

CONSUMPTION AND BACTERIAL RESISTANCE TO AMINOGLYCOSIDES AT SUDANESE UNIVERSITY HOSPITAL

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ABSTRACT

Objectives: The objective of this study was to describe patterns of antimicrobial resistance to gentamicin (Gen) and amikacin (Ak) among Gram-negative aerobic bacteria during 1-year period and to determine the association between antibiotic resistance and the consumption of Gen.

Methods: Aminoglycosides consumption at Soba University Hospital wards was measured and susceptibility of Gram-negative bacteria for the same period was evaluated. Consumption data were converted to defined daily doses (DDDs)/100 bed days based on DDD/anatomical therapeutic chemical the WHO system. The association between the frequency of strains resistant to Gen and Ak and their consumption was assessed by linear regression analysis using Spearman's correlation. The level of statistical significance was set at $p < 0.05$.

Results: A total of 973 Gram-negative isolates were identified and tested for antimicrobial susceptibility to Gen and Ak. Resistance to Gen alone was found to be 19.42%; $n=189$, resistance to Ak alone was found to be 3.08%; $n=30$, and resistance to Gen plus Ak was found to be 5.24%; $n=51$. *Pseudomonas aeruginosa* was the most resistant pathogen to Ak plus Gen (2.26%; $n=22$). A positive correlation between the increases in the use of Gen and the prevalence of bacterial resistance among hospital wards was found (correlation coefficient $r=0.6$; $p=0.04$).

Conclusion: Gen and Ak are still highly active antimicrobial agents for the treatment of aerobic Gram-negative bacteria at times of intensified resistance to other antimicrobial agents. Monitoring the use of aminoglycosides is very important too.

Keywords: Aminoglycosides, Resistance, Gram-negative bacteria, Hospital Prescribing, Sudan.

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INTRODUCTION

After seven decades in clinical use, aminoglycoside antibiotics still remain crucial drugs for the treatment of acute infections and specific conditions such as tuberculosis. They are especially effective in the containment of *Pseudomonas* bacteria in patients with cystic fibrosis, regardless of the harmful side effects that are being gradually solved by extensive research around the world. The development of bacterial resistance to aminoglycosides during therapy is rare [1], and the prevalence of resistance to aminoglycoside remained relatively low compared to cephalosporins or fluoroquinolones [2].

The most predominant resistance mechanism is through aminoglycoside-modifying enzymes. Resistance may occur when bacteria produce 16S rRNA methylases, which cause high level and broad-spectrum aminoglycoside resistance [3]. Other resistance mechanisms do exist, which can act simultaneously in the same cell [4]. These include reduced permeability by modification of outer membrane or diminished inner membrane transport [5], export outside the cell by activation of efflux pumps [6,7], adaptive resistance [8], active swarming, and a non-specific mechanism newly shown in *Pseudomonas aeruginosa* cells specially against gentamicin (Gen), which revealed adaptive antibiotic resistance against numerous antibiotics [9] and sequestration of the drug by tight binding to an altered aminoglycoside acetyltransferase [10].

Aminoglycoside-modifying enzymes catalyze the modification at -OH or -NH₂ groups of the 2-deoxystreptamine nucleus or the sugar moieties and can be acetyltransferases, nucleotidyltransferases, or phosphotransferases [11,12].

The DDD is known as the average standard daily dose of a drug used in an adult assuming 70 KGs as average weight [13]. DDD has been used extensively to compare frequencies of antimicrobial use between wards, hospitals, and even countries. A small number of studies have used the DDD methodology in children with justification [14-17].

The use of the adult Anatomical Therapeutic Chemical (ATC)/DDD methodology to compare pediatric to adult antibiotic prescribing is misleading because it does not take into account either age or weight of the child. Accordingly, the total DDD from an adult ward would be much higher than that for a neonatal ward, which makes straight judgments very challenging between hospitals or wards containing such problem. When the use of antibiotic in adult ward is negligible, and the same antibiotic is extensively used in pediatric wards such problem will not affect the significance of consumption variation. The practice mentioned above is because pediatricians are very adherent to sepsis management guidelines, which is the most prevalent disease among pediatric and neonate patients in our institution, while other practitioners always find the way to skip aminoglycosides for another option even if the culture results are positive. That is because of low knowledge about aminoglycosides, their efficacy, low resistance rate and finally the ghost of nephrotoxicity and ototoxicity.

