

# Evaluation of Five Susceptibility Test Methods for Detection of Tobramycin Resistance in a Cluster of Epidemiologically Related *Acinetobacter baumannii* Isolates

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*Acinetobacter baumannii* is a major nosocomial pathogen causing infections in critically ill patients. This organism has acquired the propensity to rapidly develop resistance to most antibiotics. At several hospitals within Cape Town, South Africa, tobramycin and colistin are frequently the only therapeutic options. Vitek2 automated susceptibility testing (AST) is used in the clinical laboratory to determine selected susceptibility profiles. The suspicion of a possible AST-related technical error when testing for susceptibility to tobramycin in *A. baumannii* precipitated this study. Thirty-nine *A. baumannii* strains isolated from clinical specimens (June to December 2006) were included in this prospective study. Tobramycin susceptibility testing results obtained by AST, disc diffusion, the epsilometer test (Etest), and agar dilution were compared to those for broth microdilution (BMD), the reference method. The tobramycin susceptibility results revealed errors in 25/39 (64%) isolates (10 very major and 15 minor errors) when AST was compared to BMD, 12/39 (31%) (2 very major and 10 minor errors) when Etest was compared to BMD, 16/39 (41%) (3 very major and 13 minor errors) when disc diffusion was compared to BMD, and 21/39 (54%) (10 very major and 11 minor errors) when agar dilution was compared to BMD. Using PCR, we detected *aac(3)-IIa*, which is associated with tobramycin resistance, in 21/25 of the discrepant isolates. Molecular typing (using pulsed-field gel electrophoresis and repetitive sequence-based PCR [rep-PCR]) showed that these isolates were genetically related. Clinical laboratories that routinely use the Vitek2 system should consider an alternative testing method for determining susceptibility to tobramycin.

*Acinetobacter baumannii* has gained increased notoriety as a highly resistant nosocomial pathogen globally. This organism has been associated with infections in immunocompromised and debilitated patients, particularly in the intensive care unit (ICU) setting (1, 2).

*A. baumannii* has the capacity for long-term survival in the hospital environment (3, 4). In addition, its remarkable capacity to acquire resistance has prompted its classification as a high-priority pathogen by the Antimicrobial Availability Task Force of the Infectious Diseases Society of America (5). Pandrug-resistant phenotypes have been isolated in many settings (6–10). In the Western Cape, South Africa, *A. baumannii* is endemic in many hospital ICUs. The majority of these organisms are multidrug resistant, retaining susceptibility to only tobramycin and colistin. Of the *A. baumannii* strains isolated from blood cultures during 2006 in the diagnostic laboratory at Groote Schuur Hospital, only 55% and 57% remained susceptible to imipenem and meropenem, respectively (our unpublished data).

The pressure on clinical diagnostic laboratories to produce rapid identification and susceptibility profiles has resulted in increasing use of automated microbiology systems, such as Vitek2 (bioMérieux, Marcy l'Etoile, France). Although there are many advantages to the use of this technology, several studies have indicated inaccurate susceptibility results ranging from false resistance to false susceptibility, especially when testing nonfermenting Gram-negative bacteria such as *A. baumannii* (11–20). These inaccuracies have a major impact on patient management as they may encourage the use of inactive antimicrobials in critically ill patients. In addition, they promote the use of broader-spectrum antibiotics if narrow-spectrum drugs are falsely reported as resistant.

A discrepancy between tobramycin susceptibility testing using manual methods and the Vitek2 automated susceptibility testing (AST) method alerted the clinical diagnostic laboratory to a possible technical error, thus precipitating this study. We conducted a prospective study to investigate the accuracy of tobramycin susceptibility testing in *A. baumannii* in comparison to validated susceptibility test methods. In addition, we hypothesized that the resistance was due to the aminoglycoside modifying enzyme, AAC(3)-IIa (aminoglycoside acetyltransferase), as the gene encoding this enzyme was previously isolated from clinical isolates of *A. baumannii* at our institution (21). The clonal relatedness of these isolates was also investigated.

