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Original Article

Laboratory based antimicrobial resistance surveillance for *Pseudomonas aeruginosa* blood isolates from South Africa

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Abstract

Introduction: Antimicrobial resistant bacterial infections are widespread globally and increases in antimicrobial resistance presents a major threat to public health. *Pseudomonas aeruginosa* is an opportunistic healthcare-associated pathogen with high rates of morbidity and mortality and an extensive range of resistance mechanisms. This study describes the antibiotic susceptibility profiles of *P. aeruginosa* isolates from patients with bacteraemia submitted by sentinel laboratories in South Africa from 2014 to 2015.

Methodology: Organism identification and antimicrobial susceptibility testing were done using automated systems. Molecular methods were used to detect common resistance genes and mechanisms.

Results: Overall the susceptibility was high for all antibiotics tested with a decrease over the two-year period. There was no change in the MIC_{50} and MIC_{90} breakpoints for all antibiotics from 2014 to 2015. The MIC_{50} was within the susceptible breakpoint range for most antibiotics and the MIC_{90} was within the susceptible breakpoint range for colistin only. Phenotypically carbapenem non-susceptible isolates harboured the following plasmid-mediated genes: *bla*$_{VIM}$ (n = 81, 12%) and *bla*$_{GES}$ (n = 6, 0.9%); *bla*$_{NDM}$ (n = 4, 0.6%) and *bla*$_{OXA-48}$ and variants (n = 3, 0.45%). Porin deletions were observed in one meropenem non-susceptible isolate only, and multi-drug resistance efflux pumps were expressed in the majority of the non-susceptible isolates investigated. *Bla*$_{VEB-1}$, *bla*$_{IMP}$ and *bla*$_{KPC}$ were not detected.

Conclusion: The prevalence of resistance to commonly used antibacterial agents was low for *P. aeruginosa* isolates and similarly, tested resistance mechanisms were detected in a relatively small proportion of isolates. Findings in this study represent baseline information for understanding antimicrobial susceptibility patterns in *P. aeruginosa* isolates from blood. Our surveillance report may assist in contributing to hospital treatment guidelines.

Key words: Antimicrobial susceptibility testing; resistance genes; carbapenemases; efflux pumps; porins.

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Introduction

Pseudomonas aeruginosa is an opportunistic nosocomial pathogen with high rates of morbidity and mortality in infected patients [1]. Treatment is challenging due to the presence of intrinsic antimicrobial resistance genes and the organism's ability to acquire genes encoding multiple resistance mechanisms [2,3]. These mechanisms of resistance often exist simultaneously and confer combined resistance to the bacterial isolate [4]. Intrinsic resistance

may be due to the production of an inducible AmpC β-lactamase; low permeability of the cell wall due to a porin loss; and upregulation of multi-drug resistant efflux pump systems. Acquired resistance is due to the acquisition of additional resistance genes on mobile genetic elements such as plasmids, transposons and bacteriophages from other organisms and due to mutations in chromosomal genes that upregulate resistance genes [5-7]. *P. aeruginosa* is naturally susceptible to carboxypenicillins, ureidopenicillins (e.g. piperacillin); some third- and fourth-generation cephalosporins (e.g. ceftazidime and cefepime), monobactams (e.g. aztreonam), carbapenems (e.g. imipenem, meropenem, doripenem), aminoglycosides and fluoroquinolones (e.g. ciprofloxacin). Resistance to penicillins and cephalosporins may be due to hyperproduction (or derepression) of Amp-C cephalosporinases which are chromosomally encoded [7-9]. Protein channels (porins) allow the uptake of nutrients and other important substances into the cell including antimicrobial agents [10]. Mutational impermeability due to the loss of OprD, a porin that forms narrow transmembrane channels in the outer membrane, results in resistance to carbapenems particularly imipenem and reduced susceptibility to meropenem. Mutations can result in porin loss, a modification in the size of the porin channel or a reduction in expression of the porin. Resistance-nodulation-division (RND) bacterial efflux pumps are a family of transporters that play an important role in virulence, stress responses and clinical resistance. These pumps are regulated by regulators (repressors or activators), modulators and two-component regulatory systems in which mutations can result in antimicrobial resistance [11]. The upregulation of efflux pumps confers resistance to a host of antimicrobial agents: MexAB-OprM upregulation confers resistance to the penicillins, fluoroquinolones, cephalosporins and to a lesser degree, meropenem; MexCD-OprJ and MexEF-OprN upregulation causes resistance to fluoroquinolones and some beta–lactams; while MexXY-OprM upregulation leads to aminoglycoside resistance [7,9,12,13]. Acquired resistance to antibiotics in the beta-lactam group including the penicillins, cephalosporins, monobactams and carbapenems is due to the acquisition of genes encoding antimicrobial hydrolysing enzymes such as the *Pseudomonas* specific enzymes (PSE) PSE-1 and PSE-4; the extended-spectrum beta-lactamases (ESBL) TEM, SHV, VEB, GES, PER and BEL types; the oxacillinases (OXA) and the metallo-beta-lactamases (MBL) NDM, IMP and VIM [7,9]. Acquired resistance to colistin may be due to the presence of the plasmid-mediated *mcr-1* gene [14].

