A RETROSPECTIVE ANALYSIS OF THE PERFORMANCE OF VITEK®2 COMPARED TO MANUAL BROTH MICRODILUTION FOR COLISTIN SUSCEPTIBILITY TESTING OF ACINETOBACTER BAUMANNII CLINICAL ISOLATES

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Declaration: Student's contribution to article and agreement of co-authors

I, **Vuyolwethu Fadana**, student number **705549**, declare that this **Research Report** is my own work and that I contributed adequately towards research findings published in the article stated below which are included in my **Research Report**.

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ABSTRACT

Studies comparing the performance of Vitek®2 against manual broth micro-dilution (mBMD) for colistin susceptibility testing have yielded conflicting results. Although the latter is the recommended reference method, it is not readily available. A retrospective analysis was performed to assess the performance of Vitek®2 against mBMD on colistin minimum inhibitory concentration determination in extensively-drug resistant A. baumannii isolates and to determine the epidemiology of these isolates at Charlotte Maxeke Johannesburg Academic Hospital. Vitek®2 performance as compared to mBMD was as follows: categorical agreement 89%, essential agreement 56%, major error rate 8% and very major error rate 55%. With these results Vitek®2 cannot be recommended as an alternative to mBMD for colistin susceptibility testing. Of the isolates analysed, 71% were from adult patients, 18% from neonates and the remainder (11%) from older paediatric patients. Differences in ward distribution between the age groups were noted. These require further investigation.

Keywords: Acinetobacter; colistin; broth microdilution; Vitek®2

NOMENCLATURE

NHLS - National Health Laboratory Service

CMJAH - Charlotte Maxeke Johannesburg Academic Hospital

MALDI-TOF MS - Matrix assisted laser desorption ionization – time of flight mass spectrometry

AST - Antimicrobial susceptibility testing

XDR - Extensively drug resistant

MIC - Minimal inhibitory concentration

CLSI - Clinical and Laboratory Standards Institute

EUCAST - European Committee on Antimicrobial Susceptibility Testing

BMD - Broth micro-dilution

mBMD - Manual BMD

FDA - Food and Drug Administration

CA - Categorical agreement

EA - Essential agreement

ME - Major error

VME - Very major error

CDW - Corporate Data Warehouse

ICU - Intensive care unit

CI - Confidence intervals

IQR - Interquartile ranges

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A retrospective analysis of the performance of Vitek®2 compared to manual broth micro-dilution for colistin susceptibility testing of *Acinetobacter baumannii* clinical isolates

Vuyolwethu Fadana, Teena Thomas, Nina von Knorring

INTRODUCTION

The genus *Acinetobacter* was discovered by Dutch microbiologist Martinus Willem Beijerinck from soil samples in 1911.[1] Since then, species within the *A. baumannii* complex have been implicated in a variety of infections including bloodstream infections, skin and soft tissue infections, respiratory tract infections and meningitis.[2, 3] Distinction between colonization and infection is often difficult in hospitalized patients as this organism is ubiquitous in the environment. It has been associated with increased mortality when isolated from critically ill patients. [4] Risk factors for infection with *A. baumannii* complex include mechanical ventilation, prolonged hospital stay, ICU admission and exposure to patients that are either infected or colonized with *A. baumannii*.[4]

Increasing antimicrobial resistance in this group of organisms and more recently, resistance to the polymyxin antibiotic, colistin, has meant that timeous identification of the infecting pathogen and determination of its antimicrobial susceptibility profile is necessary to ensure early appropriate antimicrobial therapy.[4, 5] The emergence of resistance to colistin, one of the last active agents against extensively drug -resistant (XDR) *A. baumannii* has warranted the review of available antimicrobial susceptibility testing (AST) methods. Different laboratory methods for the assessment of colistin AST amongst *A. baumannii* isolates have been evaluated. Challenges with polymyxin AST are due to its molecular nature. Its large size limits diffusion into agar with disc diffusion and gradient diffusion (Etest®) methods and the positive charge causes it to bind to the plastic material in microtiter plates used for susceptibility testing.[6]

Since 2016, the reference method for colistin AST recommended by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) is the ISO broth micro-dilution (BMD) method (ISO-

20776).[7] Owing to its laborious nature, the implementation of manual BMD (mBMD) is currently not feasible in the routine microbiology laboratory.[6] Its use has been restricted to reference centers where more experienced personnel conduct this testing. This prolongs laboratory turn-around times and may cause delays in appropriate therapy. Alternative methods therefore need to be considered. The Food and Drug Administration (FDA) recommends that for an AST method to be deemed acceptable, it must provide high categorical (CA) and essential (EA) agreement (both ≥90%) with low major error (ME) and very major error (VME) rates when compared to the reference method (<3%).[8] The use of diffusion methods for colistin susceptibility testing has been found to result in an unacceptable rate of false susceptible results (1.7-13%) when compared to BMD.[6, 9-11] Although both diffusion methods (disc diffusion and gradient diffusion) are easy to perform and are readily available to most routine laboratories, they are currently not recommended for use by the CLSI-EUCAST polymyxin breakpoints working group for colistin susceptibility testing.[12]

Vitek®2 (BioMerieux Inc, Marcy l'Etoile, France) is an automated microbial identification and AST platform that is used in most public and private laboratories within South Africa and, therefore, offers an attractive alternative to mBMD for colistin susceptibility testing. Findings from studies comparing it to mBMD have, however, shown conflicting results on its performance. Dafopoulou et al compared different colistin susceptibility testing methods (mBMD with polysorbate-80, Vitek®2, Etest, Agar dilution and MIC test strip) on 51 *Klebsiella pneumoniae* and 20 *A. baumannii* clinical isolates.[13] Eighteen of the *A. baumannii* isolates were resistant to colistin by mBMD. Assessed against mBMD, Vitek®2 resulted in CA of 85% and EA of 90% for the *A. baumannii* isolates. There were no VMEs reported. These findings were similar to those obtained by Lo-Ten-Foe et al who also found good EA (93.1%) between Vitek®2 and mBMD amongst 80 gram-negative bacteria, including 10 *Acinetobacter* spp. isolates.[14] Categorical agreement and error rates were not reported. Piewngam and Kiratisin demonstrated a low VME rate of 0.7% on 290 isolates of *A. baumannii*.[15] These findings suggest that Vitek®2 could be a viable alternative to mBMD. In contrast to these results, Vourli et al assessed two automated systems, Phoenix100 and Vitek®2, and found them to have unacceptably high VME rates when compared to mBMD (41.4% and 37.9% respectively).[16] In addition, in an undated notice BioMerieux retracted Vitek® 2 use for colistin AST owing to the CLSI-EUCAST recommendations and an in-house study showing a high VME rate. [17] The MIC distribution of the isolates and

VME rate were not specified. Due to these contradictory findings in the literature, further research into this area was warranted.

As a result, the aim of this study was to compare the performance of Vitek®2 colistin susceptibility testing to mBMD for clinical XDR *A. baumannii* complex isolates and to describe the epidemiology of these isolates at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH). We also aimed to make recommendations on the use of Vitek®2 for colistin susceptibility testing within the National Health Laboratory Services (NHLS) routine microbiology laboratories.

METHODS

Ethical considerations

Ethical approval was obtained from the University of Witwatersrand Human Research Ethics Committee (clearance certificate number M191048 MED 19-10-043).

Data collection

This was a descriptive, retrospective study. Data from patients admitted to CMJAH with positive cultures of XDR *A. baumannii* between 01 January 2017−30 June 2019 was analyzed. Epidemiological and microbiological data was extracted from the Corporate Data Warehouse (CDW), a division of the NHLS that stores national public health-care laboratory data. The epidemiological data included patient age, gender and admission ward type (intensive care unit (ICU)/non-ICU and surgical/medical). The microbiological data included the sample type that cultured the isolate, Vitek®2 and mBMD colistin MIC results.

Study setting

Charlotte Maxeke Johannesburg Academic Hospital is a tertiary care hospital in Gauteng, South Africa, with both outpatient and inpatient services. There is a bed capacity of 1088, catering for adults and children. The hospital offers medical and surgical services with various sub-specialties. In addition, there are separate adult and paediatric intensive care units.

