HIGH-LEVEL GENTAMICIN RESISTANCE AMONG CLINICAL ISOLATES IN A NIGERIAN TEACHING HOSPITAL

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ABSTRACT

High-level resistance to gentamicin (HLGR) is scarcely reported in hospitals in Nigeria. This study was therefore carried out to determine the prevalence of HLGR among clinical isolates in a Nigerian University Teaching Hospital.
Assay for resistance to gentamicin was carried out in Mueller Hinton agar (Oxoid) plates using the single concentration agar diffusion method while, HLGR strain was detected in agar plates containing ≥500µg/mL of gentamicin.
A total of 207 (25.5%) of the 811 clinical isolates were resistant to gentamicin at 10µg/mL. Isolates that were resistant to gentamicin at concentration ≥500µg/mL were reported as being high-level gentamicin resistant. In all 66(8.1%) were classified as HLGR strains. The highest prevalence of HLGR was detected among Pseudomonas aeruginosa 24(16.3%), followed by Klebsiella pneumoniae 10(13.7%), Enterococcus faecalis 6(11.5%) and Enterococcus faecium 3(7.5%). The prevalence rates of HLGR were comparable ($p>0.05$) among gram positive and gram negative bacteria. Three levels of HLGR gentamicin at 500µg/mL, 1000µg/mL and 2000µg/mL were associated with 34(4.2%), 23(2.8%), 9(1.1%) of the isolates respectively.
The findings have highlighted a high prevalence of HLGR strains among clinical isolates at tertiary care hospital in Nigeria. Hence, a regular monitoring of post-therapeutic serum levels of gentamicin should be given due consideration by physicians, and there is need for a national drug usage policy to control the use of antimicrobials in Nigeria and indeed other developing countries to reduce spread of resistant strains.

Keywords: High-Level Gentamicin Resistance, clinical isolates, Nigeria

Introduction

Resistance to antimicrobial agents among clinical isolates is a serious therapeutic problem (1,2,3). Inspite of its known ototoxic (4) and nephrotoxic (5) potentials, gentamicin has continued to top the list of antimicrobial agents used in Nigeria for the management of serious bacterial infections (Dr. Udonwa – personal communication). This choice is predicated by its broad spectrum action against many gram positive and gram, negative bacteria at concentrations between 0.3-8.0µg/ml (6). Resistance to gentamicin has been reported in all parts of the world and the mechanism of resistance is not related to a single factor (7,8,9). However, the most important mechanism is the acquisition of a transferable resistant factor which encodes the production of aminoglycoside in-activating enzymes (8,10) with six of them capable of inactivating gentamicin (11).

Though high-level resistance to gentamicin (HLGR) is well documented in many parts of the world (12,13,14) there are very scarce reports in hospitals in Nigeria. This study was therefore undertaken to provide data on high level gentamicin resistance among clinical isolates in a Nigerian University Teaching Hospital.

MATERIALS AND METHODS

Bacterial Strains

A total of 811 bacterial strains isolated from routine clinical specimens in the Microbiology Laboratory of the University of Calabar Teaching Hospital (UCTH) were used for the study. The isolates stratified into 504 gram negative and 307 gram positive bacteria were either aerobic or facultative anaerobes. The isolates were obtained from burn wounds (152), post operative wounds (219), midstream and catheter specimens of urine (225), blood cultures (60), genital swabs (23) and ear swabs (102). The bacterial strains were isolated using standard procedures and characterized to specie level using the API 20E-system (Biomereux, France). They were maintained on nutrient agar slants at room temperature until the susceptibility tests were carried out. The study was conducted between 2006 and 2007.

Assay for Resistance to Gentamicin

Mueller Hinton agar (Oxoid) plates were prepared on each day of the assay to contain 10µg of Gentamicin per mL. The plates were allowed to dry by exposure in a 37°C incubator for 30-45 minutes. A standard inoculum of each of the bacterial species was prepared to contain $1x10^5$ CFU/mL using the method described by Cheesbrough (15). Each plate was divided into 6 sectors and a loopful of over night broth culture of each organism containing 10^5 CFU/ml was inoculated on each sector. The plates were inoculated at 35°C aerobically for 24 hours after which they were examined for growth on each segment. Any bacterial strain that produced at

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least one colony was recorded as being resistant to gentamicin

Assay for High-Level Gentamicin Resistance

Bacterial species resistant to 10µg of gentamicin were further assayed for high-level resistance by the organisms. Mueller Hinton agar plates were prepared to contain 500µg, 1000µg and 2000µg of gentamicin each. The plates were prepared fresh on each day of the analysis. The inoculum and application of the inocula were carried out as described earlier (15). The inoculated plates were incubated at 35°C for 24 – 48 hours. Bacterial strains that produced colonies on these plates were categorized as high-level resistant strains.

Results

Table 1 shows the sources of the samples, the bacterial species isolated and the number of isolates that were resistant to 10µg/ml gentamicin. The highest incidence of resistant strains were found among *Pseudomonas aeruginosa* 53(36.1%), *Klebsiella pneumoniae* 19(26.0%), *Enterococcus faecalis* 11(21.2%) and *Enterococcus faecium* 8(20.0%). In all, 207 (25.0%) of all the bacterial isolates examined were resistant to gentamicin.

In table 2 is presented the distribution of high-level gentamicin resistant bacterial isolates encountered during the study. Of the 811 bacterial strains examined, 34(4.2%) were resistant to gentamicin at 500µg concentration, 23(2.8%) at 1000µg and 9(1.1%) at 2000µg. The prevalence of HLGR strains among individual species shows that 24(16.3%) of the 147 isolates of *Pseudomonas aeruginosa* were HLGR strains, the distribution of HLGR strains among other bacterial species were *Klebsiella pseumoniae* 13.7%, *Enterococcus faecalis* 11.5%, *Enterococcus faecium* 7.5%, *Proteus mirabilis* 7.1%, *E. coli* 4.0%, *S. aureus* 4.0% and coagulase negative staphylococcus 4.6%.

