Aminoglycoside Resistance in Clinical Isolates of Gram Negative Bacilli at the University Hospital of the West Indies, Jamaica: Comparison of Two Time Periods

G Reynolds-Campbell, A Nicholson, N Christian, R Hardie, J Cook

ABSTRACT

Objective: *Aminoglycosides were introduced into use over 60 years ago. The University Hospital of the West Indies (UHWI), a tertiary care teaching hospital, in Kingston, Jamaica, introduced the use of gentamicin in 1973 and amikacin in 1980. This report examined the susceptibility patterns to these agents in 1547 consecutive isolates of Gram negative bacilli (GNB) encountered between September 1 and November 30, 2011, at UHWI and compares the data with that observed previously in 1981 at the same institution.*

Methods*: The Vitek 2 (bioMeriéux, Durham, NC) was used for isolate identification, minimum inhibitory concentration determination and aminoglycoside susceptibility testing. Quality control was done using American Type Culture Collection standard strains of* E coli *(ATCC 25922) and* Pseudomonas aeruginosa *(ATCC 27853).*

Results: *Of the 1547 organisms, 267 had resistance to one or both aminoglycosides. Amikacin resistance increased from 0.6% (1981) to 7.2% [2011] ($p < 0.05$), while gentamicin resistance increased from 6.7% to 14.8% ($p < 0.05$) for the corresponding period. The majority of samples with aminoglycoside resistant organisms came from the intensive care unit and surgical inpatients. Urine samples persistently produced the largest amount of gentamicin resistant isolates.*

Conclusions: *Although there has been a statistically significant rise in aminoglycoside resistance, aminoglycosides continue to remain highly effective against approximately 83% of GNB despite continuous usage at this institution for over three decades. Continued national surveillance, implementation of infection control policies and antibiotic stewardship are all essential in retaining low resistance levels.*

Keywords: Amikacin, aminoglycosides, drug resistance, gentamicin

Resistencia a los Aminoglucósidos en Aislados Clínicos de Bacilos Gram-negativos en el Hospital Universitario de West Indies, Jamaica: Comparación de Dos Períodos de Tiempo

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RESUMEN

Objetivo: *El uso de los aminoglucósidos se introdujo hace más de 60 años. El Hospital Universitario de West Indies (HUWI), un hospital docente de atención terciaria, en Kingston, Jamaica, introdujo el uso de la gentamicina en 1973 y de la amicacina en 1980. Este informe examina los patrones de susceptibilidad a estos agentes en 1547 aislados consecutivos de bacilos gram-negativos (BGN) encontrados entre el 1 de septiembre y el 30 de noviembre de 2011, en el HUWI, y compara los datos con los observados previamente en 1981 en la misma institución.*

Métodos: *El sistema Vitek 2 (bioMeriéux, Durham, NC) fue utilizado para la identificación del aislado, la determinación de la concentración mínima inhibitoria (CIM), y las pruebas de susceptibilidad de los aminoglucósidos. El control de calidad se realizó con cepas estándar de* E coli *(ATCC 25922) y* Pseudomonas aeruginosa *(ATCC 27853) de la compañía American Type Culture Collection.*

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DOI: 10.7727/wimj.2014.040

Resultados: *De los 1547 organismos, 267 presentaban resistencia a uno o ambos aminoglucósidos. La resistencia a la amikacina aumentó de 0.6% (1981) a 7.2% [2011]* (p < 0.05), *mientras que la resistencia a la gentamicina aumentó de 6.7% a 14.8% (*p < 0.05) *para el período correspondiente. La mayoría de las muestras con microorganismos resistentes a los aminoglucósidos procedían de la unidad de cuidados intensivos y pacientes quirúrgicos hospitalizados. Las muestras de orina consistentemente produjeron la mayor cantidad de aislados resistentes a la gentamicina.*

