Amikacin-resistant *Acinetobacter* species mediated by the *aph*A6 gene associated with clinical outcome at an academic complex hospital in KwaZulu-Natal Province, South Africa

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Background. Drug-resistant *Acinetobacter* species present serious therapeutic and infection control policy challenges globally. Although aminoglycosides have played a crucial role in the treatment of infections with multidrug-resistant (MDR) *Acinetobacter* spp., recent reports indicate that these bacteria are developing resistance to aminoglycosides around the globe.

Objectives. To determine the association between amikacin resistance and clinical outcomes of patients. The minimum inhibitory concentrations (MICs) of amikacin against *Acinetobacter* spp. and genes associated with resistance were also investigated.

Methods. Clinical information from 107 patients with *Acinetobacter* spp. cultured from clinical specimens was recorded during ward rounds at an academic complex hospital in KwaZulu-Natal Province, South Africa, including clinical outcomes, history of antibiotics prescribed and microbiological investigations. The 107 *Acinetobacter* isolates were investigated for susceptibility to antimicrobial agents in use at local hospitals. Genes related to amikacin resistance (*aph*A6 and *aac*A4) were investigated by polymerase chain reaction (PCR) and sequencing. Analysis was performed on the relationship between clinical outcomes and antimicrobial resistance patterns, as well as on the amikacin MICs in resistant isolates (n=6) v. their PCR results.

Results. The majority (5/6, 83.3%) of patients with amikacin-resistant *Acinetobacter* infection were discharged, and 1/6 (16.7%) died. No underlying clinical factors were significantly associated with clinical outcome. Amikacin resistance was observed in 6/107 isolates (5.6%), with MICs of 32 μg/mL (n=3) and ≥64 μg/mL (n=3) for the amikacin-resistant isolates. All 6 of these isolates were also extensively drug-resistant (XDR). The *aph*A6 gene (797 base pair) was detected in all amikacin-resistant isolates.

Conclusions. Most tested *Acinetobacter* isolates were susceptible to amikacin, underscoring the crucial role of this antibiotic in the treatment of MDR *Acinetobacter* spp. in our hospital. The emergence of XDR isolates is of serious concern and necessitates close monitoring and surveillance.

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Acinetobacter species have emerged as major hospital-associated pathogens, which have evolved into multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains during the past decade.[1] *Acinetobacter* spp. have the capacity to acquire resistance to antimicrobial agents through genetic factors such as plasmids and pathogenicity islands,[2] resulting in resistant strains that are difficult to treat.[3] The Infectious Diseases Society of America has therefore declared *Acinetobacter* spp. among the six antimicrobial-resistant pathogens responsible for high morbidity and mortality.[3,4]

Although *Acinetobacter* spp. are common colonisers that may lead to community-acquired infection, they are also opportunistic pathogens often found in immunocompromised patients with prolonged hospitalisation.[5] Immunosuppressive therapy places cancer patients at risk of developing *Acinetobacter* infections that may result in sepsis, respiratory infections, wound infections and urinary tract infections.[3,6-8]

XDR *Acinetobacter* spp. are defined as being resistant to all the tested antimicrobials except colistin, whereas pandrug-resistant (PDR) isolates are resistant to all agents.[9] A rise in infections from XDR *Acinetobacter* spp. has been reported.[10,11] The global rise of MDR *Acinetobacter* spp. and the emergence of XDR *Acinetobacter* spp. therefore pose a major challenge to current treatment options and infection control.[12,13]

Until recently, amikacin was the most active aminoglycoside in the treatment of infections caused by *Acinetobacter* spp. in academic complex hospitals in KwaZulu-Natal Province, South Africa (SA). It remains the drug of choice for treatment of MDR *Acinetobacter* infections, yet resistance has increased in recent years.[14]

Acinetobacter spp. have several mechanisms of aminoglycoside resistance.[15,16] In general, the major mechanism in Gram-negative bacteria is enzymatic modification of the amino or hydrolol groups of the agent through aminoglycoside-modifying enzymes.