## MATERIALS AND METHODS

**Bacterial strains.** A total of 39 nonrecurring clinical isolates of *A. baumannii* were tested. These isolates were obtained from five hospitals in Cape Town, South Africa (Groote Schuur Hospital, Red Cross War Memorial Children's Hospital, Victoria Hospital, Mowbray Maternity Hospital, and GF Jooste Hospital) over a 7-month period (June 2006 to December 2006). The Groote Schuur (893 beds) and Red Cross hospitals (240 beds) are tertiary academic hospitals with superspecialist services, while the Victoria (158 beds) and GF Jooste hospitals (224 beds) are secondary-level hospitals offering general specialist care. Mowbray hospital

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(179 beds) is a secondary-level maternity hospital with tertiary level neonatal care.

The isolates were obtained from the following clinical specimens: 14 tracheal aspirates, seven sputum samples, seven pus swabs (including three swabs collected during environmental screening of two different ICUs during an outbreak investigation), four blood cultures, four urine specimens, two tissue cultures, and one fluid culture. The majority of the isolates were obtained from clinical specimens from patients in ICUs at the various hospitals (32/39). Isolates were selected which had zone diameters or MICs (as determined by Vitek2) in each of the different susceptibility categories. Fourteen isolates had MICs close to the susceptible/intermediate breakpoints (as defined by the Clinical and Laboratory Standards Institute [CLSI] [22]). The breakpoints are as follows: for zone diameters, susceptible  $\geq 15$  mm, intermediate 13 to 14 mm, and resistant  $\leq 12$  mm, and for MICs, susceptible  $\leq 4$   $\mu\text{g/ml}$ , intermediate 8  $\mu\text{g/ml}$ , and resistant  $\geq 16$   $\mu\text{g/ml}$  (22). Identification and susceptibility testing, with the exception of agar dilution, broth microdilution (BMD), and PCR, were performed on freshly isolated organisms passaged twice on 2% blood agar plates (Greenpoint Media Laboratory, NHLS). The isolates were stored on beads (Viabank VIM tubes, Abtek Biologicals Ltd.) at  $-70^\circ\text{C}$  while awaiting the remaining susceptibility and molecular testing.

**Identification.** All strains were identified by the Vitek2 Gram-negative identification card (bioMérieux, La Balme, France) according to the manufacturer's instructions at 0.6 McFarland (McF). A percent probability greater than 90% was considered an acceptable identification.

**Susceptibility test methods.** To ensure that there were no effects of agar pH and cation concentration on the aminoglycoside susceptibility testing, the susceptibility levels of all isolates were tested by disc diffusion (Oxoid, Basingstoke) and Etests (AB Biodisk, Solna, Sweden) using cation-adjusted Mueller-Hinton medium from three commercial manufacturers (bioMérieux [Randburg, South Africa], Bio-Rad Laboratories [Johannesburg, South Africa], and Greenpoint NHLS Media Laboratory [Cape Town, South Africa]) at 0.5 McF per CLSI and manufacturer's guidelines, respectively (23). These tests were performed with inoculum from the same subculture. In addition, automated susceptibility testing using the Vitek2 NO-22 susceptibility card (bioMérieux, La Balme, France) was performed at 0.6 and 1.0 McF, respectively. Although 1.0 McF is not a recommended standard for performing aminoglycoside susceptibility testing, we hypothesized that a heavier inoculum would favor expression of resistance (24). To prevent editing of MICs on the basis of other inferred resistance mechanisms, the advanced expert system (software version 2.01) was not used to analyze the raw antibiotic susceptibility data obtained. To ensure reproducible results, agar dilution and BMD were performed on the isolates in duplicate by two different scientists using cation-adjusted Mueller-Hinton medium at 0.5 McF (25). This testing was performed at the Research and Development Division of bioMérieux (La Balme, France) because the local diagnostic laboratory did not have the experience to carry out this testing. Results were interpreted using CLSI criteria (22). Broth microdilution was used as the reference method because it most closely simulated the methodology used by Vitek2.