In the last years, several studies were carried out to determine the relationship between consumption of antimicrobial agents and the development of bacterial resistance and build a hypothesis of a positive correlation. In a study, which investigated the development of resistance in Gram-negative bacteria and evaluated its association with consumption of various groups of antibiotics, a statistically significant

correlation between increase of bacterial resistance and consumption of aminoglycosides, fluoroquinolones, and cephalosporins was reported [18]. A positive correlation between increase of resistance among isolates of *Klebsiella pneumoniae* and consumption of the third-generation cephalosporins was also reported [19]. These and other studies documented that high consumption of antibiotic and high resistance rates are equivalent [20-22].

However, regardless, the recommendations of the above-mentioned study [18], resistance of *Enterobacteriaceae* to the third- and fourth-generation cephalosporins and fluoroquinolones increased even with the lower consumption of these antibiotics. This clarifies that correlation between the consumption of these two antibiotic groups and the bacterial resistance was not confirmed and considered as the rationale of the present study.

The objective of this study was to describe patterns of antimicrobial resistance to Gen and amikacin (Ak) among Gram-negative isolates during 1 year. Another objective was to determine if there was any association between antibiotic resistance and the use of Gen.

METHODS

Ethical consideration

The present study was conducted after the approval of the Soba Centre for Audit and Research in Khartoum, Sudan (RIB Number: 10122015).

The present study was conducted at Soba University Hospital (SUH) of Khartoum University, Sudan, a 500-bed health-care center providing tertiary care for patients in Khartoum, the surrounding areas and the other states of Sudan.

Pharmacy and inventory data were collected for the entire year of 2016 for only parenteral aminoglycosides (Gen and Ak) in grams as DDD/100 beds for each ward that uses aminoglycosides for therapeutic purpose only (medicine, surgery, intensive care unit [ICU], high dependency unit [HDU], obstetrics and gynecology, pediatric medicine, pediatric surgery, pediatric ICU, nursery ICU, and kwash). Raw data were entered into a spreadsheet (Microsoft Excel) and converted into DDD/100 beds using the WHO ATC system to identify antibiotics and their DDD.

The DDD is based on the average daily dose used for the main indication of the drug. The following ATC subgroups were used (an example of a reference antibiotic is given in parentheses): J01GB and aminoglycosides (Gen and Ak). The DDDs for the aminoglycosides are based on the use in severe infections.

Gram-negative microorganisms isolated from blood and urine received at the microbiology laboratory at SUH during the period of January–December 2016, were included in the study.

Specimens were inoculated on the appropriate culture media according to standard bacteriological techniques. Identification of significant bacterial growth was done using Gram staining and standard biochemical tests [23].

Antibiotic susceptibility testing was performed using Kirby-Bauer disk diffusion technique, and zone diameters were read and interpreted as per CLSI recommendations as in Table 1 [24].

Suspension of the standard control strains (*Escherichia coli*, ATCC 25922 and *P. aeruginosa*, ATCC 27853) was prepared and processed the same as the tested organisms so as to assess the validity of antibiotics,

media, and other variables [24]. A total of 973 isolates of Gram-negative bacteria were used in the analysis including *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *Proteus vulgaris*, and *Coliform*.

Statistical analysis

The participant's responses were encoded, and the data were analyzed using Statistical Package for the Social Sciences (SPSS, version 20.0, Chicago, IL, US).

Descriptive analysis was used to calculate the proportion of resistance to Gen, Ak, and Gen+Ak for each isolated strains during the study period. Linear regression analysis using Spearman's correlation was used to assess the relationship between the frequency of strains resistant to Gen and Gen consumption in the different wards under investigation. The level $p < 0.05$ was considered as the cutoff value for significance.

RESULTS

During the period between January 1 and December 31, 2016, the records of 973 isolates that were identified and tested for antimicrobial susceptibility to Gen and Ak were included in this study. A total of 703 of these isolates were tested using both Gen and Ak disks, and the rest 265 isolates were tested using Ak disks only.

The results showed that *K. pneumoniae* was the most commonly identified organism (n=432; 44.4%), followed by *P. aeruginosa* (n=281; 28.9%), *E. coli* (n = 247; 25.3%), *Coliform* (n=7; 0.7%), and *Proteus* (n=6; 0.6). Resistance to both Gen and Ak for the same isolate among the whole isolates was 5.24%; n=51.

Table 2 summarizes the types of isolates and their resistance to Ak, Gen, or AK+Gen. *P. aeruginosa* is the most resistant to both Ak+Gen (n=22) and Ak only (n=10), *K. pneumoniae* has the higher resistance to Gen only (n=94).