Amikacin is commonly used at Inkosi Albert Luthuli Central Hospital (IALCH), an academic complex hospital in Durban, KwaZulu-Natal, owing to the increasing prevalence of MDR *Acinetobacter* spp., especially for nebulisation of pneumonia and in combination with piperacillin-tazobactam for systemic infections.

Objectives

To characterise *Acinetobacter* spp. isolates and compare the clinical outcomes of infected patients with the phenotypic and genotypic characteristics of XDR *Acinetobacter* spp. at IALCH.

Methods

The study received ethical approval from the Biomedical Research Ethics Committee (BREC), College of Health Sciences, University of KwaZulu-Natal (ref. no. BE 283/12).

Study design

The study was analytical and observational experimental research that highlighted the prevalence of amikacin-resistant *Acinetobacter* spp., clinical outcomes, and association with genes *aph*A6 and *aac*A4, related to amikacin resistance.

Patients and bacterial isolates

Clinical information on 107 patients with *Acinetobacter* spp. cultured from clinical specimens was recorded during clinical ward rounds at IALCH. The information included clinical outcomes, history of antibiotics prescribed at local hospitals as part of routine management, and antimicrobial susceptibility patterns of the 107 *Acinetobacter* isolates.

The minimum inhibitory concentrations (MICs) for 60 of the 107 *Acinetobacter* isolates were investigated. Six amikacin-resistant clinical isolates of *Acinetobacter* spp. were selected for genotypic characterisation at the Microbiology Laboratory, National Health Laboratory Service, Durban.

Susceptibility testing

Susceptibility testing was performed using the VITEK 2 automated system (BioMérieux, France) with the VITEK 2 GN ID card and the VITEK 2 AST-N255 card. The MICs of the appropriate antimicrobial agents in use were determined for 60 *Acinetobacter* isolates using the Epsilometer test (E-test) (BioMérieux, France). The MIC_{90} and MIC_{50} were determined for each antibiotic agent tested against the 60 isolates. The antibiotics included amikacin, carbapenems (imipenem, meropenem), ceftazidime, ciprofloxacin, colistin and piperacillin-tazobactam. *Acinetobacter* ATCC 19606 was used as the quality control strain. The results were interpreted according to the Clinical and Laboratory Standards Institute.[17] An MIC >32 µg/mL for amikacin was considered to indicate resistance.[17]

Polymerase chain reaction and sequencing

Genomic DNA from each of 13 isolates, comprising 6 clinically amikacin-resistant strains, 3 controls and 4 known sensitive clinical isolates, was extracted using a previously described method.[18]

The presence of the genes related to amikacin resistance (*aph*A6 and *aac*A4) was further investigated by polymerase chain reaction (PCR). The MICs of amikacin (n=6) were compared with the PCR results of these resistant isolates and clinical outcome.

Clinical and laboratory data collection

Clinical and laboratory data on 107 patients are reported here. The data included demographics, underlying medical condition, type of specimen, exposure to antimicrobial agents before and after isolation of *Acinetobacter* spp., admission to intensive care units (ICUs) or other units, and clinical outcomes. The clinicians defined the type of infection. Patients who did not receive specific treatment for *Acinetobacter* spp. were classified as colonised. Clinical response to treatment was classified as successful in patients whose infection-defining signs and symptoms resolved and as failed for patients who deteriorated or whose signs and symptoms persisted.

Statistical analysis of the data

The data were captured, standardised and analysed using the Statistical Package for Social Sciences (SPSS), version 19 (IBM, USA). The association between underlying conditions and outcome was analysed using Pearson's χ^2 test. Logistic regression analysis was used to test for factors associated with survival status of patients.

Results

Susceptibility of *Acinetobacter* spp. isolates (*N*=107)

Six isolates (5.6%) that were resistant to amikacin were defined as XDR based on their antibiograms. Eighty isolates (80/107, 74.8%) were MDR. The rest were resistant to fewer than three different classes of tested agents and were therefore not classified as MDR (Table 1). Table 2 shows the antimicrobial MICs of 60 *Acinetobacter* isolates.

