Analysis and comparison of cumulative antibiograms for Charlotte Maxeke Johannesburg Academic Hospital adult intensive care and high care units, 2013 & 2017

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Abstract

Background. Infection is a common complication for patients in intensive care units (ICUs) and increasing antimicrobial resistance is a major concern. It is therefore crucial to monitor antimicrobial resistance patterns in order to support clinical decision making and antimicrobial stewardship strategies. Clinical microbiologists should provide annual cumulative antibiogram reports, which can be used to guide initial empirical antimicrobial therapy for the management of infections.
Objectives. Analyses of the cumulative antibiograms for the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) combined adult multidisciplinary ICU and high care unit (HCU) for 2013 and 2017, comparison of the antimicrobial susceptibility testing (AST) patterns between two years; and analyses of the subset of blood culture isolates.
Methods. A retrospective descriptive analysis was performed of routine bacterial and fungal culture and AST data extracted from the NHLS laboratory information system for the combined adult ICU and high care unit. Only the first diagnostic isolate of a given species per patient per year was included in the analysis. All analysis and reporting were done in accordance with the applicable Clinical and Laboratory Standards Institute guidelines.
Results. *Enterobacteriaceae* predominated in first-isolate cultures in 2013 (60%) and 2017 (56%). There was an overall decrease in extended-spectrum beta-lactamase-producing *Enterobacteriaceae* from 2013 (42%) to 2017 (30%) (p=0.013), accompanied by an increase in carbapenem-resistant *Enterobacteriaceae* from 2013 (4%) to 2017 (11%) (p=0.24). Although the total percentage of *Acinetobacter* spp. decreased in 2017 (p=0.021), the proportion of extensively drug-resistant isolates doubled to 68% in 2017 (p<0.001). The percentage of MRSA decreased significantly from 49% to 14% (p<0.001), along with a significant decrease in VRE from 17% to 0% (p=0.001). *Candida auris* increased from 0% in 2013 to 11% in 2017 (p=0.002), and non-albicans *Candida* spp. predominated (80%) in blood cultures in 2017 (p=0.023).
Conclusions. Appropriate selection of empiric antimicrobial therapy should be guided by the ICU-specific antibiogram. The recommended empiric antimicrobial therapy at he CMJAH ICU/HCU based on the antibiogram analysis, would include ertapenem to cover the *Enterobacteriaceae.* Amikacin is recommended for empiric treatment of suspected pseudomonal infections. Additional empiric antimicrobial therapy for the gram-positive organisms is not routinely advocated and empiric antifungal therapy with amphotericin B or micafungin is only appropriate in patients at high risk for invasive candidiasis.

Introduction

Infection is a common complication in patients in intensive care units (ICUs) and is associated with considerable mortality, morbidity, and increased costs.[1] Antimicrobial treatment of patients with sepsis is increasingly complicated by the alarming rates of antimicrobial resistance amongst pathogens. The ICU is often called the epicentre of antimicrobial resistance development, due to its extremely vulnerable population (reduced host defences deregulating the immune responses) with increased risks of becoming infected through multiple procedures and use of invasive devices.[2-4] Most large epidemiologic studies of infection and sepsis in ICUs have been conducted in Europe, North America, and Australia, with limited data from southern Africa.[1] With increasing antimicrobial resistance worldwide, it is crucial to monitor emerging trends in antimicrobial resistance at the local level to support clinical decision making, infection control interventions, and antimicrobial stewardship (AMS) strategies.[5,6] The most urgent and serious threats for the ICU include infections with *Enterobacteriaceae* producing extended-spectrum beta-lactamases (ESBLs), derepressed AmpC beta-lactamases and carbapenemases; extensively drug-resistant and carbapenem-resistant *Acinetobacter baumannii*; multi-drug resistant *Pseudomonas aeruginosa*; methicillin-resistant *Staphylococcus aureus* (MRSA), and azole-resistant and multidrug-resistant *Candida* spp.[7,8]

In its guide for the prevention of hospital-acquired infections, the World Health Organisation specifies that clinical microbiologists are responsible for providing annual reports of antimicrobial susceptibility patterns of pathogens. Epidemiological surveillance activities by microbiology laboratories are therefore gaining growing importance.[9,10] Monitoring of antimicrobial resistance trends is commonly performed in health care facilities using an annual summary of susceptibility rates, known as a cumulative antibiogram.[5,9] The most frequent use of a cumulative antibiogram report is to guide initial empiric antimicrobial therapy for the management of infections in patients for whom definitive microbiological results to target treatment do not yet exist. The choice of empiric antimicrobial coverage is critical in the ICU because initiation of inadequate empiric therapy has been associated with poor clinical outcomes.[3,11] Bloodstream infections represent a common complication among critically ill patients in the ICU, and a leading cause of morbidity and mortality. Early appropriate antibiotic therapy is therefore a critically important aspect of these patients.[12] Cumulative antibiograms have additional applications, including, updating periprocedural or perioperative prophylaxis recommendations, providing a rationale for antimicrobial formulary selection, surveying local resistance patterns, and identifying targets for AMS and best practices.[9,13]

No cumulative antibiogram studies have yet been published from the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) adult ICU and high care unit (HCU), nor any other ICUs in South Africa. This study was designed to provide the necessary cumulative antibiograms for the CMJAH adult ICU and HCU, and to demonstrate antimicrobial resistance changes and trends between two time periods. In 2017 the South African Department of Health mandated the implementation of AMS interventions at healthcare facilities in order to combat the emerging threat of antimicrobial resistance. Emergence of antibiotic resistance is highly correlated with selective pressure resulting from excessive use of antimicrobials in the ICU. It is therefore crucial for ICU clinicians to have regularly updated antibiograms in order to make informed decisions about empiric antibiotic choices.

