Poster number:
638
49th IDSA
Annual Meeting

Effect of Change in Aminoglycoside Usage on Aminoglycoside Resistance Patterns

C. Kosmidis, M. Giannopoulou, K. Tzanetou, A. Flountzi, A. Markogiannakis, D. Goukos, G. Petrikkos and G.L. Daikos
University of Athens Medical School, Athens, Greece

C. Kosmidis, MD
Research Laboratory of Infectious Diseases and Antimicrobial Chemotherapy, University of Athens Medical School, 17 Ag. Thoma Str, Athens 11526, Greece
Email: drkostasid@gmail.com
Tel: +302107462636
Fax: +302107462635

Abstract (modified)

Background: Aminoglycosides (AG) are important bactericidal antibiotics in an era of increasing resistance. During the last decade, we observed a change in AG prescribing patterns in our hospital. We investigated the effect of this change on AG susceptibility and the prevalence of AG resistance genes.
Methods: Enterobacteriaceae (EB) isolated from blood cultures from 1997 and 2006 were studied. Susceptibilities to streptomycin (S), spectinomycin (Sp), kanamycin (K), neomycin (Ne), gentamicin (G), tobramycin (T), netilmicin (N) and amikacin (A) were determined with the disk diffusion method. PCR was used to detect genes encoding AG modifying enzymes and methylases. Yearly AG consumption was recorded from the hospital pharmacy.
Results: For the years 1997 and 2006, AG consumption in Defined Daily Doses per 100 patient-days was: A, 3.2 and 3.1; G, 1.4 and 0.8; T, 1.2 and 0.6; N, 1.8 and 0.7; S, 1.1 and 0.3, respectively. AG resistance rates for 1997 and 2006, respectively, were: S, 45.5 and 56.1; Sp, 24.5 and 21.5; Ne 23.6 and 17.1; K, 26.4 and 25; G, 14.5 and 8.8; T, 16.4 and 18.4; N, 17.3 and 15.4; and A, 14.5 and 13.6. The percentage of AG pan-resistant strains was 77.8 and 28.6, respectively. AAC(6')-I+AAC(3)-I was most common, followed by AAC(6')-I. AAC(3)-II, AAC(6')-I+ANT(2")-I and ANT(2")-I were less common. AAC(6')-I+AAC(3)-I was the most common in 1997, whereas AAC(6')-I was the most common in 2006. In 90% of isolates carrying AAC(6')-I+AAC(3)-I, APH(3')-I was also present. ANT(3')-I and APH(3'')-Ib-APH(6)-Id were both responsible for S resistance. Genes encoding methylases were not found.
Conclusion: Reduced use of G, T and N resulted in a significant increase in G, but not T and N, susceptibility rates, as well as a significant reduction in AG pan-resistant EB. Decreased prevalence of AAC(3)-I explained these findings. Reduction in G use may preserve the usefulness of this agent against severe infections by multiresistant bacteria such as carbapenemase-producing EB.

Background

Aminoglycosides (AG) are important agents for the treatment of infections by Enterobacteriaceae (EB). In our region, carbapenemase-producing EB have become endemic, and AG are among the few available therapeutic options. In order to preserve their usefulness, it is crucial to understand the effect of AG usage patterns on AG resistance. Over the last decade, we observed a change in aminoglycoside usage patterns in our institution. We hypothesized that this may have affected resistance to AG and aimed to correlate changes in AG usage with changes in the prevalence of individual resistance mechanisms.

Methods

For the years 1997 and 2006, EB isolated from blood cultures in a tertiary-care hospital in Athens, Greece, were retrieved from the Microbiology Laboratory. One isolate per patient was used. *Serratia* strains were not included in the analysis of genes related to the GTNA phenotype, as they are known to carry the chromosomal *aac(6')-Ic* gene. Susceptibilities to streptomycin (S), spectinomycin (Sp), kanamycin (K), neomycin (Ne), gentamicin (G), tobramycin (T), netilmicin (N) and amikacin (A) were determined with disk diffusion testing. Isolates resistant to at least one of the AG were subjected to PCR for the presence of resistance genes according to phenotype. Genes encoding for 16S rRNA methylases were sought in isolates exhibiting AG pan-resistance. AG resistance genes, phenotypes and primers used are shown in **Table 1**. Primers were selected based on previous reports on the prevalence of AG resistance genes in our region (1,2). DNA extraction, PCR reaction and amplification conditions were as described previously (3).
Data on yearly consumption of G, T, N, A, and S from 1997 to 2006 were obtained from the hospital's pharmacy and recorded as Defined Daily Doses (DDDs) per 100 patient-days.