Quality control organisms included *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922, as recommended by CLSI and the manufacturer.

**Interpretation of susceptibility results.** Discordant results with the reference method (BMD) were classified as very major errors (VMEs), major errors, or minor errors (mEs). The interpretative category errors that were used were defined as follows: VME (isolate resistant by reference method but susceptible by test method), major error (isolate susceptible by reference method but resistant by test method), and mE (difference between reference method and test method differs by 1 interpretative category). For disc diffusion, the mean of the zone diameters obtained from all three media was used in the analysis. For Etests, the mode of the MICs obtained from all three media was used for comparison with BMD. For agar dilution, the single discordant result (isolate 33) was reconciled

by choosing the result which most closely matched the results of the reference method. For BMD, discrepant results were reconciled by choosing the result with the higher MIC value. This accords with standard laboratory practice, as theoretically these results would be used to influence the choice of antibiotic in critically ill patients.

**PCR for detection of *aac(3)-IIa*.** Genomic DNA was extracted using the EasyMag (bioMérieux, Durham, North Carolina) per the manufacturer's instruction. Primers (forward [F], CGC GGA AGG CAA TAA C, and reverse [R], GCT TCT CAA GAT AGG TG) described previously by Jacobson et al. were used to amplify a 786-kb fragment of *aac(3)-IIa* (21). *A. baumannii* strains designated MOS-1 and MOS-2, which were isolated from a single patient at Groote Schuur Hospital during the early 1980s, were used as positive and negative controls, respectively (21).

The master mix consisted of magnesium chloride (25 mM), 2.5 mM each deoxyribonucleotide triphosphate (dNTP), 20 pmol of each primer, and 2.5 U *Taq* polymerase in buffer made up to a final volume of 50  $\mu\text{l}$  per reaction. An initial denaturation at  $95^\circ\text{C}$  for 5 min, followed by 35 cycles of  $95^\circ\text{C}$  for 1 min,  $51^\circ\text{C}$  for 45 s, and  $72^\circ\text{C}$  for 1 min, and a final extension at  $72^\circ\text{C}$  for 5 min was carried out. The amplified fragments were separated on a 2% agarose gel by gel electrophoresis, stained with ethidium bromide, and visualized under UV light. Positive, negative, and extraction controls and a water blank were included for each gel. PCR was repeated for all negative isolates. In addition, a 16S rRNA PCR was performed on all isolates to confirm the integrity of bacterial DNA.

The amplicons from two randomly selected isolates were sequenced using the ABI PRISM BigDye Terminator v3.1 ready reaction cycle sequencing kit (Applied Biosystems) on the ABI genetic analyzer (Applied Biosystems) and compared to available *A. baumannii* sequences in the National Center for Biotechnology Information (NCBI) GenBank database using the BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST>).

**Pulsed-field gel electrophoresis.** The relatedness of the isolates was compared by pulsed-field gel electrophoresis (PFGE) with minor modifications of the previously described technique (21). Following plug preparation and cell lysis, DNA was digested with *Apal* (Roche) for 2 h, after which the DNA was separated on a 1.5% agarose gel (Bio-Rad) in  $0.5\times$  Tris buffer. Electrophoresis was carried out in a Gene Navigator PFGE machine (Amersham Biosciences AB, Uppsala, Sweden) for 23 h with the pulse time increasing from 5 to 45 s, after which it was stained with ethidium bromide, destained, and photographed using a Fotodyne Inc. UV light box and a Kodak 290 camera. The fingerprint images were analyzed by Gel Compare II software version 4.6 (Applied Maths, Sint-Martens-Latem, Belgium) using a Dice similarity index for cluster analysis and the unweighted pair group average for tree building. Banding patterns were compared with 1.5% optimization and 1.5% band position tolerance (26). All isolates with PFGE banding patterns with  $>87\%$  similarity were grouped within the same cluster (26).