Objectives

The primary study objectives were to prepare and analyse the cumulative antibiograms for the combined adult ICU and HCU for the years 2013 and 2017, and to compare the different organisms isolated and their antimicrobial susceptibility testing (AST) patterns between the two years. The secondary objective was to specifically analyse the cumulative antibiogram data for the subset of blood culture isolates.

Methods

Study setting

The CMJAH is an academic tertiary-level hospital, where the multidisciplinary ICU is a 12 bed unit and the HCU has 8 beds. Adult patients admitted to these units frequently present with severe sepsis and multiple organ dysfunction with or without septic shock, or have recently undergone complex surgery.[14]

Study design

A retrospective descriptive analysis of all the routine bacterial and fungal culture and AST data from the CMJAH adult ICU and HCU was performed. All the data was extracted from the National Health Laboratory Service (NHLS) laboratory information system (LIS). Culture and AST data from 1 January 2013 to 31 December 2013 was compared with data from 1 January 2017 to 31 December 2017. Clinical samples were tested at the NHLS microbiology laboratory based at CMJAH. The laboratory used a variety of identification and AST methodologies, which included the manual Kirby-Bauer disc diffusion and Etest (bioMérieux, Marcy l'Etoile, France), as well as the automated Microscan (Beckman Coulter, Brea, CA, USA), and Vitek 2 (bioMérieux, Marcy l'Etoile, France). AST results were interpreted according to the contemporary CLSI guidelines.[15,16] Molecular confirmation of carbapenemase production, was performed at the Antimicrobial Resistance Laboratory at the National Institute for Communicable Diseases (NICD) from 2014 onwards.

The CLSI guideline, CLSI M39-A4, was used to guide the compilation of the cumulative antibiograms, as it provides criteria for standardising and benchmarking antibiograms.[13] To eliminate the bias inherent in an 'all isolates' approach, only the first diagnostic isolate of a given species per patient per analysis period was included, as this approach has direct relevance to guiding recommendations for initial empiric therapy. Culture and susceptibility reports from samples collected for surveillance or screening were excluded.

Definitions

First-isolate: refers to the initial microbial isolate of a particular species recovered from a patient during the time period analysed regardless of body source, specimen type, or AST profile.[13]

Susceptible (S): a category where isolates were inhibited by the usually achievable concentrations of antimicrobial agent when the dosage recommended to treat the site of infection is used.[13]

MIC: minimum inhibitory concentrations are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a micro-organism after overnight incubation.[13]

Susceptible dose-dependent (SDD): means that the isolate's MIC was high, but that increased dosing of the agent has the potential to inhibit the yeast in vivo.[13]

Non-susceptible: a category used for isolates that are not inhibited by the usually achievable concentrations of antimicrobial agent with the normal dosage schedules, and includes intermediate (I) and resistant (R).[13]

AmpC: For the *Enterobacteriaceae*, non-susceptibility to cefoxitin was used as a marker of inducible ampicillin class C beta-lactamase production.[17]

ESBL: non-susceptibility to third and/or fourth generation cephalosporins was used to predict extended-spectrum beta-lactamase production.[15,16]

CRE: non-susceptibility to any of the carbapenems was a marker of carbapenem-resistant *Enterobacteriaceae* (CRE). Non-susceptibility to carbapenems may be the result of various mechanisms, including production of carbapenemases or via combinations of AmpC, ESBL and porin loss.[15,16]

CPE: Only *Enterobacteriaceae* with a confirmed carbapenemase-producing gene were defined as carbapenemase-producing *Enterobacteriaceae* (CPE).[15,16]

Definitions of multidrug-resistant and extensively drug-resistant were applied from Magiorakos *et al.*[18] to report on the AST resistance profiles of *Acinetobacter* spp. and *Pseudomonas* spp.

Statistical analysis

Statistical analyses were performed using Microsoft Excel and GraphPad (version 8.0). Categorical data were presented as percent susceptibility for each antimicrobial agent tested. The Agresti-Coull method was used to calculate confidence intervals (CIs) and the Fisher's exact test to compare differences between the two observed percent susceptible estimates from 2013 versus 2017. The *p*-values were reported as 2-tailed and values of <0.05 were considered statistically significant.

Ethical approval

The study was approved by the University of the Witwatersrand's Human Research Ethics Committee (ref. no. W-CBP-180802-3).

Results

ANALYSIS OF FIRST-ISOLATES

Of the 594 first-isolate cultures in 2013, 53% (*n*=314) were gram-negative bacteria, 33% (*n*=196) were gram-positive bacteria, and 14% (*n*=84) were *Candida* spp. In 2017, 59% (*n*=388) of the 662 first-isolate cultures were gram-negative bacteria, 30% (*n*=200) were gram-positive bacteria, and 11% (*n*=74) were *Candida* spp. The increase in the proportion of gram-negative bacteria from (53%) in 2013 to (59%) in 2017 was statistically significant (*p*=0.046). Numbers of anaerobic organisms isolated in both 2013 (*Bacteroides* spp., *n*=2) and 2017 (*Bacteroides* spp., *n*=12), were low.