Table 3 summarizes the resistance rates to Ak and Gen in SUH ward, in which the most resistance to Gen+Ak (n=17) and Ak only (n=8) was found in pediatric medicine ward. The most Gen only resistance rates were found in nursery ICU ward (n=44). A positive correlation between the increases in the use of Gen and the prevalence of bacterial resistance was found ($r=0.6$; $p=0.04$) (Table 4).

DISCUSSION

It seems from the present study that aminoglycosides are not used in our setting as frequently as β -lactam antibiotics. This is probably due to aminoglycosides toxicity and the fact that therapeutic monitoring is not available at Soba University Hospital. Nevertheless, where aminoglycosides are used in excess such as pediatric wards, the prevalence of aminoglycoside resistance was far below that of β -lactams.

The findings were consistent with Harbarth *et al.* [25], who reported low level of aminoglycoside resistance and found no significant change in overall Gen or Ak resistance among aerobic Gram-negative bacteria. The mentioned study concluded that aminoglycosides were still very useful drugs in the treatment of most cases of Gram-negative sepsis as well as other infections [2,26]. Low incidence of aminoglycoside resistance among *E. coli* and *P. aeruginosa* was also reported by Shimizu *et al.* [27], who related their finding to the low consumption of aminoglycosides. More recent studies also confirmed these findings [28], and they related it to decreased aminoglycosides consumption which increased susceptibility of *E. coli*, and *P. aeruginosa* to Gen, as well as increased susceptibility of *P. aeruginosa* to Ak [28]. The findings in this study also

Table 1: Interpretation of gentamicin and amikacin sensitivity results

Antimicrobial Agent	Disc Content/ μ g	Susceptible/mm	Intermediate/mm	Resistant/mm
Gentamicin	10	≥ 15	13-14	≤ 12
Amikacin	30	≥ 17	15-16	≤ 14

Table 2: Resistance of gentamicin, amikacin, and gentamicin+amikacin to each isolate during 2016 at Soba University Hospital

Bacteria	n (%)				Total
	Resistance to amikacin	Resistance to gentamicin	Resistance to amikacin+gentamicin	Sensitivity	
<i>Klebsiella</i>	15 (3.4)	94 (21.7)	21 (4.8)	302 (69.9)	432
<i>Pseudomonas</i>	10 (3.5)	53 (18.8)	22 (7.8)	196 (69.7)	281
<i>Escherichia coli</i>	3 (1.2)	42 (17)	8 (3.2)	194 (78.5)	247
<i>Coliform</i>	1 (14.2)	0 (0)	0 (0)	6 (85.7)	7
<i>Proteus</i>	1 (16.6)	0 (0)	0 (0)	5 (83.3)	6
Total	30 (3.08)	189 (19.42)	51 (5.24)	703 (72.25)	973

Table 3: Resistance to Gen, Ak, and Gen+Ak for each isolate during 2016 at Soba University Hospital wards

Departments	Resistance				Total
	Ak	Gen	Ak+Gen	Sensitivity	
HDU	1	7	0	42	50
ICU	0	5	3	47	55
Kwash	4	20	1	41	66
Medicine	3	10	3	57	73
Nephrology	2	8	4	35	49
NICU	4	44	4	71	123
OP	2	33	6	162	203
PICU	0	12	1	44	57
PM	8	31	17	108	164
PS	5	13	10	57	85
Surgery	0	2	1	20	23
Urology	1	4	1	18	24
OBs	0	0	0	1	1
Total	30	189	51	703	973

HDU: High dependency unit, ICU: Intensive care unit, Kwash: Kwashiorkor ward, NICU: Nursery intensive care unit, OP: Outpatients, PICU: Pediatric intensive care unit, PM: Pediatric medicine ward, PS: Pediatric surgery ward, OBs: Obstetrics and gynecology ward, Gen: Gentamicin, Ak: Amikacin

Table 4: Evaluation of Gen consumption and resistance among the wards at Soba University Hospital during 2016

Ward	Number of beds ward	Number of Gen 0.08 g consumed/year	DDD/100 beds	Resistance to Gen+Ak/Gen	Resistance to Gen+Ak/Gen %
HDU	5	30	0.78278	7	14
ICU	8	57	0.81336	8	15
Kwash	29	310	1.39461	21	32
Medicine	90	135	0.16116	13	18
NICU	27	430	1.45442	48	39
OBs	0	0	0.05708	0	0
PICU	4	60	1.36986	13	23
PM	42	650	1.41335	48	27.57
PS	32	290	1.27327	23	29
Surgery	70	105	0.15221	3	13