Methods

Table 1. Primers used in this study

Gene	Enzyme	Primer	Sequence	Reference	Phenotype
aacA$_4$	AAC(6)-Ib	aacA$_4$.F aacA$_4$.R	ATGACTGAGCATGACCTTGCG TTAGGCATCACTGCGTGTTCG	(4)	KTNA
aacA$_7$	AAC(6)-II	aacA$_7$-F aacA$_7$-R	ATGGATAGTTCGCCGCTCGT GAGGCGAATTCGGTGCATCC	(5)	KTNA
aacC$_1$	AAC(3)-Ia	aacC$_1$-F aacC$_1$-R	ATGGGCATCATTCGCACATGTAGG TTAGGTGGCGGTACTTGGGTC	(4)	G
aac(3)-II	AAC(3)-II	aac(3)-II-F aac(3)-II-R	TGAAACGCTGACGGAGCCTC GTCGAACAG GTAGCACTGAG	(6)	GTN
strA/strB	APH(3")-Ib- APH(6)-Id	StrA/strB-F StrA/strB-R	TATCTGCGATTGGACCCTCTG CATTGCTCATCATTTGATCGGCT	(7)	S
aphA1-Iab	APH(3')-I	aph(3)-I-F aph(3)-I-R	AAACGTCTTGCTCGAGGC CAAACCGTTATTCATTCGTGA	(8)	KNe
aadA	ANT(3")-I	aadA-F aadA-R	GTGGATGGCGGCCTGAAGCC AATGCCCAGTCGGCAGCG	(8)	SSp
aadB	ANT(2")-Ia	aadB-F aadB-R	ATGGACACAACGCAGGTCGC TTAGGCCGCATATCGCGACC	(4)	GTK
armA	ArmA	armA-F armA-R	CAAATGGATAAGAATGATGTT TTATTTCTGAAATCCACT	(9)	P
rmtB	RmtB	rmtB-F rmtB-R	ATGAACATCAACGATGCCCT CCTTCTGATTGGCTTATCCA	(10)	P

G: gentamicin, N: netilmicin, T: tobramycin, A: amikacin, K: kanamycin, Ne: neomycin, S: streptomycin, Sp: spectinomycin, P: pan-resistance

Results

Table 2. Aminoglycoside resistance rates by year of isolation

	1997 (n=110) % resistant	2006 (n=228) % resistant	Overall (n=338) % resistant	*p*	OR (95%CI)
Streptomycin	45.5	56.1	52.7	0.081	1.54 (0.97-2.43)
Spectinomycin	24.5	21.5	22.5	0.579	0.84 (0.49-1.44)
Neomycin	23.6	17.1	19.2	0.185	0.67 (0.38-1.17)
Kanamycin	26.4	25	25.4	0.791	0.93 (0.55-1.57)
Gentamicin	14.5	8.8	10.7	0.132	0.57 (0.28-1.14)
Tobramycin	16.4	18.4	17.8	0.761	1.15 (0.63-2.12)
Netilmicin	17.3	15.4	16	0.638	0.87 (0.47-1.60)
Amikacin	14.5	13.6	13.9	0.867	0.92 (0.48-1.77)

Results

AG consumption for the time period 1997-2006 is illustrated in **Figure 1.** A was the most frequently used agent and its use remained stable. The use of all other AG declined. Specifically, comparing 1997 and 2006, for G, T, N and S, there was a 57%, 50%, 39% and 27% decline in their use, respectively. Resistance rates to all AG tested are shown in **Table 2.** Twenty-five percent of EB isolated in 1997 could not be retrieved. Among EB resistant to at least one of the 4 clinically significant AG, resistance rates for 1997 and 2006 were: G, 88.9 and 47.6 (**p, 0.004**; OR, 0.11; 95% CI, 0.02-0.56); T, 100 and 100; N, 94.4 and 83.3 (p, 0.415; OR, 0.24; 95% CI, 0.03-2.59); and A, 88.9 and 73.8 (p, 0.308; OR, 0.35; 95% CI, 0.07-1.79), respectively.
Overall, there were 178 EB resistant to S or Sp, of which 102 (57.3%) were resistant only to S, whereas 76 (42.7%) were resistant to S and Sp. The ANT(3")-I mechanism was found in 110 (61.8%) and the APH(3")-Ib-APH(6)-Id mechanism in 78 (43.8%) isolates. Among isolates with the SSp phenotype, ANT(3")-I was detected in 41 (53.9%), both ANT(3')-I and APH(3")-Ib-APH(6)-Id were detected in 33 (43.4%) and neither gene in 2 isolates (2.6%). Among EB resistant to S but susceptible to Sp, APH(3")-Ib-APH(6)-Id was detected in 22 (21.6%), ANT(3")-I alone in 13 (12.7%), both genes in 23 (22.5%) and neither gene in 44 (43.1%). APH(3')-I was present in 22% of isolates in 1997 and 17% of isolates in 2006. This gene was present in 90% of isolates carrying the combination AAC(3)-I and AAC(6')-I (see below).
For isolates resistant to at least one of the 4 main AG (AG-resistant isolates), the resistance phenotypes were: for 1997, GTNA (14), TN (1), GTN (1), TNA (1), GTA (1) and for 2006, TNA (18), GTNA (12), GTN (5), GT (3), T (3), TA (1). The most common mechanism was AAC(6')-I (81.7%), followed by AAC(3)-I (40%), AAC(3)-II (5%) and ANT(2")-I (5%). The prevalence of AG resistance mechanisms for each year of the study is shown in **Figure 2**. The species and the number of isolates associated with each mechanism were: AAC(6')-I and AAC(3)-I : *K. pneumoniae* (12), *P. mirabilis* (8), *E. aerogenes* (2), *E. coli* (2); AAC(6')-I: *K. pneumoniae* (15), *E. coli* (2), *P. mirabilis* (1), *E. cloacae* (1), *K. oxytoca* (1); AAC(3)-II: *E. coli* (2), *K. pneumoniae* (1); AAC(6')-I and ANT(2")-I: *E. coli* (1) and *E. cloacae* (1); ANT(2")-I: *P. mirabilis* (1); AAC(6')-I+?: *K. pneumoniae* (2), *E. aerogenes* (1); unknown mechanism: *E. coli* (4), *K. pneumoniae* (1), *P. mirabilis* (1), *E. cloacae* (1). No methylase genes were found. For 1997 and 2006, 77.8% and 28.6% of AG-resistant isolates were resistant to all 4 AG, respectively.

