

# Horizontal transfer of OXA-23-carbapenemase-producing *Acinetobacter* species in intensive care units at an academic complex hospital, Durban, KwaZulu-Natal, South Africa

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**Introduction:** Carbapenemase production is an important mechanism of carbapenem resistance in *Acinetobacter* species. This study investigated the presence of the carbapenem-hydrolysing class D  $\beta$ -lactamase-encoding genes, *bla*<sub>OXA-23</sub> and *bla*<sub>OXA-58</sub>, and their association with the spread of multidrug-resistant (MDR) *Acinetobacter* species in intensive care units at an academic hospital.

**Method:** Forty-four MDR *Acinetobacter* species were confirmed using VITEK<sup>®</sup>2 and Epsilon meter tests. The *bla*<sub>OXA-23</sub> and *bla*<sub>OXA-58</sub> genes were detected by polymerase chain reaction (PCR) in twenty-four selected isolates. The *bla*<sub>OXA-23</sub> amplicons were sequenced and compared to the GenBank database. Genotypic relatedness of isolates was determined by pulsed field gel electrophoresis (PFGE). Clinical and laboratory data were analysed.

**Results:** Among the twenty-four isolates, eighteen were carbapenem resistant and six were sensitive. The *bla*<sub>OXA-23</sub> gene, but not *bla*<sub>OXA-58</sub> was detected in the eighteen resistant strains. The *bla*<sub>OXA-23</sub> amplicons showed 100% identity with the GenBank database of *bla*<sub>OXA-23</sub>. The MICs of carbapenems against *Acinetobacter* species carrying the *bla*<sub>OXA-23</sub> gene were 8 to > 16  $\mu$ g/ml. Genetic relatedness was evident among isolates of seven pairs from fourteen patients. Of these patients, twelve were in the same ICUs and two were adjacent to another ICU during the same hospitalisation period.

**Conclusion:** The selected MDR *Acinetobacter* species carried the *bla*<sub>OXA-23</sub> gene responsible for resistance to carbapenems, while molecular and clinical data analysis suggested horizontal transmission in ICUs. In addition, the PFGE typing of a diverse collection of MDR *Acinetobacter* species clones showed that isolates were related to no more than two patients, suggesting that no outbreak had occurred.

**Keywords:** *bla*<sub>OXA-23</sub> genes, carbapenem-hydrolysing class D  $\beta$ -lactamases (CHDLs), horizontal transmission, molecular surveillance

## Background

Multidrug-resistant (MDR) *Acinetobacter* species (*Acinetobacter* spp.), are both community acquired and nosocomial opportunistic infections and have been responsible for outbreaks around the globe, especially in intensive care units (ICUs).<sup>1–5</sup> The past twenty years has seen an increase in the prevalence of the pathogen<sup>6–8</sup> with MDR outbreaks reported in the United States.<sup>9–11</sup> A significant reservoir is the large number of chronically ill patients<sup>5</sup> from whom colonisation of recently hospitalised patients may take place.<sup>6,7</sup>

There has been an increase of *Acinetobacter* spp. resistance to cephalosporins and carbapenems over the years,<sup>12–14</sup> leaving clinicians with limited therapeutic options.<sup>12–14</sup> Resistance to carbapenems, which display high efficacy and low toxicity, is of global concern.<sup>12–14</sup> Surveillance reports from China determined that carbapenem resistance in *Acinetobacter* spp. doubled from 30% in 2006 to 63% in 2013.<sup>12–14</sup> South African studies revealed *Acinetobacter* spp. resistance towards carbapenems and cephalosporins,<sup>15,16</sup> specifically imipenem (86%), meropenem (86%) cefepime (90%) and ceftazidime (89%).<sup>16</sup>

The major mechanism of resistance to  $\beta$ -lactams in *Acinetobacter* spp. is carbapenem-hydrolysing class D  $\beta$ -lactamases (CHDLs), also known as OXA-type enzymes or oxacillinases.<sup>16</sup> Previous studies classified the class D carbapenemases into four subgroups: associated with OXA-23, OXA-58 as plasmid-encoded and OXA-24, OXA-40, OXA-51 as chromosomally encoded.<sup>17,18</sup> There are currently five subclasses of OXA associated with *Acinetobacter* spp.; the intrinsic chromosomal OXA-51-like, of which there are over 70 variants, and the acquired OXA-23-like, OXA-24 (OXA-40-like), OXA-58-like, and OXA-143-like.<sup>19</sup> In addition, the OXA-235, OXA-236, and OXA-237, the first representatives of a novel subclass of CHDLs, were described in *A. baumannii* strains in 2013.<sup>20</sup> Enzymes belonging to the OXA-23, OXA-24/40, OXA-48, OXA-51, OXA-58, and OXA-143 subgroups are of major clinical importance due to their wide dissemination in bacterial pathogens.<sup>21–23</sup> The genes encoding these enzymes are widespread<sup>20</sup> and have been found on both chromosome and plasmids, allowing for spread across *Acinetobacter* species.<sup>20,24</sup> The prevalence of OXA-23 can be attributed to the acquisition of genetic elements, such as plasmids and transposons, as the OXA-23 gene is located on a plasmid or chromosome.<sup>16</sup> In a