In addition, repetitive sequence-based PCR (rep-PCR) using DiversiLab (bioMérieux, Marcy l'Etoile, France) was performed by the R&D Division, bioMérieux, La Balme, France.

**Ethics.** This research received approval from the University of Cape Town Health Sciences Faculty Research Ethics Committee (REC REF 458/2006).

## RESULTS

**Characterization of clinical isolates.** All 39 isolates were identified as *A. baumannii* by the Vitek2 instrument with a high percent probability (99%).

**Tobramycin susceptibility and error rates.** The level of agreement between the different methods and the reference method (BMD) varied widely and is shown in Table 1. A significant number of VMEs (10 isolates) and mEs (15 isolates) were observed when Vitek2 AST was compared to BMD. A similar number of VMEs and mEs were detected when agar dilution was compared to BMD. Three VMEs and 13 mEs were noted with disc diffusion testing, and two VMEs and 10 mEs were detected when testing was

TABLE 1 Results from the 39 *A. baumannii* isolates showing tobramycin susceptibility testing and molecular typing results<sup>a</sup>

Isolate	Disc diffusion (mm/susceptibility)	Etest (MIC [μg/ml]/susceptibility)	Agar dilution (MIC [μg/ml]/susceptibility)	Vitek 0.6 McF (MIC [μg/ml]/susceptibility) <sup>b</sup>	Broth microdilution (MIC [μg/ml]/susceptibility)	<i>aac(3)-IIa</i> gene <sup>c</sup>	DiversiLab clone
7	0/R	256/R	128/R	≥16/R	>256/R	—	D
40	4/R	8/I	4/S	4/S	>256/R	+	C
3	3/R	32/R	16/R	8/I	256/R	—	D
4	10/R	16/R	8/I	8/I	256/R	—	C
27	0/R	64/R	32/R	≥16/R	256/R	—	D
31	12/R	16/R	16/R	4/S	256/R	+	C
18	13/I	16/R	4/S	8/I	128/R	+	C
36	13/I	8/I	16/R	4/S	128/R	+	C
38	0/R	64/R	16/R	≥16/R	128/R	—	A
10	13/I	16/R	4/S	8/I	64/R	+	C
5	11/R	8/I	8/I	8/I	32/R	+	C
6	14/I	4/S	2/S	2/S	32/R	+	C
11	13/I	16/R	4/S	2/S	32/R	—	C
12	9/R	32/R	8/I	8/I	32/R	+	C
13	11/R	32/R	4/S	4/S	32/R	+	C
17	13/I	16/R	2/S	4/S	16/R	+	C
22	16/S	8/I	2/S	≤1/S	16/R	+	C
23	21/S	2/S	≤0.5/S	≤1/S	16/R	—	B
39	15/S	8/I	2/S	2/S	16/R	+	C
8	13/I	8/I	4/S	4/S	8/I	+	C
9	15/S	8/I	2/S	2/S	8/I	+	C
14	14/I	8/I	4/S	2/S	8/I	+	C
15	15/S	8/I	4/S	4/S	8/I	+	C
20	15/S	4/S	2/S	≤1/S	8/I	+	C
26	0/R	64/R	2/S	≥16/R	8/I	+	C
32	17/S	4/S	2/S	≤1/S	8/I	+	C
33	10/R	16/R	8/I	4/S	8/I	+	C
42	14/I	8/I	4/S	2/S	8/I	+	C
29	14/I	8/I	2/S	2/S	4/S	—	C
24	22/S	2/S	≤0.5/S	≤1/S	2/S	—	C
35	21/S	2/S	≤0.5/S	≤1/S	2/S	—	B
2	19/S	2/S	≤0.5/S	≤1/S	1/S	—	B
16	23/S	2/S	≤0.5/S	≤1/S	1/S	—	A
21	15/S	2/S	≤0.5/S	≤1/S	1/S	—	A
28	23/S	1/S	≤0.5/S	≤1/S	1/S	+	A
30	20/S	2/S	≤0.5/S	≤1/S	1/S	—	A
34	23/S	1/S	≤0.5/S	≤1/S	1/S	—	A
37	21/S	2/S	≤0.5/S	≤1/S	1/S	—	B
41	21/S	2/S	≤0.5/S	≤1/S	1/S	—	C