Correlation coefficient $r=0.7$; $P=0.02$. HDU: High dependency unit, ICU: Intensive care unit, Kwash: Kwashiorkor ward, NICU: Nursery intensive care unit, PICU: Pediatric intensive care unit, PM: Pediatric medicine ward, PS: Pediatric surgery ward, OBs: Obstetrics and gynecology ward, Gen: Gentamicin, Ak: Amikacin, DDD: Defined daily dose

confirmed that higher susceptibility rates that can be maintained and even increased by reduction of consumption and monitoring of use as well. Additional studies attributed the decreased rates of resistance to Ak in some Gram-negative bacteria to decreased consumption of this antimicrobial, while no correlation was found between the decreased consumption and decreased resistance rates to Gen [29]. This partial agreement may be related to lack of aminoglycoside consumption and antimicrobial resistance studies in Gram-negative bacteria as reported by the researchers. However, the investigators concluded that decreased rates of resistance to Ak and tobramycin in some of the Gram-negative bacteria studied were associated with decreased consumption of those antimicrobials.

Furthermore, another supportive study found a positive correlation between consumption and resistance to Gen in *K. pneumoniae* and *E. coli* and Ak in *E. coli*, while no relationship was found between consumption of the third- and fourth-generation cephalosporins and

resistance to ceftazidime or between fluoroquinolone consumption and resistance to ciprofloxacin [21]. These results may suggest that the relationship between resistance and antibiotic administration cannot be affected by only the decrease in consumption, but it is most likely determined by additional factors such as horizontal clonal spread of the identical multiresistant isolates and recombination processes such as conjugation of bacterial plasmids [21].

In Sudan, high resistance rates to other antimicrobials, especially cephalosporins, were reported. These antimicrobials were extensively used because they are highly promoted and easily accessible [30,31]. Furthermore, an Indian study of prevalence and antimicrobial susceptibility patterns of bacterial isolates reported the sensitivity of Gram-negative bacteria to Gen (90.20%) and Ak (89.14%), while the resistance of Gram-negative bacteria to cefuroxime (75%) and cefazolin (79.25%) [32]. These high resistance rates were most probably due to extended-spectrum beta-lactamases and metallo-beta-lactamases [33].

Therefore, it is suggested that low consumption of aminoglycosides must be the explanation for the low resistance rates. It is worth noting that aminoglycosides are not well promoted in Sudan, and their side effects make them unpopular to health-care professionals. Unavailability of monitoring blood level tests also worsens the condition even more.

In this study, a great variation in Gen and Ak consumption among adults and pediatrics wards was identified. During a whole year, only one patient in obstetrics and gynecology ward received Gen, while excessive use in NICU and Pediatric Medicine was reported, taking into account that one Gen ampoule can be shared between many pediatric patients. This practice did not affect our statistical analysis and DDD/100 beds calculation, because the DDD/100 beds for Gen for pediatric wards remained higher than adult wards due to the great variation in consumption between adult and pediatric wards.

The study has several limitations. Firstly, the parameter of defined daily dose (DDD) has been used in paediatric patients, which makes straight judgments very challenging between hospitals, or wards since accurate comparison between adult dosing and paediatric dosing patterns cannot be made. Secondly, there were no consumption data for Ak because it is not supplied as a free lifesaving drug from the government and the patient has to buy it from outside the hospital, so correlation between consumption and resistance was done for Gen only. Thirdly, the absence of previous similar studies does not provide a comparison scale. In addition, the lack of organization in the documentation system within the institutions and the short study period of this research, all act as limitations. Lastly, the small number of wards (only 10 reported) using aminoglycosides for therapeutic purposes leads the correlation to be quite sensitive when sample size is taken into consideration.

CONCLUSION

This study suggests that the decreasing incidence of Gen resistance in some Gram-negative bacteria was associated with decreased consumption levels of this antimicrobial agent. The results of this study also suggest that the practice of monitoring the use and decreasing the consumption of aminoglycosides is important in hospitals that have a high prevalence of aminoglycoside resistance. Further studies involving larger patient numbers and more hospitals in Sudan are also recommended to provide a wider view on the status of resistance to aminoglycosides in the whole

country. Taking the above findings together, aminoglycosides remain effective and affordable antibacterial drugs for patients and can substitute for expensive and newer drugs such as carbapenems, especially in developing countries.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS' CONTRIBUTIONS

All of the authors contributed significantly in study conception and design, drafting manuscript, and critical revision. However, acquisition of data was one of the major roles of Mrs. Bakheit.

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