Table 1. Drug resistance patterns of *Acinetobacter* spp. isolates from clinical specimens (*N*=107)

Antibiotic susceptibility	*n* (%)
MDR *Acinetobacter* spp.	80 (74.8)
XDR *Acinetobacter* spp.*	6 (5.6)
PDR *Acinetobacter* spp.	0
Amikacin resistant*	6 (5.6)
Resistant to <3 tested agents (not MDR)	15 (14.0)
Total	107

MDR = multidrug resistant; XDR = extensively drug resistant; PDR = pandrug resistant.
*Same *Acinetobacter* spp.

Table 2. MIC_{50} and MIC_{90} of the *Acinetobacter* spp. isolates from clinical specimens (*N*=60)

			MICs (CLSI) (µg/mL)	
Antibiotics	MIC_{50}, (µg/mL)	MIC_{90}, (µg/mL)	Sensitive	Resistant
CST	0.25	0.5	<0.5	>0.5
IMP	24	>32	<1	>4
MEM	24	>32	<1	>4
TZP	>256	>256	16	>32
AK	8	16	16	>64
CIP	>32	>32	0.5	4
CAZ	>16	>16	16	>16

MIC = minimum inhibitory concentration; CLSI = Clinical and Laboratory Standards Institute; CST = colistin; IMP = imipenem; MEM = meropenem; TZP = piperacillin-tazobactam; AK = amikacin; CIP = ciprofloxacin; CAZ = ceftazidime.

Among the 6 amikacin-resistant isolates, the MICs of amikacin ranged between 32 and ≥64 µg/mL (Table 2).

Detection of *aph*A6 and *aac*A4 genes

Six cases with amikacin-resistant *Acinetobacter* spp. were identified. The clinical characteristics and outcome of those 6 patients and MICs of tested antibiotics (*n*=6) are shown in Table 3.

PCR amplification allowed for detection of the *aph*A6 gene (797 base pair (bp)) from the 6 amikacin-resistant *Acinetobacter* spp. clinical isolates (Fig. 1). However, the *aac*A4 gene (489 bp) was not present in these isolates (Fig. 2.).

Phenotypic and genotypic analysis of the amikacin-resistant *Acinetobacter* spp.: Correlation of antibiogram with *aph*A6 and *aac*A4 genes

The MICs of amikacin and other tested drugs are shown in Table 2. The 6 amikacin-resistant strains were sensitive only to colistin and therefore defined as XDR *Acinetobacter* spp. (Table 3). These 6 strains were phenotypically resistant and showed the presence of the *aph*A6 gene but not the *aac*A4 gene (Figs 1 and 2).

Demographic features, clinical characteristics and outcomes of all patients with infections due to *Acinetobacter* spp. (*N*=107)

Clinical data were analysed using simple descriptive analysis. The demographic data on patients with *Acinetobacter* infection (*N*=107) are shown in Table 4. *Acinetobacter* spp. were most commonly isolated from adult patients in non-ICU wards and in neonates or paediatric patients.

Underlying diseases

Acinetobacter spp. were cultured most commonly in adults presenting with trauma and injury, and in paediatric patients with congenitally abnormal organs. Trauma was predominant overall. Retroviral disease, cancer and other conditions showed little risk of colonisation and infection (Table 4). No statistically significant differences ($p>0.05$ (0.151)) were observed between children and adults with medical and surgical conditions with regard to the presence of *Acinetobacter* infections.