Isolate identification and antimicrobial susceptibility testing

Microbiology services within the hospital are provided by the NHLS. The identification of isolates within the institution was performed by either Vitek®2 GN ID card or matrix assisted laser desorption ionization - time of flight - mass spectrometry. These methods are unable to differentiate species within the *A. baumannii* complex. Routine antimicrobial susceptibility testing was performed using Kirby-Bauer disc diffusion susceptibility method or Vitek®2 AST-N256 card according to the manufacturer's instructions. Isolates that had AST by the former method were excluded from the study. Quality control strains are utilised and isolate purity plates are inoculated during performance of Vitek®2 AST. Isolates are processed for mBMD when colistin therapy is considered, such as for clinically significant XDR *A. baumannii* complex isolates.

Manual BMD is performed on subcultures by trained personnel with appropriate controls according to ISO-20776. Microtiter plates with 96 wells are prepared in advance with cation adjusted Mueller Hinton broth growth medium and doubling dilutions of colistin in wells 1-10. Wells 11 and 12 are reserved for negative and positive growth control. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 are utilized for quality control of plates after preparation. One of these is also utilized for daily quality control. A standardized inoculum of test organism (5×10^5 CFU/mL) is inoculated into the colistin containing wells and the positive growth control well. A purity plate is also inoculated. Plates are incubated at 35±2ºC for 20-24 hours. For a valid run, the purity plate should have pure growth, there should be no growth in the negative growth control well and there should be adequate growth in the positive growth control well. The MIC is determined by visual inspection and is defined as the colistin concentration with complete inhibition of growth. Isolates that are colistin-resistant by mBMD are sent to a reference laboratory for mcr 1-5 testing. However, this data is not presented in this study.

Data analysis

All XDR *A. baumannii* isolates cultured between 01 January 2017−30 June 2019 from any sample type were analyzed. Isolates were allocated to the ward at the time of sample collection. Those that were obtained from outpatient departments or without a ward type specified, were excluded. Repeat blood culture isolates from the same patient were included if

the isolate was cultured at least two weeks after the initial blood culture. Repeat samples from the same patient for sample types other than blood cultures, such as tracheal aspirates, sputum, swabs, fluid aspirates, tissue biopsies and urine, were included if they were obtained at least one month after the initial culture. Intravenous central venous catheter tips were not included as they are processed in a separate laboratory using a different automated AST platform.

All isolates with mBMD results with/without Vitek®2 results were used for epidemiological analysis. Microsoft excel 2016 was used for data analysis. For the distribution of epidemiological data parametric/non-parametric statistical methods were applied. Data with a Gaussian distribution was described by means of 95% confidence intervals (95% CI), whereas for non-parametric data, medians with interquartile ranges (IQR) were used. The following age categories were analysed: neonates ≤1 month, older children >1 month -13 years and adults ≥14 years

Only isolates with colistin susceptibility results from both Vitek®2 and mBMD were used to assess performance of Vitek®2. The performance of Vitek®2 colistin susceptibility testing was determined by assessing categorical agreement, essential agreement, very major and major error rates in comparison to mBMD colistin susceptibility testing, according to the FDA recommendations.[8] . CLSI recommends breakpoints for colistin of ≤2 μg/mL as intermediately-susceptible and ≥4 μg/mL as resistant in *A. baumannii* isolates. [18]

The following equations were employed to assess agreement and error rates:

- Categorical agreement = (number of isolates correctly classified by Vitek®2 as either colistin-susceptible or resistant in comparison to mBMD / total number of isolates tested) X 100
- Essential agreement = (number of isolates within one doubling dilutions of the mBMD MIC on Vitek®2 / total number of isolates tested) X 100
- Major error rate = (number of falsely resistant isolates on Vitek®2 / number of susceptible isolates by mBMD) X 100

- Very Major error rate = (number of falsely susceptible isolates by Vitek®2 / number of resistant isolates by mBMD) X 100

RESULTS

Data for 523 isolates was obtained from all samples types that cultured XDR *A. baumannii* and were submitted for colistin mBMD. Hundred and thirteen isolates were removed as they were duplicates or had other exclusion criteria. Of the 410 remaining isolates, 337 (82%) isolates had both Vitek®2 and mBMD MIC results (Figure 1).

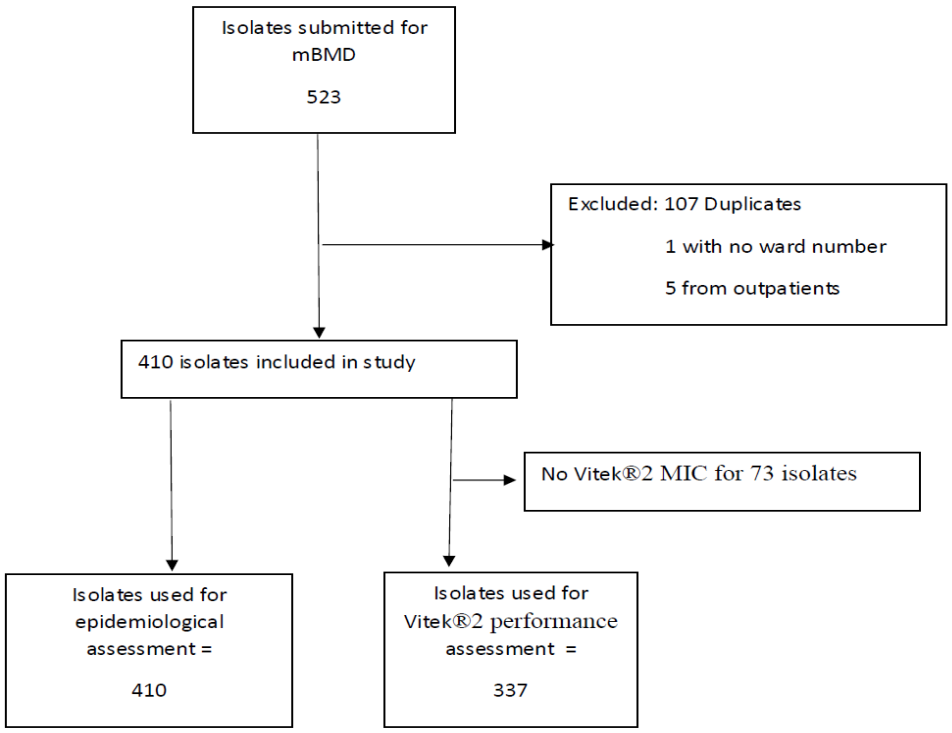
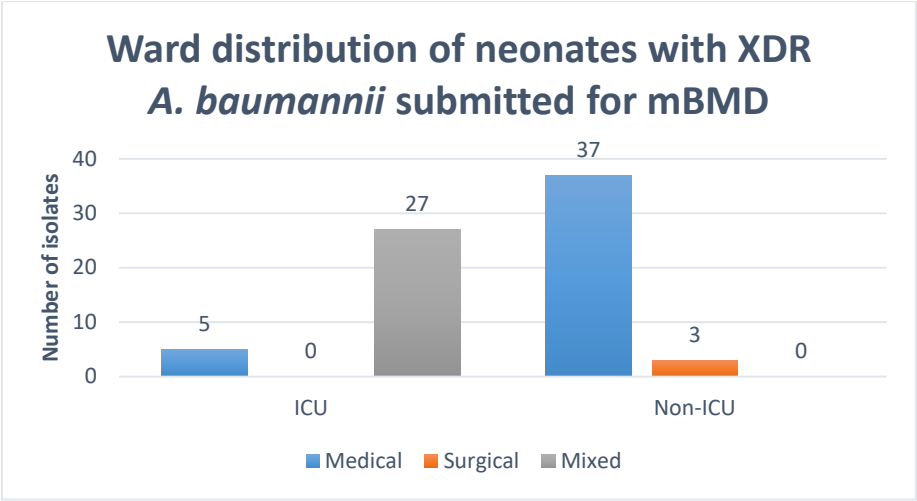


Fig. 1. Number of isolates utilised for epidemiological and Vitek®2 assessment (01 Jan 2017-30 Jun 2019)

The number of samples submitted for mBMD was low at the beginning of the study period but subsequently increased over time. Excluding isolates without a specified age (3%; 13/410), the majority of isolates were from adult patients (280/397 - 71%; mean age of 43 years, 95% CI: 41 – 44). Amongst the paediatric population, 61% (72/117) of isolates were

from neonates (median age of 9 days, IQR 5 - 14). The ratio of males to females across the different ages groups was 0.9:1 in neonates, 1.3:1 in other children and 1:1 in adults.

