

Original Article

Laboratory information system for reporting antimicrobial resistant isolates from academic hospitals, South Africa

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Abstract

Introduction: We aimed to evaluate the appropriateness of Digital Innovation South Africa (DISA)-based laboratory information system (LIS) for assessing the prevalence, patterns and trends of antimicrobial resistance, and associated demographic factors.

Methodology: A retrospective analysis was conducted on routine data of blood culture isolates of *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. These isolates were collected by the National Health Laboratory Services between July 1, 2005 and December 31, 2009 at seven tertiary public hospitals. Factors associated with antimicrobial resistance were analysed using multivariate logistic regression.

Results: Information on 9969 isolates was available, of which 3942 (39.5%), 4466 (44.8%) and 1561 (15.7%) were *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, respectively. Reporting of resistance across antibiotics tested was highest in patient age group less than 5 years old. Methicillin resistant *Staphylococcus aureus* was 39% on average. There was a significant increasing trend of *Klebsiella pneumoniae* resistance to ciprofloxacin (32.6% to 64.9%, $p < 0.001$), cotrimoxazole (67.5% to 81.6%, $p < 0.001$) and cefotaxime-ceftriaxone (55.5% to 73.2%, $p < 0.001$) over the study period. *Pseudomonas aeruginosa* resistance to meropenem showed a significant increasing trend from 2006 (27.5%) to 2009 (53.9%) ($p < 0.001$). Age group < 5 years, female gender, hospital location, year of infection were significantly associated with antimicrobial resistance.

Conclusions: The percentages of antimicrobial resistance were high and showed a significant increasing trend among individual agents over the duration of the study e.g. ciprofloxacin, cotrimoxazole among others. Continued surveillance of antimicrobial resistance among bloodstream hospital-acquired infections should be strengthened.

Key words: Laboratory information systems; trends; antimicrobial resistance; bacterial pathogens; nosocomial infections; surveillance.

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Introduction

The extent of antimicrobial drug resistance has accentuated the need for continued surveillance [1-3]. Resistance in bacterial pathogens to conventional antimicrobials has become a global problem, particularly in those causing hospital associated infections, emphasizing the relevance to systematically monitor patterns and trends of antimicrobial resistance over time [4-6].

Improved information systems for the reporting of antimicrobial resistance would facilitate timely implementation of appropriate interventions including review of antimicrobial prescription policies and treatment guidelines that would strengthen prudent use of antimicrobials, [7-9] to preserve the effectiveness of currently available agents [6].

Surveillance networks such as the European Antimicrobial Resistance Surveillance System -EARSS (Europe) [10] and the National Nosocomial Infections Surveillance System-NNIS (USA) [11] have been established over the years and they provided reliable antimicrobial susceptibility data, described prevalent resistance patterns and monitored emerging antimicrobial resistance [12]. However, there is a scarcity of data from most developing countries regarding the levels of antimicrobial resistance, even among nosocomial pathogens. A recent systematic review showed evidence of resistance to commonly used antimicrobial drugs in the South African population [13].

The proportion of methicillin-resistant *Staphylococcus aureus* (MRSA) was 35% while

Klebsiella pneumoniae showed increasing resistance to third generation cephalosporins or isolates producing extended-spectrum beta-lactamases (ESBLs) from 33% to 49%, and from 18% to 28% for fluoroquinolones in academic hospitals from 1999 and 2007. Resistance among *Pseudomonas aeruginosa* isolates to ciprofloxacin was 43% [13]. The results presented in this paragraph are from published papers about information gathered systematically from various laboratory settings in South Africa.

Antimicrobial resistance is a main reason for failure of empirical treatment and clinicians rely on guidelines based on surveillance for antimicrobial resistance [2,14,15].

Local antimicrobial resistance patterns serve as a guide for empirical treatment [14]. In addition, antimicrobial resistance surveillance data may guide public health interventions to control the development of antimicrobial resistance and spread of resistant pathogens in hospitals [6]. Data on resistance patterns would augment infection control measures and promote improved antimicrobial prescribing habits among clinicians [4].

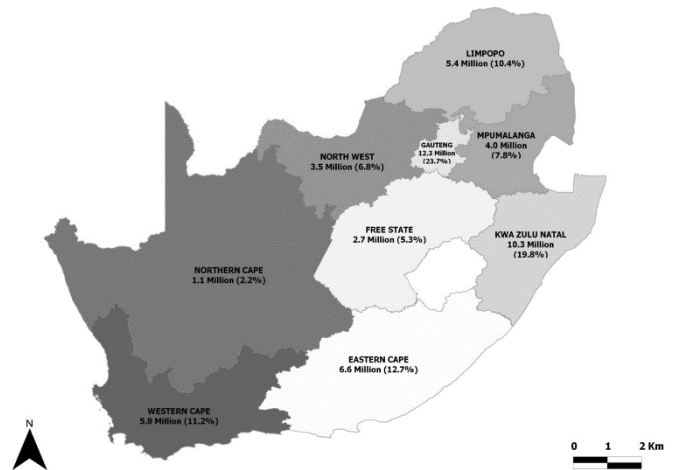
The present study aims to record and analyse data from the laboratory information system (LIS) relating to antimicrobial resistance prevalence, patterns and temporal trends, as well as demographic factors associated with antimicrobial resistance among three selected pathogens, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, causing blood stream infections in patients admitted at tertiary public hospitals in South Africa.

Methodology

This was a retrospective analysis of isolates from routine blood culture testing and their antimicrobial susceptibility data reported from 2005-2009 by the National Health Laboratory Service (NHLS) and extracted from the Corporate Data Warehouse (CDW) situated at the corporate office of the NHLS at Sandringham, South Africa. The study was approved by the Human Research Ethics Committee of the, University of the Witwatersrand, approval number M10625.

Seven tertiary public hospitals were included in the study. From Gauteng Province four hospitals associated with Universities of the Witwatersrand (Wits) and Pretoria (UP) were included. These were: Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), Chris Hani Baragwanath Hospital (CHBH), and Helen Joseph Hospital (HJH) (Wits). From Free State Province Universitas Hospital (UH) associated with

Figure 1. Map of the Republic of South Africa showing geographical location of hospitals included in the study.



University of Free State (UFS) was included; and from Western Cape Province, Groote Schuur Hospital (GSH) and Tygerberg Hospital (TH) associated with Universities of Cape Town (UCT) and Stellenbosch (SUN) were included. These are all reputable specialist referral hospitals that have functioning laboratories with good quality assurance practices of laboratory methods likely to yield reliable results as well as computerised laboratory systems interfaced to the CDW in Johannesburg from which data for this study were extracted. The map (Figure 1) highlights geographical location of hospitals in relation to the three provinces from which data of bacterial isolates were obtained.

The NHLS academic laboratories used the automated BacTAlert system (Biomérieux, Marcy-l'Étoile, France) for blood culture investigations and automated MicroScan supplied (Siemens, Munich, Germany) or Vitek 2 systems (Biomérieux, Marcy-l'Étoile, France) or conventional biochemical methods for identification of pathogens. Antibiotic susceptibility testing was interpreted following the Clinical Laboratory Standards Institute (CLSI) guidelines [16]. Various methods were used including testing by disk diffusion technology such as the Kirby-Bauer and Etest methods or automated testing using MicroScan or Vitek 2 systems.