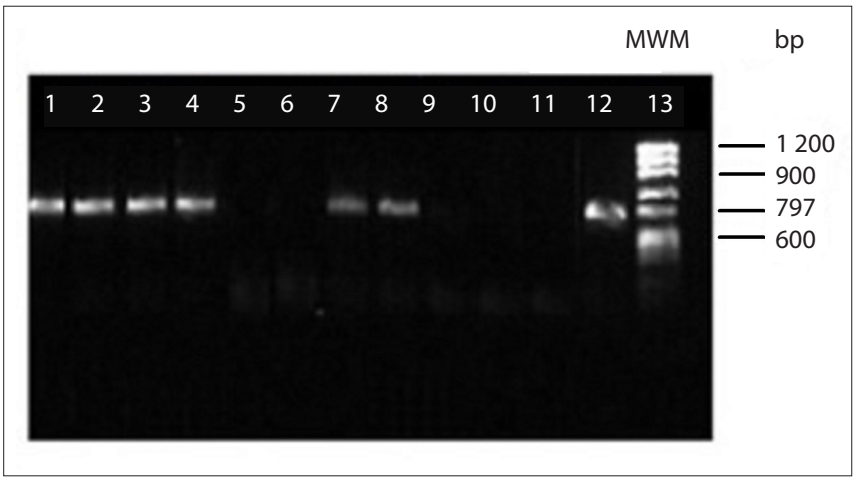

Fig. 1. Polymerase chain reaction for detection of the amikacin-resistant aph*A6 gene of* Acinetobacter *spp. Lanes 1 - 4 and 7 - 8: amikacin-resistant strains (9, 11, 15, 31, 42, 51) (*aph*A6 gene detected); lanes 5, 6, 9, 10: amikacin-sensitive strains (8, 20, 25, 60) (*aph*A6 gene bands absent); lane 11: negative control; lane 12: positive control; lane 13: MWM. (MWM = molecular weight marker; bp = base pair.)*

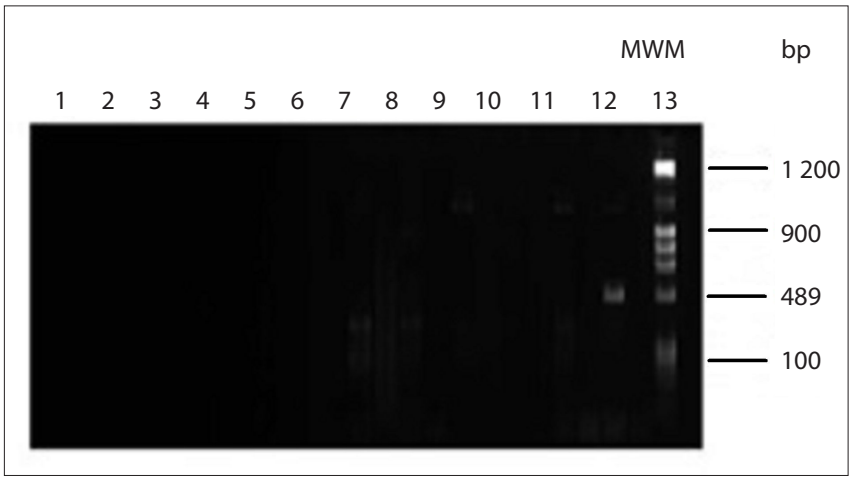

Fig. 2. Polymerase chain reaction for detection of the amikacin-resistant aac*A4 gene of* Acinetobacter *spp. Gene absent in all tested isolates. Lanes 1 - 11: isolates; lane 12: positive control; lane 13: MWM. (MWM = molecular weight marker; bp = base pair.)*

Table 3. Patients' clinical characteristics and outcome, and MICs of other antimicrobial agents tested against amikacin-resistant *Acinetobacter* spp. (*N*=6)

	MIC (µg/mL)											
Isolates	IMP	MEM	AK	TZP	CAZ	CIP	CST	Ward	Specimen	Days in hospital	Treated with	Outcome
AK-R	>16	>16	>64	>128	64	>4	<0.5	LW	BC	15	TZP + AK/MEM + CST	Discharged
AK-R	>16	>16	>64	>128	64	>4	<0.5	ICU	Pus	23	TZP + AK	Died
AK-R	>16	>16	>64	>128	64	>4	<0.5	ICU	ETA	28	CST	Discharged
AK-R	>16	>16	32	>128	64	>4	<0.5	HCU	BC	35	CST	Discharged
AK-R	8	>16	32	>128	64	>4	1	PU	Pus	43	None	Discharged
AK-R	>16	>16	32	>128	64	>4	0.5	VU	Pus	29	None	Discharged

MIC = minimum inhibitory concentration; AK-R = amikacin resistant; IMP = imepenem; MEM = meropenem; AK = amikacin; TZP = piperacillin-tazobactam; CAZ = ceftazidime; CIP = ciprofloxacin; CST = colistin; LW = labour ward; ICU = intensive care unit; HCU = high-care unit; PU = plastic unit; VU = vascular unit; BC = blood culture; ETA = endotracheal aspirate; None = no antibiotics given.