Figure 2 illustrates the distribution of admission ward types for the different age groups with positive cultures. Amongst all age groups, the majority of isolates were from non-ICU wards with differences in distribution between medical and surgical wards noted between the age groups. Samples from non-ICU surgical wards were more prevalent amongst the adult age group, whereas samples taken in non-ICU medical wards predominated among the pediatric group.



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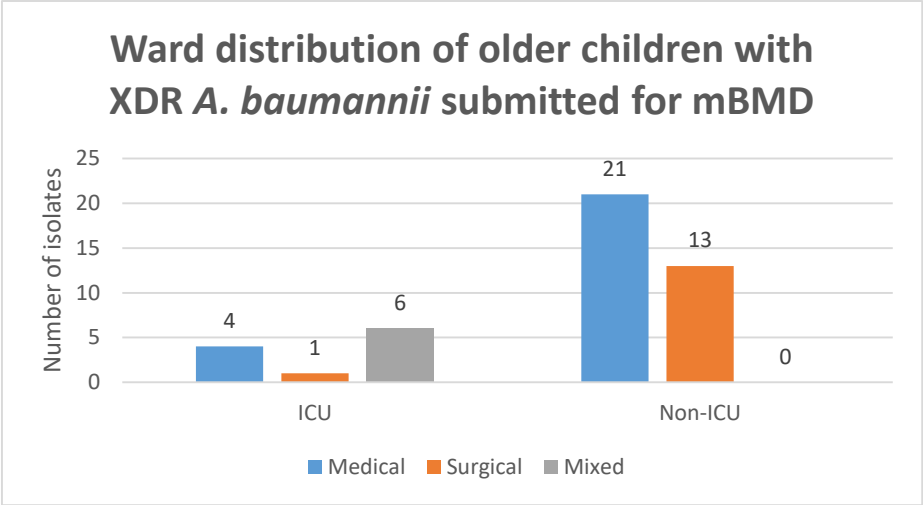

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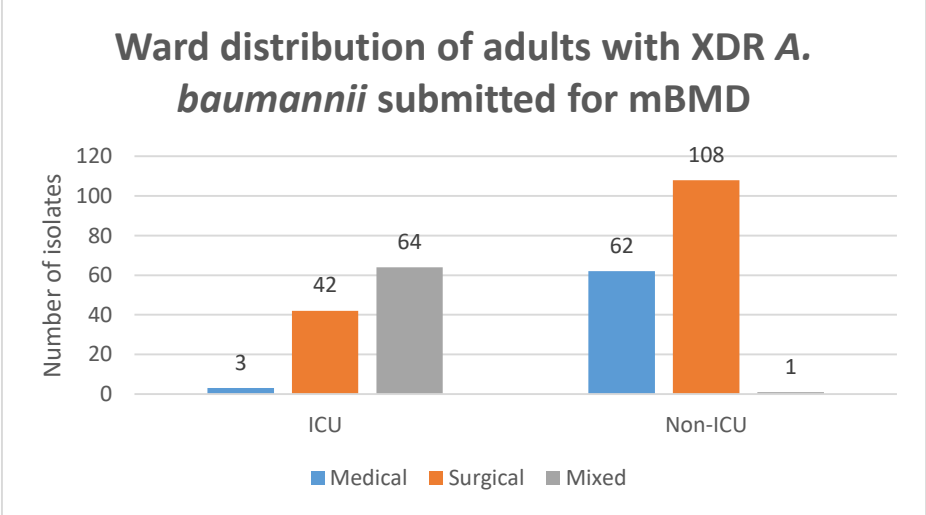

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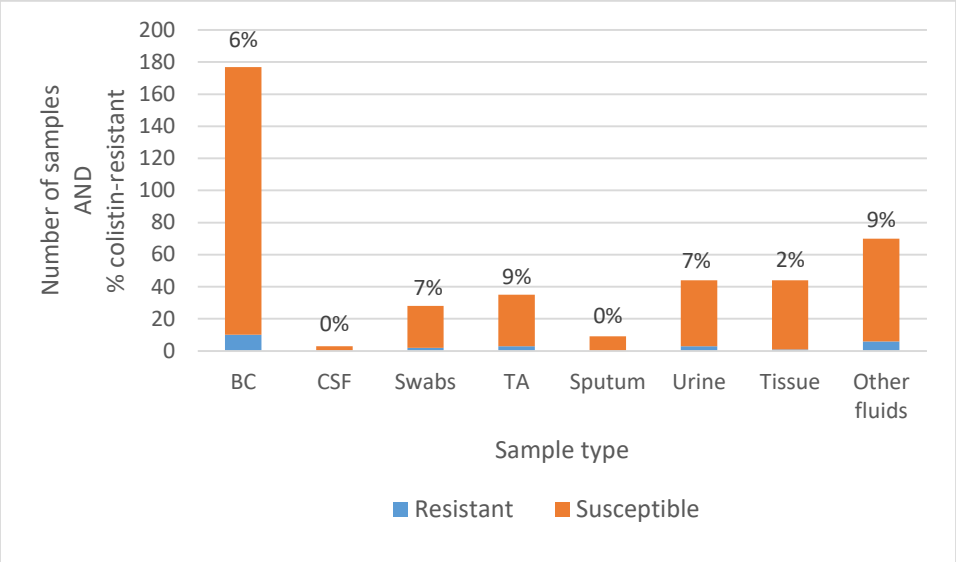
201 Fig. 2. Ward distribution of neonates, children and adults with XDR *A. baumannii* submitted
202 for mBMD

Regarding the sample types submitted, the majority of isolates were cultured from blood culture samples (177/410; 43%). Twenty-five (25/410; 6%) colistin-resistant isolates were identified by mBMD in total (Figure 3). The highest percentage of colistin-resistant isolates was from tracheal aspirates and other fluids.



BC blood culture; CSF cerebrospinal fluid; TA tracheal aspirate

Fig. 3. Distribution of colistin-resistant and susceptible XDR *A. baumannii* isolates by sample type (n=410)

The majority of the colistin-resistant isolates were from adult patients (17/25; 68%). Non-ICU wards predominated (16/25; 64%), with most isolates coming from surgical wards (11/25; 44%) (Figure 4).

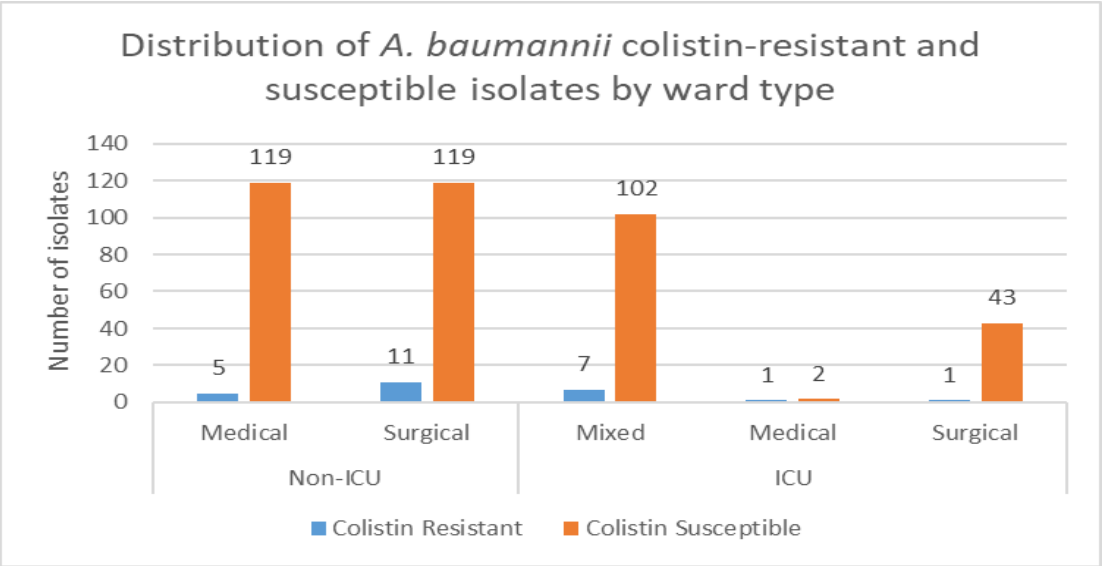


Fig. 4: Distribution of *A. baumannii* colistin-resistant and susceptible isolates by ward type

Of the 337 isolates with colistin susceptibility results available from both Vitek®2 and mBMD, 20 (6%) were resistant to colistin by mBMD (Table 1). Vitek®2 was found to have a categorical agreement of 89% (300/337) and an essential agreement of 56% (190/337) with mBMD. The very major error rate was 55% (11/20) and the major error rate was 8% (26/317).