In South Africa there have been reports of multi-drug resistant *P. aeruginosa* infections causing outbreaks in various hospitals [15-17] justifying the need for national surveillance of this pathogen in order to monitor antimicrobial resistance trends. A national surveillance programme as outlined and advocated by the World Health Organisation (WHO) will assist in creating awareness among clinicians and the general public on the appropriate use of antibiotics [18]. The Antimicrobial Resistance Laboratory (AMRL) at the Centre for Healthcare-associated infections, Antimicrobial Resistance and Mycoses (CHARM) utilising the GERMS-SA platform has therefore established a laboratory-based antimicrobial resistance surveillance system for nosocomial pathogens. In this study the antibiotic susceptibility profiles of *P. aeruginosa* isolates from patients with bacteraemia and positive blood cultures were determined. Isolates were obtained from 12 sentinel sites from four provinces in South Africa. Various resistant phenotypes and genotypes of *P. aeruginosa* from 2014 to 2015 were characterised to determine antimicrobial susceptibility profiles and common resistance mechanisms among pathogenic strains.

Methodology

Patient selection

P. aeruginosa blood culture isolates were submitted to the Centre for Healthcare-associated infections, Antimicrobial Resistance and Mycoses (CHARM) at the National Institute for Communicable Diseases (NICD) from 12 public healthcare sector academic centers in four provinces in South Africa: Gauteng, KwaZulu-Natal, Free State and the Western Cape. Demographic information was obtained from the laboratory request form. A three-week exclusion period was applied to avoid duplicate isolates of the same organism from the same patient. Audit cases were defined as those cases that were identified according to the public healthcare sector Corporate Data Warehouse (CDW) but not received for processing in the laboratory. The CDW houses records of patient details and laboratory results.

Phenotypic methods

When isolates were received in the AMRL, organism identification was confirmed using automated systems (VITEK II (bioMèrieux, Marcy-l'Etoile, France) and/or the Microflex MALDI-ToF (Bruker Daltonik, GmbH). To resolve a conflict in the organism

identification between the sending and reference laboratories, 16s rRNA sequencing was performed (Inqaba Biotec, Pretoria, South Africa). Antimicrobial susceptibility testing (AST) was performed using the MicroScan Walkaway system (Siemens, Sacramento, CA, USA). Susceptibility results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [19]. The Sensititre instrument (Trek Diagnostic Systems, Cleveland, Ohio, USA) was used for colistin susceptibility and was interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [20].

Genotypic methods

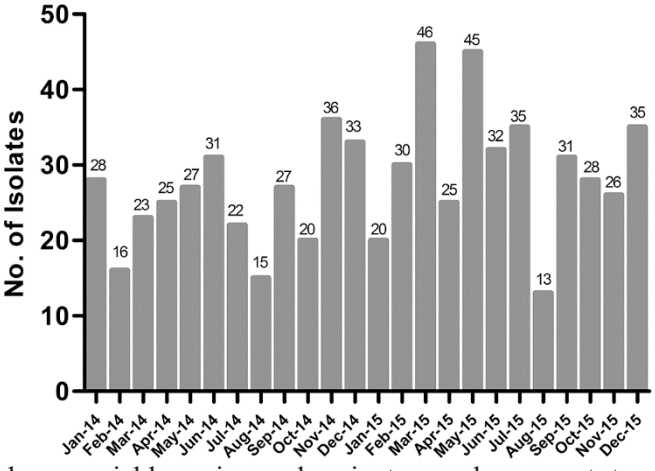
Molecular resistance mechanisms were determined for different isolates based on the phenotypic susceptibility results. In brief, for those isolates displaying:

