen type | No. of specimens | Acinetobacter species | No. isolated (%) | No. CRAB reported (%) | No. CRAB recovered | Pseudomonas aeruginosa | No. isolated (%) | No. CRPA reported (%) | No. CRPA recovered |
|---------------|------------------|-----------------------|------------------|-----------------------|--------------------|-----------------------|------------------|-----------------------|--------------------|
| Blood         | 7343             | 412 (5.6)             | 4 (0.97)         | 2                     | 127 (1.72)         | 0                     | 0                |
| Sputum        | 908              | 119 (13.1)            | 1 (0.84)         | 1                     | 73 (8.03)          | 7 (9.6)               | 4                |
| Pus swab      | 350              | 7 (2)                 | 0                | 0                     | 5 (1.2)            | 4 (80)                | 4                |
| Tracheal aspirate | 221      | 344 (155.7)           | 11 (3.2)         | 10                    | 207 (93.7)         | 15 (7.2)              | 10               |
| Ear swab      | 177              | 117 (66.1)            | 7 (5.98)         | 4                     | 13 (7.3)           | 2 (15.4)              | 2                |
| Throat swab   | 160              | 13 (8.1)              | 3 (23)           | 1                     | 13 (8.1)           | 3 (23.1)              | 1                |
| Catheter tip  | 94               | 58 (61.7)             | 1 (1.7)          | 1                     | 45 (47.9)          | 5 (8.9)               | 4                |
| BAL fluid     | 16               | 7 (43.8)              | 2 (26.6)         | 2                     | 5 (41.3)           | 0                     | 0                |
| Total         | 9269             | 1077 (11.6)           | 29 (2.7)         | 21                    | 488 (5.3)          | 36 (7.4)              | 25               |

**Table 1** Clinical specimens from which CRAB and CRPA were recovered

**Table 2** Carbapenemase activity among CRPA and CRAP isolates (n = 46)

| MHT          | MHT (%) | MBL activity | EDTA-DST (%) | IMP hydrolysis (%) | MEM hydrolysis (%) | KPC (n = 28) | KPC (%) |
|--------------|---------|--------------|--------------|--------------------|--------------------|--------------|---------|
| CRPA (25)    | 10 (40) | 15 (60)      | CRPA (25)    | 18 (72)            | 7 (28)             | 25 (100)     | 0       |
| CRAB (21)    | 7 (33)  | 14 (67)      | CRAB (21)    | 10 (48)            | 11 (52)            | 21 (100)     | 0       |
| Total: 46    | 17      | 29           | 46           | 28                  | 18                  | 46           | 28      |

CRPA Carbapenem resistant Pseudomonas aeruginosa, CRAB Carbapenem resistant Acinetobacter baumanii, EDTA-DST EDTA disk synergy test, IMP Imipenem, MEM Meropenem, KPC Klebsiella pneumoniae carbapenemase, +ve Positive, -ve Negative
(88%), cefepime (75%), piperacillin/tazobactam (63%), and amikacin (50%), Table 4. Most of the CRAB and CRPA isolates were MDR (resistance to three or more classes of antimicrobials) however, all were susceptible to colistin. Furthermore, the susceptibility profiles of isolates carrying a single OXA-type gene (either \( \text{bla}_{\text{OXA-23}} \) or \( \text{bla}_{\text{OXA-24}} \)) were not different from profiles of isolates that carried two genes concurrently.

Similarly, \( \text{bla}_{\text{VIM}} \)-positive CRPA isolates showed high level resistance to gentamicin (100%), ceftazidime (89%), amikacin (72%), cefepime (72%), piperacillin/tazobactam (56%) and ciprofloxacin (50%); 94% of the isolates were MDR but susceptible to colistin. As well, all CRAB and CRPA isolates that were positive for both \( \text{bla}_{\text{VIM}} \) and class 1 integrons were highly resistant to the tested antibiotics but the susceptibility profiles were not different from isolates that did not carry class 1 integrons, Table 5.

### Transfer experiments

To demonstrate the potential for mobility of carbapenemase encoding genes in the hospital environment, conjugation experiments were successfully performed. Donors were CRPA and CRAB isolates that were positive for both \( \text{bla}_{\text{VIM}} \) and class 1 integrons while the recipient was sodium azide resistant \( \text{E. coli} \) strain J53. PCR assays showed that 25 and 39% of CRPA and CRAB donors respectively, successfully transferred the \( \text{bla}_{\text{VIM}} \) genetic element to \( \text{E. coli} \). Additional file 1: Figure S1.

