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Cross-resistance between some aminoglycoside antibiotics

J.A. ONAOLAPO

Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharm. Sciences, A.B.U., Zaria.

Abstract

The cross-resistance between the aminoglycoside antibiotics, gentamycin, tobramycin, kanamycin and streptomycin was tested using clinical isolates of *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* obtained from Ahmadu Bello University Teaching Hospital Zaria, Nigeria (ABUTH).

The antibiotics can be classified into two groups: the kanamycin and streptomycin group on one hand to which isolates easily acquired resistance, and the amikacin, gentamycin and tobramycin group on the other to which the isolates did not easily develop resistance. There seems not to be any hard and fast rule that can be applied to the cross-resistance between these aminoglycoside antibiotics. The isolates varied markedly in their pattern of cross-resistance towards the different antibiotics.

Resume

Les antibiotiques peuvent se classer en 2 groupes, a savoir le groupe de la kanamycine et de la streptomycurin d'une part, groupe coutre lequel les isolants acquierent facilement de la resistance, at le groupe de l' amikacine, de la gentamycine et de la tobramycine, d' autre part. Contre ce dermier groups, les isolates n' acquierent pas facilement de resistance. Il ne semble pas y avoir de regles strictes qui puissent s' appliquer a la resistance croises entre ces antibiotiques aminoglycoside. Les isolates ont varie de mainere remarquable dans leur structure de la resistance croisee a l' egard des divers antibiotiques.

Introduction

Cross-resistance is a phenomenon in which a bacterium which has developed resistance to an agent shows a corresponding increase in resistance to other agents chemically related to the initial agent[1]. Cross-resistance can arise as a result of therapeutic selection, transduction and episomal transferance by conjugation[2].

The phenomenon of cross-resistance is important in clinical chemotherapy because it can reduce the therapeutic value of many groups of antibiotics like the penicillins, tetracyclines and the aminoglycosides. This becomes even more serious when cross resistance occurs with chemically unrelated antibiotics.

The aim of this report is to find the pattern of cross-resistance between amikacin and tobramycin which are not in use in Nigeria and the commonly used gentamycin, kanamycin and streptomycin. This will help to determine whether organisms which are resistant to any of the commonly used aminoglycoside antibiotics in ABUTH will become resistant to the members of this group which are not in use in this Hospital, thus giving a useful guide in the prescription of this group of antibiotics especially in life threatening infections.

Materials and methods

Bacterial Species

The organisms used were *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus,* all of which were obtained as isolates from surgical and medical wards of ABUTH. The isolates were purified by streaking on nutrient agar. Pure colonies so obtained were identified by streaking on MacConkey agar, 0.03% cetrimide agar and mannitol salt agar to select *E. coli, Ps. aeruginosa* and *Staphid aureus* respectively. Pure cultures were stored on nutrient agar slopes at 4S.

Antibiotic (discs and powders)

10μg discs of amikacin, gentamycin, tobramycin and streptomycin and 30μg discs of kanamycin were obtained from Oxoid Ltd. (U.K.). Amikacin and Kanamycin in pure powders from Bristol Myers Laboratory (U.K.); gentamycin and streptomycin powders from Chemical Industrial Dev. Co. Cairo (Egypt); and Tobramycin powder from Eli-Lily (Italia).

Media

Mueller Hinton Agar (MHA) and Nutrient Broth and agar (Difco) were used.

Resistance Studies

Fifty isolates each of *E. coli; Ps. aeruginosa* and *Staph. aureus* were tested for sensitivity to amikacin, gentamycin, tobramycin, kanamycin and streptomycin

using the disc sensitivity method of Bauer *et al.* 1966[3]. Ten isolates each, found to be sensitive to all the tested aminoglycoside antibiotics were selected. In addition, those isolates which were naturally resistant to streptomycin and kanamycin were also selected by the sensitivity method[3].

The sensitive isolates were exposed separately to gradient concentrations of amikacin, gentamycin, tobramycin, streptomycin and kanamycin until they became resistant. This was achieved by pouring 20mls of melted and cooled MHA at 48°C containing standard antibiotic solution into a petridish in a tilted position so that one end of the dish was just covered. After this layer had solidified, another aliquot of the same volume as before but without antibiotic solution was poured on the top of the first layer while the plate was standing level. The plates were incubated at 37°C for 18 hrs to ensure complete upward diffusion of the antibiotics resulting in a uniform concentration gradient.