234 Table 1: Colistin minimum inhibitory concentration results for XDR *A. baumannii* isolates by

235 both Vitek®2 and mBMD

		Vitek®2 MIC								
		<0,5	0,5	1	2	4	8	16	>16	Total number of isolates
	<0,125	0	0	0	0	0	0	0	1	1
	0,25	24	0	0	0	0	0	0	1	25
	0,5	154	0	3	1	0	0	0	15	173
	1	96	0	1	0	1	0	0	7	105
	2	11	0	0	1	0	0	0	1	13
	CLSI-EUCAST break-point									
mBMD MIC	4	3	0	2	1	0	0	0	2	8
	8	1	0	0	1	0	0	0	0	2
	16	0	0	0	0	0	0	0	0	0
	32	0	0	0	0	0	0	0	0	0
	64	0	0	0	0	0	0	0	1	1
	>64	3	0	0	0	0	1	0	5	9
	Total number of isolates	292	0	6	4	1	1	0	33	337

Break-point | Essential agreement | Categorical agreement | Major errors | Very major errors

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DISCUSSION

The recommended reference method for colistin susceptibility testing, mBMD according to ISO-20776, is difficult to implement in routine microbiology laboratories. Due to financial constraints and technical competencies required, only one NHLS laboratory is currently able to offer mBMD in South Africa. This is likely to have a negative impact on patient care due to delays in turn-around-times of results. In contrast, Vitek®2 is available in most NHLS microbiology laboratories and would have been a favorable alternative. However, our study demonstrates unacceptable performance, with 11 of 20 (55%) colistin-resistant isolates having false-susceptible results on Vitek®2. Vitek®2 also reported some isolates with mBMD colistin MIC >64 mg/L as susceptible. This has the potential for patients infected with colistin-resistant isolates not detected by Vitek®2 receiving colistin treatment, leading to detrimental effects. This extremely high VME rate is in contrast with other studies as mentioned earlier. [13, 15] Those studies included fewer isolates of *A. baumannii* compared to our study. Other authors however found similarly high VMEs. [16] In addition to the unacceptable VME rate, the CA, EA and the ME rate with Vitek®2 were also unacceptable according to the FDA requirements for an AST testing platform. This is in keeping with a study by Pfennigwerth et al that also found poor essential agreement (75.9%) with Vitek®2 on testing Enterobacterales, despite better categorical agreement (90.5%).[19] Based on these results Vitek®2 cannot be recommended as an alternative and attempts need to be made to make mBMD more readily available and easier to implement until other testing options become available.

Matuschek et al evaluated 5 recently developed commercial BMD systems - SEMPA1 (custom Sensititre® plate, Thermo Fisher Scientific, EastGrinstead, UK), MICRONAUT-S and MICRONAUT MIC-Strip (MERLINDiagnostika Gmbh, Bornheim, Germany), SensiTest (Liofilchem, Roseto degli Abruzzi, Italy) and UMIC (Biocentric, Bandol, France). These were evaluated against mBMD using various gram- negative organisms including 22 *Acinetobacter spp* isolates.[20] They demonstrated overall better performance compared to our findings with Vitek®2. The majority of platforms had acceptable CA and EA (>90%), with much lower error rates than obtained in this study. Interestingly, there were no falsely-susceptible *A. baumannii* isolates. These methods may offer an alternative to mBMD and further research on their performance is required.

Our findings also provide insight into the epidemiology of XDR *A. baumannii* in our setting. The distribution of XDR *A. baumannii* isolates within CMJAH appears to vary based on the age group assessed. There was a predominance of these isolates from medical wards amongst the paediatric population and surgical wards amongst the adults. In our setting, all wards are managed by the same infection prevention and control (IPC) team and contact precautions are initiated for all patients with multidrug-resistant (MDR) and XDR isolates regardless of the ward type. Compliance to infection prevention and control measures may vary between different wards. Differences in antibiotic prescribing practices cannot be excluded as another driving factor for the differences in the ward areas that were affected.

The majority of colistin-resistant isolates were obtained from the adult population and predominantly from the non-ICU surgical wards. These wards also had the highest overall number of XDR *A. baumannii* isolates. However, admission rates for different units were not accessible for this study and this is required for a more comprehensive interpretation of the data. This does nonetheless highlight the possible relationship between colonization/infection with XDR isolates and infection/colonization with colistin-resistant isolates. Patients having prior colistin-susceptible isolates have been found to get infected/colonized with colistin-resistant isolates on exposure to colistin.[21] We postulate that wards with higher numbers of patients colonized/ infected with XDR *A. baumannii* isolates have higher usage of colistin. This antibiotic pressure then drives emergence of colistin-resistant isolates in those units. Appropriate antimicrobial stewardship and IPC practices could mitigate this. The proportion of colistin-resistant and susceptible isolates between most of the sample types was similar and as such, the sample type cannot be used to predict resistance.

LIMITATIONS

XDR *A. baumannii* isolates that were cultured and not submitted for mBMD were not included in this study. Submission of isolates for mBMD within the institution is dependent on a number of factors including whether the isolates are considered clinically significant or if colistin is used for treatment. Isolates from intravenous catheters were also not included. These factors may have had an effect on the data that is presented. The inaccessibility to hospital patient admission data in the different units over the study period limited interpretation of the epidemiological data and comparison of the different age groups. A full

retrospective patient record review would also be required to comprehensively assess the factors resulting in differences between the different ward types and age groups.

Only a small number of colistin-resistant isolates were obtained. Analysis of a larger number of resistant isolates with a wider MIC distribution is required for a more robust evaluation. In addition, the distinction between colonization and infection in the patients with positive cultures of XDR *A. baumannii* was beyond the scope of this study, as a result, the clinical significance of these isolates could not be determined.

Strengths of this study included the large number of XDR *A. baumannii* isolates that were available for analysis compared to other studies. In addition, the retrospective design allowed for the findings to be a reflection of what one would expect in routine clinical practice.

CONCLUSION

Based on the results of this study, Vitek®2 cannot be recommended as an alternative to mBMD for colistin AST in our setting. Further studies are required to determine if the commercially-available colistin BMD methods are a cost effective option with acceptable analytical performance. In addition, the semi-automated platforms such as Vitek®2, should be optimized for colistin AST. Factors driving the differences in ward distribution amongst the different age groups still need to be elucidated. The use of colistin as a driver for the development of resistance in this population cannot be excluded and requires further study. Ongoing monitoring of colistin resistance is also required.

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APPENDIX 1: APPROVED RESEARCH PROTOCOL

TITLE

A retrospective analysis of the performance of Vitek®2 compared to manual broth micro-dilution for colistin susceptibility testing of *Acinetobacter baumannii* clinical isolates

INVESTIGATORS

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BACKGROUND

The genus *Acinetobacter* was discovered by Dutch microbiologist Martinus Willem Beijerinck from soil samples in 1911 (1). As a gram-negative bacillus predominantly found in the environment, it was initially thought to be of low pathogenicity to humans. However, *Acinetobacter* species have now established themselves as an important cause of community and more commonly hospital acquired infections (1,2). Species within the *A. baumannii* complex have been implicated as causes of blood stream infections, skin and soft tissue infections, respiratory tract infections and meningitis (2,3). Distinction between colonization and infection is often difficult in hospitalized patients as this organism is commonly cultured from this environment. They have nonetheless been associated with increased mortality when isolated from critically ill patients (3).

Increasing antimicrobial resistance, and more recently resistance to the polymyxin antibiotic, colistin, has meant that timeous identification of the infecting pathogen and determination of its antimicrobial susceptibility profile is necessary to ensure early appropriate antimicrobial therapy (4,5). Currently, bacterial identification within the National Health Laboratory Services' (NHLS) microbiology laboratory at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) utilizes both the Vitek®2 (Vitek®2, BioMerieux) automated system and matrix assisted laser desorption ionization – time of flight mass spectrometry (MALDI-TOF MS; Vitek®MS, BioMerieux). In addition, Vitek®2 is also able to provide antimicrobial susceptibility testing (AST) results for this organism.