Conclusiones: *Aunque ha habido un aumento estadísticamente significativo de la resistencia a los aminoglucósidos, éstos continúan siendo altamente eficaces contra aproximadamente el 83% de los BGN, a pesar de su uso continuo por más de tres décadas en la institución referida. La vigilancia nacional continua, la implementación de políticas de control de la infección, así como la administración cuidadosa y responsable de los antibióticos son esenciales para retener niveles de resistencia bajos.*

Palabras claves: Amikacina, aminoglucósidos, resistencia a los medicamentos, gentamicina

West Indian Med J 2015; 64 (2): 88

INTRODUCTION

Aminoglycosides are produced naturally by the Actinobacteria, order Actinomycetales which includes the genera Streptomyces and Micromonaspora. They are often used in synergistic combination with other antimicrobial agents and provide a valuable therapeutic option for treating multidrug resistant bacterial infections. β-lactam or glycopeptide antibiotics (cell wall active agents) are commonly used with aminoglycosides as they facilitate entry into the bacterial cytoplasmic space (1, 2).

Aminoglycosides offer a broad spectrum of antibacterial activity covering both Gram positive and Gram negative organisms. Some of the members in this group including amikacin, gentamicin and tobramycin are also antipseudomonal. Amikacin has activity against *Nocardia*, rapid growing mycobacteria and is also reserved for gentamicin resistant Gram negative bacilli [GNB] (2). Coverage of anaerobes is negligible as they lack the specific respiration dependent transport mechanism for the uptake of these antibiotics (3).

The 30S ribosomal subunits associated with cell membranes are the target of the aminoglycosides (2). Binding to the aminoacyl site (A-site) of the 30S subunit, they trigger acceleration in uptake and intracellular accumulation of the antibiotic, leading to impaired protein synthesis (4). This prevents proper formation of the initiation complex of peptide synthesis (binding of mRNA, fMet tRNA, and the 50S subunit association). Aminoglycosides also act by impairing proof-reading of the mRNA, altering the elongation of the nascent chain. The aberrant chains formed, when inserted into the cell membrane, lead to altered permeability and further stimulation of aminoglycoside uptake (2, 5).

Aminoglycoside antibiotics have been in use for more than 60 years and various mechanisms of bacterial resistance have been recognized in these organisms which are shared by clonal or horizontal transfer to varying species (6). This problem led to the development of successive agents in this group to combat resistant strains of organisms. Gentamicin has been in use at the University Hospital of the West Indies (UHWI) since 1973 whilst amikacin, which was introduced in the 1980s, is usually reserved for serious infections or those due to gentamicin resistant strains. Two previous studies by Bodonaik *et al* (7, 8) reported aminoglycoside resistance over a 20-year period. The aim of this study is to re-examine the prevalence of resistance to amikacin and gentamicin at the UHWI, assessing the trends in resistance since 1981 in two time periods, so as to allow for more informed decisions on the empiric use of these agents in the severely ill patient. Rising resistance levels and novel resistance mechanisms occurring worldwide dictate this need.

SUBJECTS AND METHODS

A total of 1547 consecutive GNB isolates from clinical samples were collected from the laboratory of the Department of Microbiology, The University of the West Indies, Mona, between September 1 and November 30, 2011. The samples were from both inpatients and outpatients across all the hospital services and their sources included urine, sputum, wound swabs and blood cultures. The Vitek 2 (bioMeriéux, Durham, NC) was used for identification, minimum inhibitory concentration (MIC) determination/antibiotic susceptibility testing. The results were interpreted according to Clinical Laboratory Standards Institute (CLSI) criteria (9). American Type Culture Collection (ATCC) standard strains *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were used as quality controls. Resistance patterns were compared to published data from 1981. This study received ethical approval from the University Hospital of the West Indies/University of the West Indies/Faculty of Medical Sciences Ethics Committee. Samples were used anonymously therefore consent was not necessary. There was no risk to patients or study investigators as universal precautions and appropriate laboratory procedures were adhered to.