2012 study, Liakopoulos *et al.* reported the prevalence of OXA-23 in Greece from 2010 to 2011 to be 95%,<sup>25</sup> while Koh *et al.* reported that 91% of *Acinetobacter* spp. isolates from Singapore produced carbapenemase and carried the *bla*<sub>OXA-23</sub> gene.<sup>26</sup> Previous studies conducted at Pretoria Academic Hospital revealed the high prevalence of OXA-51 (83%, 99%) and of OXA-23 at (59%, 77%) in 2013 and 2015, respectively.<sup>16, 18</sup> Isolates of carbapenem resistant *Acinetobacter* spp. are often extensively drug-resistant (XDR), since they are susceptible to one or two agents only.<sup>27</sup> Additionally, the recent rise not only in XDR but also pandrug-resistant (PDR) *Acinetobacter* spp.<sup>22</sup> is of global concern. Due to the problem of resistance and antimicrobial availability, the Infectious Diseases Society of America determined that *Acinetobacter* spp. is a particularly concerning pathogen.<sup>28</sup> Moreover, the Centers for Disease Control and Prevention has highlighted the importance of MDR *Acinetobacter* spp. transmission in nosocomial and community acquired infections.<sup>5</sup> In our setting, the latter half of 2008 discovered the emergence of *Acinetobacter* spp. clinical strains with resistance to multiple classes of antimicrobials, including carbapenems, piperacillin-tazobactam, ceftazidime and fluoroquinolones. Hence, treatment options were restricted to salvage agents like colistin and amikacin.

In this study, we investigated a representative sample of *Acinetobacter* spp. from Inkosi Albert Luthuli Hospital (IALCH) from 2013 to 2014. We determined the presence of two carbapenem resistance genes using PCR, sequencing and correlated the MIC of the carbapenems with the genes. We also investigated the clinical data of patients, including ward of admission, site of specimen, prescribed antibiotics and outcome. Nosocomial spread of the strains was investigated through pulsed field gel electrophoresis (PFGE).

## Methods

### Study setting

The study was conducted at the Department of Medical Microbiology, University of KwaZulu-Natal (UKZN)/National Health Laboratory Service (NHLS). Ethics approval was obtained from the Biomedical Research Ethics Committee, UKZN (Reference No BE 283/12).

The stored *Acinetobacter* spp. ( $n = 60$ ) isolates had previously been isolated from the patients hospitalised at IALCH from January 2013 to January 2014. Of the sixty, forty-four MDR *Acinetobacter* spp. were confirmed by using VITEK 2 (BioMérieux, France). The MICs of imipenem and meropenem were determined using the VITEK 2 and Epsilometer tests (E-test) (BioMérieux, France).

Using the antibiogram, four XDR and fourteen MDR isolates with the same sensitivity patterns were selected for molecular investigation. PCR, sequencing and PFGE typing were used to investigate carbapenem-hydrolysing class D  $\beta$ -lactamase production, the presence of the *bla*<sub>OXA-23</sub> and *bla*<sub>OXA-58</sub> genes, as well as the association between drug resistance and presence of the genes.

The *A. baumannii* ATCC 19606 strain was used as quality control for antimicrobial susceptibility tests and molecular methods. The *bla*<sub>OXA-23</sub> and *bla*<sub>OXA-58</sub> carried *Acinetobacter* spp. isolates obtained from the National Institute for Communicable Diseases (NICD) served as controls for PCR, sequencing and PFGE.

### Drug susceptibility testing and MIC

The stored isolates were confirmed to be MDR *Acinetobacter* species. Identification and antibiotic susceptibility testing (AST) of MDR *Acinetobacter* spp., including MICs, were performed using the VITEK® 2 GN Card automatic method (BioMérieux, France) and the Epsilometer test (E-test®) (BioMérieux, France) as per the guidelines from the Clinical and Laboratory Standards Institute (CLSI), 2013.<sup>29</sup>

Carbapenem-resistant *Acinetobacter* spp. were resistant to both imipenem and meropenem with MICs of 8 µg/ml, whereas carbapenem-susceptible *Acinetobacter* spp. possessed a MIC of < 1 µg/ml and carbapenem-intermediate *Acinetobacter* spp. a MIC of 1-2 (<4) µg/ml.<sup>29</sup>

### Detection of *bla*<sub>OXA-23</sub> and *bla*<sub>OXA-58</sub>

Eighteen MDR *Acinetobacter* spp. belonging to the same antibiogram groups were selected for amplification by PCR to detect the carbapenem resistance genes *bla*<sub>OXA-23</sub> and *bla*<sub>OXA-58</sub>. Resistance mediating genes were assessed for the presence of polymorphisms with the Big Dye Terminator v3.1 Cycle Sequencing kit (Life Technologies, ThermoFisher Scientific, South Africa).