The emergence of resistance to colistin, one of the last active agents against extensively drug - resistant (XDR) *A. baumannii,* has warranted the review of available AST methods. By definition, XDR *A. baumannii* isolates are resistant to all but one or two classes of antibiotics (6). Different laboratory methods for the assessment of colistin AST amongst *A. baumannii* isolates have been assessed. These include dilution and diffusion based AST methods which are either manually performed or automated. Dilution methods (e.g. broth micro-dilution) are based on the inhibition of growth of a standard inoculum of bacteria by varying concentrations of antimicrobial agent suspensions. The lowest concentration of antimicrobial agent used with visible inhibition of bacterial growth is termed the Minimal Inhibitory Concentration (MIC). Certain automated methods, e.g. Vitek®2, utilize a standard

suspension of antibiotic and monitor the rate of bacterial growth, using algorithms to determine the MIC, thus providing a calculated MIC. Diffusion based methods [e.g. disc and gradient diffusion (E-test®)] rely on diffusion of an antimicrobial agent into agar and inhibition of growth of the test organism that has been inoculated onto the agar. Problems with polymyxin AST are a result of its molecular nature. Its size limits diffusion into agar and its positive charge causes it to bind to the plastic material in microtiter plates used for susceptibility testing (7).

Since 2016 the recommended reference method for colistin AST by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) is by the ISO-broth micro-dilution (BMD) method (ISO-20776) (8). Owing to its laborious nature, the implementation of manual BMD (mBMD) is currently not feasible in the routine microbiology laboratory (7). mBMD use has been restricted to reference centers where more experienced personnel conduct this testing. Alternative methods therefore need to be considered. The Food and Drug Administration (FDA) recommends that for an AST method to be deemed acceptable, it must fulfill the following criteria when compared to a reference method (9):

- Categorical agreement: Both methods result in the same susceptibility category for ≥90% of tested isolates.
- Essential agreement: For ≥90% of isolates, the MIC with the tested method is within ± 1 two-fold dilution of the reference method's MIC.
- Major error rate: The rate of false resistant isolates with the tested method should be <3%.
- Very major error rate: The rate of false susceptibility with the tested method should be <3% (10).

Various studies have compared different methods of colistin susceptibility testing. Diffusion methods, including disc diffusion and gradient diffusion, have been found to have unacceptable performance when compared to mBMD (8,10). Comparing disc diffusion on 200 isolates of gram-negative bacteria (including 60 *A. baumannii* isolates), Gales et al found disc diffusion to have an unacceptable false susceptibility rate of 5% (11). This is postulated to be due to poor diffusion of polymyxins into the agar (12). E-test® has also been found to have a high false susceptibility rate (35%) when compared to mBMD (10). Although both

methods are readily available to most routine laboratories, they are currently not recommended by the CLSI-EUCAST polymyxin breakpoints working group for colistin susceptibility testing (8).

Dafopoulou et al compared different colistin susceptibility testing methods to mBMD on 51 *Klebsiella pneumoniae* and 20 *A. baumannii* clinical isolates (10). Eighteen of the *A. baumannii* isolates were resistant to colistin by mBMD. Assessed against mBMD, Vitek®2 resulted in categorical agreement of 85% and essential agreement of 90% for the *A. baumannii* isolates. In contrast, E-test® was found to have an unacceptable false susceptibility rate (35%) and poor categorical agreement to mBMD (65%). Although Vitek®2 did not fulfill all four FDA requirements, it performed better than the other methods tested. Lo-Ten-Foe et al also found good essential agreement (93.1%) between Vitek®2 and mBMD amongst *A. baumannii* isolates (13).

The above mentioned studies only included a small number of colistin resistant *A. baumannii* isolates. In contrast to their findings, a study performed by Vourli et al comparing the automated platforms Phoenix100 and Vitek®2 to mBMD found the two automated systems to have unacceptably high very major error rates (41.4% and 37.9% respectively) (14). This study included 117 clinical isolates of *A. baumannii*, with 25% of the isolates being colistin resistant.

As mentioned above, the method currently recommended by the CLSI-EUCAST polymyxin breakpoints working group is mBMD. This method, however, is not easy to implement in a routine microbiology laboratory. Vitek®2 systems are widely available in most microbiology laboratories in the private and public sectors in South Africa but findings from studies comparing it to mBMD have had conflicting results. Further studies in this area are therefore required in order to ascertain whether this could be a viable alternative in our setting.

AIMS AND OBJECTIVES

Aims:

- To assess the performance of Vitek®2 colistin susceptibility testing in comparison to mBMD for clinical XDR *A. baumannii* isolates.

- To formulate recommendations on use of Vitek®2 for colistin susceptibility testing within the NHLS routine microbiology laboratories.

- To describe the epidemiology of XDR *A. baumannii* isolates cultured during the period of 01 January 2017 – 30 June 2019 within Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), a tertiary institute in Johannesburg, South Africa.

Objectives:

- To determine performance of Vitek®2 colistin susceptibility testing by assessing categorical agreement, essential agreement, very major and major error rates in comparison to mBMD colistin susceptibility testing

- To analyze the following parameters in patients colonized or infected with XDR *A. baumannii* at CMJAH: patient age, patient gender, ward areas where the isolates were cultured from and sample types from which the isolates were cultured. Allocation of the infection to a particular ward will be based on culturing the isolate from the patient after the patient had been admitted to that ward for ≥ 48 hours duration.

STUDY DESIGN AND METHODS

This will be a descriptive, retrospective study. Data inclusion criteria will include:

- XDR *A. baumannii* isolates cultured between 01 January 2017 and 30 June 2019 from any specimen type with both Vitek®2 and mBMD colistin susceptibility results

- Only samples obtained from patients admitted at CMJAH will be included.

- Repeat blood culture isolate/s from the same patient will only be included if the isolate is cultured at least two weeks after the initial blood culture.

• Repeat specimens from the same patient for sample types other than blood cultures (e.g. tracheal aspirates, sputum, swabs, fluid aspirates, tissue biopsies and urine) will only be included if they are cultured at least one month after the initial culture.

On average about 15 *A. baumannii* isolates are submitted for mBMD per month. The expected number of isolates for the specified time period is therefore 400-450.

DATA COLLECTION

Data will be extracted from the NHLS laboratory information system - TrakCare. Data collected will include (also see appendix 1 for data collection sheet):

- Patient date of admission to the ward
- Date of sample registration
- Sample type
- Vitek®2 MIC
- BMD MIC
- Patient age
- Patient gender
- Ward number
- Type of ward (medical/surgical/ICU/non-ICU)

DATA ANALYSIS

Microsoft excel and statistica version 13.05.0.17 will be used for data analysis and the following analysis will be made:

• Data distribution will be assessed and parametric/non-parametric statistics will be used depending on the distribution of the data.

• The following equations will be used to assess agreement and error rates:

Categorical agreement = (number of isolates correctly classified by Vitek®2 as either colistin susceptible or resistant / Total number of isolates tested) X 100

Essential agreement = (number of isolates within 2 doubling dilutions of the BMD MIC by Vitek®2 / total number of isolates tested) X 100

Major error rate = (number of false resistant isolates by Vitek®2 / number of susceptible isolates by BMD) X 100

Very Major error rate = (number of false susceptible isolates by Vitek®2 / number of resistant isolates by BMD) X 100

ETHICS

Ethical approval for the study has been obtained from the University of Witwatersrand Human Research Ethics Committee (Clearance certificate number: M191048 MED 19-10-043 – see attached). Approval from the NHLS research and academic affairs office to access the Trakcare data and the Chief Executive Officer's office at CMJAH to utilize the data has been obtained (see attached).

BUDGET

Stationary and printing material at an approximated total cost of R2000 and publication fees (depending on the accepting journal) will be provided by the Department of Clinical Microbiology and Infectious Diseases, Witwatersrand University. No further costs will be incurred.

STUDY OUTCOMES

The researcher aims to accomplish the following with this study:

- Obtain a master of medicine degree in clinical pathology
- Learn research skills that will be valuable for future research projects
- Obtain a publication in a peer reviewed journal

LIMITATION/PROBLEMS

This study will only include XDR *A. baumannii* isolates as these are the isolates submitted for colistin mBMD. The epidemiology described will therefore be for this subset of organisms and not a reflection of all *A. baumannii* infections.

An additional limitation may include incomplete demographic data.