<sup>a</sup> PCR of *aac(C)-IIa* and DiversiLab results. S, sensitive; I, intermediate, R resistant.<sup>b</sup> Shading indicates very major errors; underlining indicates minor errors.<sup>c</sup> +, *aac(3)-IIa* gene present; —, *aac(3)-IIa* gene absent.

performed by Etest. Irrespective of the testing method, no major errors were detected (Table 2).

There were three discordant results when we used Vitek2 at 1.0 McF versus 0.6 McF. However, the results did not show improve-

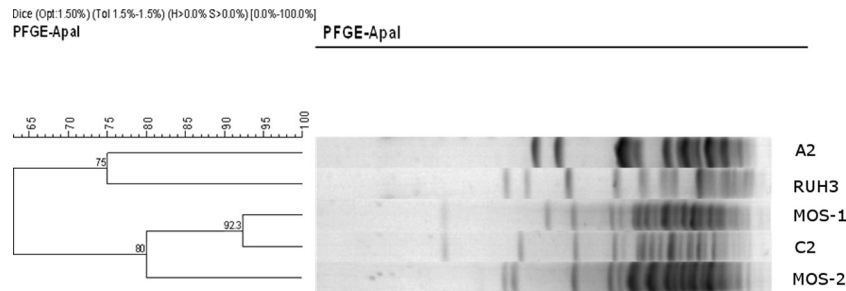
ment with a higher inoculum; instead, two additional VMEs would have been detected if 1.0 McF was used for testing.

The specimen types from which *A. baumannii* was isolated and which gave rise to VMEs included three tracheal aspirates, two sputum samples, two tissue samples, two environmental swabs (from an outbreak investigation), and a blood culture. The minor errors were detected from the following specimen types: seven tracheal aspirates, three urine specimens, two sputum samples, two pus swabs, and one sterile fluid. There was no evidence of an association between specimen type and error rate; however, numbers were small.

**Detection of *aac(3)-IIa*.** Previous research at our institution had shown that the prevalent mechanism of tobramycin resistance among *A. baumannii* isolates was an aminoglycoside-modifying enzyme encoded by the *aac(3)-IIa* gene (21). Twenty-one of the 25 isolates that demonstrated discordant results (either sensitive or

TABLE 2 Error rates from comparison of the different test methods with BMD for susceptibility testing of the 39 *A. baumannii* isolates

Method	% (no.) of:			Total error rate [% (no.)]
	Very major errors	Major errors	Minor errors	
Disc diffusion	7.69 (3)	0 (0)	33.33 (13)	41.02 (16)
Etest	5.13 (2)	0 (0)	25.64 (10)	30.77 (12)
Agar dilution	25.64 (10)	0 (0)	28.21 (11)	53.85 (21)
Vitek2	25.64 (10)	0 (0)	38.46 (15)	64.10 (25)



**FIG 1** Results of PFGE comparing 2 representative isolates of the most common DiversiLab clones, A and C, and MOS-1. A2, isolate 16 (fully susceptible strain without *aac(3)-IIa*; DiversiLab clone A); C2, isolate 32 (intermediately susceptible strain with *aac(3)-IIa*; DiversiLab clone C); RUH3, control strain; MOS-1, clinical strain with *aac(3)-IIa*; MOS-2, clinical strain without *aac(3)-IIa*.

intermediate by Vitek2 but resistant by BMD) were found to contain the *aac(3)-IIa* gene (Table 1).

Analysis of the sequencing data of the amplicons from two isolates (17 and 28) showed that they were 100% identical to the sequence of the *aac(3)-IIa* gene (GenBank accession number M62833).