### Discussion

Antimicrobial drug resistance (AMR) is a well-recognized global problem and high rates of resistance to first-line antibiotics among Gram-negative bacteria have been reported in Uganda [22]. As such, routine surveillance studies are necessary to monitor antibiotic resistance trends in low-income countries where antibiotic selection pressure is likely to be high.

This study has revealed that the prevalence of CRAB and CRPA in clinical specimens from Mulago Hospital remains relatively low and comparable to recently reported rates for carbapenem resistant Gram negative bacteria in Uganda [1, 9, 23]. Importantly, almost all the CRAB and CRPA isolates were from the ICU. Furthermore, \( \text{bla}_{\text{VIM}} \) and \( \text{bla}_{\text{OXA-23}} \) were the most prevalent carbapenemase genes with the former being detected in all CRAB and CRPA isolates. As such, \( \text{bla}_{\text{VIM}} \) and \( \text{bla}_{\text{OXA-23}} \) are the genes mediating carbapenem resistance in CRAB and CRPA in the Mulago Hospital ICU. Again, these findings are consistent with a recent study at Mulago Hospital and other settings in developing countries where similar studies have been done [1–3, 9, 24]. In addition to the \( \text{bla}_{\text{VIM}} \) gene, we also detected \( \text{bla}_{\text{OXA-23}} \), \( \text{bla}_{\text{OXA-24}} \) and \( \text{bla}_{\text{OXA-51}} \) genes in CRAB. Unlike \( \text{bla}_{\text{OXA-23}} \)- and \( \text{bla}_{\text{OXA-24}} \)-like genes, \( \text{bla}_{\text{OXA-51}} \) naturally occurs in \text{Acinetobacter baumannii} [1, 6, 8]. Furthermore, while in the previous study at Mulago [1] the detection of \( \text{bla}_{\text{OXA-51}} \)-like gene in CRAB was reported, it was not detected in this study. However, this observation is in agreement with studies by Minandi et al. 2011 [25] and Wu et al. 2015 [26] who discussed the transition from carriage of \( \text{bla}_{\text{OXA-51}} \)-like to \( \text{bla}_{\text{OXA-23}} \)-like and \( \text{bla}_{\text{OXA-24}} \)-like genes. Furthermore, through conjugation experiments we demonstrated the potential for horizontal gene transfer of the \( \text{bla}_{\text{VIM}} \) gene. Transfer rates in our study were low but in agreement with another study [27] which reported similar transfer rates. We have therefore provided evidence suggestive of mobility for carbapenem resistance genes in the Mulago

| Isolate No. | Year of isolation | IMP | MEM | CN | PTZ | CAZ | AK | FEP | CIP | CT |
|------------|------------------|-----|-----|----|-----|-----|----|-----|-----|----|
| 4268       | 2015             | R   | R   | R  | R   | R   | R  | R   | R   | S  |
| 2932       | 2015             | R   | R   | R  | R   | R   | R  | R   | S   | R  |
| 4643       | 2015             | R   | R   | R  | I   | R   | S  | I   | R   | S  |
| 1566.3     | 2015             | R   | R   | S  | R   | R   | S  | R   | S   | S  |
| 1566       | 2016             | R   | R   | R  | I   | R   | S  | R   | I   | S  |
| 2753.1     | 2016             | R   | R   | R  | I   | R   | I  | R   | R   | S  |
| 4264.2     | 2017             | R   | R   | R  | R   | R   | R  | R   | S   | S  |
| 4264.1     | 2017             | R   | R   | R  | R   | R   | R  | R   | S   | S  |
| % R        |                  | 100 | 100 | 88 | 63  | 100 | 50 | 75  | 88  | 0  |