### DNA extraction of *Acinetobacter* spp. isolates<sup>30, 31</sup>

Genomic DNA, from each of twenty-seven isolates, comprising eighteen clinical MDR strains, three controls and six sensitive clinical isolates, was extracted from an overnight culture using a loopful of colonies suspended in 500 µl of 1x TE buffer, pH 8.5 (Tris EDTA) (Capital Laboratory Supplies, Durban, South Africa). Cell lysis and protein digestion were performed using a combination of 10% sodium dodecyl sulfate and 10 mg/ml proteinase K (Capital Laboratory Supplies, Durban, South Africa). Proteins were precipitated with 10% cetyltrimethyl ammonium bromide (CTAB)/4% NaCl and polysaccharides were extracted by the addition of 24:1 chloroform-isoamyl alcohol (Capital Laboratory Supplies, Durban, South Africa). The DNA was precipitated with isopropanol, washed with 70% cold ethanol (Capital Laboratory Supplies, Durban, South Africa) and dissolved in an appropriate volume of 1xTE buffer, pH 8. The DNA was electrophoresed in a 1% agarose gel (Capital Laboratory Supplies, Durban, South Africa) to determine quality and quantity. The extracted genomic DNA was stored at -20 °C (Defy Ltd, Multimode, South Africa) until further analysis.

### Amplification of the *bla*<sub>OXA-23</sub> and *bla*<sub>OXA-58</sub> genes

The *bla*<sub>OXA-23</sub> and *bla*<sub>OXA-58</sub> gene regions were amplified using primers (Roche Diagnostics, Randburg, South Africa) specific to the up and downstream regions of the gene sequences (Table 1). The PCR master mix consisted of 5 µl template DNA, 10x PCR

**Table 1:** Primer sequences and the corresponding annealing temperatures

Primers	Sequence (5' to 3')	Annealing temperature (°C)	Reference
<i>bla</i> <sub>OXA-23</sub> Forward	TCTGGTTGTACG-GTTCAGC	53	[32]
<i>bla</i> <sub>OXA-23</sub> Reverse	AGTCTTTC-CAAAAATTTTG	53	[32]
<i>bla</i> <sub>OXA-58</sub> Forward	ATGAAATTAT-TAAAAATTTGAGT-TTAG	55	[32]
<i>bla</i> <sub>OXA-58</sub> Reverse	TTATAAATAAT-GAAAAACACCCAAC	55	[32]

buffer, 0.2 µM of each primer, 2.5 mM dNTPs, 1 U Taq DNA Polymerase (ThermoFisher Scientific, United States), 1.5 µM MgCl and nuclease free water, with a total volume of 25 µl. The initial denaturation steps were performed for 5 min at 95 °C, followed by 40 cycles of 20 s at 95 °C, 1 min at 55 °C and 30 s at 72 °C. This was followed by a final extension step of 5 min at 72 °C. Both positive and negative controls were used during the PCR amplification process. PCR products were electrophoresed on a 1.5% agarose gel (Capital Laboratory Supplies, Durban, South Africa) with markers of known molecular weights (ThermoFisher Scientific, Waltham, Massachusetts) and visualised under UV light.

#### Purification of PCR products

The PCR product were purified using 2 U Shrimp Alkaline Phosphatase (SAP) (AEC-Amersham, Little Chalfont, United Kingdom) and 10 U Exonuclease (Amersham, Little Chalfont, United Kingdom) as per manufacturer's instructions. After the addition of 2 U of SAP and 10 U of Exonuclease I, the PCR tubes were briefly incubated at 32 °C for 30 min and then at 80 °C for 15 min in a water bath to deactivate the enzymes.

#### DNA sequencing of *bla*<sub>OXA-23</sub> amplicons

Gene sequencing was performed with the ABI Big Dye Terminator v 3.1 Cycle Sequencing kit (ThermoFisher Scientific, Waltham, Massachusetts). The sequenced products were separated by capillary array electrophoresis using the ABI 3500 Genetic Analyser (ThermoFisher Scientific, Waltham, Massachusetts).

The National Center for Biotechnology Information (NCBI) database was accessed and the sample sequences were aligned and compared to a reference sequence using the Basic Local Alignment Search Tool (BLAST) (<https://www.ncbi.nlm.nih.gov/>).

#### Genotypic relatedness of clinically selective *Acinetobacter* spp. determined by PFGE

Macro-restriction analysis of multidrug-resistant *Acinetobacter* spp. obtained from IALCH was performed using an adaptation of the method by Seifert *et al.*,<sup>33</sup> and Sader *et al.*,<sup>34</sup> Pure isolates from blood agar plates were grown in brain heart infusion (BHI) broth for 15-18 hr at 37 °C in a shaking incubator (Vacutec, South Africa) for plug preparation. A volume of broth corresponding to 1 optical density (OD) at 600 nm was used to make a 1% PFGE agarose plug for each isolate. DNA was extracted within the agarose blocks by cell lysis over 18 hours. Plug slices for each isolate were digested with 20U *Apal* restriction endonuclease (New England Biolabs, USA) for 4 hr at 37 °C. The restricted DNA was electrophoresed on a 1% Pulsed-Field Electrophoresis Gel (BioRad, United States) in 0.5X Tris-Borate-EDTA buffer (Merck, South Africa) at 6 V/cm with a 5-25 sec switch time for 24 hours using the Chef-DR 3 system (BioRad, United States).