**Relatedness of isolates.** The majority of clinical strains were isolated from various ICUs from four different hospitals. The relatedness of the isolates was determined by rep-PCR using the DiversiLab (bioMérieux) system. The isolates clustered into four clones, with the majority of isolates clustering in cluster C (Table 1). Seventeen of the cluster C isolates were isolated from Groote Schuur Hospital, with four of these isolates from non-ICU wards. Nine isolates from cluster C were isolated from Red Cross Hospital, with a single isolate from Mowbray Maternity Hospital. The results of the DiversiLab analysis confirmed the presence of a predominant clone that was not confined to a single hospital. In addition, isolates 3, 4, and 11 (which lacked the AAC(3')-IIa enzyme but were resistant to tobramycin) were scattered across clusters C and D, confirming that tobramycin resistance was not linked to a single clone at our hospitals.

PFGE was performed to compare a representative of the two commonest clones (C and A) with the dominant strains that had previously been isolated at our institution (Fig. 1). The MOS-1 and MOS-2 strains were isolated from the same patient during the same period of hospitalization at Groote Schuur Hospital (21). Isolate C2 was highly similar to MOS-1, a tobramycin-resistant strain with the *aac(3)-IIa* gene, that has been present in Cape Town, South Africa, since the early 1980s (21). Although it was not our intention to do a formal outbreak investigation, 8 of 11 isolates (including 2 environmental swabs) from Red Cross Hospital's ICU belonged to cluster C, confirming a possible outbreak in the unit. A2 represented a strain that was susceptible to tobramycin by all test methods. This strain was not related to cluster C by PFGE, confirming the results obtained by DiversiLab.

## DISCUSSION

Inaccuracies with AST have been reported in the literature, particularly among nonfermenters (11–20). Few studies have been performed to evaluate tobramycin susceptibility testing using AST in *A. baumannii*. A recent study found that up to one-third of *A. baumannii* isolates ( $n = 107$ ) tested were incorrectly reported as susceptible to amikacin by the Vitek2 instrument (11). The manufacturers of the Vitek2 and other automated systems suggest ei-

ther nonreporting or confirmation of the susceptibility result by manual methods in these cases.

After suspecting a possible error in tobramycin susceptibility testing by AST, we determined the susceptibilities of 39 isolates by multiple manual and automated methods. An overall category agreement error rate of <10% was considered an acceptable performance of a susceptibility test method, which included  $\leq 1.5\%$  VMEs and  $\leq 3.0\%$  major errors (27). Our study found a high total error rate of 64% in isolates tested by Vitek2, with 25% of these being VMEs. The use of a higher concentration of inoculum when performing AST did not have an effect on reducing the error rates.

Although CLSI recognizes the agar dilution method as a reference method, a high error rate (54% total errors) was detected when testing tobramycin. Eight out of 10 of the isolates tested by agar dilution with VMEs were confirmed to possess the *aac(3)-IIa* gene, which confers resistance to tobramycin. There have been no studies reported in the recent literature that have particularly evaluated the accuracy of the agar dilution test method for tobramycin. Further studies to confirm our results are required.

Errors occurred with manual testing methods (disc diffusion and Etests) as well, with the total rates being 41 and 31%, respectively. The Etest had the most acceptable performance (2% VMEs, no major errors). A study at the San Antonio Military Medical Center (San Antonio, TX) revealed errors with manual and automated susceptibility testing of tobramycin in *A. baumannii* (11). Very major errors were detected in 13.1% of isolates tested by Vitek2, compared to 2.8% VMEs when disc diffusion and Etests were compared to BMD (11). Akers et al. also noted VMEs with tobramycin susceptibility testing using other automated systems (11). We detected a much higher rate of VMEs when testing using Vitek2 (25%) but similar rates when testing using disc diffusion and Etests (3% and 2%, respectively).