Quality control bacteria, comprising standard internationally recognised strains for susceptibility testing were used at each participating site. It is standard practice worldwide for quality control procedures to be used for drug susceptibility testing, including the use of dedicated international strains such as *S. aureus* ATCC 25923 strain for Gram-positive bacteria and *E. coli* ATCC 25922 strain and *P. aeruginosa* ATCC 27853

[16] for Gram-negative bacteria, as well as standardization of inoculum size and incubation period. Only single isolates recorded by LIS from a bacteraemia episode was included in the present analysis to avoid bias introduced by reporting susceptibility testing on multiple specimens per patient. Bamford *et al.*, outlined details of methods of susceptibility testing of blood culture isolates in use by participating microbiology laboratories for the selected organisms [17].

Data were extracted on all blood culture isolates of *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* reported within the study period by DISA-LIS at the NHLS. Susceptibility data reported by DISA were extracted from the CDW data repository by running queries using structured query language (SQL) from several database servers. All blood culture data of bacterial isolates reported to NHLS between July 1, 2005 and December 31, 2009

were included. All relevant demographic and microbiological factors included in the analysis were extracted. Percentages of resistance were calculated according to the number of resistant isolates divided by the total number of blood culture isolates for each organism. All susceptibility data were collected of isolates including patients’ demographic and geographic characteristics including age, gender, province, name of hospital and year of data collection.

Data were assessed for completeness and analysed using Stata version 13 (StataCorp Limited, College Station, Texas, USA). Univariate analysis was performed to describe the frequency distribution of the selected pathogens as well as their resistance to antimicrobial agents. Associations between resistance and various potential risk factors (province, organism, age, gender, hospital wards and specimen collection year) were analysed using Pearson chi-square test for categorical variables. A multivariate logistic regression

Table 1. Distribution of Demographic and Geographical characteristics

Characteristic	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>
Age	n/N * (%)	n/N * (%)	n/N * (%)
< 5	1224 (31.1)	1673 (37.5)	444 (28.4)
5-9	95 (2.4)	60 (1.3)	34 (2.2)
10-19	231 (5.9)	194 (4.3)	67 (4.3)
20-29	504 (12.8)	439 (9.8)	223 (14.3)
30-39	598 (15.2)	611 (13.7)	235 (15.1)
40-49	482 (12.2)	458 (10.3)	188 (12.0)
50-59	354 (9.0)	421 (9.4)	151 (9.7)
60-69	270 (6.9)	336 (7.5)	126 (8.1)
≥70	184 (4.7)	274 (6.1)	93 (6.0)
Gender			
Male	2185 (57.4)	2421 (56.0)	858 (57.0)
Female	1619 (42.6)	1902 (44.0)	648 (43.0)
Hospital			
Charlotte Maxe JAH	611 (15.5)	670 (15.0)	304 (19.5)
Chris Hani Bara	1120 (28.4)	1382 (30.9)	454 (29.1)
Helen Joseph	374 (9.5)	268 (6.0)	109 (7.0)
Steve Biko PAH	438 (11.1)	786 (17.6)	307 (19.7)
Universitas	173 (4.4)	261 (5.8)	67 (4.3)
Groote Schuur	556 (14.1)	531 (11.9)	135 (8.7)
Tygerberg	670 (17.0)	568 (12.7)	185 (11.9)
Province			
Gauteng	2543 (64.5)	3106 (69.6)	1174 (75.2)
Free State	173 (4.4)	261 (5.8)	67 (4.3)
Western Cape	1226 (31.1)	1099 (24.6)	320 (20.5)
Year			
2005	335 (8.5)	344 (7.7)	133 (8.5)
2006	965 (24.5)	974 (21.8)	355 (22.7)
2007	849 (21.5)	1002 (22.4)	358 (22.9)
2008	922 (23.4)	1124 (25.2)	347 (22.2)
2009	871 (22.1)	1022 (22.9)	368 (23.6)

*The proportions (%) are number of isolates for each characteristic (n) / total number of isolates for each individual pathogen (N). The total number of isolates for each characteristic were *Staphylococcus aureus* = 3942; *Klebsiella pneumoniae* = 4466; *Pseudomonas aeruginosa* = 1561, except for gender where the total number of isolates were: *Staphylococcus aureus* = 3804; *Klebsiella pneumoniae* = 4323; *Pseudomonas aeruginosa* = 1506 due to missing data on gender.

model was used to investigate independent predictors of antibiotics-specific resistance as well as composite resistance based on a set of antibiotics. Two-sided *p* values of < 0.05 were considered significant.

Results

Demographic and geographical characteristics of bacteraemia episodes

There were 9969 selected pathogen isolates linked to single bacteraemic episodes within the study period of which 3942 (39.5%) were *Staphylococcus aureus*, 4466 (44.8%) *Klebsiella pneumoniae* and 1561 (15.7%) *Pseudomonas aeruginosa*. Of nine age-related groups, the less than 5 years age group had the most bacteraemic episodes linked to the three pathogens: 1224 (31.1%) of *Staphylococcus aureus*, 1673 (37.5%) of *Klebsiella pneumoniae* and 444 (28.5%) of *Pseudomonas aeruginosa*. A second bacteraemic peak occurred in the 30 – 39 years age groups related to the three pathogens: 598 *Staphylococcus aureus* (15.2%), 611 *Klebsiella pneumoniae* (13.7%) and 235 *Pseudomonas aeruginosa* (15.1%) with slightly lower figures for the 20-29 years age group and further slightly reduced figures for adults aged 40 – 69 years. The lowest figures were in the 5 - 9 years, 10 - 19 years, and > 70 years age groups, ranging from 95 – 231 (2.4% – 5.9%) for *Staphylococcus aureus*, 60 -274 (1.3% - 6.1%) for *Klebsiella pneumoniae* and 34 - 93 (2.2% - 6.0%) for *Pseudomonas aeruginosa*.

There were more bacteraemic episodes caused by each of the three pathogens in males than in females. The proportion of bacteraemic episodes in relation to numbers of admissions and duration of patients' stay in hospital was not available in the CDW database for comparison of frequency of organism-specific bacteraemic episodes between hospitals. However, considering the relative percentages of organism-specific episodes in each hospital, *Staphylococcus aureus* episodes at HJH (49.8%), TH (47.1%) and GSH (45.5%) were higher than mean of 40.3% of all 7 hospitals; *Klebsiella pneumoniae* episodes at UH (52.1%) and SBPAH (51.3%) were much higher than the 7-hospital mean of 45.5%; and *Pseudomonas aeruginosa* episodes at SBPAH (20.1%) and CMJAH (19.2%) were higher than the 7-hospital mean of 15.2%. This may suggest possible excess of *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* cases at the named hospitals. The numbers of episodes per annum for the respective pathogens for the period 2006-2009 varied from 871 to 965 (mean 902) for *Staphylococcus aureus*, 974 to 1124 (mean

1030.5) for *Klebsiella pneumoniae* and 347 – 368 (mean 357) for *Pseudomonas aeruginosa* (Table 1).