Cluster analysis was performed according to criteria outlined in Van Belkum *et al.*<sup>35</sup> and a type was defined as PFGE banding patterns differing by 4 or less bands. The results were interpreted according to the Tenover criteria.<sup>36</sup>

#### Clinical and laboratory data collection

The clinical characteristics, namely type of specimen, ward of admission, prescribed antibiotics and patient outcome, were documented from the patient records. In addition, the laboratory data were recorded from the laboratory computer system. Both

the clinical and laboratory data were verified during wards rounds and both were analysed.

#### Statistical analysis of clinical characteristics and patient outcomes

Data was captured and analysed using the Statistical Package for Social Sciences (IBM® SPSS Statistics version 19). Results were presented using descriptive statistics such as frequency and percentage. The association between underlying patient condition and response to antibiotic agents was determined using the Pearson chi-square test or Fisher's exact test. Logistic regression was used to test for factors associated with patient mortality.

## Results

#### Drug susceptibility tests and MICs

During the study period, forty-four of the sixty stored *Acinetobacter* spp. isolates were confirmed MDR by the MICs. The selected isolates were resistant to carbapenems, with MIC values for imipenem and meropenem at 8 to > 16 µg/ml as determined by the Vitek (BioMérieux, France) automatic system and confirmed with E-test (BioMérieux, France). The control isolate (ATCC 19606) showed sensitivity to imipenem and intermediate sensitivity to meropenem, with MICs of 0.25 and 1 µg/ml respectively. The *bla*<sub>OXA-23</sub> positive control isolate was resistant to imipenem and meropenem with a MIC of > 16 µg/ml for both. The *bla*<sub>OXA-58</sub> positive control strain was resistant to imipenem and meropenem with a MIC of 8 and 4 µg/ml respectively (Table 2).

#### Clinical characteristics and patient outcomes

The clinical characteristics and patient outcomes were analysed in those patients whose isolates showed the presence of the *bla*<sub>OXA-23</sub> gene (*n* = 18). Among the eighteen isolates, four (22%) were XDR isolates which possessed the same antibiogram and fourteen (78%) were resistant to all agents except amikacin and colistin (Table 3).

#### Amplification result of the *bla*<sub>OXA-23</sub> and *bla*<sub>OXA-58</sub> genes by PCR (*n* = 24)

The *bla*<sub>OXA-58</sub> gene was not detected in any of the isolates tested. The *bla*<sub>OXA-23</sub> gene was detected in 18 clinical isolates, but not in 6 carbapenem sensitive *Acinetobacter* spp. isolates (Figure 1, A-D). The majority (17 out of 18) of *Acinetobacter* spp. isolates carrying the carbapenemase gene exhibited high MICs (>16 µg/ml) to carbapenems (Table 2).

**Table 2:** MICs of imipenem and meropenem for *Acinetobacter* spp. isolates from the Central Hospital in the academic complex and the occurrence of the corresponding carbapenemase gene

Number of isolates ( <i>n</i> = 24)	IMP	MEM	<i>bla</i> <sub>OXA23</sub> + or -	<i>bla</i> <sub>OXA58</sub> + or -
17	>16	>16	+	-
1	8	>16	+	-
6	<0.25	<0.25	-	-
* <i>bla</i> <sub>OXA23</sub>	>16	>16	+	-
** <i>bla</i> <sub>OXA58</sub>	8	4	-	+
ATCC 19606	<0.25	1	-	-

Key: +: detected; -: not detected; IMP: Imipenem; MEM: Meropenem; \*: positive control for *bla*<sub>OXA-23</sub> known *Acinetobacter* spp.; \*\*: positive control for *bla*<sub>OXA-58</sub> known *Acinetobacter* spp.