RESULTS

Of the 1547 isolates of GNB collected over the three-month period, 267 showed resistance to one or more aminoglycoside. A total of 229/1547 (14.8%) of the GNB were resistant to gentamicin, the most commonly used aminoglycoside at the

UHWI, while 113/1547 (7.3%) showed resistance to amikacin (Table 1). Seventy-five of these 113 GNB were also resistant to gentamicin (Table 2). This is a significant increase when compared to gentamicin (6.7%) and amikacin (0.6%) resistance rates in 1981 (Table 1). Of note, all six of the amikacin resistant isolates in the 1981 study were also resistant to gentamicin. For isolates resistant to both antibiotics, this rate increased from 8.6% in 1981 to 28.1% in 2011 [$p \leq 0.05$] (Table 2).

Acinetobacter spp represented 9.3% of the isolates and showed 27.6% resistance to gentamicin. Of the 29 *Providencia* sp isolated, 16 (55.2%) showed gentamicin resistance, whilst *E coli,* which accounted for 24.0% of the study population, showed a 15.6% resistance to gentamicin. *Pseudomonas aeruginosa*, a common GNB pathogen, had gentamicin resistance rates of 5.1%, while the 1981 data showed rates of 18.5% (Fig. 1).

Table 1: Gentamicin and amikacin resistance among Gram negative bacilli at the University Hospital of the West Indies (1981 and 2011)

	Total		Number (%) resistant to:					
			Gentamicin			Amikacin		
Organisms	**1981**	**2011**	**1981**	**2011**	***p*-values**	**1981**	**2011**	***p-v*alues**
Escherichia coli	249	366	1 (0.4)	57 (15.6)	0.0001	0 (0)	39 (10.7)	0.0001
Klebsiella pneumoniae	226	205	9 (3.9)	35 (17.1)	0.0001	0 (0)	15 (7.3)	0.0001
Enterobacter sp	116	128	5 (4.3)	8 (6.3)	0.5768	0 (0)	6 (4.7)	0.5246
Proteus mirabilis	82	112	2 (2.4)	14 (12.5)	0.0154	0 (0)	3 (2.7)	0.1902
Proteus sp	47	13	2 (4.2)	2 (15.4)	0.0001	0 (0)	2 (15.4)	0.0630
Pseudomonas aeruginosa	178	238	33 (18.5)	12 (5.1)	0.0001	2 (1.1)	3 (1.3)	0.6340
Providencia sp	15	29	2 (13.3)	16 (55.2)	0.0077	2 (13.3)	0 (0)	0.1110
Serratia	22	36	8 (36.6)	2 (5.6)	0.0042	0 (0)	1 (2.8)	0.6207
Citrobacter sp	29	49	3 (10.3)	1 (2.0)	0.1422	0 (0)	1 (2.0)	0.6282
Acinetobacter sp	49	145	2 (4.0)	40 (27.6)	0.0002	2 (4.0)	10 (6.9)	0.3764
Others	17	226	3 (17.7)	42 (18.6)	0.6117	0 (0)	33 (14.6)	0.0763
All isolates	**1030**	**1547**	**70 (6.7)**	**229 (14.8)**	**0.0001**	**6 (0.6)**	**113 (7.3)**	**0.0001**

Table 2: Comparison of resistance to gentamicin, amikacin and both aminoglycosides among isolates collected in 1981 and 2011 using 2 × 2 contingency tables for calculation of p-values

Total number (total number resistant to aminoglycosides)		**Number (%) resistant to**					
		Gentamicin only		**Amikacin only**		**Both gentamicin and amikacin**	
1981	**2011**	**1981**	**2011**	**1981**	**2011**	**1981**	**2011**
1030 (70)	1547 (267)	64 (91.4)	154 (57.7)	0 (0)	38 (14.2)	6 (8.6)	75 (28.1)
P-values		0.0001		0.0002		0.0002	