Table 1. Summary of the total and resistant gram-negative isolates (first-isolate results), 2013 versus 2017

	2013				2017				2013 *vs.* 2017
Organism	***n* isolates (% of total[*])**	***n* isolates (% of group[†])**	***n* (%) resistant isolates**	**95% CI**	***n* isolates (% of total[*])**	***n* isolates (% of group[†])**	***n* (%) resistant isolates**	**95% CI**	***p*-value[‡]**
Total *Enterobacteriaceae*	187 (60)			54 – 65	219 (56)			51 – 61	0.442
Total AmpC			29 (16)	11 – 21			27 (12)	9 - 17	0.388
Total ESBL			79 (42)	35 – 49			66 (30)	24 - 37	**0.013**
Total CRE			8 (4)	2 - 8			23 (11)	7 - 15	**0.024**
***Klebsiella* spp.**		69 (37)		30 – 44		85 (39)		33 - 45	0.758
AmpC			3 (4)	1 – 13			4 (4)	1 – 10	1.000
ESBL			42 (61)	49 – 72			30 (34)	25 – 45	**0.001**
CRE			4 (6)	2 - 14			16 (18)	11 - 27	**0.029**
E. coli		58 (31)		25 – 38		69 (32)		26 - 38	1.000
AmpC			2 (3)	0 – 12			3 (3)	0 - 11	1.000
ESBL			17 (29)	19 – 42			23 (32)	22 - 44	0.848
CRE			1 (2)	0 - 10			0 (0)	0 - 6	0.457
***Enterobacter* spp.**		30 (16)		11 - 22		34 (16)		11 - 21	0.892
AmpC			10 (33)	19 - 51			15 (41)	26 - 58	0.801
ESBL			7 (23)	12 - 41			10 (26)	14 - 43	0.193
CRE			0 (0)	0 - 13			7 (18)	8 - 34	0.483
Other *Enterobacteriaceae*[§]		30 (16)		11 - 22		31 (14)		10 - 19	0.676
AmpC			10 (33)	19 - 51			9 (26)	13 - 43	0.582
ESBL			7 (23)	12 - 41			7 (19)	9 - 37	0.762
CRE			0 (0)	0 - 13			3 (6)	1 - 22	0.492
Total non-fermentative GNB	124 (39)			34 - 45	149 (38)			34 - 43	0.815
***Acinetobacter* spp.**		65 (52)		44 - 61		57 (38)		31 - 46	**0.021**
MDR			41 (62)	49 - 72			9 (14)	7 - 26	**<0.001**
XDR			23 (34)	23 - 46			40 (68)	55 - 79	**<0.001**
***Pseudomonas* spp.**		52 (42)		34 - 51		65 (44)		36 - 52	0.807
MDR			8 (13)	6 - 26			7 (9)	4 - 19	0.559
XDR			1 (0)	0 - 8			5 (6)	2 - 15	0.128
Total non-fermenters[¶]		7 (6)		3 - 11		27 (18)		13 - 25	**0.002**
Burkholderia spp.		3 (2)		1 - 7		21 (14)		9 - 21	**<0.001**
TOTAL GNB[**]	314 (53)			49 - 57	388 (59)			55 - 62	**0.046**

CI = confidence interval, ESBL = extended-spectrum beta-lactamase, CRE = carbapenem-resistant *Enterobacteriaceae*, MDR = multidrug-resistant, XDR = extensively drug-resistant, GNB = gram-negative bacilli

[*]Total GNB

[†]Groups are: Total *Enterobacteriaceae*, Total non-fermentative GNB

[‡]Statistically significant values in bold

[§]2013: *Proteus* spp. (*n*=10), *Serratia* spp. (*n*=7), *Citrobacter* spp. (*n*=6), *M. morganii* (*n* =5), *P. stuartii* (*n* =1), *Salmonella* spp. (*n* =1); 2017: *Proteus* spp. (*n* =12), *M. morganii* (*n* =9), *Citrobacter* spp. (*n* =5), *S. marcescens* (*n* =1), *H. alvei* (*n* =1), *Salmonella* spp. (*n* =1), *R. ornithinolytica* (*n* =1), *Pantoa* spp. (*n* =1)

[¶]2013: *S. maltophilia* (*n*=5), *B. cepacia* (*n*=2); 2017: *S. maltophilia* (*n*=6), *Burkholderia* spp. (*n*=21)

[**]2013: *Bacteroides spp.* (*n* =2), *H. influenzae* (*n* =1) were additional gram-negatives added to total; 2017: *Bacteroides* spp. (*n* =12), *H. influenzae* (*n* =8) were additional gram-negatives added to total

Enterobacteriaceae

The *Enterobacteriaceae* made up the largest proportion of first-isolate cultures in 2013 (60%) and 2017 (56%) (Table 1). Of the *Enterobacteriaceae* isolated in 2013 and 2017, there were 3 main genera: *Klebsiella* spp., *Escherichia coli*, and *Enterobacter* spp. Of the panel of antimicrobial agents tested, the only significant change was an overall decrease in susceptibility to piperacillin/tazobactam from 75% in 2013 to 64% in 2017 (*p*=0.017). The statistically significant decrease in ESBL-producing isolates from 42% in 2013, to 30% in 2017 (*p*=0.013) was accompanied by a significant increase in the number of CRE from 4% in 2013, to 11% in 2017 (*p*=0.024). Only 4 of the 8 CRE were sent for genotyping in 2013, of which 1 was a bla_{VIM}, 1

was a *bla*$_{IMP}$, and 2 tested negative for carbapenenase genes. In 2017, 21 of the 23 CRE were sent for genotyping. The predominant carbapenamase was *bla*$_{OXA-48}$ and its variants (*n*=17). Three *bla*$_{NDM}$ and 1 *bla*$_{VIM}$ were also detected.