**Table 3:** Clinical characteristics and outcomes of the patients with *Acinetobacter* spp. isolates with *bla*<sub>OXA-23</sub> gene

	Clinical outcomes (n = 18)	
	Deceased, n (%) 7(39%)	Discharge n (%) 11(61%)
Antibiotics	Resistance n (%)	Resistance n (%)
Ceftazidime	7(100%)	11(100%)
Ciprofloxacin	7(100%)	11(100%)
Piperacillin-tazobactam	7(100%)	11(100%)
Imipenem	7(100%)	11(100%)
Meropenem	7(100%)	11(100%)
Amikacin	3(17%)	1(6%)
Colistin	0	0
	<i>p</i> > 0.05 (0.288)	
ICUs	5(28%)!	9(50%)!
Non-ICUs	2(11%)	2(11%)
	<i>p</i> < 0.05 (0.001)!	
	<i>p</i> > 0.05 (0.515)	
Sterile specimens	5(28%)	6(33%)
Non-sterile specimens	2(11%)	5(28%)
	<i>p</i> > 0.05 (0.417)	
Monotherapy	3(17%)	5(28%)
Combination therapy	4(22%)	6(33%)
	<i>p</i> > 0.05 (0.648)	
*Horizontal transfer (n = 14; 7 pairs)		
**Both in one pair	4(22%)	6(33%)
***One patient in one pair	2(11%)	2(11%)
	<i>p</i> > 0.05 (0.643)	

Key: \*: PFGE typing showed horizontal transfer that strains were related from no more than two patients and thus no outbreak occurred during the study period.

\*\*\*: Among the 7 pairs of patients, 2 pairs in deceased and 3 pairs in discharged.

\*\*\*: one patient in each pair, in deceased and in discharged.

! *p* < 0.05: the outcomes in ICU was statistically significance 28% in ICU deceased whereas 50% survived.

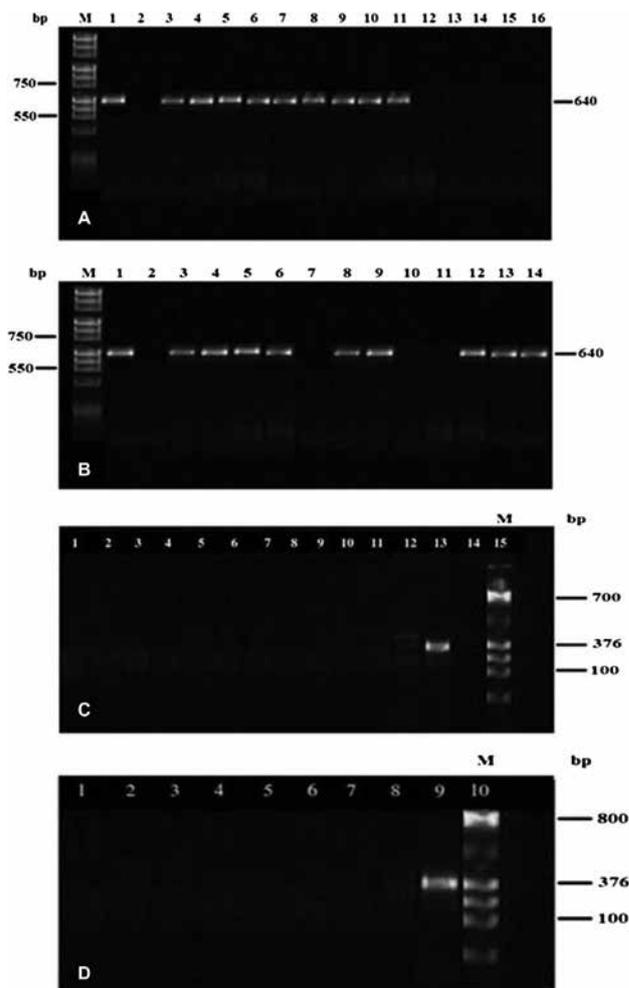
Sterile specimen (BC, blood culture; CSF, cerebrospinal fluid); Non-sterile specimen (ETA, endotracheal aspirate; pus; CT, catheter tip); ICUs (N-ICU, Neonatal intensive care unit; ICUT, intensive care unit trauma); Non-ICUs (LW, Labour ward; Neurosurgical ward; High Care unit, Surgical unit, Vascular unit).

### Sequencing finding of *bla*<sub>OXA-23</sub> amplicon

Sequencing of the *bla*<sub>OXA-23</sub>-like amplicon of 18 *Acinetobacter* spp. isolates revealed 100% identity with that from the GenBank database

### Correlation between PCR results and MICs of MDR *Acinetobacter* spp. (n = 18)

Table 2 shows the MICs of the eighteen MDR *Acinetobacter* spp. isolates, correlated with the presence of the CHDL genes. The *bla*<sub>OXA-23</sub> gene was detected in all the isolates which were phenotypically resistant to carbapenems, with MICs of 8 to > 16 µg/ml for imipenem and meropenem. This gene was not detected in the four carbapenem sensitive strains tested (Figure 1A).