Distribution of antimicrobial resistance rates among selected pathogens

Resistance to various antimicrobial agents was observed between isolates of the three pathogenic bacteria that were studied. In the case of *Staphylococcus aureus*, resistance was not detected for linezolid during the period of this study (0 out of 70) and minimal resistance (1 out of 865) was recorded as for vancomycin and 8.5% (33/388) to fusidic acid (Table 2). For ampicillin, and by inference benzyl penicillin, *Staphylococcus aureus* resistance was 95.7% (3322/3471), while for cloxacillin the overall percentage resistance was 15.4% (588/3828) with considerable variation between hospitals, the highest being 37.0% and 43.0% for GSH and TH respectively. Of the 827 isolates tested for gentamicin susceptibility, 428 (51.8%) were recorded as resistant.

For *Klebsiella pneumoniae*, resistance was encountered in very low numbers of isolates to meropenem and imipenem (~ 0.1% out of > 3000 isolates tested) and 2.0% (50/2474) for etrapenem. High percentages of cephalosporin resistance were recorded i.e. 66.0% (2238/3390) for cefotaxime/ceftriaxone. Cephamycin resistance was lower at 52.0% (357/687) despite a better in vitro susceptibility for cefoxitin. Resistance to ampicillin/amoxicillin was almost complete at 99.5% and for co-amoxiclav it was (64.8%), while for piperacillin-tazobactam resistance was recorded in 1820/2745 (66.3%) of *Klebsiella pneumoniae* isolates. Resistance to amikacin was 26.4% (695/2631) while the figures for gentamicin (58.7%) and tobramycin (80.4%) were appreciably higher (Table 2).

Among *Pseudomonas aeruginosa* isolates, resistance to beta-lactam antibiotics was lowest in the case of ceftazidime at 20.0% (287/1421) and 36.0% (367/1021) for cefepime, while 31.9% of *Pseudomonas aeruginosa* isolates were resistant to piperacillin-tazobactam. Resistance to the carbapenem antibiotics, meropenem and imipenem was 44.4% and 46.7% respectively. Resistance to the aminoglycoside agents varied from 29.4% (236/804) to 48.2% (364/755) for *Pseudomonas aeruginosa* isolates in the case of amikacin and tobramycin respectively. Resistance to ciprofloxacin was reported in 35.1% (343/976) of *Pseudomonas aeruginosa* isolates, while resistance to colistin was found in 4 of 212 isolates tested, 2 each from CHBH and TH respectively (Table 2).

Table 2. Antimicrobial resistance patterns of selected blood borne infections, by hospital, during 2005 – 2009 period

Organism/drug	Total	CMJAH	CHB	HJ	SBPAH	UH	GSH	TH	p-value
<i>Staphylococcus aureus</i>	% (n/N)**								
Ampicillin	95.7 (3,322/3,471)	96.8 (481/497)	98.8 (941/952)	97.4 (295/303)	88.2 (365/414)	92.1 (140/152)	94.7 (482/509)	96.0 (619/644)	0.85
Cloxacillin	15.4 (588/3,828)*	4.2 (25/595)*	0.4 (4/1,103)*	3.8 (14/366)*	5.0 (21/422)	30.4 (51/168)	37.0 (193/522)	43.0 (280/652)	< 0.001
Vancomycin	0.12 (1/865)	0.0 (0/34)	0.0 (0/11)	0.0 (0/25)	0.0 (0/37)	0.0 (0/108)	0.0 (0/337)	0.3 (1/313)	0.94
Gentamicin	51.8 (428/827)	40.0 (6/15)	25.0 (3/12)	66.7 (6/9)	22.2 (2/9)	50.0 (1/2)	46.6 (132/283)	55.9 (278/497)	0.02
Erythromycin	34.5 (802/ 2,326)	40.5 (104/257)	26.6 (92/346)	53.6 (59/110)	19.0 (65/343)	40.9 (54/132)	29.7 (153/515)	44.1 (275/623)	< 0.001
Linezolid	0.0 (0/70)	0.0 (0/6)	0.0 (0/4)	0.0 (0/3)	0.0 (0/1)	0.0 (0/32)	0.0 (0/15)	0.0 (0/9)	Na
Clindamycin	32.4 (650/2,005)	17.9 (40/224)	23.6 (77/326)	32.1 (27/84)	14.6 (50/343)	33.1 (47/142)	28.2 (147/522)	72.0 (262/364)	< 0.001
Rifampicin	39.9 (194/486)	25.0 (3/12)	0.0 (0/14)	55.6 (5/9)	-	30.0 (3/10)	32.1 (95/296)	60.7 (88/145)	< 0.001
Fusidic acid	8.5 (33/388)	11.1 (1/9)	9.1 (1/11)	0.0 (0/3)	0.0 (0/3)	25.0 (2/8)	0.7 (2/269)	31.8 (27/85)	< 0.001
Cotrimoxazole	29.9 (577/1,930)	36.8 (89/242)	30.9 (100/324)	27.9 (22/79)	50.0 (3/6)	28.5 (43/151)	24.6 (128/521)	31.6 (192/607)	0.02
<i>Klebsiella pneumoniae</i>	% (n/N)**								
Ampicillin_Amoxy	99.5 (3,769/ 3,789)	99.8 (617/618)	99.5 (1298/1305)	100.0 (253/253)	99.3 (739/744)	98.4 (246/250)	99.3 (277/279)	99.7 (339/340)	0.16
Amoxiclav	64.8 (2,550/3,936)	64.0 (336/525)	73.0 (869/1,190)	64.4 (154/239)	59.2 (439/741)	55.7 (113/203)	52.7 (263/499)	69.8 (376/539)	< 0.001
Imipinem	0.1 (4/3,059)	0.0 (0/386)	0.2 (2/863)	0.0 (0/156)	0.0 (0/568)	0.0 (0/221)	0.2 (1/493)	0.3 (1/372)	0.79
Meropenem	0.1 (5/3,046)	0.0 (0/397)	0.1 (1/890)	0.0 (0/162)	0.2 (1/565)	0.0 (0/141)	0.2 (1/495)	0.5 (2/396)	0.66
Ertapenem	2.0 (50/2,474)	2.6 (9/349)	2.8 (18/652)	3.5 (5/145)	0.8 (4/533)	4.6 (10/220)	0.8 (3/376)	0.5 (1/199)	0.01
Cefazolin	86.3 (1,864/2,161)	94.8 (349/368)	97.1 (789/813)	90.9 (140/154)	65.2 (329/505)	77.8 (168/216)	0.0 (0/2)	86.4 (89/103)	< 0.001
Ceftazidime	82.0 (2,428/2,962)	91.1 (346/380)	95.5 (799/837)	91.7 (144/157)	69.1 (425/615)	91.3 (94/103)	58.7 (289/492)	87.6 (331/378)	< 0.001
Cefuroxime	73.8 (2,437/3,301)	82.3 (354/430)	93.3 (746/800)	81.4 (144/177)	62.6 (423/676)	61.6 (138/224)	59.5 (297/499)	67.7 (335/495)	< 0.001
Cefoxitin	52.0 (357/687)	65.6 (40/61)	73.0 (65/89)	42.9 (21/49)	49.3 (213/432)	37.5 (3/8)	23.8 (10/42)	83.3 (5/6)	< 0.001
Cefotaxime/ceftriaxone	66.0 (2,238/3,390)	58.3 (342/587)	65.1 (822/1,263)	58.0 (148/255)	68.1 (425/624)	92.5 (124/134)	59.9 (161/269)	83.7 (216/258)	< 0.001
Cefepime	79.8 (2,364/2,963)	92.9 (315/339)	94.4 (794/841)	92.5 (135/146)	69.8 (396/567)	55.7 (107/192)	59.5 (292/491)	84.0 (325/387)	< 0.001
Piperacillin-tazobactam	66.3 (1,820/2,745)	75.5 (247/327)	83.9 (590/703)	79.8 (138/173)	69.7 (430/617)	51.6 (96/186)	29.6 (137/463)	65.9 (182/276)	< 0.001
Gentamicin	58.7 (2,242/3,820)	53.2 (289/543)	69.5 (726/1,045)	55.2 (123/223)	53.2 (387/723)	56.7 (136/240)	57.8 (289/500)	54.0 (292/541)	< 0.001
Tobramycin	80.4 (1,564/1,946)	89.6 (233/260)	95.9 (561/585)	89.6 (103/115)	93.0 (40/43)	60.0 (102/170)	55.9 (264/272)	86.7 (261/301)	< 0.001
Amikacin	26.4 (695/ 2,631)	21.3 (62/291)	33.8 (162/479)	21.9 (28/128)	40.2 (471/674)	17.2 (33/192)	14.9 (74/498)	17.6 (65/369)	< 0.001
Ciprofloxacin	51.8 (1,380/2,666)	48.5 (191/394)	55.6 (424/763)	77.7 (139/179)	47.6 (280/588)	43.1 (56/130)	46.5 (133/286)	48.2 (157/326)	< 0.001
Nalidixic-acid	83.8 (586/699)	93.2 (82/88)	95.4 (313/328)	85.9 (73/85)	70.2 (33/47)	-	53.2 (67/126)	72.0 (18/25)	0.001
Nitrofurantoin	92.3 (262/284)	75.0 (6/8)	80.0 (4/5)	95.5 (63/66)	100.0 (47/47)	66.7 (2/3)	88.9 (112/126)	96.6 (28/29)	0.02
Chloramphenicol	72.3 (704/974)	89.2 (173/194)	95.9 (372/388)	82.9 (68/82)	100.0 (1/1)	-	29.2 (90/308)	0.0 (0/1)	0.01
Colistin	1.7 (4/230)	0.0 (0/16)	0.0 (0/2)	2.5 (1/40)	2.6 (1/38)	-	0.0 (0/116)	0.0 (0/18)	0.03
Cotrimoxazole	73.8 (2,547/ 3,451)	89.2 (173/194)	95.9 (372/388)	82.9 (68/82)	100.00 (1/1)	-	29.2 (90/308)	0.0 (0/1)	< 0.001