A total of 48 of the 504(9.5%) gram negative bacteria tested were HLGR strains while, 18 of the 307(5.9%) gram positive bacteria tested were HLGR. There was no significant difference in the prevalence of HLGR among Gram-positive and Gram-negative strains ($p>0.05$).

Discussion

The problems of antimicrobial resistance particularly among hospital-acquired bacterial pathogens are enormous especially in the third world developing countries (2,3,16). In this study we report HLGR prevalence rate of 8.1 percent among clinical isolates of bacteria at the University of Calabar Teaching Hospital. This finding highlights the magnitude of therapeutic failures associated with the use of gentamicin in our centre. This high prevalence rate may be connected with usage of sub-optimal doses of gentamicin as routine monitoring of post-therapeutic antibiotic blood levels is not usually requested by physicians (17). In addition, it has been observed that a large number of patients are treated empirically by some physicians with stat dose of gentamicin (280mg). In this study, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* contributed to 24(16.3%) and 10(13.7%) of the HLGR strains detected. These two organisms are very often associated with various stages of burn wound infections (18) which are often treated with topical gentamicin preparations. It is therefore not surprising that these organisms top the list of HLGR strains in this study. The prevalence of HLGR strains among gram negative (9.5%) and gram positive (5.9%) organisms is comparable in this finding. Similar findings have also been reported in the study of Schmitz *et al.* (12) and Fugita *et al.* (18).

In another study, Wiland *et al.* (13) reported a high prevalence rate of HLGR Enterococci isolated from bacteremic patients. Similarly, Arellano *et al.* (19) reported 14.4% and 25% of HLGR *E. faecalis* and *E. faecium* respectively from patients in a tertiary care facility in Mexico. In this study, a prevalence of 11.5% and 7.5% of *Enterococcus faecalis* and *Enterococcus faecium* respectively has been reported. Enterococci is traditionally resistant to gentamicin as documented in this study (*E. faecium* 80.0% and *E. faecalis* 78.8% resistance respectively) at gentamicin level of 10µg/ml. Enterococcus has been cited as one important cause of hospital-acquired infections (19) hence, Gentamicin in a synergistic combination with a cell wall-active antibiotics (such as ampicillin and vancomycin) have remained the drug of choice in the treatment of enterococcal infections in humans, (20,21). However, a study in India had identified HLGR enterococci to gentamicin at 500µg concentration, and intermediate resistant to vancomycin at MIC 8 µg/mL (22). The study emphasized the reliability of MIC over agar-disk diffusion method in determining low level vancomycin resistance for reason of anticipated synergistic action of the combined therapy for serious infections like endocarditis, meningitis or possibly other serious infections in immunodeficient patients.

Conclusion

The findings in this study highlight a high prevalence of HLGR among clinical isolates in the University of Calabar Teaching Hospital, a tertiary care facility in Nigeria. These finding have a far reaching implications in the use of gentamicin in management of hospital and community-acquired infections caused by these organisms and in the spread of resistant strains. Therefore, regular monitoring of post-therapeutic serum levels of gentamicin should be given due consideration by physicians while, a large scale surveillance survey of HLGR strains in Nigeria is hereby advocated for future research. In addition, a national drug policy to control the indiscriminate use of gentamicin in Nigeria is advocated particularly, where alternative antimicrobials are indicated.

Table 1: Sources, bacterial isolates and no. (%) resistance to gentamicin (10µg)

Samples	No. organisms isolates from each sample source								Total No. isolated from each sample source (%)
	Ps. aurogi-nosa	*Esch. coli*	*Staph. aureus*	*Coag. -ve Staph*	*Kleb. pneumoniae*	*Prot. mirabilis*	*E. faecalis*	*E. faecium*	
Burns	51	43	24	-	19	25	11	9	182
Post operative wounds	45	57	63	-	21	18	8	7	219
Mid stream/catheter urine	14	65	11	51	20	21	25	18	225
Blood culture	-	19	23	6	-	6	5	1	60
Genital swabs	-	-	10	8	-	-	3	2	23
Ear swabs	37	15	19	-	13	15	-	3	102
Total No. of each bacterial Species isolated	147	199	150	65	73	85	52	40	811
Total No. of each bacterial species	53(36.1)	25(1.6)	7(11.3)	7(10.8)	19(26.0)	13(15.7)	41(78.8)	32(80.0)	207(25.5)

Table 2: Prevalence of high-level gentamicin resistant clinical isolates at UCTH, Calabar

Bacterial isolates		No. (%) resistant to Gentamicin at the following concentrations				*No. (%) of HLGR*
		10µg	500µg	1000µg	2000µg	
Pseudomonas aeruginosa (n=147)		53(36.1)	11	8	5	24(16.3)
Escherichia coli (n=199)		25(12.6)	6	3	-	8(4.0)
Staphylococcus aureus (n=150)		17(11.3)	3	2	1	6(4.0)
Coagulase negative Staph. (n=65)		7(10.8)	2	1	-	3(4.6)
Klebsiella pneumoniae (n=73)		19(26.0)	5	3	2	10(13.7)
Proteus mirabilis (n=85)		13(15.7)	3	2	1	6(7.1)
Enterococcus faecalis (n=52)		41(78.8)	3	3	0	6(11.5)
Enterococcus faecium (n=40)		32(80.0)	2	1	0	3(7.5)
Total (%)	811	207(18.9)	34(4.2)	23(2.8)	9(1.1)	66(8.1)

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