**Figure 1 A, B, C and D:** Detection of the Oxacillinase genes (*bla*<sub>OXA-23</sub> and *bla*<sub>OXA-58</sub> genes) in *Acinetobacter* spp. in a 1.5% agarose gel following amplification by PCR. Phenotypic resistant *Acinetobacter* spp. (MDR *Acinetobacter* spp., 18 isolates). Key: Figure 1A: *bla*<sub>OXA-23</sub> amplification product of 640 bp. M, molecular weight marker (mw); lane 1, positive control (PC); lane 2, negative control (NC); lanes 3-11, phenotypic resistant *Acinetobacter* spp. (9 isolates); lanes 12-15, (four isolates were phenotypic sensitive *Acinetobacter* spp.) lane 16, ATCC 19606 isolate was included. Figure 1B: *bla*<sub>OXA-23</sub> amplification product of 640 bp. M, molecular weight marker (mw); lane 1, positive control (PC); Lane 2, negative control (NC); Lanes 3-6, 8, 9 and 12-14, phenotypic resistant *Acinetobacter* spp. (9 isolates); Lanes 10, 11 (two isolates phenotypic sensitive *Acinetobacter* spp.); Lane 7, ATCC19606 isolate was included. Figure 1C *bla*<sub>OXA-58</sub> amplification product of 376 bp. Lanes 1-7, phenotypic MDR *Acinetobacter* spp., lane 8, ATCC19606, Lane 9, positive control; lane 10, molecular weight markers. Figure 1D: *bla*<sub>OXA-58</sub> amplification product of 376 bp. Lanes 1-11, phenotypic MDR *Acinetobacter* spp. (*bla*<sub>OXA-58</sub> gene was not detected) lanes 13, positive controls; lane 14, negative control; Lane 15, molecular weight marker (376 bp); ATCC 19606 isolate was included.

### Genotypic relationship between clinically selective *Acinetobacter* spp. determined by PFGE (n = 20)

Twenty *Acinetobacter* spp. isolates (including controls) were selected according to clinical characteristics for genotyping using PFGE (Figure 2).<sup>37</sup> The control isolates (ATCC 19606) in lane 26, lane 27 (OXA-23) and lane 28 (OXA-58) showed a fingerprinting pattern different to the rest of the isolates, indicating that the technique was suitably discriminatory for the investigation of their spread (Table 4).



**Figure 2:** Pulsed field gel electrophoresis results of the OXA-23 producing *Acinetobacter* spp. Key: Lane 26: ATCC 19606; Lane 27: OXA-23, lane 28: OXA-58 strains controls lanes 2, 14, 29, ladders (*Salmonella braenderup* H9812 used for the DNA ladder); lanes 3-13, isolates 13, 15, 16, 17, 24, 25, 29, 30, 19, 21, 26; lanes 20, isolate 6 (failed); lanes 21- 25, isolates 11, 12, 10, 2, 18.

**Table 4:** PFGE typing interpretation ( $n = 20$ )

No. of pairs (patients)	Typical no. of fragment difference (pulsotypes)	Admission date	Specimens	Wards	Epidemiologic interpretation / Comments*
1	0	Within a week	BC-CSF	ICU2A-ICU2 B	Indistinguishable horizontal transfer
1	0	Same date	CSF-ETA	ICU2A	Indistinguishable horizontal transfer
1	1	Within a week	BC- BC	ICU2B- NICU	Closely related horizontal transfer
2	2	Same date	BC- CVPtip CSF-Pus	ICU2A x 2**	Closely related horizontal transfer
1	2	Same date	ETA-Pus	ICUT	Closely related horizontal transfer
1	3	Within a week	ETA-ETA	VU	Closely related horizontal transfer
4	5	Within a week	CVPtip-CSFCVPtip-BCCVPtip-PusCVPtip-ETA	ICU2A x 4**	Possible related
4	6	Within a week	BC-PusBC-ETA	ICU2A-ICUTICU2A-VU x 2**ICU2B-NICU	Possible related
7	$\geq 7$	>one week	ETA-ETAETA-CSFETA-PusCSF-Pus	ICU2A- NICUICU2A-VU x 4**VU-VU, VU-ICUT	Unrelated

Key: PFGE typing interpretation according to Tenover criteria.<sup>36</sup>

\*One pair (2 patients) (horizontal transfer between 2 patients/one pair).

\*\*Pairs; 2 pairs of patients in ICU2A and horizontal transfer in each pair, possible related in each pair x 4 pairs in ICU2A, possible related in each pair x 2 pairs in ICU2A and VU, unrelated in each pair x 4 pairs; VU, vascular unit; BC, Blood culture.

CSF, cerebrospinal fluid, ETA, endotracheal aspirate; CVP tip, central venous pressure line tip.

Two clusters consisting of two isolates each (30 and 19 in lanes 10 and 11) and (24 and 25 in lanes 7 and 8) were indistinguishable and identified as horizontal transfer between each pair (Table 4; Figure 2). According to the Tenover criteria,<sup>36</sup> isolates that demonstrate no band differences are regarded as indistinguishable, and 2–3 band differences are closely related, and are therefore most likely related to each other and interpreted as horizontally transmitted. Isolates with 4–6 band differences are possibly related. The interpretation of PFGE typing results according to the Tenover criteria are shown in Table 4. Based on this interpretation, MDR *Acinetobacter* spp. were most likely horizontally transferred among seven pairs of patients: six pairs in ICUs and one pair in the Vascular unit (VU).