- Carbapenem resistance were selected for the detection of carbapenemases *bla*$_{NDM}$, *bla*$_{IMP,}$ *bla*$_{VIM;}$ *bla*$_{OXA-48}$ and its variants, *bla*$_{KPC}$ and *bla*$_{GES}$ as well as porin (OprD) deletion.
- Resistance to any of the beta-lactams were selected for the detection of the ESBL, *bla*$_{VEB-1}$.
- Resistance to the fluoroquinolones and/or aminoglycosides and/or β-lactams (pipercillin, cefepime, ceftazidime) were selected for testing for the expression of the efflux pumps MexAB-OprM, MexXY-OprM, MexCD-OprJ and MexEF-OprN.

the DNA was extracted using a crude boiling method. Half a 1 µL loop-full of subculture was resuspended in Tris-EDTA (ethylenediamine tetra-acetic acid) buffer and heated at 95ºC for 25 minutes. The bacterial cells were lysed and the supernatant containing the DNA was harvested and stored at -70ºC for further screening.

Bla$_{NDM}$, *bla*$_{IMP,}$ *bla*$_{VIM,}$ *bla*$_{OXA-48}$ and its variants, *bla*$_{KPC}$ and *bla*$_{GES,}$ were screened for using a multiplex real-time polymerase chain reaction (PCR) (LightCycler 480 II, Roche Applied Science, Penzberg, Germany; LightCycler 480 Probes Master kit, Roche Diagnostics, Indianapolis, USA) and the individual LightMix Modular carbapenemase kits (Roche Diagnostics, Indianapolis, USA). The G-Storm (Somerton Biotechnology Centre, Somerton, UK) thermal cycler was used for the conventional singleplex PCRs of *bla*$_{VEB-1}$ and OprD porin deletions using the Qiagen Multiplex PCR kit (Qiagen, Nordrhein-Westfalen, Germany) with previously published primers [12,21]. Because the OprD region is a hypervariable region and variants may be present, two PCRs may have been required i.e. in cases of the first PCR not amplifying the target, an alternate PCR for this gene was performed according to a previous publication [12]. The presence of this gene following both PCRs indicates the absence of a porin deletion i.e. there was no reduction of the transmembrane channels (porins). The absence of this gene following both PCRs indicates a reduction of the transmembrane channels and hence a deletion of the porin, OprD potentially indicating that resistance to the cabapenems may be attributed to this.

Figure 1. Monthly distribution of viable *P. aeruginosa* isolates collected over the two- year period, 2014 and 2015 (n = 669).



To determine the expression of the Mex efflux pumps, RNA was extracted using the Roche High Pure RNA Isolation kit (Roche Diagnostics, Indianapolis, USA) followed by cDNA synthesis using the Roche Transcriptor First Strand cDNA Synthesis kit (Roche Diagnostics, Indianapolis, USA). Conventional PCR was performed on the G-Storm thermal cycler using the Qiagen Multiplex PCR kit and previously published primers [22].

Amplified products were separated on a 2% agarose gel.

Statistical Analysis

Data were captured in a Microsoft Access database and exported to Microsoft Excel which was used for data analysis. Demographic data of patients from whom isolates were obtained were summarised and descriptive statistics were analysed using GraphPad Prism (version 5.01 for Windows, GraphPad Software, La Jolla California USA).

Ethics

Permission to conduct this study was obtained from the Human Research Ethics Committee (Medical)

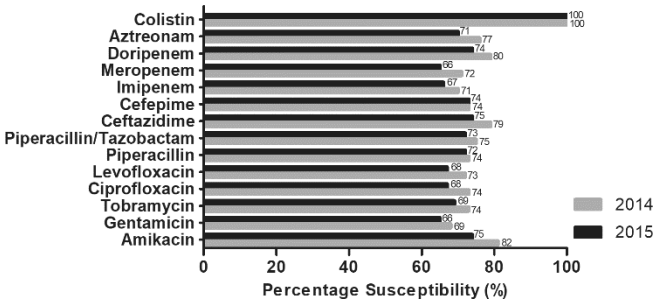
R14/49, University of Witwatersrand, Johannesburg, South Africa, Clearance certificate M10464.