IMP Imipenem, MEM Meropenem, CN Gentamicin, PTZ Piperacillin/tazobactam, CAZ Cefazidime, AK Amikacin, FEP Cefepime, CIP Ciprofloxacin, CT Colistin, R Resistant, I Intermediate, S Susceptible. 4264.2, 4268 & 4264.1 carried both \( \text{bla}_{\text{OXA-23}} \)-like & \( \text{bla}_{\text{OXA-24}} \)-like genes.
Table 5 Susceptibility profiles of CRAB and CRPA positive for bla<sub>VIM</sub> plus class 1 integrons

| Isolate No. | Year of isolation | Colistin resistant Acinetobacter baumannii |
|-------------|------------------|------------------------------------------|
|             |                  | IMP | MEM | CN | PTZ | CAZ | AK | FEP | CIP | CT |
| 4268*       | 2015             | R R | R R | R R | R R | R R | R R | R R | S   | S  |
| 2932*       | 2015             | R R | R R | R R | R R | S R | R S | S R | S   | S  |
| 4643*       | 2015             | R R | R R | R R | R R | S R | S R | S R | S   | S  |
| 1566.3*     | 2015             | R R | S R | R R | S R | S R | S R | S R | S   | S  |
| 2932.2*     | 2015             | R R | R R | S R | R S | S R | S R | S R | S   | S  |
| 4722.1      | 2015             | R R | I R | S R | I R | S R | S R | S R | S   | S  |
| 5730.2*     | 2015             | R R | R R | I R | R R | R R | R R | R R | S   | S  |
| 1566*       | 2016             | R R | R R | S R | S R | S R | S R | S R | S   | S  |
| 2753.1      | 2016             | R R | R R | I R | I R | S R | S R | S R | S   | S  |
| 1688*       | 2016             | R R | R R | I R | S R | I I | S I | S I | S   | S  |
| 2026.2      | 2016             | R R | R R | S I | S I | S I | S I | S I | S   | S  |
| 4264.2*     | 2017             | R R | R R | R R | R R | R R | R R | R R | S   | S  |
| 5730a       | 2015             | R R | R R | R R | R R | R R | R R | R R | S   | S  |
| 4786        | 2015             | R R | R R | R R | R R | R R | R R | R R | S   | S  |

Of note, carriage of bla<sub>VIM</sub> was associated with high resistance to carbapenems and other classes of antimicrobials [25, 26]. While bla<sub>VIM</sub> carriage in this setting has been reported before [3, 28, 29], a high prevalence of bla<sub>VIM</sub> is worrying as this genetic element renders carbapenems (imipenem & meropenem) ineffective [3, 28–31], narrowing treatment options to colistin & polymyxin B that have been associated with drastic side effects e.g. nephrotoxicity, neurotoxicity, etc. [3, 29–31]. As such, a narrow window remains before total antimicrobial resistance is seen in these bacterial isolates as they continue to be subjected to antibiotic pressure in the hospital [32–35].

Unlike in the previous study [1] we did not detect the three MBL genes bla<sub>SPM</sub>, bla<sub>NDM</sub> and bla<sub>IMP</sub> that have been associated with high resistance to carbapenems especially to imipenem [36, 37]. Further, the high prevalence of bla<sub>VIM</sub> in CRAB and CRPA in this study is suggestive of the role of the accessory genome in evolution of carbapenem resistance in our setting. bla<sub>VIM</sub> is characterized as an acquired MBL gene translocated between bacteria via class 1 integrons, in horizontal gene transfer mechanisms mostly involving conjugative plasmids [28, 38–40].

There were certain limitations in this study. We screened isolates for colistin susceptibility using the agar diffusion method instead of the recommended broth microdilution or the Etest methods. Further studies should take this into consideration given that poor agar dilution characteristics can limit the predictive accuracy of the disk diffusion method.
Conclusions

\textit{bla}_{VIM} and \textit{bla}_{OXA-23} are the most prevalent carbapenemase genes among CRAB and CRPA isolates at the Mulago Hospital ICU. There is need to improve infection control practices at the hospital to curb the transmission of the isolates in the hospital.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12879-019-4510-5.

Additional file 1: Figure S1. A representative image showing donors and trans-conjugants screened for presence of \textit{bla}_{VIM} gene. Lanes: L, 100 bp DNA ladder; D, 382 bp \textit{bla}_{VIM} fragment in donors (CRAB or CRPA isolate); T, 382 bp \textit{bla}_{VIM} fragment in trans-conjugant (E. coli J53, recipient); P, \textit{bla}_{VIM} carrying strain (positive control); JSJ, un conjugated \textit{E. coli J53} (negative control recipient).