Table 2 (continued). Antimicrobial resistance patterns of selected blood borne infections, by hospital, during 2005 – 2009 period.

Organism/drug	Total	CMJAH	CHB	HJ	SBPAH	UH	GSH	TH	p-value
<i>Pseudomonas aeruginosa</i>	% (n/N)**								
Imipinem	46.7 (334/715)	31.0 (36/116)	57.6 (83/144)	55.6 (15/27)	59.12 (107/181)	29.4 (15/51)	24.5 (24/98)	55.1 (54/98)	< 0.001
Meropenem	44.4 (319/718)	27.5 (33/120)	61.2 (93/152)	59.3 (16/27)	58.1 (104/179)	33.3 (9/27)	18.6 (19/102)	44.1 (45/102)	< 0.001
Ceftazidime	20.1 (287/1,431)	10.4 (30/288)	21.2 (89/419)	9.7 (10/103)	39.5 (118/299)	33.3 (9/27)	13.1 (17/130)	8.5 (14/165)	0.001
Cefepime	36.0 (367/1,021)	25.3 (41/162)	36.6 (108/295)	18.9 (11/58)	45.8 (120/262)	32.6 (15/46)	24.3 (26/107)	50.6 (46/91)	< 0.001
Piperacillin-tazobactam	31.9 (452/1,419)	13.1 (36/264)	29.5 (118/400)	13.6 (14/103)	64.0 (178/278)	18.5 (12/65)	33.3 (40/120)	30.2 (54/179)	< 0.001
Gentamicin	34.3 (461/1,343)	19.8 (49/249)	38.0 (130/342)	28.7 (27/94)	41.87 (121/289)	26.3 (15/57)	30.3 (40/132)	43.9 (79/180)	< 0.001
Tobramycin	48.2 (364/ 755)	32.2 (47/146)	57.2 (123/215)	42.3 (22/52)	79.5 (58/73)	26.5 (13/49)	25.2 (30/119)	70.3 (71/101)	< 0.001
Amikacin	29.4 (236/804)	19.4 (31/160)	32.6 (70/215)	50.0 (22/44)	45.2 (56/124)	18.8 (19/48)	21.4 (22/103)	23.6 (26/110)	< 0.001
Ciprofloxacin	35.1 (343/976)	21.43 (39/182)	43.7 (86/197)	46.9 (30/64)	45.3 (121/267)	29.7 (11/37)	35.5 (27/76)	18.9 (29/153)	0.01
Colistin	1.9 (4/212)	0.0 (0/22)	4.6 (2/44)	0.0 (0/5)	0.0 (0/107)	-	0.0 (0/19)	13.3 (2/15)	0.01

*Susceptibility testing at certain sites underreported resistance to cloxacillin; CMJAH = Charlotte Maxeke Johannesburg Academic Hospital; SBPAH = Steve Biko Pretoria Academic Hospital; CHB = Chris Hani Baragwanath Hospital; HJ = Helen Joseph Hospital; UH = Universitas Hospital; GSH = Groote Schuur Hospital; TH = Tygerberg Hospital.

Table.3. Trends of Antimicrobial resistance rate of selected blood born infections by Year.