In order to isolate resistant mutants, a fairly heavy suspension of the test organism (*E. coli, Ps. aeruginosa* and *Staph. aureus*) were prepared from 24hr culture. Three streaks each were made on different plates each containing a different concentration of each antibiotic. The plates were incubated at 37°C for 72 hrs. After incubation, one or more of isolated colonies were transferred into nutrient broth and incubated at 37°C for a long enough period until growth was obtained. The cultures thus obtained were then restreaked on freshly prepared gradient agar plates, the concentration of the antibiotic in the plates being increased as permitted by the increased tolerance of the organism.

After many transfers, pure resistant colonies each isolate were transferred to nutrient broth, a the minimum inhibitory concentrations (M.I.Cs) the antibiotics against the resistant isolates we determined using standard tube dilution method.

Cross resistance between the tested antibioti was also studied using the disc sensitivity t method of Bauer *et al.* [3]

Results

After the exposure of the isolates to the antibioti those mutants which had M.I.Cs about equal to greater than ten times that before exposure we taken to be resisant. The results were as presented Table 1. The increases in the M.I.Cs of the antibioti against the resistant isolates differ depending on t antibiotics and the isolates.

For streptomycin, the increase in its M.I. against resistant *E. Coli, Ps. aeruginosa* and *Sta aureus* was greater than 16, 17, and 160 fol respectively after only three transfers compared wi those of the sensitive isolates. The correspondi increase for kanamycin was more than 16, 9, and 1 folds after four transfers. For Amikacin, there we 64, 8.4 and 60 folds increase in its MIC against *coli, Ps. aeruginosa* and *Staph. aureus* respective after five transfers compared with sensitive cel whereas there were only 10, 12, 11 and 8, 10, 10 fo increases in the M.I.Cs of gentamycin and tobramyc respectively against these organisms after the san number of transfers compared with the sensitive cells.

Table 1: M.I.Cs in mcg/ml of amikacin, tobramycin, gentamycin, kanamycin and streptomycin against resistant isolates

Antibiotic	*E. coli*		*Ps. aeruginosa*		*Staph. aureus*	
	A	B	A	B	A	B
Amikacin (after 5 transfers)	0.1258	8.0	1.1875	10.0	0.03357	2.0
Tobramycin (after 5 transfers)	0.0629	7.0	1.0625	10.0	0.01678	1.65
Gentamycin (after 5 transfers)	0.9375	9.5	1.750	20	0.1875	2.0
Kanamycin (4 transfers)	6.250	> 100	26.5625	> 250	0.6250	> 100
Streptomycin (3 transfers)	5.625	> 100	6.250	> 100	0.625	> 100

A = Average of M.I.Cs of 10 sensitive isolates (mcg/ml) B = Average of M.I.Cs of 10 resistant isolates (mcg/ml)

The cross-resistance between these aminoglycoside antibiotics are also shown in Tables 2 and 3.

Table 2: Sensitivity of amikacin, gentamycin and tobramycin resistant isolates to some other aminoglycoside antibiotics expressed in percentages

Number of Isolates Tested	Amikacin		Gentamycin		Tobramycin		Kanamycin		Streptomycin	
	S	R	S	R	S	R	S	R	S	R
E. coli 10*	NT	NT	40	60	20	80	0	100	0	100
(10)	0	100	NT	NT	0	100	0	100	0	100
[10]	0	100	0	100	NT	NT	0	100	0	100
Ps. Aeruginosa 10*	NT	NT	0	100	0	100	0	100	0	100
(10)	0	100	NT	NT	0	100	0	100	0	100
[10]	0	100	0	100	NT	NT	0	100	0	100
Staph. aureus 10*	NT	NT	50	50	0	100	30	70	50	50
(10)	0	100	NT	NT	0	100	0	100	80	20
[10]	0	100	40	60	NT	NT	0	100	20	80

S = sensitive; R = resistant; NT = not tested
* = isolates trained to become resistant to amikacin
( ) = isolates made to become resistant to gentamycin
[ ] = isolates made to become resistant to tobramycin

Table 3: Sensitivity of kanamycin and streptomycin resistant isolates to the aminoglycoside antibiotics expressed in percentages

Number of Isolates Tested	Amikacin		Gentamycin		Tobramycin		Kanamycin		Streptomycin	
	S	R	S	R	S	R	S	R	S	R
E. coli										
17Δ	100	0	100	0	100	0	NT	NT	5.9	94.1
25▲	100	0	100	0	100	0	36	74	NT	NT
10[ ]	100	0	100	0	100	0	NT	NT	60	40
10[ ]	100	0	100	0	100	0	70	30	NT	NT
Ps. aeruginosa										
16Δ	100	0	100	0	100	0	NT	NT	31.3	68.7
100Δ	100	0	100	0	100	0	90	10	NT	NT
100[ ]	100	0	100	0	100	0	NT	NT	80	20
100■	100	0	100	0	100	0	80	20	NT	NT
Staph. aeureus										
2Δ	100	0	100	0	100	0	NT	NT	0	100
52▲	100	0	100	0	100	0	96.2	3.8	NT	NT
10[ ]	100	0	100	0	100	0	NT	NT	80	20
10■	100	0	100	0	100	0	80	20	NT	NT