Table 4. Demographic and clinical data on patients with *Acinetobacter* spp. cultured from clinical specimens (*N*=107)

	<1 year (*N*=20), *n*	Paediatric >1 year (*N*=8), *n*	Adult (*N*=79), *n*	*p*-value
Sex				
Male	12	6	46	
Female	5	1	31	
NA	3	1	2	
Ward				
ICU, paediatric	5	1	-	
Paediatric surgery	1	1	-	
Neonatal	14	-	-	
Paediatric oncology	-	2	-	
Paediatric medical unit	-	1	-	
Trauma	-	2	-	
NA	-	1	6	
ICU, adult	-	-	18	
Non-ICU	-	-	55	
Underlying disease				0.151 (>0.05)
RVD	5	-	7	
Abnormal organ (congenital)	10	-	-	
Respiratory disease	2	1	-	
Sepsis	3	-	-	
Cancer	-	2	3	
Surgical	-		17	
Medical	-	2	20	
Injury/trauma	-	3	32	
Antibiotic history				0.018 (<0.05)
CZT	1		11	
CZT + combination	-		1	
AK (nebulisation)	1	2	11	
Others (TZP, CIP, MEM)	17	4	30	
No antibiotics given	1	2	26	
Outcome				0.942 (>0.05)
Discharged (67/107, 62.6%)	10	8	49	
Died (23/107, 21.5%)	6	-	17	
NA (17/107, 15.9%)	4	-	13	

NA = not available; ICU = intensive care unit; RVD = retroviral disease; CZT = colistin; AK = amikacin; TZP = piperacillin-tazobactam; CIP = ciprofloxacin; MEM = meropenem.

Antibiotic use

Tazocin (piperacillin-tazobactam), ciprofloxacin and meropenem were used in most cases. Colistin monotherapy and colistin combinations were not commonly used. Analysis revealed that infections with *Acinetobacter* spp. were treated mostly with a piperacillin-tazobactam and amikacin combination, while for XDR strains colistin monotherapy or other combinations were used according to the specific characteristics of individual cases (Table 4). Use of colistin, combinations and amikacin differed significantly between adult and paediatric patients ($p<0.05$ (0.018)).

Clinical outcome

The majority of the patients (67/107, 62.6%) were discharged, but mortality was high at 21.5% ($n=23$) (Table 4). Clinical outcome was not significantly associated with age ($p>0.05$ (0.942)).

Clinical characteristics and outcomes of patients infected with amikacin-resistant *Acinetobacter* spp. (*n*=6)

All 6 patients with amikacin-resistant *Acinetobacter* infections were hospitalised in different units for >2 weeks (21 - 43 days) with chronic illness (Table 3). Two isolates were obtained from blood culture, 3 from pus swabs and 1 from an endotracheal aspirate. Two patients with significant *Acinetobacter* infections were treated with colistin, while 2 with colonisation received no antibiotics. One of the 6 died, and 5 recovered and were discharged (Table 4).

Discussion

Despite *Acinetobacter* spp. being classified by the Infectious Diseases Society of America a decade ago as one of the six most important MDR micro-organisms in hospitals worldwide,[3,4,19] drug-resistant *Acinetobacter* spp. still present a serious therapeutic and infection control challenge. Increasing antimicrobial resistance among *Acinetobacter*, resulting in the evolution of XDR and PDR strains, has been documented globally.[12]

The present study revealed the presence of amikacin-resistant *Acinetobacter* spp. at IALCH, with 6 (5.6%) of 107 isolates being amikacin resistant and sensitive only to colistin, defined as XDR *Acinetobacter* spp.