Results

A total of 956 *P. aeruginosa* isolates were identified for the period of January 2014 to December 2015. Of these 686 were received in the laboratory, 17 of which were non-viable and one was missing/broken. The remaining 270 isolates were audit cases. This report analysed a total of 669 isolates that were received. Figure 1 demonstrates the monthly distribution of viable isolates collected over the two-year period. Majority of the isolates were submitted by Gauteng (n = 347, 52%), followed by the Western Cape (n = 237, 35%), KwaZulu-Natal (n = 57, 9%) and the Free State (n = 28, 4%). Demographic data was limited. Males accounted for 52% (n = 349) and females accounted for 45% (n = 306) with unknown sex information for the remaining 14 patients. Patient age ranged from < 1 year to 88 years with a median age of 36.9 years (n = 649). Age was unknown in 20 patients. Majority of the patients were from adult wards (71%, n = 72) followed by 25% (n = 170) from paediatric wards. Polymicrobial bloodstream infection was noted in 87 (13%) of the patients with various combinations of Gram-positive and Gram-negative organisms.

Overall the susceptibility rate was high for all antibiotics tested (Table 1). A breakdown of the susceptibility by antibacterial agent and year is presented in Figure 2; overall the susceptibility rates decreased over the two-year period but were not significant (p = 0.8148). Susceptibility to the aminoglycosides ranged from 69% to 82% in 2014 and 66% to 75% in 2015. The susceptibility to all three aminoglycoside antibiotics decreased from 2014 to 2015. This was also observed for ciprofloxacin which was 74% in 2014 and decreased to 68% in 2015. There was a decrease in susceptibility of 2% for piperacillin and piperacillin/tazobactam from 2014 to 2015. Decreases in susceptibility were also seen for the third and fourth generation cephalosporins from 2014 to 2015 (approximately 4% for ceftazidime and an inconsequential change of 0.16% for cefepime).

Figure 2. Percentage susceptibility to antibacterial agent, 2014 and 2015.



Percentages have been rounded off; For 2015, results for aztreonam were missing for 4 isolates (0.6%); One isolate could not be retrieved for colistin susceptibility testing on the Sensititre instrument.

Table 1. Antibiotic MIC_{50} and MIC_{90} breakpoints for *P. aeruginosa* isolates (n=669).

	Susceptible isolates (n)	Susceptibility (%)	2014		2015		MIC interpretive breakpoints (µg/mL)*	
			MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	Susceptible	Resistant
Antibacterial agent*								
Aminoglycosides								
Amikacin	522	78	≤ 8	> 32	≤ 8	> 32	≤ 16	≥ 64
Gentamicin	485	67	4	> 8	4	> 8	≤ 4	≥ 16
Tobramycin	477	71	≤ 2	> 8	≤ 2	> 8	≤ 4	≥ 16
Fluoroquinolones								
Ciprofloxacin	471	70	≤ 0.5	> 2	≤ 0.5	> 2	≤ 1	≥ 4
**Levofloxacin	467	70	≤ 1	> 4	≤ 1	> 4	≤ 2	≥ 8
Extended Spectrum-Beta-Lactams								
Piperacillin	488	73	≤ 8	> 64	≤ 8	> 64	≤ 16	≥ 128
Piperacillin/Tazobactam	495	74	≤ 8	> 64	≤ 8	> 64	≤ 16/4	≥ 128/4
Ceftazidime	513	77	2	> 16	2	> 16	≤ 4	≥ 16
Cefepime	494	74	4	> 16	4	> 16	≤ 2	≥ 16
Carbapenems								
Imipenem	459	69	≤ 1	> 8	≤ 1	> 8	≤ 2	≥ 8
Meropenem	458	69	≤ 1	> 8	≤ 1	> 8	≤ 2	≥ 8
Doripenem	514	77	≤ 1	> 4	≤ 1	> 4	≤ 2	≥ 8
Aztreonam	491	74	4	16	4	> 16	≤ 8	≥ 32
***Colistin	668	100	≤ 2	≤ 2	≤ 2	≤ 2	≤ 2	≥ 8