Klebsiella spp. were the most frequent gram-negative bacteria isolated in both years. In 2013, 61% of all *Klebsiella* spp. were ESBL producers and this decreased to 34% in 2017 (p=0.001) due to the accompanying increase in CRE from 6% in 2013 to 18% in 2017 (p=0.029). In comparison with 2013, *Klebsiella* spp. demonstrated higher susceptibility to amoxicillin-clavulanic acid (p=0.008), cefepime (p=0.03) and trimethoprim-sulfamethoxazole (p=0.006) in 2017 (Fig. 1A). Of the aminoglycosides, *Klebsiella* spp. were most susceptible to amikacin in both years. In 2013 and 2017, more than 50% of the *Klebsiella* spp. were non-susceptible to third generation cephalosporins. *Klebsiella* spp. were all susceptible to tigecycline in 2017, but no comparison could be made with 2013 as tigecycline was not tested.

E. coli was the second most prevalent *Enterobacteriaceae* isolated in both years (Table 1). Susceptibility to the third and fourth generation cephalosporins was greater than 60% (Fig. 1B). Amikacin was the most susceptible aminoglycoside for *E. coli*. The only antimicrobial agents with susceptibilities below 50% in both years were ampicillin, amoxicillin-clavulanic acid and trimethoprim-sulfamethoxazole.

Enterobacter spp. were the third most frequently isolated *Enterobacteriaceae* in the study years. *Enterobacter* spp. produce an inducible AmpC beta-lactamase, and expression of this enzyme confers resistance to broad-spectrum cephalosporins including cefotaxime, ceftazidime, and ceftriaxone. More than 50% of the isolates were susceptible to the fourth generation cephalosporin, cefepime (Fig. 1C). Again, amikacin was the most susceptible aminoglycoside for the *Enterobacter* spp.

The remaining *Enterobacteriaceae* that were isolated had less than 30 isolates per genus, and were thus grouped together as 'other *Enterobacteriaceae*' for the purposes of analysis. In this group, more than 80% of isolates were susceptible to the aminoglycosides, as well as ciprofloxacin in both 2013 and 2017 (Fig. 1D).

Non-fermentative gram-negative bacteria

In 2013, *Acinetobacter* spp. were the predominant non-fermenters (52%), followed by *Pseudomonas* spp. (42%) (Table 1). In contrast, in 2017, *Pseudomonas* spp. predominated (44%), followed by *Acinetobacter* spp. (38%). The remaining non-fermentative gram-negative bacteria isolated had numbers below 30 per genus. However, due to their intrinsic multidrug-resistance, they were included in the analysis. In 2013, 5 *Stenotrophamonas maltophilia* and 2 *Burkholderia cepacia* were isolated, and in 2017, 21 *Burkholderia* spp. and 6 *S. maltophilia* were isolated.

The overall reduction in the number of *Acinetobacter* spp. in 2017 was statistically significant (p=0.021). In 2013, 62% were MDR compared with only 14% in 2017 (p<0.0001). This was due to the significant doubling of the percentage of XDR *Acinetobacter* spp., from 34% in 2013, to 68% in 2017 (p<0.001). There was a significant increase in non-susceptibility in 2017 to ceftazidime (p=0.008), gentamicin (p=0.016), and tobramycin (p<0.001) (Fig. 2A). In 2017, more than 60% of all antimicrobials tested were non-susceptible for *Acinetobacter* spp., and susceptibility to the carbapenems, meropenem and imipenem, was less than 20%. Tigecycline was susceptible in 89% of *Acinetobacter* spp. in 2017, but no comparison could be made with 2013 as tigecycline was not tested.

There were limited changes in *Pseudomonas* spp. between the 2 study years. A significant reduction in the susceptibility of piperacillin/tazobactam was observed, from 92% in 2013 to 78% in 2017 (p=0.04) (Fig. 2B). There was a significant increase in number of *Burkholderia* spp. from 2013 to 2017 (p<0.001). Both *S. maltophilia* and *Burkholderia* spp. had very high non-susceptibility rates to multiple antimicrobials (>69%) (Fig. 2C), which is in keeping with their intrinsically resistant phenotype.

Gram-positive bacteria

Of the 196 gram-positive bacteria isolated in 2013, 58% were *Staphylococcus* spp. and 37% were *Enterococcus* spp. (Table 2). Similarly, in 2017, of the 200 gram-positive bacterial isolates, 64% were *Staphylococcus* spp. and 28% were *Enterococcus* spp. For the remaining 5% (*n*=10) of gram-positive organisms isolated in 2013 and 8% (*n*=16) in 2017, AST analysis was omitted due to the small number of isolates.