### Correlation between clinical characteristics and laboratory results (n = 44)

Of the forty-four stored MDR *Acinetobacter* spp. isolates, thirty-eight (86%) and forty-four (100%) were sensitive to amikacin and colistin respectively (Table 5). The MICs of both imipenem and meropenem against OXA-23 carrying *Acinetobacter* spp. were high (8 to > 16 µg/ml) (Table 2). The clinical characteristics and outcomes of patients with *Acinetobacter* spp. producing *bla*<sub>OXA-23</sub> gene are shown in Table 3. Fourteen (78%) of the eighteen strains showed identical antibiograms sensitive to amikacin and colistin. Four (22%) showed the same phenotypic antibiogram and were only sensitive to colistin, therefore defined as XDR. The fourteen (78%) colistin and amikacin sensitive isolates were obtained from patients in ICUs and the remaining four (22%) from non-ICUs.

Eleven (61%) of the eighteen OXA-23 carrying MDR *Acinetobacter* spp. cultured were from sterile sites, namely blood cultures and CSF, while seven (39%) were from non-sterile sites such as ETA, pus, catheterised urine and catheter tip.

Eight (45%) patients were treated with monotherapy and ten (55%) with combination therapy. Eleven patients (61%) were discharged. The seven (39%) patients who demised were mostly admitted at ICUs among the patients with OXA-23-carrying *Acinetobacter* spp. (Table 3). Of the seven pairs of patients from whom the horizontally transferred strains were cultured from ICUs (six pairs) and the vascular unit (one pair), both in two pairs demised and three pairs were discharged. Of the remaining two pairs of patients, one of pair demised and one of other pair was discharged (Table 3).

The results clearly indicate there are several pulsotypes of *Acinetobacter* spp. within wards studied at IALCH (Table 4). There were four distinct pulsotypes identified suggested that there were horizontal transfer of organisms between the ICUs (NICU, ICU2A and ICU2B), horizontal transfer of the organisms between ICUs (ICUT and ICU2A and within ICU2A itself) and there was horizontal transfer of organisms between the ICU2A and the non-ICU (Vascular unit) ward. There were several isolates which were unrelated to the four main pulsotypes. No ascertain could be made regarding how they were acquired in the patients. However, the PFGE typing showed diversity in these collection of MDR *Acinetobacter* spp. clones, where isolates were related from not more than two patients.

### Discussion

All tested *Acinetobacter* spp. (n = 44) were MDR, defined in the current study as resistant to three or more classes of drugs or carbapenem,<sup>27</sup> and inclusive of XDR, defined as MDR *Acinetobacter* spp. resistant to all agents except colistin. Previous studies confirmed that *Acinetobacter* spp. are resistant to many antibiotics,<sup>2-4, 12, 13, 38</sup> and susceptibility tests revealed that the strains displayed the colistin-only-sensitive (COS) profile. Multidrug-resistance of *Acinetobacter* spp. is a major challenge and treatment options for these infections are limited.

Table 5: MICs of appropriate antibiotics (n = 44)

No. of isolates (n = 44)	MICs µl/mL							Specimen	Ward
	TZP	AK	CAZ	CIP	IMP	MEM	CST		
21	>128	16	64	>4	>16	>16	<0.5	BC	ICU 2A,2B
3*	>128	>64	64	>4	>16	>16	<0.5	ETA	ICU2A
3	>128	8	64	>4	>16	>16	<0.5	BC	ICU2B
2	>128	8	16	>4	>16	>16	<0.5	TIP	ICU2B
1	>128	4	8	>4	>16	>16	<0.5	BC	ICU2B
1	>128	8	8	2	>16	>16	<0.5	ETA	ICU2A
1	>128	16	64	>4	8	>16	<0.5	ETA	ICU2A
2	>128	<2	64	>4	>16	>16	<0.5	PDF	ICUT
1	>128	8	8	>4	>16	>16	<0.5	PUS	ICUT
1	>128	16	64	>4	8	>16	1	URINE	ICUT
1*	>128	64	64	>4	8	>16	1	PUS	Non-ICU
2	>128	8	32	>4	>16	>16	<0.5	CSF	Non-ICU
2*	>128	64	64	>4	>16	>16	<0.5	BC	Non-ICU
1	>128	16	2	>4	8	>16	<0.5	PF	Non-ICU
1	>128	16	8	<0.25	>16	>16	<0.5	ETA	Non-ICU
1	>128	4	64	>4	>16	>16	<0.5	FLUID	Non-ICU

Key: TZP, piperacillin-tazobactam; AK, amikacin; CAZ, ceftazidime; CIP, ciprofloxacin.

IMP, imipenem; MEM, meropenem; CST, colistin.

\*, XDR-*Acinetobacter* spp. (resistant to all except colistin).

Non-ICUs (High Care unit, Vascular unit, Labour ward, Neurosurgery unit, Orthopaedic unit).