Percentages have been rounded off; * Clinical Laboratory Standards Institute guidelines, 2016 [17]; For 2015, results for aztreonam were missing for 4 isolates (0.6%); **Note: Levofloxacin is generally not used as an anti-pseudomonal agent, particularly in treating bacteraemias; ***Note: One isolate could not be retrieved for colistin susceptibility testing on the Sensititre instrument.

Table 2. Antimicrobial susceptibility overview for 669 *P. aeruginosa* isolates and genotype results for non-susceptible isolates.

		Carbapenemase								Expression of efflux pumps			
Antibiotic	**Total no. of Isolates n, (%)**	**OXA-48 and variants n, (%)**	**NDM n, (%)**	**VIM n, (%)**	**IMP n, (%)**	**GES n, (%)**	**KPC n, (%)**	**VEB-1 n, (%)**	**OprD deletion n, (%)**	**MexAB-OprM n, (%)**	**MexCD-OprJ n, (%)**	**MexXY-OprM (n), %**	**MexEF-OprN (n), %**
Imipenem													
Susceptible	459 (69)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Nonsusceptible	210 (31)	3 (0.45)	4 (0.6)	81 (12)	0	6 (0.9)	0	0	0	NT	NT	NT	NT
Meropenem													
Susceptible	458 (69)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Nonsusceptible	211 (32)	3 (0.45)	4 (0.6)	78 (11.7)	0	6 (0.9)	0	0	1 (0.15)	NT	NT	NT	NT
Doripenem													
Susceptible	514 (77)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Nonsusceptible	155 (23)	3 (0.45)	3 (0.45)	76 (11)	0	5 (0.75)	0	0	0	NT	NT	NT	NT
Piperacillin													
Susceptible	488 (73)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Nonsusceptible	181 (27)	NT	NT	NT	NT	NT	NT	0	NT	137 (23)	137 (23)	132 (22)	136 (23)
Ceftazidime													
Susceptible	513 (77)	NT	NT	NT	NT	NT	NT	0	NT	NT	NT	NT	NT
Nonsusceptible	156 (23)	NT	NT	NT	NT	NT	NT	0	NT	126 (21)	126 (21)	122 (20)	125 (21)
Cefepime													
Susceptible	494 (74)	NT	NT	NT	NT	NT	NT	0	NT	NT	NT	NT	NT
Nonsusceptible	175 (26)	NT	NT	NT	NT	NT	NT	0	NT	133 (22)	133 (22)	130 (22)	131 (22)
Aztreonam													
Susceptible	490 (74)	NT	NT	NT	NT	NT	NT	0	NT	NT	NT	NT	NT
Nonsusceptible	174 (26)	NT	NT	NT	NT	NT	NT	0	NT	NT	NT	NT	NT
Ciprofloxacin													
Susceptible	468 (70)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Nonsusceptible	199 (30)	NT	NT	NT	NT	NT	NT	NT	NT	150 (25)	150 (25)	146 (24)	146 (24)
Levofloxacin													
Susceptible	467 (70)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Nonsusceptible	202 (30)	NT	NT	NT	NT	NT	NT	NT	NT	150 (25)	150 (25)	146 (24)	146 (24)
Amikacin													
Susceptible	522 (78)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Nonsusceptible	147 (22)	NT	NT	NT	NT	NT	NT	NT	NT	110 (18)	NT	105 (18)	NT
Gentamicin													
Susceptible	449 (67)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Nonsusceptible	220 (33)	NT	NT	NT	NT	NT	NT	NT	NT	165 (28)	NT	141 (24)	NT
Tobramycin													
Susceptible	479 (72)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Nonsusceptible	190 (28)	NT	NT	NT	NT	NT	NT	NT	NT	139 (23)	NT	133 (22)	NT

Only isolates that produced a non-susceptible phenotypic (AST) result was selected for genotypic testing. Percentages have been rounded off; NT: not tested, denotes that the phenotypic (AST) result did not warrant the genotypic assay or a susceptible result was obtained; For the porin OprD, a deletion denotes a negative result i.e. OprD was not detected; OXA: oxacillinase, NDM: New Delhi Metallo-beta-lactamase, VIM: Verona Intergron-encoded Metallo-beta-lactamase, IMP: Imipenem Metallo-beta-lactamase, GES: Guiana Extended-spectrum beta-lactamase, KPC: *Klebsiella pneumoniae* carbapenemase, VEB: Vietnam Extended-spectrum beta-lactamase, Opr: Outer membrane porin protein, Mex: multidrug efflux.