Table 2. Summary of the total and resistant gram-positive isolates (first-isolate results), 2013 versus 2017

	2013				2017				2013 *vs.* 2017
Organism	***n* isolates (% of total[*])**	***n* isolates (% of group[†])**	***n* (%) resistant isolates**	**95% CI**	***n* isolates (% of total[*])**	***n* isolates (% of group[†])**	***n* (%) resistant isolates**	**95% CI**	***p*-value[‡]**
Total *Staphylococcus* spp.	114 (58)			51 - 65	128 (64)			57 - 70	0.257
S. aureus		49 (43)		34 - 52		44 (34)		27 - 43	0.187
MRSA			24 (49)	36 - 63			6 (14)	6 - 27	**<0.001**
Coagulase-negative staphylococcus		65 (57)		48 - 66		84 (66)		57 - 73	0.078
MR CoNS			54 (83)	72 - 90			54 (72)	54 - 74	**0.016**
Total *Enterococcus* spp.	72 (37)			30 - 44	56 (28)			22 - 35	0.068
E. faecalis		20 (28)		19 - 39		19 (34)		23 - 47	0.562
E. faecium		50 (69)		58 - 79		36 (64)		51 - 76	0.573
VRE			12 (17)	10 - 27			0 (0)	0 - 8	**0.001**
Other gram-positive cocci[§]	10 (5)			3 - 9	16 (8)			5 - 13	0.311
TOTAL GRAM-POSITIVE COCCI	196 (33)			29 - 37	200 (30)			27 - 34	0.301

CI = confidence interval, MRSA = methicillin-resistant *S. aureus*, MR CoNS = methicillin-resistant coagulase-negative staphylococcus, VRE = vanvomycin-resistant enterococcus
[*]Total gram-positive cocci
[†]Groups are: Total *Staphylococcus spp.*, Total *Enterococcus* spp., Other gram-positive cocci
[‡]Statistically significant values in bold
[§]2013: *S. agalactiae* (*n*=1), *S. anginosus* (*n*=3), *S. pneumoniae* (*n*=2), *S. pyogenes* (*n*=1) , *S. viridans* (*n*=3); 2017: *S. anginosus* (*n*=5), *S. pneumoniae* (*n*=4), *S. viridans* (*n*=7)

The *Staphylococcus* spp. made up the largest proportion of the first-isolate gram-positive cultures in both 2013 (*n*=114) and 2017 (*n*=128). The number of MRSA decreased significantly in 2017 to 14% ($p<0.001$). Additionally, the susceptibility of *S. aureus* to rifampicin also increased significantly in 2017 ($p=0.005$) (Fig. 3A). All *S. aureus* isolates were susceptible to vancomycin and linezolid in both study years. The vast majority of CoNS were resistant to cloxacillin. However, there was a significant reduction in the number of methicillin-resistant CoNS, from 83% in 2013 to 72% in 2017 ($p=0.016$) (Fig. 3B). The susceptibility of CoNS to trimethoprim-sulfamethoxazole also increased significantly in 2017 ($p=0.028$).

E. faecium predominated in both years, hence only one third of *Enterococcus* spp. were susceptible to ampicillin (Fig. 3C). In 2013, 17% of the *Enterococcus* spp., isolated were vancomycin-resistant enterococci (VRE). This reduced significantly in 2017, where no VRE were isolated ($p=0.001$). All the *Enterococcus* spp. in both study years were susceptible to linezolid.

Candida spp.

Table 3 summarises the different *Candida* spp. isolated in both the study years. *Candida albicans* was the predominant species isolated in both 2013 (64%) and 2017 (65%). *C. albicans* remained 100% susceptible to the 2 routinely tested azole antifungals, fluconazole and voriconazole, in both years. There were 2 significant changes in the species distribution in the study years: *C. parapsilosis* decreased from 7% in 2013 to 0% in 2017 ($p=0.030$), and *C. auris* increased from 0% in 2013 to 11% in 2017 ($p=0.002$). Overall susceptibility of non-albicans *Candida* spp. (NAC) to the azole antifungals remained under 50% for both years (Fig. 4). Micafungin and amphotericin B were 100% susceptible in 2017, but no comparison could be made with 2013 as these antifungals were not tested.

Table 3. Summary of the *Candida* spp. isolated (first-isolate results), 2013 versus 2017

	2013		2017		2013 vs. 2017
Organism	**Total *n* (%) isolates**	**95% CI**	**Total *n* (%) isolates**	**95% CI**	***p*-value***
C. albicans	54 (64)	54 - 72	48 (65)	53 - 75	1.000
Non-albicans *Candida* spp.	30 (36)	26 - 46	26 (35)	25 - 47	1.000
C. auris	0 (0)	0 - 5	8 (11)	2 - 20	**0.002**
C. glabrata	11 (13)	7 - 22	11 (15)	8 - 25	0.820
C. krusei	4 (5)	2 - 12	4 (5)	2 - 14	1.000
C. parapsilosis	6 (7)	3 - 15	0 (0)	0 - 6	**0.030**
C. tropicalis	3 (4)	1 - 10	2 (3)	0 - 10	1.000
Other *Candida* spp.	6 (7)	3 - 15	1 (1)	0 - 8	0.122
TOTAL CANDIDA	84 (14)	12 - 17	74 (11)	9 - 14	0.125