Antibiotics tested	Total	2005	2006	2007	2008	2009	p-value
<i>Staphylococcus aureus</i>	% (n/N) **						
Ampicillin	95.7 (3,322/3,471)	96.2 (305/317)	95.9 (869/906)	94.9 (755/795)	95.6 (688/720)	96.2 (705/733)	0.774
Cloxacillin	15.4 (588/3,828)*	22.2 (74/334)	19.0 (182/960)	14.5 (123/848)	14.4 (118/817)	10.5 (91/869)	0.042
Vancomycin	0.1 (1/865)	0.0 (0/106)	0.4 (1/288)	0.0 (0/212)	0.0 (0/145)	0.0 (0/114)	0.735
Gentamicin	51.8 (428/827)	53.6 (59/110)	45.5 (141/310)	46.5 (87/187)	67.8 (97/143)	57.14 (44/77)	< 0.001
Erythromycin	34.5 (802/2,326)	40.1 (95/237)	40.3 (242/601)	29.6 (182/614)	31.2 (148/475)	33.8 (135/399)	< 0.001
Clindamycin	32.4 (650/2,005)	37.1 (79/213)	38.0 (194/511)	26.3 (145/552)	32.2 (125/388)	31.4 (107/341)	< 0.001
Rifampicin	39.9 (194/486)	32.0 (24/75)	35.5 (59/166)	34.6 (44/127)	66.7 (46/69)	42.9 (21/49)	< 0.001
Fusidic acid	8.5 (33/388)	11.7 (8/68)	6.2 (9/146)	5.3 (6/114)	16.3 (7/43)	17.7 (3/17)	0.070
Cotrimoxazole	29.9 (577/1,930)	33.3 (67/201)	33.1 (168/508)	24.9 (128/514)	29.6 (117/395)	31.1 (97/312)	0.042
<i>Klebsiella pneumoniae</i>	% (n/N) **						
Amoxiclav	64.8 (2,550/3,936)	60.0 (189/315)	60.1 (527/877)	61.6 (559/908)	70.1 (629/897)	68.8 (646/937)	< 0.001
Imipinem	0.1 (4/3,059)	0.4 (1/255)	0.0 (0/663)	0.0 (0/653)	0.1 (1/736)	0.3 (2/732)	0.377
Meropenem	0.2 (5/3,046)	0.0 (0/255)	0.0 (0/683)	0.3 (1/669)	0.1 (1/714)	0.4 (3/725)	0.363
Ertapenem	2.0 (50/2,474)	2.4 (4/166)	2.7 (11/410)	2.7 (14/529)	1.2 (8/665)	1.9 (13/704)	0.350
Ceftazidime	82.0 (2,428/2,962)	77.2 (180/233)	79.0 (512/648)	78.0 (533/683)	85.5 (591/691)	86.6 (612/707)	< 0.001
Cefuroxime	73.8 (2,437/3,301)	65.5 (188/287)	70.2 (512/729)	69.3 (516/745)	79.3 (593/748)	79.3 (628/792)	< 0.001
Cefoxitin	52.0 (357/687)	13.9 (11/19)	35.7 (51/143)	62.7 (133/212)	69.4 (120/173)	52.5 (42/80)	< 0.001
Cefotaxime-ceftriaxone	66.0 (2,238/3,390)	55.5 (127/229)	56.9 (376/661)	62.4 (498/798)	72.2 (618/856)	73.2 (619/846)	< 0.001
Cefepime	79.8 (2,364/2,963)	73.2 (164/224)	76.5 (484/633)	76.6 (518/676)	84.7 (599/707)	82.9 (599/723)	< 0.001
Piperacillin-tazobactam	66.3 (1,820/2,745)	58.6 (123/210)	61.7 (383/621)	62.5 (422/675)	72.3 (457/632)	71.7 (292/407)	< 0.001
Gentamicin	58.7 (2,242/3,820)	52.4 (176/336)	53.9 (498/923)	51.5 (468/909)	66.5 (535/805)	66.7 (565/847)	< 0.001
Tobramycin	80.4 (1,564/1,946)	75.8 (116/153)	81.3 (377/464)	79.3 (318/401)	82.8 (360/435)	79.7 (393/493)	0.375
Amikacin	26.4 (695/2,631)	21.2 (54/255)	22.3 (152/683)	32.0 (197/615)	25.9 (138/531)	28.2 (154/547)	< 0.001
Ciprofloxacin	51.8 (1,380/2,666)	32.6 (69/212)	40.5 (231/570)	48.7 (299/614)	58.5 (397/679)	64.9 (384/591)	< 0.001
Chloramphenicol	72.3 (704/974)	62.8 (81/129)	69.2 (189/273)	67.2 (160/238)	67.2 (158/208)	92.1 (116/126)	< 0.001
Colistin	1.7 (4/230)	3.6 (1/28)	0.0 (0/22)	7.7 (1/13)	0.0 (0/13)	1.3 (2/154)	0.401
Cotrimoxazole	73.8 (2,547/3,451)	67.5 (201/298)	69.7 (556/798)	69.1 (547/792)	77.5 (605/781)	81.6 (638/782)	< 0.001
<i>Pseudomonas aeruginosa</i>	% (n/N) **						
Imipinem	46.7 (334/715)	47.5 (29/61)	31.3 (47/150)	47.3 (79/167)	46.4 (70/151)	58.6 (109/186)	< 0.01
Meropenem	44.4 (319/718)	48.2 (27/56)	27.5 (42/153)	45.2 (76/168)	47.8 (76/159)	53.9 (98/182)	< 0.01
Ceftazidime	20.1 (287/1,431)	17.1 (21/123)	11.8 (39/330)	21.5 (73/339)	23.5 (69/294)	24.6 (85/345)	< 0.01
Cefepime	36.0 (367/1,021)	36.6 (34/93)	26.3 (55/209)	32.1 (87/271)	41.5 (85/205)	43.6 (106/243)	< 0.01
Piperacillin-tazobactam	31.9 (452/1,419)	13.1 (36/264)	29.5 (118/400)	13.6 (14/103)	64.0 (178/278)	18.5 (12/65)	< 0.01
Gentamicin	34.3 (461/1,343)	39.0 (48/123)	21.7 (73/336)	37.5 (50/133)	45.6 (108/237)	36.4 (107/294)	< 0.01
Tobramycin	48.2 (364/755)	38.7 (24/62)	40.2 (68/169)	60.2 (112/186)	54.9 (89/162)	40.3 (71/176)	< 0.01
Amikacin	29.4 (236/804)	25.7 (18/70)	16.2 (29/179)	31.8 (62/195)	30.7 (60/163)	34.0 (67/197)	< 0.01
Ciprofloxacin	35.1 (343/976)	25.9 (28/108)	21.3 (49/230)	36.3 (86/237)	36.8 (77/209)	53.7 (103/192)	< 0.01
Colistin	1.9 (4/212)	0.0 (0/7)	4.6 (1/22)	0.0 (0/56)	1.9 (1/54)	2.7 (2/73)	0.67

* Due to suppression pattern at certain site numbers are underestimated

Table 4. Univariate and multivariate analysis of factors associated with antimicrobial drug resistance among the selected blood culture infections.