Abbreviations

ATCC: American Type Culture Collection; \textit{bla}: \beta-lactamase; BLAST: Basic Local Alignment Search Tool; CLSI: Clinical laboratory Standards Institute; CRAB: Carbapenem Resistant Acinetobacter baumannii; CRPA: Carbapenem Resistant Pseudomonos aeruginosa; EDTA: Ethylene Diamine Tetra Acetic acid; ENT: Ear, Nose and Throat; ICU: Intensive Care Unit; IMP: Imipenemase; KPC: \textit{Klebsiella pneumoniae} carbapenemase; MBL: Metallo-\beta-lactamase; MHA: Mueller Hilton Agar; MHT: Modified Hodges Test; NDM: New Delhi Metallo-\beta-lactamase; OXA: Oxacillinase; PCR: Polymerase Chain Reaction; SIM: Sulphur Indole Motility; SPM: Sao Paulo Metallo-\beta-lactamase; TSI: Triple Sugar Iron; VM: Verona integron encoded Metallo-\beta-lactamase

Acknowledgements

We thank staff at the Clinical Microbiology and Molecular biology laboratories, College of Health Sciences, Makerere University, for technical and administrative assistance.

Author contributions

DPK conceived the study, provided technical guidance, supervised the work, reviewed the final manuscript and provided the laboratory supplies for the molecular assays. CFN participated in study design, provided technical assistance including supervision and manuscript reviews. DA designed the study, performed the microbiology & molecular assays, analyzed the data and drafted the manuscript in partial fulfillment of the requirements for the award of the degree of Master of Science (Immunology & Clinical Microbiology) of Makerere University. MO provided technical assistance during performing of the molecular assays. MLJ & HK provided samples and research support (i.e. laboratory supplies for the microbiology assays). GM, IS, & RM participated in the writing and manuscript reviews. All authors reviewed and approved the final manuscript.

Funding

This work was supported in part by the DELTAS Africa Initiative (grant # 107743/2/15/2), The DELTAS Africa Initiative is an independent funding scheme of the African Academy of Sciences (AAS), Alliance for Accelerating Excellence in Science in Africa (AESA), and supported by the New Partnership for Africa’s Development Planning and Coordinating Agency (NEPAD Agency) with funding from the Welcome Trust (Grant no. 107743) and the UK Government. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. DPK was a recipient of a Postdoctoral Fellowship from the Makerere University Infection & Immunity Programme (MUII-Plus) at the MRC/UAVR & LSHTM Uganda Research Unit, Entebbe.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Ethical approvals were obtained from the Higher Degrees Research and Ethics Committee of the School of Biomedical Sciences (approval number: SBS-509), Makerere University. The Ethics Committee waived the requirement for informed consent as the investigated isolates were obtained from clinical specimens referred to the diagnostic laboratory as part of routine care.

Consent for publication

Not applicable.

Competing interests

David Kateete is an editorial board member for BMC Infectious Diseases.