TIMELINE

	Jun	**Jul**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**
Literature review										
Preparing protocol										
Ethics application										
Protocol assessment										
Data collection										
Data analysis										
Writing up paper										

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APPENDIX 1

UNIQUE NUMBER	AGE	GENDER	ADMISSION DATE	WARD					DATE OF SAMPLE REGISTRATION	SAMPLE TYPE	VITEK		BMD	
				NUMBER	MEDICAL	SURGICAL	ICU	NON-ICU			MIC	INT	MIC	INT

APPENDIX 2: ETHICS CLEARANCE CERTIFICATE

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr Vuyolwethu Fadana

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M191048 MED 19-10-043

NAME: (Principal Investigator)	Dr Vuyolwethu Fadana
DEPARTMENT:	School of Pathology Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	A retrospective analysis of the performance of Vitek®2 compared to manual broth micro-dilution for colistin testing of susceptibilty Acinobacter baumanni clinical isolates
DATE CONSIDERED:	25/10/2019
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Teena Thomas
APPROVED BY:	Dr C Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	03/12/2019

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report** The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed In this case the study was initially reviewed October and will therefore be due in the month of October each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

03/12/2019
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX 3: PLAGIARISM FORM AND REPORT

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Vuyiswetha Faona (Student number: 705549) am a student registered for the degree of MMed in Clinical Anatomy in the academic year 2021.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature: _____ Date: 05/08/2021

705549:AJLM_MANUSCRIPT.doc x

by Vuyolwethu Fadana

Submission date: 28-Apr-2021 04:10PM (UTC+0200)
Submission ID: 1572373423
File name: nments_f923fc8b-5e9e-4a69-86e3-2ee9b7ff9fcb_AJLM_MANUSCRIPT.docx (155.81K)
Word count: 3620
Character count: 21777

AFRICAN JOURNAL OF LABORATORY MEDICINE MANUSCRIPT

A retrospective analysis of the performance of Vitek®2 compared to manual broth micro-dilution for colistin susceptibility testing of *Acinetobacter baumannii* clinical isolates

Vuyolwethu Fadana, Teena Thomas, Nina von Knoring

ABSTRACT

Background: The recommended method for colistin antimicrobial susceptibility testing (AST), manual broth micro-dilution (mBMD), is difficult to implement and therefore not readily available. In contrast, Vitek®2, an automated microbial identification and AST platform is available in most microbiology laboratories in South Africa. Assessment of its performance against mBMD for colistin AST has, however, shown conflicting results.

Objectives: To assess the performance of Vitek®2 against mBMD colistin minimum inhibitory concentration determination in extensively-drug resistant (XDR) *A. baumannii* isolates and study the epidemiology of these isolates.

Methods: Colistin mBMD results of XDR *A. baumannii* isolates from patients admitted at Charlotte Maxeke Johannesburg Academic Hospital between 01 January 2017 and 30 June 2019 were compared to the Vitek®2 results. Additional data was extracted to determine the distribution of XDR *A. baumannii* within the institution.

Results: Of the 410 isolates submitted for colistin mBMD, 337 had Vitek®2 results. Vitek®2 performance as compared to mBMD was as follows: categorical agreement 89%, essential agreement 56% with major error rate of 8% and very major error rate of 55%. Of the analysed isolates, the majority were from adult patients (71%), the remainder from infants (25%) and older paediatric patients (4%). Most isolates were from non-ICU wards (62%) with surgical wards dominating in adults and medical wards in the paediatric population.

Conclusion: Vitek®2 demonstrated unacceptable performance when compared to mBMD and cannot be recommended for colistin AST. Factors driving the difference in ward distribution between the age groups require further investigation.

Key words: Acinetobacter; colistin; broth microdilution; Vitek®2

INTRODUCTION

The genus *Acinetobacter* was discovered by Dutch microbiologist Martinus Willem Beijerinck from soil samples in 1911.[1] Since then, species within the *A. baumannii* complex have been implicated in a variety of infections including blood stream infections, skin and soft tissue infections, respiratory tract infections and meningitis.[2, 3] Distinction between colonization and infection is often difficult in hospitalized patients as this organism is ubiquitous in the environment. It has been associated with increased mortality when isolated from critically ill patients.[4] Risk factors for infection with *A. baumannii* complex include mechanical ventilation, prolonged hospital stay, ICU admission and exposure to patients that are either infected or colonized with *A. baumannii*.[4]

Increasing antimicrobial resistance in this group of organisms and more recently, resistance to the polymyxin antibiotic, colistin, has meant that timeous identification of the infecting pathogen and determination of its antimicrobial susceptibility profile is necessary to ensure early appropriate antimicrobial therapy.[4, 5] The emergence of resistance to colistin, one of the last active agents against extensively drug -resistant (XDR) *A. baumannii* has warranted the review of available antimicrobial susceptibility testing (AST) methods. Different laboratory methods for the assessment of colistin AST amongst *A. baumannii* isolates have been assessed. Challenges with polymyxin AST are due to its molecular nature. Its large size limits diffusion into agar with disc diffusion and gradient diffusion (Etest) methods and the positive charge causes it to bind to the plastic material in microtiter plates used for susceptibility testing.[6]

Since 2016, the reference method for colistin AST recommended by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) is the ISO broth micro-dilution (BMD) method (ISO-20776).[7] Owing to its laborious nature, the implementation of manual BMD (mBMD) is currently not feasible in the routine microbiology laboratory.[8] Its use has been restricted to reference centers where more experienced personnel conduct this testing. This prolongs laboratory turn-around times and may cause delays in appropriate therapy. Alternative

methods therefore need to be considered. The Food and Drug Administration (FDA) recommends that for an AST method to be deemed acceptable, it must provide high categorical (CA) and essential (EA) agreement (both ≥90%) with low major error (ME) and very major error (VME) rates when compared to the reference method (<3%).[8] The use of diffusion methods for colistin susceptibility testing has been found to result in an unacceptable rate of false susceptible results (1.7-13%) when compared to BMD.[6, 9, 10-11] Although both diffusion methods (i.e. disc diffusion and gradient diffusion) are easy to perform and are readily available to most routine laboratories, they are currently not recommended for use by the CLSI-EUCAST polymyxin breakpoints working group for colistin susceptibility testing.[12]

Vitek®2 (BioMerieux Inc., Marcy l'Etoile, France) is an automated microbial identification and AST platform that is used in most public and private laboratories within South Africa and, therefore, offers an attractive alternative to mBMD for colistin susceptibility testing. Findings from studies comparing it to mBMD have, however, shown conflicting results on its performance. Daifopoulou et al compared different colistin susceptibility testing methods (mBMD with polysorbate-80, Vitek®2, Etest, Agar dilution and MIC test strip) on 51 *Klebsiella pneumoniae* and 20 *A. baumannii* clinical isolates.[13] Eighteen of the *A. baumannii* isolates were resistant to colistin by mBMD. Assessed against mBMD, Vitek®2 resulted in CA of 85% and EA of 90% for the *A. baumannii* isolates. There were no VMEs reported. These findings were similar to those obtained by Lo-Ten-Foe et al who also found good EA (93.1%) between Vitek®2 and mBMD amongst 80 gram-negative bacteria, including 10 *Acinetobacter* spp. isolates.[14] Categorical agreement and error rates were not reported. Piewngam and Kiratisin demonstrated a low VME rate of 0.7% on 290 isolates of *A. baumannii*.[15] These findings suggest that Vitek®2 could be a viable alternative to mBMD. In contrast to these results, Vourli et al assessed two automated systems, Phoenix100 and Vitek®2, and found them to have unacceptably high VME rates when compared to mBMD (41.4% and 37.9% respectively).[16] In addition, in an undated notice BioMerieux retracted Vitek® 2 use for colistin AST owing to the CLSI-EUCAST recommendations and an in-house study showing a high VME rate.[17] The MIC distribution of the isolates and VME rate were not specified. Due to these contradictory findings in the literature, further research into this area was warranted.

As a result, the aim of this study was to compare the performance of Vitek®2 colistin susceptibility testing to mBMD for clinical XDR *A. baumannii* isolates and to describe the epidemiology of these isolates. We also aimed to make recommendations on the use of Vitek®2 for colistin susceptibility testing within the National Health Laboratory Services (NHLS) routine microbiology laboratories.

METHODS

Ethical considerations

Ethical approval was obtained from the University of Witwatersrand Human Research Ethics Committee (clearance certificate number M191048 MED 19-10-043).