Characteristic	<i>Staphylococcus aureus</i>		<i>Klebsiella pneumoniae</i>		<i>Pseudomonas aeruginosa</i>	
	UOR (95% CI)	AOR (95% CI)	UOR (95% CI)	AOR (95% CI)	UOR (95% CI)	AOR (95% CI)
Age						
< 5	1.01 (0.79-1.27)	1.74 (1.33-2.28)	1.51 (1.21-1.88)	1.49 (1.19-1.87)	0.87 (0.63-1.20)	0.83 (0.58-1.19)
5-9	0.52 (0.29-0.93)	0.66 (0.35-1.26)	1.70 (0.93-3.12)	1.58 (0.86- 2.91)	1.19 (0.58- 2.44)	1.28 (0.60-2.74)
10-19	0.79 (0.56-1.11)	0.84 (0.56-1.25)	1.01 (0.71-1.42)	0.94 (0.66-1.34)	0.96 (0.56- 1.67)	0.95 (0.53-1.70)
20-29	1 (0.79-1.27)	1	1	1	1	1
30-39	1.19 (0.96-1.48)	1.25 (0.94-1.67)	1.17 (0.905-1.50)	1.15 (0.89-1.48)	1.26 (0.87-1.82)	1.25 (0.85-1.85)
40-49	0.90 (0.70-1.15)	0.93 (0.68-1.27)	0.89 (0.68- 1.16)	0.89 (0.68- 1.16)	0.92 (0.62-1.36)	0.91 (0.60-1.38)
50-59	0.82 (0.62-1.09)	0.80 (0.56-1.13)	0.90 (0.68-1.18)	0.88 (0.66-1.16)	1.00 (0.66-1.51)	0.97 (0.62-1.50)
60-69	0.93 (0.68-1.27)	0.96 (0.66-1.39)	0.95 (0.71-1.27)	0.92 (0.69-1.23)	1.08 (0.70-1.67)	1.08 (0.67-1.72)
≥ 70	0.68 (0.46-1.00)	0.78 (0.50-1.23)	0.82 (0.60-1.11)	0.78 (0.57 -1.07)	1.02 (0.63-1.66)	1.01 (0.61-1.70)
Gender						
Male	1	1	1	1	1	1
Female	0.99 (0.85-1.15)	0.97 (0.82-1.14)	1.17 (1.03-1.33)	1.13 (1.00-1.29)	1.03 (0.84-1.27)	0.99 (0.80-1.24)
Hospital						
CMaxeke JAH	1	1	1	1	1	1
CHani Bara	0.41 (0.31-0.55)	0.41 (0.30-0.56)	1.22 (1.01-1.48)	1.08 (0.89-1.32)	1.92 (1.40-2.62)	1.87 (1.34-2.62)
Helen Joseph	0.95 (0.68-1.33)	1.26 (0.88-1.80)	1.09 (0.81-1.46)	1.23 (0.91-1.67)	1.66 (1.05-2.65)	1.48 (0.91- 2.39)
SBPAcademic	0.83 (0.60-1.15)	1.00 (0.72-1.41)	0.95 (0.77-1.18)	0.95 (0.76-1.18)	5.43 (3.84-7.68)	5.16 (3.62-7.36)
Universitas	2.55 (1.76-3.70)	3.08 (2.10-4.52)	1.56 (1.14-2.134)	1.39 (1.01-1.91)	1.80 (1.04-3.11)	1.60 (0.91-2.79)
Groote Schuur	2.98 (2.29- 3.89)	3.78 (2.85-5.01)	1.04 (0.82-1.31)	1.10 (0.86-1.39)	2.33 (1.53- 3.55)	2.08 (1.35-3.21)
Tygerberg	4.10 (3.18-5.29)	4.75 (3.6- 6.20)	1.25 (0.99-1.58)	1.12 (0.88-1.42)	3.20 (2.18-4.70)	3.02 (2.04-4.47)
Province						
Gauteng	1		1	---	1	---
Free State	3.69 (2.65-5.12)	---	1.44 (1.09-1.90)	---	0.85 (0.51-1.40)	---
Western Cape	5.13 (4.37- 6.02)	---	1.05 (0.91-1.21)	---	1.32 (1.03-1.69)	---
Year						
2005	1	1	1	1	1	1
2006	0.82 (0.63-1.07)	0.72 (0.54-0.97)	0.93 (0.72-1.20)	0.90 (0.69-1.17)	0.48 (0.32-0.72)	0.52 (0.33-0.80)
2007	0.67 (0.51-0.89)	0.61 (0.45-0.82)	0.88 (0.68-1.13)	0.87 (0.67-1.13)	0.84 (0.56-1.25)	0.79 (0.51-1.21)
2008	0.56 (0.43-0.74)	0.48 (0.35-0.65)	1.27 (0.99-1.64)	1.29 (0.99-1.68)	1.21 (0.81-1.80)	1.20 (0.78-1.84)
2009	0.44 (0.33-0.58)	0.39 (0.28-0.53)	1.27 (0.99-1.65)	1.27 (0.98-1.66)	1.13 (0.76-1.68)	1.11 (0.72-1.70)

CI, confidence interval; UOR, unadjusted odds ratio; AOR, adjusted odds ratio.

Patterns of Staphylococcus aureus resistance

The frequencies of resistance to clindamycin (72%) and rifampicin (60.7%) in *Staphylococcus aureus* at TH were high. The LIS recorded resistance to vancomycin as < 0.5% and MRSA as 43% being highest at TH, range 0.4% - 43%. Resistance to cotrimoxazole ranged 25% - 37% and gentamicin resistance ranged from 40% - 67% across all hospitals. There was scanty data for other drugs to make any meaningful analysis (Table 2).

Trends of Staphylococcus aureus resistance

The total number of reported cloxacillin-resistant *Staphylococcus aureus* declined progressively from 182 to 91 during the period 2006 to 2009 as did the ratios of resistant to susceptible isolates (expressed in percentages) which might be explained by the lack of standardized recording in LIS of susceptibility to cefoxitin and cloxacillin. A decline in MRSA in recent years has been reported in Scottish and other European hospitals due to rigorous infection control measures such as simple hand washing before touching patients, eating food and after using the toilet. The total numbers of resistant isolates of *Staphylococcus aureus* but not the ratios of resistant to susceptible cultures, also showed steady declines in the case of gentamicin, clindamycin and rifampicin resistance (Table 3).

Demographic factors associated with Staphylococcus aureus resistance

The age-group < 5 years was significantly associated with *Staphylococcus aureus* resistance to antimicrobials. Children < 5 years were 74% more likely to have had incidence of *Staphylococcus aureus* resistant isolates (AOR 1.74, CI 1.33 - 2.28) compared to the 20 - 29 years age-group. There was a significant association between antimicrobial resistance and hospital location. *Staphylococcus aureus* isolates at UH were appreciably more likely to be resistant to antimicrobials, (AOR 3.08, CI 2.10 - 4.52); *Staphylococcus aureus* isolates from Groote Schuur hospital were 3.8 times more likely to be resistant to antimicrobials (AOR 3.78, CI 2.85 - 5.01). At Tygerberg hospital, *Staphylococcus aureus* isolates were 4.8 times more likely to be resistant to antimicrobials (AOR 4.75, CI 3.60 - 6.20). In general *Staphylococcus aureus* isolates from UH, GSH, TH were significantly more likely to be resistant to antimicrobials (Table 4).

Patterns of Klebsiella pneumoniae resistance

For the 4.5 - year study period, the carbapenems covered the widest range of *K. pneumoniae* isolates.

Cephalosporins resistance in *Klebsiella pneumoniae* was high but varied widely e.g. cefotaxime/ceftriaxone resistance was 50.0% - 65.1% from five of the seven hospitals, 83.7% and 92.5% for the other remaining two hospitals. Cefepime resistance was high in the three Johannesburg hospitals (92.5% - 94.4%) compared with 55.75 - 84.0% for the other remaining four hospitals.

Carbapenem and colistin resistance for *Klebsiella pneumoniae* was very low: imipenem and meropenem resistance 0.1% each and ertapenem 2.0%; colistin resistance was 1.9%. Resistance rates for co-amoxiclav in *Klebsiella pneumoniae* isolates averaged at 64.8% for the seven hospitals. At four of the seven hospitals resistance to cotrimoxazole was in excess of 70%. The mean resistance rates for aminoglycosides were amikacin 26.4%, gentamicin 58.7% and tobramycin 80.4%.

Ciprofloxacin resistance rates in *Klebsiella pneumoniae* at the seven hospitals were ~ 50%. Piperacillin-tazobactam resistance in *Klebsiella pneumoniae* was high with a mean resistance of 66.3% and rates varying from 29.6% at GSH to 83.9% at CHB similar to cefepime (Table 2).