In our setting, amikacin is commonly used with piperacillin-tazobactam as a second-line treatment option and amikacin nebulisation for pneumonia cases as general antibiotic policy. Fortunately, 101 (94.4%) of 107 *Acinetobacter* spp. isolates were

highly sensitive to amikacin. In the past, aminoglycosides have played a crucial role in the treatment of infections with MDR *Acinetobacter* spp. However, Lee *et al.*[20] reported that *Acinetobacter* were developing resistance to aminoglycosides around the globe. The current study showed that amikacin-resistant *Acinetobacter* isolates at IALCH carried the *aph*A6 gene but not the *aac*A4 gene. At 5.6%, the prevalence was significantly lower in our local setting than in Korea, according to the 2009 Korean Nationwide Surveillance on Antimicrobial Resistance (KONSAR) study, where amikacin-resistant *Acinetobacter* spp. increased to 48%.[20]

Our data analysis identified a potential emerging challenge to treatment and clinical management that was elucidated by phenotypic and genotypic characterisation of *Acinetobacter* spp. The study highlights the crucial role of standard amikacin use, as can be seen by the MIC_{50} and MIC_{90} of amikacin within the sensitive range, while the MIC_{50} and MIC_{90} of imipenem, ciprofloxacin, ceftazidime and piperacillin-tazobactam in the tested isolates were within the highly resistant range (Table 2).

Treatment of MDR *Acinetobacter* spp. infection usually requires the use of appropriate drugs such as piperacillin-tazobactam plus amikacin, ciprofloxacin, ceftazidime, carbapenem, colistin and tigecycline based on the local antibiogram or individualised microbiological results. Infections with *Acinetobacter* spp. were mostly treated with piperacillin-tazobactam plus amikacin, whereas colistin monotherapy or combinations were used for XDR *Acinetobacter* spp. according to the individual case.

Previous studies[1,21,22] have reported MDR *Acinetobacter*-associated sepsis to be most common in ICU patients. The present study showed that *Acinetobacter* infections were common in both non-ICU and ICU wards. Infections in the ICU were mainly associated with trauma cases. All isolates were cultured from the specimens after 21 - 43 days of hospitalisation and prior to amikacin exposure.

Infection with *Acinetobacter* spp. was most prevalent in patients aged 25 - 60 years, and in non-ICU, trauma and postoperative paediatric units. Trauma cases were predominant overall, because *Acinetobacter* spp. are part of the skin flora and an environmentally acquired organism. Moreover, in the present study, patients in the academic hospital with retroviral disease, cancer and other clinical conditions were not prone to colonisation and infection, possibly because of strict infection prevention and control measures in all high-care units.

The majority of the 107 patients were treated with antibiotics such as piperacillin-tazobactam, amikacin, ciprofloxacin and meropenem according to the local protocol. However, colistin monotherapy, drug combinations and the combination of amikacin with tazocin were used significantly more often in adult patients than paediatric patients ($p<0.05$ (0.018)). Infection with XDR *Acinetobacter* spp. was treated with colistin monotherapy or combinations according to the individual case based on consultation between the clinician and the microbiologist. Our study highlighted that colistin is a key therapeutic option for the treatment of infections with XDR *Acinetobacter* spp. This finding also indicates the need to enhance infection prevention and control measures and antibiotic stewardship programmes.

As far as we are aware, this study is the first to describe detailed clinical and molecular characteristics of amikacin-resistant *Acinetobacter* spp. at IALCH, a public academic hospital in KwaZulu-Natal. Molecular analysis suggested a potential mechanism of amikacin resistance to be the presence of the *aph*A6 gene.

Underlying clinical diseases were not significantly associated with clinical outcome in patients with *Acinetobacter* spp. infections.

A surveillance report for 2016 from the SA private sector[23] showed that 47% and 37% of *A. baumannii* isolates were non-susceptible to the aminoglycosides gentamicin and amikacin, respectively. Although the proportion resistant to amikacin has increased in private hospitals, the above study did not include molecular analysis. Molecular epidemiological studies are required when investigating transmission dynamics, which will in turn inform intervention strategies to prevent the spread of drug-resistant strains. Infection prevention and control should also aim to identify reservoirs and sources of infection to recognise and prevent further spread of MDR, XDR and PDR *Acinetobacter* spp.

Declaration. None.

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Conflicts of interest. None.

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