This study has demonstrated that carbapenem drugs are no longer active against selected MDR *Acinetobacter* spp. at local setting (Inkosi Albert Luthuli Academic Complex hospital). The data revealed isolates with high susceptibility to amikacin (86%) and colistin (100%) among the forty-four tested MDR *Acinetobacter* spp. isolates. Therefore, these agents are the mainstay, last resort antibiotics for MDR *Acinetobacter* spp. in the local setting. In this current situation, there are no standardised guidelines for the management for MDR- and XDR-*Acinetobacter* spp. infection.

*Acinetobacter* spp. isolates with the same sensitivity pattern in the antibiograms were selected for molecular investigation, namely four XDR and fourteen MDR isolates. A group of (*bla*<sub>OXA-23</sub> encoded) OXA-23-carbapenemase-producing MDR and XDR *Acinetobacter* species were detected in MDR *Acinetobacter* spp., but no sensitive isolates were identified at ICUs in an academic complex.

The *bla*<sub>OXA-23</sub> gene is believed to be responsible for the mechanism of carbapenem antibiotic resistance in *Acinetobacter* spp.<sup>39</sup> The OXA-23 gene of carbapenem resistant *Acinetobacter* spp. was first reported in 1985 in Scotland<sup>40</sup> and subsequent outbreaks of OXA-23-producing *A. baumannii* occurred in various locations around the world.<sup>39,40</sup>

MDR *Acinetobacter* spp. represents a high-risk global and local infection control challenge.<sup>40</sup> In one study, the gene encoding OXA-23 was found in plasmids, facilitating its spread among *Acinetobacter* species.<sup>14</sup> In this study, the spread of *bla*<sub>OXA-23</sub> carried *Acinetobacter* species was demonstrated by PFGE typing. These findings suggest horizontal transfer between the pairs of patients in ICUs and the vascular unit (Table 4). Moreover, it should be noted that an isolate in the neurology ward adjacent to the ICU unit showed possible relatedness. Thus, it is possible that the establishment of clones in different wards does not account for clonal transmission in hospitals.

The OXA-23 producing MDR *Acinetobacter* spp. isolates were cultured from both sterile and non-sterile clinical specimens, hence the recommendation for aseptic handling of specimen collection. In addition, hand washing practices need to be audited due to this investigation confirming the dramatic rise of multiple clones with *bla*<sub>OXA-23</sub> producing MDR *Acinetobacter* spp. and to control the spread in the local setting. Awareness of the development and existence of drug resistant organisms plays a crucial role in optimising infection control practices, establishing antimicrobial stewardship programs, and establishing active regional surveillance systems. This study also has shown that a correlation between the MDR phenotype and genes related to carbapenem resistance. MDR *Acinetobacter* spp. with resistant MICs to either of the carbapenem agents may be associated with isolates producing the plasmid-mediated or chromosome-mediated gene encoding *bla*<sub>OXA-23</sub>.

During this study time, the antibiograms of all these isolates were phenotypically identical for more than three strains in ICUs. However, the PFGE typing demonstrated a diversity in the MDR *Acinetobacter* spp. clones, which suggests that isolates were related from not more than two patients per cluster; and, therefore, no outbreak had occurred based on the PFGE typing interpretation. Therefore, isolates with the same antibiogram never-the-less need to be typed genotypically and, at the very least, their MIC values should be checked.

According to data analysis of clinical characteristics, patient outcomes and laboratory data, the clinical outcomes had no association with the following factors: resistance patterns of

*Acinetobacter* spp. cultured, ICU versus non-ICU wards, sterile sites versus non-sterile sites, and monotherapy versus combination therapy. There was no statistical significance ( $p > 0.05$ ) in each analysis (Table 3). This may be due to the small sample size, which is the main limitation of our study. Continuous surveillance of antibiotic resistance genes in MDR *Acinetobacter* spp. is crucial for epidemiological purposes and to prevent further dissemination of these genes. In addition, it is necessary to monitor the clinical prevalence and spread of antibiotic resistance genes associated with *Acinetobacter* spp. Future research should include the detection of other resistance genes, as well as determining the genetic relatedness of *Acinetobacter* spp. isolates in other hospitals in KZN.

In conclusion, this study discovered that the main carbapenem resistance mechanism of *Acinetobacter* spp. was due to OXA-23 carbapenemase activity. Although the isolates were spread in ICUs and other ICU related units, there did not seem to be an outbreak according to the demographic clinical data, MICs and PFGE typing (Table 4). Therefore, it is likely that no outbreak had occurred.

This is the first report on epidemiological and molecular observations of *Acinetobacter* spp. with the detection of the *bla*<sub>OXA-23</sub> gene in MDR *Acinetobacter* species. Molecular typing of the selected isolates showed that MDR *Acinetobacter* species carried the *bla*<sub>OXA-23</sub> gene responsible for resistance to carbapenems (MICs 8 to > 16 µg/ml). The outcomes provided support for local infection prevention and control management guidelines as part of the antibiotic stewardship programme. Continued molecular surveillance of local epidemiological information and antibiotic resistance surveillance are crucial for infection prevention and control, and is essential in the standard management at the hospital.

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