Twenty-one of the 25 isolates that demonstrated discordant results (either sensitive or intermediate by Vitek2 but resistant by BMD) in our study were found to contain the *aac(3)-IIa* gene (Table 1). This confirmed a possible mechanism of resistance to tobramycin. This is not unexpected, as a worldwide study of *A. baumannii* (which included isolates from South Africa) has shown that another AAC(3) enzyme, AAC(3)-I, was the commonest aminoglycoside-modifying enzyme (AME) present, accounting for resistance to aminoglycosides in 50% (12/24) of *Acinetobacter* spp. tested (28). Numerous AMEs have been isolated from *A. baumannii*. The *aac(3)-IIa* gene confers resistance to gentamicin, tobramycin, dibekacin, netilmicin, and sisomicin. This resistance profile is common among the *Enterobacteriaceae* (29). The



AAC(3)-IIa enzyme is the commonest resistance mechanism in the group exhibiting this resistance profile, accounting for 84.8% of isolates (29). This aminoglycoside resistance profile has been detected in 21.3% of *Acinetobacter* spp. (29).

Akers et al. concluded that the AME genotype was an inadequate predictor of the aminoglycoside phenotype, suggesting that multiple resistance mechanisms were operating simultaneously (11). This contrasted with our study, which showed a good correlation between the presence of the *aac(3)-IIa* gene and tobramycin resistance, with the gene being present in 12/19 (63%) isolates with tobramycin MICs of  $\geq 16$ .

Seven isolates that were resistant to tobramycin by BMD lacked the *aac(3)-IIa* gene (Table 1). Five of these isolates exhibited tobramycin MICs of  $\geq 128$   $\mu\text{g/ml}$ . These isolates likely have other mechanisms of resistance to tobramycin, such as AAC-6' or ANT-2'', combinations of AMEs, efflux pumps, or other resistance mechanisms that were not explored further as this was not the focus of our study. Importantly, though, the failure of Vitek2 to detect these resistant isolates suggests a wider failure of the system to detect tobramycin resistance.

Alarming, Akers et al. showed that susceptibility to tobramycin by BMD and disc diffusion was retained in the presence of a potentially inactivating AME gene in 16 (18%) isolates due to other AMEs, including combinations such as *aph(3')-Ia* and *ant(2'')-Ia*, *aac(6')Ih* and *aph(3')-Ia* (11). We found that just a single isolate that was susceptible by all test methods harbored the *aac(3)-IIa* gene. This finding may be explained by a so-called cryptic gene, a phenotypically silent DNA sequence. This hypothesis may be supported by similar findings in *Salmonella* spp., where it has been shown that a 60-kb deletion upstream of an aminoglycoside resistance gene, *aac(6')-Iy*, encoding 6'-N-acetyltransferase type I in a strain of *Salmonella enterica* subsp. *enterica* serotype Enteritidis, BM4362, resulted in the gene being highly expressed. This gene was silent in the progenitor strain, BM4361 (30). However, as this gene was not the focus of our study, we did not investigate it further.

One hypothesis for the discrepant Vitek2 results may be that the catalytic activity of the AAC(3)-IIa enzyme is too slow to be detected by rapid automated susceptibility test methods, particularly with a poor substrate like tobramycin (31). Whether this has implications on the clinical outcome of patients treated with tobramycin has yet to be determined.

The results of the PFGE suggest that it is unlikely that the tobramycin-resistant strains had recently emerged due to the increased use of tobramycin at our hospitals, as the clone had been present in the hospital since the early 1980s.

**Conclusion.** To the best of our knowledge, this is the first study in South Africa that has assessed the ability of Vitek2 to detect *A. baumannii* isolates with reduced susceptibility to tobramycin. Tobramycin, tigecycline, and colistin are the only antibiotics available in the public health sector in South Africa that remain effective against multidrug-resistant strains of *A. baumannii*. Thus, accurate susceptibility testing remains critical.

The data from our study confirm the limitations of both automated and manual tobramycin susceptibility test methods. Vitek2 appears to be unreliable for the detection of tobramycin resistance in *A. baumannii*. It appears that manual methods, such as Etest, may be more reliable for susceptibility testing when tobramycin is considered for use as a potential therapeutic agent.

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