Data collection

A descriptive, retrospective data analysis of patients admitted to Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) with positive cultures of XDR *A. baumannii* was conducted. Epidemiological and microbiological data was extracted from the Corporate Data Warehouse (CDW), a division of the NHLS that stores national public health-care laboratory data. The epidemiological data included patient age, gender and admission ward type (i.e. intensive care unit (ICU)/non-ICU and surgical/medical). The microbiological data included the sample type that cultured the isolate, Vitek®2 and mBMD colistin MIC results.

Data analysis

All XDR *A. baumannii* isolates cultured between 01 January 2017−30 June 2019 from any specimen type were analyzed. Isolates that were obtained from outpatient departments or without a ward type specified, were excluded. Repeat blood culture isolates from the same patient were included if the isolate was cultured at least two weeks after the initial blood culture. Repeat specimens from the same patient for sample types other than blood cultures (e.g. tracheal aspirates, sputum, swabs, fluid aspirates, tissue biopsies and urine) were included if they were obtained at least one month after the initial culture. Intravenous central venous catheter tips were not included as they are processed in a separate laboratory using a different automated AST platform. All isolates with mBMD results with/without Vitek®2 results were used for epidemiological analysis. Only isolates with colistin susceptibility results from both Vitek®2 and mBMD were used to assess performance of Vitek®2. The

performance of Vitek®2 colistin susceptibility testing was determined by assessing categorical agreement, essential agreement, very major and major error rates in comparison to mBMD colistin susceptibility testing, according to the FDA recommendations.[8]

Microsoft excel 2016 was used for data analysis. For the distribution of epidemiological data parametric/non-parametric statistical methods were applied. Data with a Gaussian distribution was described by means of 95% confidence intervals (95% CI), whereas for non-parametric data, medians with interquartile ranges (IQR) were used. The following equations were employed to assess agreement and error rates:

- Categorical agreement = (number of isolates correctly classified by Vitek®2 as either colistin susceptible or resistant in comparison to mBMD / total number of isolates tested) X 100
- Essential agreement = (number of isolates within one doubling dilutions of the mBMD MIC on Vitek®2 / total number of isolates tested) X 100
- Major error rate = (number of falsely resistant isolates on Vitek®2 / number of susceptible isolates by mBMD) X 100
- Very Major error rate = (number of falsely susceptible isolates by Vitek®2 / number of resistant isolates by mBMD) X 100

RESULTS

Data for 523 isolates was obtained from all specimen types that cultured XDR *A. baumannii* and were submitted for colistin mBMD. Hundred and thirteen isolates were removed as they were duplicates or had other exclusion criteria. Of the 410 remaining isolates, 337 (82%) isolates had both Vitek®2 and mBMD MIC results (Figure 1).

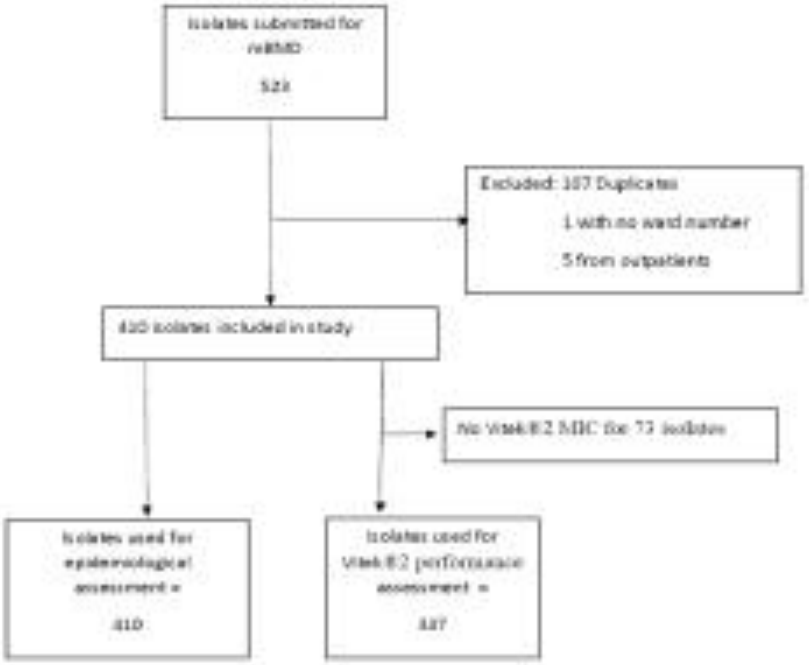


Fig. 1. Number of isolates utilised for epidemiological and Vitek®2 assessment (01 Jan 2017-30 Jun 2019)

The number of samples submitted for mBMD was low at the beginning of the study period but subsequently increased over time. Excluding isolates without a specified age (3%; 13/410), the majority of isolates were from adult patients ≥14 years (280/397 - 71%; mean age of 43 years, 95% CI: 41 – 44). Amongst the paediatric population ≤13 years, 61% (72/117) of isolates were from neonates (median age of 9 days, IQR 5 - 14). The ratio of males to females across the different ages groups was 0.9:1 in neonates, 1.3:1 in other children and 1:1 in adults.

Figure 2 illustrates the distribution of admission ward types for the different age groups (neonates ≤1 month, children >1 month -13 years and adults ≥14 years) with positive cultures. Amongst all age groups, the majority of isolates were from non-ICU wards with differences in distribution between medical and surgical wards noted between the age groups.

Samples from non-ICU surgical wards were more prevalent amongst the adult age group, whereas samples taken in non-ICU medical wards predominated among the pediatric group.

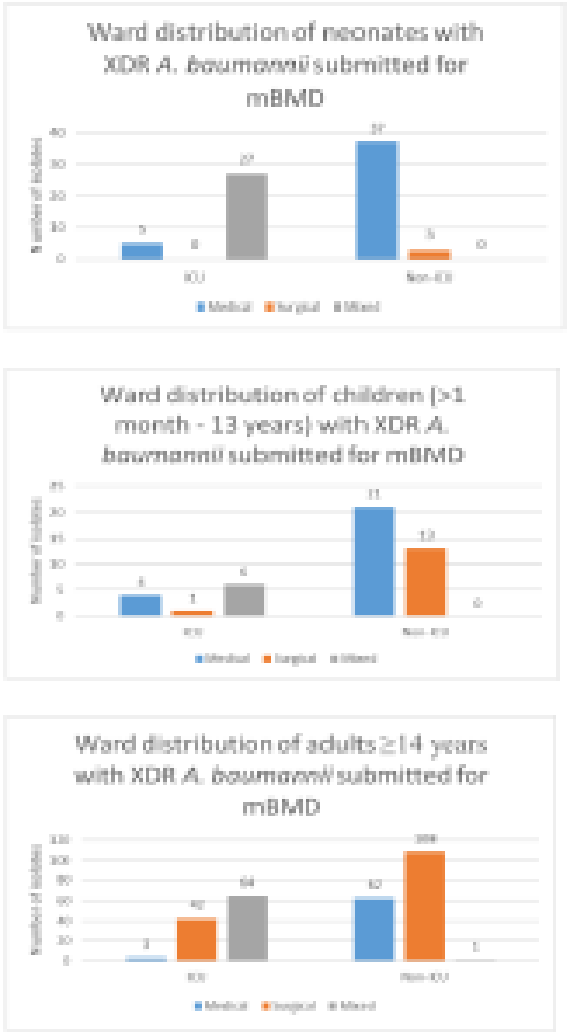
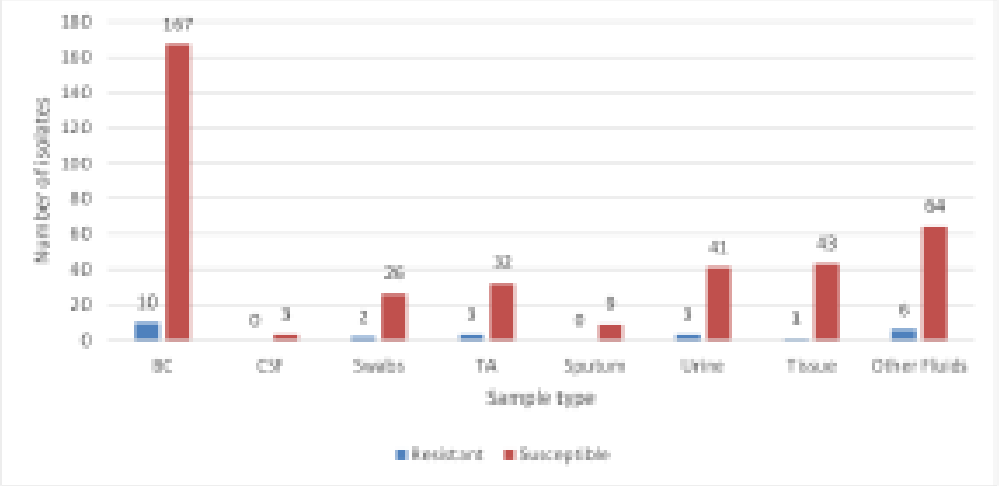


Fig. 2. Ward distribution of neonates, children (>1 month - 13 years) and adults ≥14 years with XDR *A. baumannii* submitted for mBMD

Regarding the sample types submitted, the majority of isolates were cultured from blood culture samples (177/410; 43%) and most of the colistin-resistant isolates were from this

sample type. Twenty-five (25/410; 6%) colistin-resistant isolates were identified by mBMD in total (Figure 3).