Decreases in susceptibility were observed for the carbapenems (approximately 5% for imipenem and 6% for meropenem and doripenem). There was approximately a 6% susceptibility decrease for aztreonam. All isolates were fully susceptible to colistin; however, one isolate could not be retrieved and was not tested on Sensititre for colistin susceptibility. There was no change in the MIC_{50} and MIC_{90} values for all antibiotics from 2014 to 2015 and the MIC_{50} was within the susceptible breakpoint range for most antibiotics with the exception of cefepime. The MIC_{90} was within the susceptible breakpoint range for colistin only. With the exception of ceftazidime, cefepime, imipenem and meropenem, which were within the resistance breakpoint range, these MIC_{90} breakpoint values corresponded to the intermediate resistance category for the remaining antibiotics (Table 1).

Table 2 demonstrates the overall numbers and percentages of the non-susceptible isolates per resistance genotype. Percentages were calculated from the total number of isolates (n = 669). The exceptions were as follows: for aztreonam results for five isolates were not included and percentages were calculated out of a total of 664; for the efflux pump PCRs, a total of 70 samples were excluded from the analysis (sufficient RNA could not be extracted for 65 samples although the RNA extraction procedure was repeated, the isolates for two samples could not be retrieved and a further three samples were not processed) and percentages were calculated as the percentage of the total number of isolates tested (n = 599).

Among resistant isolates, the highest proportion of resistance was attributable to the expression of efflux pumps (18-25%) and the lowest to porin deletion (0.15%). Carbapenemases (*bla*$_{NDM}$, *bla*$_{OXA-48}$ and variants, *bla*$_{VIM}$ and *bla*$_{GES}$) accounted for 14%. *Bla*$_{IMP}$, *bla*$_{KPC}$ and *bla*$_{VEB-1}$ were not detected in any of the isolates (Table 2).

Of the 669 isolates, 234 (35%) were phenotypically carbapenem non-susceptible and 94 (14%) produced a carbapenemase: *bla*$_{NDM}$ (n = 4, 0.6%); *bla*$_{OXA-48}$ and variants (n = 3, 0.45%); *bla*$_{VIM}$ (n = 81, 12%) and *bla*$_{GES}$ (n = 6, 0.9%) (Table2). A combination of two genes was expressed in three isolates (*bla*$_{NDM}$ and *bla*$_{OXA-48}$ and variants, n = 1; *bla*$_{NDM}$ and *bla*$_{VIM}$, n = 1; *bla*$_{VIM}$ and *bla*$_{GES}$, n = 1).

For outer membrane impermeability, only one isolate displaying reduced susceptibility to meropenem had a porin deletion. This isolate did not possess any of the other carbapenem resistance mechanisms tested. Interestingly, OprD mutation did not confer imipenem resistance in any of the isolates, indicating that this is an uncommon resistance mechanism to carbapenems among our invasive *P. aeruginosa* isolates (Table 2).

MexAB-OprM and MexCD-OprJ expression were detected in, 223 and 202 isolates, respectively. For MexXY-OprM, a total of 202 isolates were screened, 197expressed the efflux pump and five did not, however these five isolates expressed the three other efflux pumps. For MexEF-OprN, a total of 223 isolates were screened, 219 expressed the efflux pump and four did not; however, these four isolates expressed the remaining three efflux pumps.