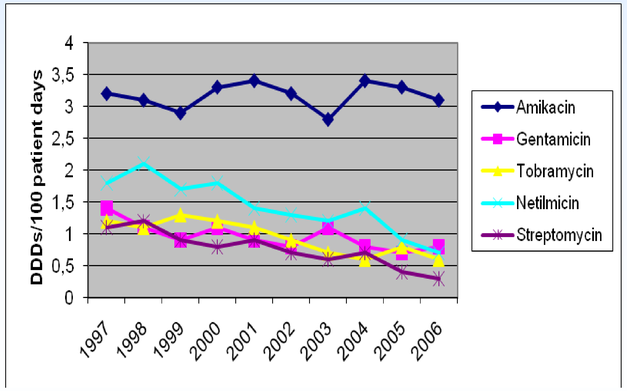

Figure 1. Aminoglycoside consumption during the study years

Results

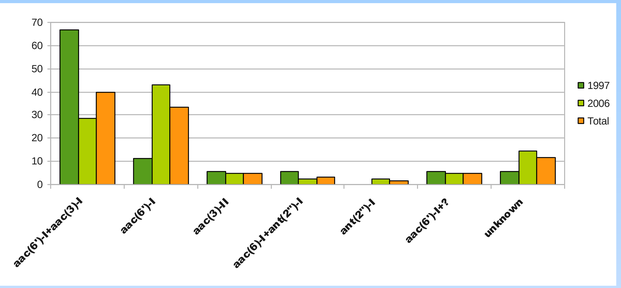

Figure 2. Prevalence of resistance mechanisms

Conclusion

- Reduction in G use during the study period resulted in a reduction in G resistance rates, most prominent among AG-resistant EB.
- Reduction in N and T use did not result in reduced N and T resistance because of persistence of the AAC(6')-I mechanism.
- Following reduction in G use, AAC(6')-I became the dominant mechanism, replacing AAC(6')-I+AAC(3)-I.
- A significant reduction in AG-pan-resistant EB was seen.
- Despite decreasing S use, S resistance rate increased, likely because of carriage of ANT(3'')-I gene in integrons.
- Reduction in G use may preserve the usefulness of this agent against severe infections by multiresistant bacteria such as carbapenemase-producing EB.

References

1) Miller GH et al, J Chemother 1995;7 Suppl 2:31-44
2) Miller GH et al, Clin Infect Dis 1197;24 Suppl 1:46-62
3) Daikos GL et al, Antimicrob Agents Chemother 2007;51:2366-72
4) Hujer KM et al, Antimicrob Agents Chemother 2006;50:4114-4123
5) Garza-Ramos U et al, Antimicrob Agents Chemother 2008;52:2943-6
6) Sandvang D and Aarestrup FM, Microb Drug Resist 2000;6:19-27
7) Sunde M and Norstrom M, J Antimicrob Chemother 2005;56:87-90
8) Gebreyes WA and Thakur S, Antimicrob Agents Chemother 2005;49:503-11
9) Galimand M et al, Antimicrob Agents Chemother 2003;47:2565-71
10) Yan JJ et al, J Antimicrob Chemother 2004;54:1007-12