BC blood culture; CSF cerebrospinal fluid; TA tracheal aspirate

Fig. 3. Distribution of colistin-resistant and susceptible XDR *A. baumannii* isolates by sample type (n=410)

The majority of the colistin-resistant isolates were from adult patients (17/25; 68%). Non-ICU wards predominated (16/25; 64%), with most isolates coming from surgical wards (11/25; 44%) (Figure 4).

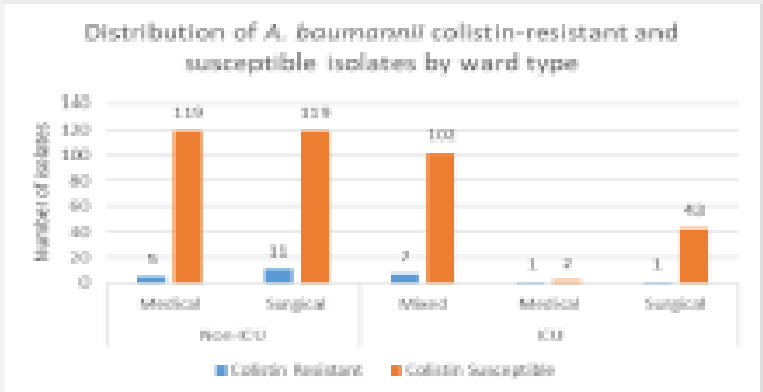


Fig. 4: Distribution of *A. baumannii* colistin-resistant and susceptible isolates by ward type

Of the 337 isolates with colistin susceptibility results available from both Vitek®2 and mBMD, 20 (6%) were resistant to colistin by mBMD. Vitek®2 was found to have a categorical agreement of 89% (300/337) and an essential agreement of 55% (184/337) with mBMD. The very major error rate was 55% (11/20) and the major error rate was 8% (26/317). Most of the very major errors occurred close to the colistin breakpoint of 2 µg/mL (Table 1).

Table 1: Colistin minimum inhibitory concentration results for XDR *A. baumannii* isolates by both Vitek®2 and mBMD

		Vitek®2 MIC								
		≤0,5	0,5	1	2	4	8	16	>16	Total number of isolates
	≤0,125	0	0	0	0	0	0	0	1	1
	0,25	24	0	0	0	0	0	0	1	25

DISCUSSION

Our findings provide insight into the epidemiology of XDR *A. baumannii* in our setting. The distribution of XDR *A. baumannii* isolates within CMJAH appears to vary based on the age group assessed. There was a predominance of these isolates from medical wards amongst the paediatric population and surgical wards amongst the adults. In our setting, all wards are managed by the same infection prevention and control (IPC) team and contact precautions are initiated for all patients with multidrug-resistant (MDR) and XDR isolates regardless of the ward type. Differences in antibiotic prescribing practices cannot be excluded as another driving factor for the differences in the ward areas that were affected. Although ICU admission has been cited as a risk factor for infection with MDR isolates,[19] this was not

evident in this study. Most isolates were from non-ICU wards in all age groups. However, prior ICU admission was not assessed.

The majority of colistin-resistant isolates were obtained from the adult population and predominantly from the non-ICU surgical wards. These wards also had the highest overall number of XDR *A. baumannii* isolates. This highlights the possible relationship between colonization/infection with XDR isolates and development of colistin resistance. Patients having prior colistin-susceptible isolates have been found to develop colistin resistance on exposure to colistin.[39] We postulate that wards with higher numbers of patients colonized/ infected with XDR *A. baumannii* isolates are more likely to be exposed to colistin and subsequently develop resistance. Appropriate antimicrobial stewardship and IPC practices could mitigate this. The proportion of colistin-resistant and susceptible isolates between the different sample types was similar and as such, the sample type cannot be used to predict resistance.

The recommended reference method for colistin susceptibility testing, mBMD according to ISO-20776, is difficult to implement in routine microbiology laboratories. Due to financial constraints and technical competencies required, only one NHLS laboratory is currently able to offer mBMD in South Africa. This is likely to have a negative impact on patient care due to delays in turn-around-times of results. In contrast, Vitek®2 is available in most NHLS microbiology laboratories and would have been a favorable alternative. However, our study demonstrates unacceptable performance, with 11 of 20 (55%) colistin-resistant isolates having false-susceptible results on Vitek®2. Most of these isolates had a minimum inhibitory concentration (MIC) close to the susceptible breakpoint, suggesting that automated systems may have poor performance close to this value. Vitek®2 also reported some isolates with mBMD colistin MIC >64 mg/L as susceptible. This has the potential to result in inappropriate antimicrobial therapy and adverse patient outcomes. This extremely high VME rate is in contrast to previously conducted studies as mentioned earlier. However, those studies included fewer isolates of *A. baumannii* compared to our study. In addition to the unacceptable VME rate, the CA, EA and the ME rate with Vitek®2 were also unacceptable according to the FDA requirements for an AST testing platform. This is in keeping with a study by Pfennigwerth et al that also found poor essential agreement (75.9%) with Vitek®2

on testing Enterobacterales, despite better categorical agreement (90.5%).[20] Based on these results Vitek®2 cannot be recommended as an alternative and attempts need to be made to make mBMD more readily available and easier to implement until other testing options become available.

Matuschek et al evaluated 5 recently developed commercial BMD systems - SENPAI (custom Sensititre plate, Thermo Fisher Scientific, EastGrinstead, UK), MICRONAUT-S and MICRONAUT MIC-Strip (MERLINDiagnostika Gmbh, Bornheim, Germany), SensiTest (LioFilchem,Roseto degli Abruzzi, Italy) and UMIC (Biocentric, Bandol, France). These were evaluated against mBMD using various gram- negative organisms including 22 *Acinetobacter spp* isolates.[21] They demonstrated overall better performance compared to our findings with Vitek®2. The majority of platforms had acceptable CA and EA (>90%), with much lower error rates than obtained in this study. Interestingly, there were no falsely- susceptible *A. baumannii* isolates. These methods may offer an alternative to mBMD and further research on their performance is required.

LIMITATIONS

XDR *A. baumannii* isolates that were cultured and not submitted for mBMD were not included in this study. Submission of isolates for mBMD within the institution is dependent on a number of factors including whether the isolates are considered clinically significant or if colistin is used for treatment. This may have had an effect on the data that is presented.

Only a small number of colistin-resistant isolates were obtained. Analysis of a larger number of resistant isolates with a wider MIC distribution is required to confirm our findings. However, the few numbers of these isolates highlights the impressive role of the antimicrobial stewardship and IPC practices in our setting. In addition, the distinction between colonization and infection in the patients with positive cultures of XDR *A. baumannii* was beyond the scope of this study, as a result, the clinical significance of these isolates could not be determined.

Strengths of this study included the large number of XDR *A. baumannii* isolates that were available for analysis compared to other studies. In addition, the retrospective design allowed for the findings to be a reflection of what one would expect in routine clinical practice.

CONCLUSION

Based on the results of this study, Vitek®2 cannot be recommended as an alternative to mBMD for colistin AST in our setting. Further studies are required to determine if the commercially-available colistin BMD methods are a cost effective option with acceptable analytical performance. In addition, the semi-automated platforms such as Vitek®2, should be optimized for colistin AST. Factors driving the differences in ward distribution amongst the different age groups still need to be elucidated. The use of colistin as a driver for the development of resistance in this population cannot be excluded and requires further study. Ongoing monitoring of colistin resistance is also required.

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