Trends of Klebsiella pneumoniae resistance

There were marked rises in ciprofloxacin resistance (32.6% in 2005 to 64.9% in 2009, $p < 0.001$) and cotrimoxazole resistance (67.5% in 2005 to 81.6% in 2009, $p < 0.001$). High rates of cephalosporin resistance were maintained or slight increases were seen over the 2005 to 2009 period e.g. ceftazidime resistance: 77.2% - 86.6%; cefotaxime resistance: (55.5% - 73.2%). There were high rates of aminoglycoside resistance showing a slight rise of resistance over this period. (*i.e.* amikacin 21.2 - 28.2 %; gentamicin 52.4 - 66.7%; tobramycin 75.8 - 79.7%). There were significant differences in rate of *Klebsiella pneumoniae* resistance by year of study among most of the antibiotics except for carbapenems, nitrofurantion, tobramycin and colistin $p > 0.05$ (Table 3).

Demographic factors associated with Klebsiella pneumoniae resistance

For *Klebsiella pneumoniae*, age-group < 5 years was significantly associated with antibiotic resistance with children < 5 years being 49% more likely to have *Klebsiella pneumoniae* resistant isolates (AOR 1.49, CI 1.19 - 1.88) compared to the 20-29 years age-group. Females were more likely to have resistant *Klebsiella pneumoniae* isolates than males (AOR 1.13, CI 1.00 - 1.29). There was a significant association between antimicrobial resistance and hospital location.

Klebsiella pneumoniae isolates at UH were 39% more likely to be resistant to antimicrobials (AOR 1.39, CI 1.01 - 1.91); even though *Klebsiella pneumoniae* isolates from HJ, GSH and TH were more likely to be resistant to antimicrobials, this was not statistically significant hence not reported in detail here. *Klebsiella pneumoniae* isolates reported in 2008 and 2009 were more likely to be resistant to antimicrobials; however this was not statistically significant (Table 4).

Patterns of Pseudomonas aeruginosa resistance

The mean ceftazidime resistance rate in *Pseudomonas aeruginosa* was 20.1% and 36.0% for cefepime. Carbapenem resistance in *Pseudomonas aeruginosa* was 46.7% and 44.4% respectively for imipenem and meropenem and 31.9% for piperacillin-tazobactam. The antibiotic most active against *Pseudomonas aeruginosa* isolates for the study period was colistin with a resistance rate of 1.9% (range 0% - 13.3%). Colistin resistance was non-existent in *Pseudomonas aeruginosa* in four of the seven hospitals. The mean ciprofloxacin resistance was 35.1% and for amikacin, gentamicin and tobramycin resistance rates in *Pseudomonas aeruginosa* were 29.4%, 34.3% and 48.2% respectively (Table 2).

Trends of Pseudomonas aeruginosa resistance

The range of ciprofloxacin resistance was 25.9% - 53.7% over the period 2005 – 2009; moderate increases in aminoglycoside resistance among *Pseudomonas aeruginosa* isolates over the study period were observed (amikacin 25.7% – 39.1%, gentamicin 21.7% – 53.7%, and tobramycin 38.7% - 60.2%). Cephalosporin resistance equally showed moderate rise i.e. ceftazidime 17.1% - 24.6% and cefepime 36.6% – 43.6%. Carbapenems resistance rate showed moderate rises ~45% - ~55% for imipenem and meropenem resistance over the 2005 – 2009 periods (Table 3).

Demographic factors associated with Pseudomonas aeruginosa resistance

For the 5 years study period, hospital location was associated with antibiotic resistance. SBPAH (AOR 5.16, CI 3.62 - 7.36), GSH (AOR 2.08, 1.35 - 3.21), TH (AOR 3.02, 2.04 - 4.47) were significantly associated with antibiotic resistance among *Pseudomonas aeruginosa* isolates. At UH, *Pseudomonas aeruginosa* isolates were 60% more likely to be resistant to antimicrobials; however, this was not statistically significant (AOR 1.60, CI 0.91 - 2.79). Even though *Pseudomonas aeruginosa* isolates reported in 2008 and 2009 were more likely to be resistant to antimicrobials,

the association was not statistically significant (Table 4).

Discussion

This study led to a detailed and systematic data analysis of the LIS in reporting antimicrobial susceptibility of isolates from blood culture over a 4.5-year period to assess possibility for reporting of trends and patterns of resistance from all isolates in the public tertiary hospitals in South Africa. A total of 9969 isolates belonging to *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* had drug susceptibility results reported by the NHLS between July 1, 2005 and December 31, 2009. The number of isolates of all three pathogens for 2005 (first year of CDW-based surveillance) were considerably smaller as we started with data collection in July that year.

Staphylococcus aureus and *Klebsiella pneumoniae* were the most common pathogens and contributed 84.3% of the total magnitude of blood stream infections among the three selected pathogens reported within this period. This is in keeping with previous studies that have shown *Staphylococcus aureus* to be the predominant cause of blood stream infections [4,5,18]. More isolates of these pathogens were reported from males compared with females and from children below the age of 5 years. The relationship of higher incidence of blood stream infections among males has been documented in previous studies [4,19]. As much as this study found higher incidence of blood stream infections among children, other studies in Canada and the USA have found smaller proportion of isolates from children [20]. There were more isolates reported from Chris Hani Baragwanath Hospital which is the largest hospital in the country and services a greater population of Soweto. Antimicrobial susceptibility was done to assess rates of resistance to various antibiotics amongst the three common pathogens associated with in-hospital acquisition.

The proportion of *Klebsiella pneumoniae* resistant isolates (defined as isolates resistant to one or more antibiotics) was higher among females while *Staphylococcus aureus* and *Pseudomonas aeruginosa* rates were similar. The proportion of *Staphylococcus aureus* resistant isolates was highest at Tygerberg Hospital, *Klebsiella pneumoniae* was highest at Universitas Hospital and *Pseudomonas aeruginosa* was highest at Steve Biko Pretoria Academic Hospital. There were more resistant isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa* reported from the Western Cape and more resistant isolates for *Klebsiella pneumoniae* reported from Free State province. The

proportion of *Staphylococcus aureus* resistant isolates was higher in 2005; *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were higher in 2008.

No recent studies in South Africa on the frequency of bacteraemic pathogens have documented comparable information. This study used blood culture data that represent invasive pathogens and therefore excludes organisms that merely colonize non-sterile sites and may be present in specimens such as pus swabs. Such data could serve to guide prescription habits and form the basis of a robust national surveillance monitoring system able to regularly document similarities and differences in antimicrobial resistance between different hospitals both locally and internationally.

Antibiotics with the broadest spectrum against *Staphylococcus aureus* were vancomycin and linezolid. Vancomycin was still active against nearly all *Staphylococcus aureus* isolates with resistance rate showing < 0.1% across all the 7 hospitals. This is consistent with previous data which reported that vancomycin was still an active agent against *Staphylococcus aureus* including MRSA [13,21-24]. Frequencies of clindamycin resistance (72%), erythromycin resistance (44.1%) and rifampicin (60.7%) among *Staphylococcus aureus* isolates at TH were relatively high. These might be linked to macrolide or as a result of inducible clindamycin resistance among erythromycin resistant strains. Simultaneous resistance to erythromycin and clindamycin among *Staphylococcus aureus* isolates could be a result of erythromycin resistance methylase genes (*erm* genes), while erythromycin resistance not crossed to clindamycin is consistent with the presence of *msrA* gene. The variation in susceptibility of erythromycin-resistant *Staphylococcus aureus* to clindamycin as observed in this study among the seven tertiary public hospitals might be an indication of epidemiological variation in the two mechanisms of resistance that was mentioned above [25,26].