The antimicrobial susceptibility profiles of isolates were compared to their genotypic results to establish how phenotypic resistance correlated with genotypic data (Table 2). For isolates with acquired resistance mechanisms, those positive for *bla*$_{VIM}$ showed the best correlation i.e. 81 of the 210 imipenem non-susceptible isolates, 78 of the 211 meropenem non-susceptible isolates and 76 of the 155 doripenem non-susceptible isolates harboured the *bla*$_{VIM}$ gene. For isolates having intrinsic resistance mechanisms, there was a good correlation between phenotype and genotype for efflux pump resistance mechanisms; for example, of 181 piperacillin non-susceptible isolates, 137 expressed MexAB-OprM and MexCD-OprJ; 132 expressed MexXY-OprM and 136 expressed MexEF-OprN (Table 2). It should be noted that expression levels and not over-expression were investigated.

When considering all mechanisms of resistance tested in non-susceptible isolates collectively, antimicrobial resistance could be solely attributed to a single mechanism of resistance for the following: 0.7% (4/599) to the presence of a carbapenemase (*bla*$_{OXA-48}$ and variants, 1/599; *bla*$_{VIM}$, 3/599) and 25% (148/599) to the expression of efflux pumps. A combination of carbapenemase and efflux pumps resistance mechanisms accounted for resistance in 12% (71/599) of the isolates and a combination of porin deletion and efflux pumps in 0.17% (1/599) of the isolates tested.

Table 3. Distribution of resistance genes/mechanisms per province.

Province	OXA-48 and variants (n)	NDM (n)	VIM (n)	GES (n)	OprD deletion (n)	MexAB-OprM (n)	MexCD-OprJ (n)	MexXY-OprM (n)	MexEF-OprN (n)
Gauteng	-	-	48	4	-	106	96	94	104
Free state	-	1	9	-	1	13	12	12	13
KwaZulu- Natal	2	1	3	1	-	16	14	14	16
Western Cape	1	2	21	1	-	88	80	77	86

The expression of efflux pumps was the predominant mechanism in all provinces (Table 3).

Discussion

Overall, majority of the isolates were from Gauteng (52%). This province constituted the largest number of sentinel sites which are also large academic centers. Delays in the receipt of appropriate antibiotics in patients with pseudomonas bacteraemia has been shown to be a risk factor for mortality and therefore it is important to have knowledge of the antimicrobial susceptibility profile of this pathogen [23]. The MIC_{50} and MIC_{90} have not changed from 2014 to 2015 and the MIC_{50} was within the susceptible breakpoint range for most antibiotics. With the exception of colistin, the MIC_{90} of the remaining antibacterial agents was within the intermediate or resistance breakpoint range. It should be noted that the methodology used to report antimicrobial susceptibilities may have a limitation as the Microscan Walkaway system gives MIC breakpoints and not actual MIC values for certain antibiotics. The overall susceptibilities for all antibiotics were relatively high for antibacterial agents tested (ranging from 66% to 100%). High susceptibility rates were also seen in systemic antibiotics in a previous South African study which investigated *P. aeruginosa* strains isolated from wound infections from paediatric burn patients in a 36-month study period. Apart from piperacillin/tazobactam (63.9%), cefepime (82.0%), ciprofloxacin and ceftazidine (80.3% each), the other antimicrobial agents (tobramycin, gentamicin, amikacin, imipenem and meropenem) had more than 90% sensitivity [24].This was also seen in a number of other studies in other countries investigating *P. aeruginosa* isolated from burn patients admitted into burn units over 1-year to 5-year study periods [25-29]. A Lithuanian study in bacteraemic patients showed similar findings to ours with low and relatively low resistance (ranging from 8.5% for amikacin to 39.4% for gentamicin) observed to the antibiotics tested. Other antibiotics tested included ciprofloxacin, piperacillin, ceftazidime, imipenem and meropenem. It should be noted that the sample size was small (n = 80) [30]. Another study in India [31] investigating 126 *P. aeruginosa* strains isolated from various sources showed varying degrees of resistance to different antimicrobials with no isolate being resistant to imipenem and meropenem, possibly due to the fact that these antibiotics are not administered in the hospital. Resistance was seen in a relatively small proportion of isolates (ranging from 18.3% for amikacin to 36.5% for cefoperazone) and other antimicrobial agents such as ciprofloxacin (31.7%). High resistance rates were seen for piperacillin (53.9%) [31]. This demonstrates that resistance patterns can vary depending on the use of antibiotics in the healthcare setting as this latter study has shown that *P. aeruginosa* is becoming resistant to antibiotics that are commonly used in the hospital.