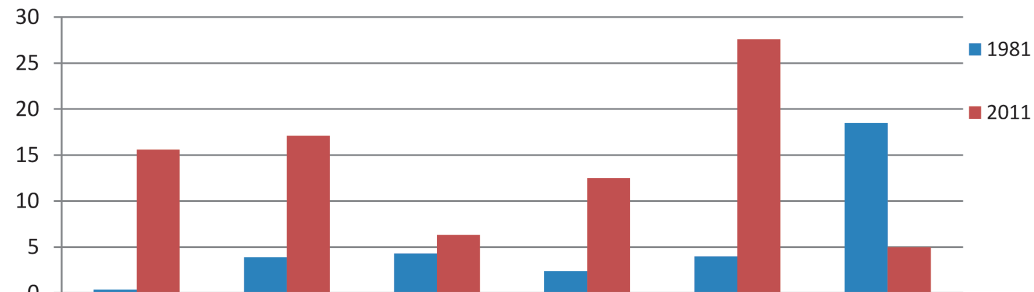
Table 3: Origin of gentamicin resistant isolates in the various clinical service areas of the University Hospital of the West Indies

Category	**Number (%) of total**		**Clinical service area/number (%) of category**											
			Surgery		**Medicine**		***Obs & Gynae**		**Paediatrics**		****ICU**		**Others**	
	1981	**2011**	**1981**	**2011**	**1981**	**2011**	**1981**	**2011**	**1981**	**2011**	**1981**	**2011**	**1981**	**2011**
Inpatients	64 (91.4)	150 (65.5)	39 (60.9)	56 (37.3)	5 (7.8)	23 (15.3)	5 (7.8)	3 (2.0)	3 (4.7)	14 (9.3)	10 (15.6)	42 (28)	2 (3.2)	12 (8.0)
Outpatients	6 (8.6)	79 (34.5)	4 (66.6)	2 (2.5)	1 (6.7)	0 (0)	0 (0)	0 (0)	1 (16.7)	2 (2.5)	0 (0)	0 (0)	0 (0)	75 (95.0)
Total	**70 (100)**	**229 (100)**	**43 (61.4)**	**58 (25.3)**	**6 (8.6)**	**23 (10.0)**	**5 (7.1)**	**3 (1.3)**	**4 (5.8)**	**16 (7.0)**	**10 (14.3)**	**42 (18.3)**	**2 (2.8)**	**87 (38.0)**

*Obstetrics and gynaecology; **Intensive care unit

Urine samples were the source of the majority (48.0%) of gentamicin resistant strains, followed by wounds/burn site swabs (24.0%) and sputum, 12.7% (Fig. 2). The highest percentage of aminoglycoside resistant GNB was found amongst the surgical (37.3%) and ICU (28.0%) inpatients followed by the medicine service, which showed a marked increase in the number of inpatients with gentamicin resistant organisms moving from 7.8% in 1981 to 15.3% in 2011 (Table 3).

compared to rates in India (71.0%), Greece (77.7%) and the United Kingdom (99.2%), especially in their *Pseudomonas* spp populations (10–13). Gentamicin resistance rates at UHWI in *Pseudomonas aeruginosa* showed a decline of 13.4% over the two study periods. While the cause of this decline was not a part of the current study, the increased availability and use of other anti-pseudomonal agents at the hospital might have played a role in this trend.



	E.coli	*K. pneumoniae*	*Enterobacter spp.*	*Proteus mirabilis*	*Acinetobacter spp.*	*Pseudomonas aeruginosa*
1981	*0.4*	*3.9*	*4.3*	*2.4*	*4*	*18.5*
2011	*15.6*	*17.1*	*6.3*	*12.5*	*27.6*	*5*

Fig. 1: Changes in the percentage of gentamicin resistance for 1981 and 2011.