CI = confidence interval
*Statistically significant values in bold

ANALYSIS OF BLOODSTREAM ISOLATES

Table 4 summarises the first-isolate organisms from blood cultures. Of the gram-negative blood culture isolates, the *Enterobacteriaceae* predominated in both 2013 (62%) and 2017 (55%). All the members of the *Enterobacteriaceae* isolated each year were analysed together as a group to allow for sufficient numbers. *Klebsiella* spp., *E. coli* and Enterobacter spp. were the predominant species in both years. There was an overall decrease in ESBL-producing *Enterobacteriaceae* from 52% in 2013, to 32% in 2017 (p=0.032). Of all the antimicrobial agents tested, there was only a significant increase in the susceptibility of gentamicin, from 41% in 2013 to 63% in 2017 (p=0.013).

Table 4. Summary of bloodstream isolates (total and resistant), 2013 (n=178) versus 2017 (n=270)

	2013				2017				2013 *vs.* 2017
Organism	***n* isolates (% of total*)**	***n* isolates (% of group†)**	***n* (%) resistant isolates**	**95% CI**	***n* isolates (% of total*)**	***n* isolates (% of group†)**	***n* (%) resistant isolates**	**95% CI**	***p*-value‡**
Total *Enterobacteriaceae*§		56 (62)		51 - 71		77 (55)		47 - 63	0.343
ESBL			29 (52)	39 - 64			25 (32)	23 - 44	**0.032**
CRE			4 (7)	2 - 17			7 (9)	4 - 18	0.760
Total non-fermentative GNB¶		35 (38)		29 - 49		63 (45)		37 -53	0.343
MDR			10 (29)	16 - 45			4 (9)	3 - 21	**0.005**
XDR			10 (29)	16 - 45			17 (37)	24 - 51	1.000
Burkholderia spp.		1 (3)		0 - 16		15 (24)		15 - 36	**0.009**
TOTAL GNB	91 (51)			44 - 58	140 (52)			46 - 58	0.923
Total *Staphylococcus* spp.		44 (66)		54 - 76		86 (78)		70 - 85	0.080
S. aureus		8 (12)		6 - 22		20 (18)		12 - 27	0.653
MRSA			3 (38)	13 - 70			2 (2)	0 - 7	0.335
Coagulase negative staphylococcus		36 (54)		42 - 65		66 (60)		51 - 69	0.653
MR CoNS			30 (83)	68 - 93			41 (62)	29 - 47	**0.040**
Total *Enterococcus* spp.		23 (34)		24 - 46		24 (22)		15 - 30	0.080
VRE			2 (9)	1 - 28			0 (0)	0 - 4	0.234
TOTAL GPC	67 (38)			31 - 45	110 (41)			35 - 47	0.554
C. albicans		11 (58)		36 - 77		4 (20)		7 - 42	**0.023**
Non-albicans *Candida* spp.ǀ		8 (42)		23 - 64		16 (80)		58 - 93	**0.023**
TOTAL CANDIDA	19 (11)			7 - 16	20 (7)			5 - 11	0.234

CI = confidence interval, ESBL = extended-spectrum beta-lactamase, CRE = carbapenem-resistant *Enterobacteriaceae*, GNB = gram-negative bacteria, MDR = multidrug-resistant, XDR = extensively drug-resistant, MRSA = methicillin-resistant *S. aureus*, MR CoNS = methicillin-resistant coagulase-negative staphylococcus, VRE = vanvomycin-resistant enterococcus, GPC = gram-positive cocci
*Total *n* 2013 = 178, total *n* 2017 = 270
†Groups are: Total gram-negative bacteria, Total gram-positive cocci, and Total Candida
‡Statistically significant values in bold
§2013: *Klebsiella* spp. (*n*=25), *E. coli* (*n*=18), *E. cloacae* (*n*=5), *Citrobacter* spp. (*n*=2), *S. marcescens* (*n*=2), *M. morganii* (*n*=1), *P. mirabilis* (*n*=1), *P. stuartii* (*n*=1), *Salmonella* spp. (*n*=1); 2017: *Klebsiella* spp. (*n*=33), *E. coli* (*n*=21), *E. cloacae* (*n*=15), *M. morganii* (*n*=3), *Proteus* spp. (*n*=2), *C. freundii* (*n*=1), *P. agglomerans* (*n*=1), *Salmonella* spp. (*n*=1)
¶2013: *A. baumannii* (*n*=19), *Pseudomonas* spp. (*n*=13), *S. maltophilia* (*n*=2), *B. cepacia* (*n*=1); 2017: *Acinetobacter* spp. (*n*=26), *Pseudomonas* spp. (*n*=18), *S. maltophilia* (*n*=3), *Burkholderia* spp. (*n*=14), *Alkaligenes* spp. (*n*=1)
ǀ2013: *C. albicans* (*n*=11), *C. glabrata* (*n*=5), *C. parapsilosis* (*n*=2), *C. kefyr* (*n*=1); 2017: *C. glabrata* (*n*=9), *C. albicans* (*n*=4), *C. auris* (*n*=5), *C. krusei* (*n*=2)

All the non-fermentative gram-negative bacteria isolated each year were analysed together as a group to allow for sufficient numbers. In 2013, 29% of the non-fermenters were MDR compared with 9% in 2017 (p=0.005), however there was a non-significant increase in the percentage of XDR non-fermenters from 29% in 2013, to 37% in 2017. A significant decrease in the susceptibility of tobramycin, from 54% in 2013 to 19% in 2017 (p=0.005) was noted and there was a significant increase in number of *Burkholderia* spp. from 3% in 2013 to 24% in 2017 (p=0.009).