In our study the overall proportion of resistance to antibacterial agents was relatively low for all isolates (susceptible and non-susceptible) and similarly resistance mechanisms were detected in a small proportion of isolates tested: carbapenemases (96/669, 14%), porin deletion (1/669, 1.5%) and efflux pumps (148/599, 25%). Thus efflux pumps were the predominant mechanism of resistance in our study. While transmission of antimicrobial resistance on plasmids (and other mobile genetic elements) is a concern, *P. aeruginosa* has the ability to develop resistance while the patient is on antimicrobial treatment resulting in mutational changes in the chromosome [32], a possible explanation for our finding which was also shown in a previous study where an increase of efflux-mediated resistance was observed during antibiotic treatment in patients diagnosed with hospital-acquired pneumonia [33]. Interestingly, although bla_{IMP}, bla_{KPC} and bla_{VEB-1} were not detected in any of the isolates in our study, they have been reported in *P. aeruginosa* in various studies [34-39].

When only non-susceptible isolates were considered, a total of 94 (40%) of the 234 carbapenem non-susceptible isolates expressed carbapenemases, three of which expressed a combination of two genes; one (0.4%) of the 234 carbapenem non-susceptible isolates displayed a reduction of the outer-membrane channel porin, OprD. Isolates non-susceptible to one or more of the following antibiotics: ciprofloxacin, levofloxacin, amikacin, gentamicin, cefepime, ceftazidime and piperacillin expressed a minimum of two of the efflux pumps tested which is not surprising as these are intrinsic mechanisms of resistance.

Varied resistance mechanisms may be evident because of the organism's highly adaptable nature. It is able to alter its properties in response to environmental changes and can grow on a wide variety of substrates. It has a large genome (6.26Mbp) and encodes 5567 genes. This considerably large genetic capacity may influence its ability to develop resistance particularly with excessive antibiotic usage [6]. This was evident by the combination of resistance mechanisms observed (carbapenemases and efflux pumps (12%) and porin deletion and efflux pumps (0.17%)). The phenotypic predictions were not entirely accurate in the

carbapenem non-susceptible group where 59% of the isolates did not harbour a carbapenemase. Furthermore, only one isolate in the carbapenem non-susceptible group displayed a porin deletion indicating that the phenotypic data does not reliably support the genotypic data. However, reduced expression of OprD was not investigated and carbapenem non-susceptibility may be due to carbapenemase variants and other carbapenemase types that were not screened for. Genotypic efflux pump results correlated in most part with phenotypic resistance and in this instance, the phenotypic data does to an extent support the genotypic data. This is not surprising as some of these are expressed constitutively at low levels. Correlation therefore differs for the mechanism of resistance investigated and the phenotypic data are not predictive of the resistance mechanism i.e. the antimicrobial resistance pattern is not specific for any resistance mechanism.

A potential limitation is that we investigated efflux pump expression only and did not quantify levels of expression to determine upregulation of efflux pumps. However, no correlation between the level of transcription and resistance in *P. aeruginosa* clinical isolates was observed in some studies and therefore the measurement of expression level is not always essential for routine diagnosis [22,40,41]. Other limitations include the following: not all possible mechanisms of resistance for all antibiotics were investigated; due to the lack of patient demographic and clinical information, it was not possible to establish accurate trends in race, ward type and clinical outcome and we were not able to differentiate between community-associated and healthcare-associated infection; and information on source of infection was not available.

Conclusion

This two-year surveillance study describes the antimicrobial susceptibility profiles and resistance mechanisms in *P. aeruginosa* isolates from patients with bacteraemia. Furthermore, this study demonstrated the presence of multiple resistance genes/mechanisms in four provinces in conjunction with the antimicrobial susceptibility profiles of the *P. aeruginosa* isolates. We established baseline data on the distribution of different mechanisms of resistance in *P. aeruginosa*. These data can be used as a reference for antibiotic resistance patterns and resistance mechanisms in invasive *P. aeruginosa* isolates from public South African hospitals. The information will be useful in guiding policy for antimicrobial stewardship committees and hospital formularies, and for the development of national treatment guidelines.

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