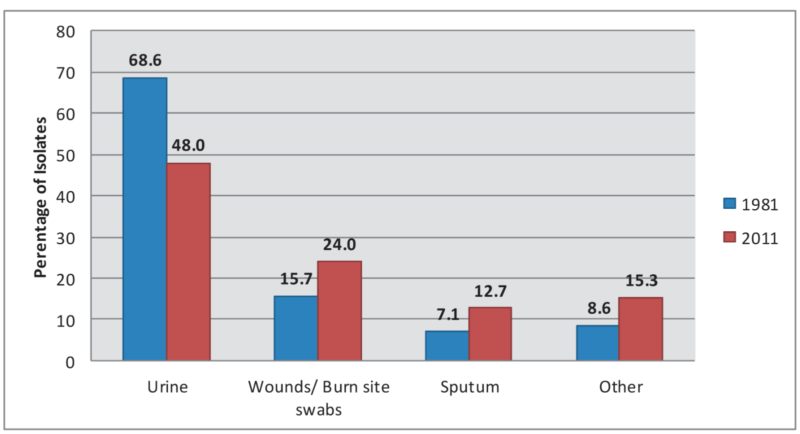


Fig. 2: Clinical sources of the gentamicin resistant strains.

DISCUSSION

Resistance to the commonly used aminoglycosides, gentamicin and amikacin, has risen by 10.5% over the past 30 years at the UHWI in Kingston, Jamaica. Gentamicin is the aminoglycoside of first choice at the UHWI, and resistance to this agent rose from 6.7% to 14.8% ($p < 0.0001$). While this increase is cause for concern, this level of resistance is low when

Gentamicin use at the UHWI has continued since its inception in the 1970s, even with the introduction of other classes of antimicrobials such as the carbapenems and glycopeptides. Its synergistic capabilities with other antibiotics has been relied upon heavily to provide therapy for most cases of sepsis, empiric or proven. Amikacin use is usually reserved for gentamicin resistant infections, which may play a role in the relatively lower rates of amikacin resistance seen at the UHWI. This practice must be enforced as a significant increase in amikacin resistance (by 6.7%) was noted in this study. Organisms such as *E coli* and *Proteus,* which in 1981 showed no amikacin resistance at the UHWI, now have rates of 10.7% and 15.4%, respectively. In comparison, GNB resistance rates to amikacin may be as high as 49.7% in Turkey, 50.0% in Greece and 55.1% in *Pseudomonas* spp in India (14–17). The UHWI prevalence data suggest that although the rise in aminoglycoside resistance has been slow over a 30-year period, there is a need to follow the policies for judicious use of these agents to maintain their efficacy in the hospital.

Surgical and intensive care unit inpatients had the highest rates (37.3% and 28.0%, respectively) of gentamicin resistant isolates from the 2011 data. Patients from these services are more likely to have surgical wound site infections and to be catheterized and bedridden for protracted periods, increasing the chances of developing nosocomial GNB infections involving the urinary and respiratory tracts. This was further highlighted by the fact that GNB from urine provided the majority of gentamicin resistant isolates for the study (48.0%), with *E coli* being the most frequently isolated organism. This was followed by isolates originating from wound/burn swabs (24.0%).

The transfer of genes responsible for antibiotic resistance between species of bacteria proves to be a major area of concern, as studies have shown that without the administration of the right empiric agent within 48 hours of presumed sepsis, there can be an increase in the mortality rate by up to three times (18). Inevitably, this will cause a deleterious impact on patients from both a clinical and economic standpoint as there are increases in length of hospital stay and use of already limited resources (19). It is therefore imperative that protocols be put in place to limit unnecessary use of antibiotics by first identifying true infections, conducting active surveillance, implementing eradication procedures for these multidrug resistant-GNB, and to enforce all antibiotic stewardship and infection control procedures within institutions (20).

Despite continuous usage for over three decades at the UHWI, aminoglycosides have retained their usefulness, as approximately 83.0% of GNB still remain susceptible to gentamicin and amikacin in this hospital. The study underlines the importance of periodic surveillance of antibiotic resistance and the application of data thus generated, in the management of infections in hospitals.

ACKNOWLEDGEMENTS

The authors thank Dr Nabin Bodonaik for his assistance in review of his previous publication from 1983 that contributed to the intellectual content of this study.

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