Of the gram-positive bacteria isolated from blood culture, CoNS predominated in both 2013 (54%) and 2017 (60%). There was a significant reduction in the number of methicillin-resistant CoNS, from 83% in 2013 to 62% in 2017 (p=0.040).

Of all the *Candida* spp. isolated from blood cultures in 2013, *C. albicans* was the predominant species isolated (58%), however in 2017, the NAC predominated (80%) and this difference was statistically significant (p=0.023). In keeping with this change, there was a significant reduction in the overall susceptibility of the bloodstream *Candida* spp. to fluconazole, from 63% in 2013 to 20% in 2017 (p=0.01). There was a significant reduction in the overall susceptibility of the bloodstream *Candida* spp. to fluconazole, from 63% in 2013 to 20% in 2017 (p=0.01).

Discussion

This is the first study describing the cumulative antibiogram results for the CMJAH multidisciplinary adult ICU and HCU. The findings from this study provide important epidemiological information. The pertinent findings from this study include the predominance of *Enterobacteriaceae* in 2013 and 2017. Of concern, was the overall increase in CRE, XDR *A. baumannii* and *Burkholderia* spp. The significant reduction in both MRSA and VRE is noteworthy. Amongst the *Candida* spp. isolated, the emergence of multidrug-resistant *C. auris* and a predominance of NAC in bloodstream isolates in 2017 reflects current global epidemiology.[19]

The ESKAPE pathogens (*E. faecium, S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa*, and *Enterobacter* spp.) represent the 6 most common multidrug-resistant pathogens threatening patients within ICUs globally.[20] These ESKAPE pathogens were also prevalent bacterial isolates in the CMJAH ICU/HCU. The predominance of *Enterobacteriaceae* in this unit is similar to the findings of the global EPIC II study.[1] The decrease in percentage of ESBL-producing *Enterobacteriaceae* was coupled with a significant increase in percentage of CRE from 4% to 11%, a finding that could not be corroborated with other studies. The majority of the CPE (65%) were *Klebsiella* spp, and the predominant carbapenamase was $bla_{OXA\text{-}48}$ and its variants (81%), which is in keeping with published national surveillance data from South Africa.[21,22] The number of $bla_{OXA\text{-}48}$ isolates may be an underestimate of the true prevalence in this unit, as $bla_{OXA\text{-}48}$ isolates could have appeared susceptible to carbapenems on routine AST and would therefore not have been sent for genotyping.[21]

Also in keeping with published data, *Klebsiella* spp. were the most frequent gram-negative bacteria isolated in both years.[22] Despite the overall ESBL decrease, *Klebsiella* spp. remained the predominant ESBL-producing *Enterobacteriaceae* in both years. The reasons for the increase in susceptibility of 3 antimicrobials in 2017, amoxicillin-clavulanic acid, cefepime, and trimethoprim-sulfamethoxazole, is not known. The CLSI cefepime breakpoints were lowered between 2013 and 2017, so one would have expected an increase in cefepime resistance over the study years.[15,16] Imipenem and meropenem had the highest susceptibilities (97-98%) of all the antimicrobial agents tested in both years. Ertapenem still had high susceptibility rates (93%) and would therefore be the most appropriate choice for empiric treatment of *Enterobacteriaceae* in cases of suspected nosocomial infection. For patients in septic shock with a suspected CRE infection, combination empiric therapy is associated with improved survival.[23] The addition of amikacin would be recommended, as amongst the CRE in 2017 specifically, 83% (19/23) of the isolates were susceptible to amikacin.

The overall prevalence of non-fermentative gram-negative bacteria did not differ between the study years. There was however, a significant increase in *Burkholderia* spp. over the 2 years, with most isolates (71%) being recovered from blood culture. This trend has also been seen elsewhere in ICU patients without cystic fibrosis.[24] There was a surprising decrease in the overall percentage of *Acinetobacter* spp. These bacteria can be found in the natural environment as well as occurring as commensals of the skin and secretions of patients. The true prevalence of infection caused by *Acinetobacter* spp. is difficult to assess as there are no guidelines to assist in differentiating between isolates that cause infection versus colonisation.[25] The increasing resistance of *Acinetobacter* spp. from 2013 to 2017, was evidenced by the doubling of XDR isolates in 2017. This increasing resistance is also in keeping with local and international published data.[22,25]

Susceptibility patterns of *Pseudomonas* spp. remained stable over the 2 study years, with a significant decrease only in the susceptibility of piperacillin/tazobactam, to under 80%, making this agent inappropriate for the empiric therapy of nosocomial *Pseudomonas* spp. infections. The carbapenems were the least susceptible antimicrobials tested for *Pseudomonas* spp. Amikacin had the highest susceptibility (94%) of all the antimicrobial agents tested in 2017, and would therefore be the agent of choice for empiric treatment of suspected nosocomial pseudomonal infections.