Received: 14 February 2019 Accepted: 24 September 2019

Published online: 16 October 2019

References

1. Kateete DP, Nakanjako R, Namugenyi J, Erume J, Joloba ML, Najjuka CF. Carbapenem resistant Pseudomonas aeruginosa and Acinetobacter baumannii at Mulago Hospital in Kampala, Uganda (2007–2009). SpringerPlus. 2016;5(1):1308.
2. Manerezhe RIZH, Nicol MP, Kaba M. The spread of carbapenemase producing bacteria in Africa: a systematic review. J Antimicrob Chemother. 2015;70(1):23–40.
3. Amudhan MS, Sekar U, Kamalanathan A, Balaraman S. \textit{bla}MP and \textit{bla}VIM Mediated Carbapenem Resistant in Pseudomonos and Acinetobacter Species in India. J Infect Dev Ctries. 2012;6(11):577–62.
4. Antunes NT, Lamoceaux TL, Toth M, Stewart NK, Frase H, Vakulenko SB. Class D \beta-Lactamases: Are They All Carbapenemases? Antimicrob Agents Chemother. 2014;58(4):2119–25.
5. Poirel L, Pitout JD, Nordmann P. Carbapenemases: Molecular Diversity and Clinical Consequences. Future Microbiol. 2007;2(5):501–12 Future Medicine.
6. Leski TA, Bangura U, Jimmy DH, Ansumana R, Lizewski SE, Li RW, Stenger DA, Taitt CR, Vora GJ. Identification of \textit{bla} (OXA-51-Like), \textit{bla} (OXA-\textit{VIM}), \textit{bla} (DIM-1), and \textit{bla} (MM) Carbapenemase Genes in Hospital Enterobacteriaceae. J Clin Microbiol. 2013;51(7):2435.
7. Albracht WC, Angstwurm M, Bader L, Gartner D. Carbapenemase producing bacteria in intensive care units. Infection. 1999;27(Suppl 2):S19–23.
8. Turton JF, Ward ME, Woodford N, Kaufmann ME, Pike R, Livermore DM, Pitt TL. The Role of \textit{ISAba1} in Expression of OXA Carbapenemase Genes in Acinetobacter Baumannii. FEMS Microbiol Lett. 2006;258(1):72–7.
9. Ooko D, Asimwe BB, Katabazi FA, Kato L, Najjuka CF, Prevalence and Characterization of Carbapenem-Resistant Enterobacteriaceae Isolated from Mulago National Referral Hospital, Uganda. PLoS One. 2015;10(8):e0135745.
10. Christine F, Najjuka DP, Kajumbula HM, Joloba ML, Essack SY. Antimicrobial susceptibility profiles of Escherichia coli and \textit{Klebsiella pneumoniae} isolated from outpatients in urban and rural districts of Uganda. BMC Res Notes. 2016;9:235.
11. Parajuli NP, Acharya SP, Mishra SK, Parajuli K, Rijal BP, Pokhrel BM. High Burden of Antimicrobial Resistance among Gram Negative Bacteria Causing Healthcare Associated Infections in a Critical Care Unit of Nepal. Antimicrob Resist Infect Control. 2017;6:67 London: BioMed Central.
12. Wikler AM. Performance Standards for Antimicrobial Susceptibility Testing. Sixteenth Informational Supplement. Clin Lab Stand Inst. 2006;16:1M–100–5.
13. UK Standards for Microbiology Investigations. 2016. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/554654/B__6021.pdf.
14. Asthana S, Mathur P, Tak V. Detection of carbapenemase production in gram-negative bacteria. J Lab physicians. 2014;6(2):69.
15. Pitout JDD, Gregson DB, Poirel L, McCleary JA, Le P, Church DL. Detection of Pseudomonos Aeruginosa Producing Metallo-\beta-Lactamases in a Large Centralized Laboratory. J Clin Microbiol. 2005;43(7):3129–35.
16. Rastegar Lari A, Azimi L, Rahbar M, Fallah F, Alaghehbandan R. Phenotypic Detection of \textit{Klebsiella pneumoniae} Carbapenemase among Burns Patients: First Report from Iran. Burns. 2013;39:1:174–6.
17. Moura A, Henriques I, Ribeiro R, Correia A. Prevalence and Characterization of Integrons from Bacteria Isolated from a Slaughterhouse Wastewater Treatment Plant. J Antimicrob Chemother. 2007;60(6):1243–50.
18. Walsh TR, Weeks J, Livermore DM, Toleman MA. Dissemination of NDM-1 Positive Bacteria in the New Delhi Environment and Its Implications for
19. Yan J-J, Wu S-M, Tsai S-H, Wu J-J, Su I-J. Prevalence of SHV-12 among Clinical Isolates Of Klebsiella pneumoniae Producing Extended-Spectrum ß-Lactamases and Identification of a Novel AmpC Enzyme (CMY-8) in Southern Taiwan. Antimicrob Agents Chemother. 2000;44(6):1438–42.

20. Evans BA, Harmouda A, Tovmer KJ, Amyes SGB. Occurrence and their association with particular epidemic lineages of Acinetobacter baumannii. Clin Microbiol Infect. 2008;14(3):268–75.

21. Totten JS, Gabriell SN, Valderen C, Kaufmanme, PTT TL. Use of sequence-based typing and multiple PCR to identify clonal lineages of outbreak strains of gram-negative Acinetobacter baumannii. J Clin Microbiol. 2007;45(8):2871–15.

22. Kajumbula H, Fujita AW, Mbabazi O, Najjuka C, Izale C, Akampurira A, Aisu S, Lamberde M, Walwema B, Bahr NC, et al. Antimicrobial drug resistance in blood culture isolates at a tertiary hospital, Uganda. Emerg Infect Dis. 2018; 24(1):174–5.