S = sensitive; R = resistant
Δ = isolates which were naturally resistant to kanamycin
▲ isolates which were naturally resistant to streptomycin
[ ] = isolates made to become resistant to kanamycin
■ = isolates made to become resistant to streptomycin

Discussion

When the isolates were made to become resistant to the aminoglycoside antibiotics, the tested antibiotics can be classified into two groups. The first group consist of streptomycin and kanamycin, to which the isolates easily acquired resistance. Infact isolates which were naturally resistant to these antibiotics were isolated. The other group comprises the amikacin, gentamycin and tobramycin group to which resistant cells only appeared after a very long exposure to these antibiotics.

There seems not to be any hard and fast rule that can be applied to the cross-resistance between the aminoglycoside antibiotic reported here. The isolates vary markedly in their pattern of cross-resistance towards the different aminoglycoside antibiotics. With *Ps. aeruginosa*, there is complete cross-resistance between gentamycin, amikacin, and tobramycin, all the 10 isolates which acquired resistance to any of them became resistant to the other. There is however partial cross-resistance between gentamycin, amikacin or tobramycin on one hand and kanamycin or stretomycin on the other.

Using *E. coli* and *Staph. aureus*, there is a partial cross-resistance between gentamycin and the other four antibiotics. However a high percentage (60%) of both amikacin-resistant *E. coli* and tobramycin-resistant *Staph. aureus* also became resistant to gentamycin. There is no marked decrease in the sensitivity of kanamycin and streptomycin resistant isolates to amikacin, gentamycin and tobramycin. This is of significance in clinical therapy. Similar results have been reported in other parts of the world. It has been shown that there is a complete cross-resistant between amikacin and other aminoglycoside antibiotics[4]. Also *Staph. aureus* trained to grow in higher concentration of gentamycin was found to acquire cross-resistance to other aminoglycoside antibiotics[5,6].

This report has also shown that the cross-resistance of the isolates to kanamycin and streptomycin is incomplete, however majority of the isolates which acquired resistance to kanamycin in most cases with a few exception were also resistant to streptomycin. Similar results have also been reported[4].

A possible explanation of these results is that development of resistance may not be by elaboration of enzymes only, since most of the cross-resistance were unidirectional (partial). Other mechanisms like reduced penetration of the antibiotics into the bacterial cell or alteration of ribosomal site may play an important role. For example *Staph. aureus* and *E. coli* which acquired resistance to amikacin were still found to be sensitive to gentamycin, kanamycin and streptomycin.

The partial cross-resistance between kanamycin and streptomycin may be due to differences in their ribosomal sites of action.

In conclusion any amikacin, gentamycin and tobramycin resistant isolate may not respond to any member of this group of antibiotics, while both kanamycin and streptomycin resistant isolate may still respond to amikacin, gentamycin and tobramycin. Also organisms that are resistant to gentamycin will not likely respond to amikacin and tobramycin despite the fact that these antibiotics are not in use in Nigeria.

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References

1. Garrod LP, Lambert HP, O' Grady F, Waterworth PMC. Drug resistance in antibiotic and chemotherapy. 4th Edn. London Churchill Livingstone 1973, pp 257-272.
2. Franklin TJC. In: Hugo WB, Russell AD, eds. Pharmaceutical Microbiology. 4th Edn. London Blackwell Scientific Publications 1987 pp 203-225.
3. Bauer AW, Kirby WM, Sherris JC, Turch M. Anbitiotic susceptibility testing by a standardised single disc method. Amer. J. Clin. Path. 1966; 45: 493-497.
4. Price KE, Defuria MD, Pursiano TA. Amikacin and aminoglycoside with marked activity against antibiotic resistant clinical isolates. J. Inf. Dis. 1976; 134 suppl. 249-260.

5. Pittman MA, Stone HH, Kolb L. Use of gentamycin sulfate for pre-operative. Bowel sterilisation Antimicrob. Ag. Chem. 1964, 4: 160-163.

6. Lacey RW. Antibiotic resistance plasmids of *Staph. aureus* and their clinical importance. Bacteriol. 1975 Rev. 39: 1-32.

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