Susceptibility of *Acinetobacter* spp. to the carbapenems was very low (<20%), but remained constant, and susceptibility was highest to tigecycline in 2017. For the non-fermenters on blood cultures, there was a very high non-susceptibility rate to the panel of antimicrobials tested and in 2017, the most susceptible agent was ceftazidime, at 62%. Empiric therapy with meropenem or imipenem will not provide adequate cover for either *Acinetobacter* spp. or *Pseudomonas* spp. For *Acinetobacter* spp. infections assessed as being clinically significant, optimal therapy will likely require the use of colistin for patients with septic shock. Reliable colistin AST was however not available in the study years.[22] New AST methods for colistin (broth microdilution) have been implemented in the laboratory, and colistin AST results would need to be studied going forward.

Amongst the gram-positive isolates, *Staphylococcus* spp. predominated in both study years. CoNS are normal skin commensals and are frequently isolated from clinical specimens. Determining whether an isolate of CoNS represents a true infection or colonisation is very difficult, and there are no simple criteria with sufficient specificity to assist with this decision.[26] On a positive note, the proportion of MRSA and VRE isolates decreased significantly. A similar decline in MRSA was also reported in another South African surveillance study.[22,27] CoNS are commonly implicated in catheter-related bloodstream infections and this unit treats these infections by removal of the infected catheter, without the use of vancomycin. Empiric use of vancomycin is therefore infrequent in this unit and this may have played a role in the decreased rates of MRSA and VRE. In 2017, all of the gram-positive cocci isolated were 99-100% susceptible to vancomycin and linezolid, making these agents appropriate for empiric therapy where gram-positive organisms are suspected to be significant.

Although *C. albicans* remained the predominant yeast isolated overall, in both 2013 (64%) and 2017 (65%), it could have been considered a commensal in many cultures (e.g. the respiratory tract, urine, and skin). There was a concerning increase in the percentage of multi-drug resistant *C. auris* in 2017, from 0% to 17%. This is consistent with the dramatic increase of *C. auris* elsewhere in South Africa and globally, over the past 4 years.[19] *Candida* spp. made up the minority of blood culture isolates in both years. However, as evidenced by numerous studies, blood cultures have a low sensitivity, despite being the gold standard for the definitive diagnosis of candidaemia.[28] *C. albicans* was the predominant species isolated (58%) from blood cultures in 2013, however, of concern was that NAC predominated (80%) in 2017, which is in keeping with the global epidemiological shift of *Candida* spp.[19] As a result of this shift in epidemiology, the azoles are no longer the agents of choice for empiric therapy of *Candida* spp. infections. Susceptibilities to micafungin and amphotericin B were 100% in 2017. These antifungals should be considered the agents of choice for empiric antifungal therapy in patients at high risk for invasive candidiasis, depending on the local availability and guideline recommendation.

There were several limitations to this study. Firstly, because of the retrospective nature of the data analysis, real-time changes in AST or emergence of resistance are not reflected. Secondly, clinical data were not available to distinguish between hospital-acquired and community-acquired infections, and true infection versus colonisation. Thirdly, in order to reduce the bias that may be present in an all-isolates approach, a first-isolate approach was used, as recommended by CLSI. This approach may however underestimate the resistance rate of nosocomial infections. Fourthly, the laboratory changed automated AST methods from Microscan in 2013 to Vitek 2 in 2017, and this could have affected AST results. In addition, this study was not able to analyse or report on colistin AST as new testing methods were recommended in 2017 by CLSI and the European Committee on Antimicrobial Susceptibility Testing (EUCAST), and these had not yet been routinely implemented by the microbiology laboratory during the study years. Lastly, tigecycline, amphotericin B and micafungin were not tested in 2013, so no comparison could be made for these antimicrobials.

This study provides the necessary/required cumulative antibiogram data for the CMJAH adult ICU and HCU, which can be used by the clinicians to guide their empiric selection of antimicrobial therapy. The current NHLS LIS was not primarily designed as a research or surveillance tool,[29] so computer software limitations and data extraction methods need to be further refined in order to make the provision of annual cumulative antibiograms an achievable task by the clinical microbiology laboratory.

Conclusion

Management of infections in ICU patients is an evolving challenge because of the ever-present threat of resistant isolates. The appropriate selection of empiric antibiotic therapy should be guided by ICU-specific antibiograms. Based on this unit's antibiogram, empiric antimicrobial therapy should always cover the *Enterobacteriaceae*, and the agent of choice would be ertapenem. Amikacin is recommended for empiric treatment of suspected pseudomonal infections. Additional empiric antimicrobial therapy for the gram-positive organisms is not routinely advocated, as the majority of isolates in this study were CoNS. In 2017, most *S. aureus* isolated were methicillin-susceptible. Ertapenem is active against methicillin-sensitive *S. aureus* and will offer coverage in the setting of empiric use. There was a low incidence of culture-proven candidaemia in this study, therefore empiric antifungal therapy with amphotericin B or micafungin would only be appropriate in patients at high risk for invasive candidiasis with accompanying clinical signs suggestive of such infections.

In order to adequately implement AMS as a tool to combat antimicrobial resistance in ICUs nationally, further prospective multi-centred epidemiological studies are needed at multidisciplinary ICUs across South Africa.

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