23. Kateete DP, Nakanjako R, Okeke ML, Najjuka CF. Genotypic diversity among multidrug resistant Pseudomonas aeruginosa and Acinetobacter species at Mulago Hospital in Kampala, Uganda. BMC Res Notes. 2017;10(1):284.

24. Mushi MF, Ihnawanie C, Bwanga F. Carbapenemase Genes among Multidrug Resistant Gram Negative Clinical Isolates from a Tertiary Hospital in Mwanza, Tanzania. BioMed Res Int. 2014;2014:303104.

25. Fabrizia Minandri SDA, Antunes LCS, Pourcel C, Principe L, Petrosillo N, Visca P. Evidence of Diversity among Epidemiologically Related Carbapenemase-Producing Acinetobacter Baumannii Strains Belonging to International Clonal Lineage II. Am Soc Microbiol. 2011;50(3):590–7.

26. Weyjuan Wu YH, Lu J, Lu Y, Wu J, Liu Y. Transition of blaOXA-58-like to blaOXA-23-like in Acinetobacter Baumannii Clinical Isolates in Southern China: An 8-Year Study. PLoS One. 2015;10(9):e0137174.

27. Poonsuk K, Tribuddharat C, Chuanchuen R. Class 1 Integrons in Pseudomonas aeruginosa and Acinetobacter baumannii isolated from clinical isolates. Southeast Asian J Trop Med Public Health. 2012;43(2):376.

28. Anna Michaliska-Falkowska PTS, Grzes PWH, Haushild O, Ojdana EATD. Emergence of Pseudomonas Aeruginosa with Class 1 Integron Carrying blaVIM-2 and blaVIM-4 in the University Clinical Hospital of Białystok (Northeastern Poland). Postepy Hig Med Dosw. 2017;71:589–94.

29. Khosravi AD, Mihani F. Detection of Metallo-ß-lactamases producing Pseudomonas Aeruginosus Strains Isolated from Burn Patients in Ahwaz, Iran. Diagn Microbiol Infect Dis. 2018;80(1):125–8 Elsevier.

30. Michalopoulos AS, Tsiodras S, Reiss K, Mentzelopoulou S, Falagas ME. Colistin treatment in patients with icu-acquired infections caused by multidrug-resistant gram-negative bacteria: the renaissance of an old antibiotic. Clin Microbiol Infect. 2005;11:115–21.

31. Falagas ME, Kasiakou SK, Saravolatz LD. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. Clin Infect Dis. 2005;40(9):1333–41.

32. Baquer Ø, Alvarez-Ottega C, Martinez JL. Ecology and evolution of antibiotic resistance. Environ Microbiol Rep. 2009;1(6):469–76 Wiley Online Library.

33. Davies JDD. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev. 2010;74(3):417–76 Wiley Online Library.

34. Chevereau G, Dravecká M, Batur T, Guvenek A, Ayhan DH, Toprak E, Bollenbach T. Quantifying the determinants of evolutionary dynamics leading to drug resistance. PLoS Biology. 2015;13(11):e1002299 Public Library of Science.

35. Bollenbach T. Antimicrobial interactions: mechanisms and implications for drug discovery and resistance evolution. Curr Opin Microbiol. 2015;27:1–8.

36. Toleman MA, JJB B, Nizam SA. Extensively Drug-Resistant New Delhi Metallo-ß-Lactamase-Encoding Bacteria in the Environment, Dhaka, Bangladesh, 2012. Emerg Infect Dis. 2015;21(6):1027–30.

37. Sidra Shan SS, Ahmad K. Detection of blalmp Gene in Metallo-ß-Lactamase Producing Isolates of Imipenem Resistant Pseudomonas Aeruginosa; an Alarming Threat. J Microbiol Res. 2015;6(1):75–80.

38. Xu Z, Li L, Shilliff ME, Alam MJ, Yamasaki S, Shi L. Occurrence and Characteristics of Class 1 and 2 Integrons in Pseudomonas Aeruginosa Isolates from Patients in Southern China. J Clin Microbiol. 2009;47(1):230–4.

39. Naa T, Miakami Y, Inai T, Poiré L, Nordmann P. Characterization of InI3, a Class 1 Plasmid- and Composite Transposon-Located Integron of Escherichia coli ‘Which Carries an Unusual Array of Gene Cassettes. J Bacteriol. 2001; 183(2):235–49.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.