The highest rates of MRSA were observed at Tygerberg and Groote Schuur hospitals in the Western Cape as opposed to Universitas hospital in the Free State province, while data from CMJAH, SBPAH, CHB, HJ in Gauteng province for cloxacillin showed gross underreporting hence were unreliable and were thus not presented in the results. The variation in rates of MRSA observed in hospitals in Western Cape and Free State provinces is consistent with previous EARSS reports [19] that showed marked geographical variation in prevalence of MRSA. In our situation the variation might potentially be due to differences in specimen

collection, carriage rates or hospital infection control policies and practices as well as prescription policies between different hospitals and provincial Departments of Health [4]. It is thus crucial to further assess the reliability of routine laboratory data generated by the LIS for monitoring antimicrobial resistance to ascertain if the observed rates of antimicrobial resistance are not due selection bias.

The most active antibiotics against *Klebsiella pneumoniae* in this study were the carbapenems. These data are similar to those shown by Zhanel *et al.* [24]. Cephalosporin, fluroquinolones and aminoglycosides showed high resistance across all sites. β -lactams, excluding carbapenems were the least active antibiotics over the 4.5-year study period with resistance rate increasing in all sites and in keeping with previous review findings done in South Africa [13]. Low levels of carbapenems resistance shows that there is evidence of emergence of carbapenemase-mediated resistance among *Klebsiella pneumoniae* isolates. Nordmann *et al* reported that *Klebsiella pneumoniae* that produces *Klebsiella pneumoniae* carbapenemase (KPC) have globally spread across hospitals [27]. Bogdanovich *et al*, reported cases of *Klebsiella pneumoniae* - carbapenemase producing isolates that showed emerging resistance to colistin [28] a reserved antibiotic for the treatment of multidrug resistant Gram-negative sepsis that resulted from failed treatment with carbapenems. These therapeutic agents have shown to have the most favourable outcomes in the treatment of bacteraemic ESBL-producing *Klebsiella pneumoniae* infections [29]. There was a significant trend of *Klebsiella pneumoniae* resistance to ciprofloxacin and cotrimoxazole while meropenem showed a significant increasing trend of resistance from 2006 to 2009, no particular resistance trend was observed for other antibiotics [21].

Pseudomonas aeruginosa resistance was evident across most of the drug classes showing high resistance to carbapenems, cephalosporin, fluroquinolones and aminoglycosides. Even though Adam *et al* in a study done among Canadian hospitals reported that resistance was encroaching to these drug classes, the resistance rate shown in this study is far higher compared to the findings of Adam *et al*. This is a significant finding denoting that geographical location does play a role in development of antimicrobial resistance, and therefore might mean that due to rapid increase and high level of intercontinental mobility, such as global air travel, resistant clones are bound to rapidly disseminate across different countries and regions [4,21,30,31].

The rates of aminoglycoside resistance among *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* isolates was varied with amikacin showing low resistance and tobramycin showing higher resistance. As shown above, among *Klebsiella pneumoniae* isolates, the mean resistance for amikacin was 26.4%, gentamicin 58.7% and tobramycin 80.4% whereas for *Pseudomonas aeruginosa* the mean resistance for amikacin, gentamicin and tobramycin were 29.4%, 34.3% and 48.2% respectively. Such observed differences in resistance patterns could be due to differences in aminoglycoside modifying enzymes; prescription patterns or variation in quality of infection control practices in these hospitals, although geographical differences in the occurrence of individual aminoglycoside resistance determinants might also play a role. This emphasizes the fact that the prudent use of aminoglycosides as well as implementation of effective infection control practices are essential in limiting the development and continued spread of aminoglycoside resistance among these pathogens [32]. The only consistently active antibiotic against *Pseudomonas aeruginosa* for the study period was colistin which had resistance rate of 1.9%. This is similar to findings of previous studies that also showed a similar pattern of high activity of colistin against *Pseudomonas aeruginosa* [21,33].

Several demographic factors were found to be significantly associated with antimicrobial resistance. For *Staphylococcus aureus* factors were: age-group < 5 years; hospital location (UH, TH, GSH) and year of infection. Factors associated with *Klebsiella pneumoniae* resistance were age-group < 5 years, female gender and hospital location (UH). The only factor significantly associated with *Pseudomonas aeruginosa* resistance was hospital location (CHB, SBPAH, GSH and TH). There is however no known reason that could explain such underlying associations.

Limitations of the study

This study had several limitations which are related to the analysis of routine laboratory data. No clinical data were available hence any determination of the impact of antimicrobial resistance on clinical outcomes could not be made. Such data are essential as their availability would help in making detailed risk factor analysis evaluating the potential impact of inappropriate antimicrobial therapy on outcome of patients with bacteraemia episode caused by either of the three selected pathogens. Secondly, the accurate prevalence of blood stream infection caused by the selected pathogens could not be ascertained as there

was no data on negative blood cultures in the CDW repository for the study period. Such data would be useful to give precise estimates of the magnitude of blood stream infection caused by such organisms, as this would help direct strategic planning of service delivery, medication procurement as well as intensity of hospital infection control procedures. Thirdly, susceptibility testing methods for individual antibiotics varied across sites for individual pathogens with other NHLS laboratories testing certain specific agents more than other sites which might have led to differences in estimation of resistance rates among those agents. Fourthly, while using the first specimen only is one approach to surveillance, a limitation of such an approach is the possibility of missing the occurrence of acquired resistance during the illness. This may not be captured by the surveillance system. In addition although susceptibility testing figures were used to assess rates of resistance among the three pathogens associated with hospital acquisition, our results do not differentiate between community and hospital acquired infections and there is therefore the potential for underestimation of resistance rates in general.

Another important limitation is that no data were available on admission date for each patient/blood culture specimen; hence no accurate description of community versus nosocomial acquired bacteraemia could be made. Lastly, the use of ‘resistance to any antimicrobial agent tested’ as a method of estimating overall resistance rate might have led to erroneous estimation of resistance among the antibiotics tested.

Conclusion

There are problems in retrieving information on AST from the current LIS. Estimated rates of antimicrobial resistance observed in this study, are a matter of serious concern, especially with regard to *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. It was encouraging to see that other antimicrobial agents are still very active against the selected pathogens. Firstly, the rate of vancomycin resistance is almost negligible (0.1%, only 1 of 865 isolates-one case at Tygerberg hospital in 2006) and linezolid resistance among *Staphylococcus aureus* isolates was not detected in this study. Secondly, carbapenems (ertapenem, imipenem and meropenem) and colistin remains highly active against *Klebsiella pneumoniae* and thirdly, that colistin is highly active against *Pseudomonas aeruginosa*. The extent of antimicrobial resistance in *Pseudomonas aeruginosa* is alarming and is aggravated by the fact that colistin the only remaining strongly

effective agent against resistant isolates is both oto- and nephrotoxic.

Therefore ongoing structured prospective surveillance to monitor the burden of bloodstream infections and their resistance profile is essential to better monitor trends and patterns of resistance to nosocomial infections at national level. Such data would enhance the knowledge of the magnitude of the problem regarding antimicrobial resistance and will form evidence upon which policies and practice aimed at containing antimicrobial resistance can be generated. In addition the analysis presented in this manuscript provides the type of assessment that has to be used to develop empirical treatment guidelines.

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