SURVEYING AMINOGLYCOSIDE-RESISTANCE MECHANISMS: A TOOL FOR THE DEVELOPMENT OF NEOGLYCOSIDES

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INTRODUCTION AND PURPOSE

Achaogen is pursuing the development of next-generation aminoglycosides (AGs), termed neoglycosides. Existing AGs show potent bactericidal activity against a wide spectrum of aerobic Gram-positive and Gram-negative pathogens but resistance (R) has developed in the form of permeability/efflux changes, ribosomal methyltransferases (RMT), and particularly AG modifying enzymes (AMEs).[1,2] Neoglycosides are designed to overcome common mechanisms of R that decrease susceptibility to older AGs, e.g., amikacin (AMK), gentamicin (GEN) and tobramycin (TOB). Given that 15 years had passed since the last extensive survey of AMEs,[3] we conducted a survey of AG-R and multidrug resistant (MDR) Gram-negative organisms within the recent SENTRY collections.

METHODS

Isolates were tested by CLSI broth microdilution methods per M7-A7 [2006]. Quality control was performed utilizing *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. Targeted concentration ranges were those published in the current CLSI M100 document.

Survey of the AG-R population in the 2005 SENTRY collection

- Organisms studied demonstrated varying antibiogram profiles, with R to at least 1 AG (AMK, GEN, TOB), selected to reflect the R patterns to these AGs within the entire 2005 SENTRY collection. Minimum inhibitory concentrations (MICs) of AMK, Apramycin (APR), Arbekacin (ARB), Fortimicin (FOR), GEN, Kanamycin (KAN), Neomycin (NEO), Netilmicin (NET), and TOB were determined.
- All organisms (total 407) were collected from patients hospitalized in geographically diverse regions during 2005 and 2006: North America (141 strains, 35%), Latin America (130 strains, 32%), and Europe (136 strains, 33%). The organisms tested consisted of 262 isolates of Enterobacteriaceae [*E. coli* (50), *Klebsiella* spp. (60), *Enterobacter* spp. (50), *Citrobacter* spp. (24), *Serratia* spp. (22), *P. mirabilis* (32), *Morganella* spp. (10), Other Indole positive Proteae (13), *Salmonella* spp. (1)], 95 isolates of *P. aeruginosa* and 50 isolates of *Acinetobacter* spp.

Survey of the MDR population in the 2007 SENTRY collection

- Organisms demonstrated varying antibiogram profiles, but all were defined as MDR with specific R to at least one AG (AMK, GEN, TOB) and ciprofloxacin, and either ceftazidime or ceftriaxone, selected to reflect the R patterns to these agents within the MDR subset of the 2007 SENTRY collection. MICs were determined for the same agents as in the 2005 study, with the exception that KAN was replaced with isepamicin (ISE).
- All organisms (total 301) were collected from patients hospitalized in geographically diverse regions during 2007: North America (51 strains, 37.9%), Latin America (68 strains, 22.6%), Europe (68 strains, 22.6%), and Asia-Pacific (114 strains, 37.9%). The organisms tested consisted of 164 isolates of Enterobacteriaceae [*E. coli* (48), *Klebsiella* spp. (49), *Enterobacter* spp. (25), *Citrobacter* spp. (11), *Serratia spp.* (11), *P. mirabilis* (10), Indole-positive Proteae (10)], 79 isolates of *P. aeruginosa* and 58 isolates of *Acinetobacter* spp.

RESULTS

An overview of the results from the 2 studies is presented in Tables 1 and 2. Given the selection criteria, it is unsurprising that the study populations exhibit significant R to AMK, GEN, and TOB. However, other less commonly used AGs also demonstrate decreased activity against these strains. This is indicative of AG-R mechanisms (AGRM) that apply across the spectrum of existing AGs.

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Table 1: AG susceptibility of AG-R subset of 2005 SENTRY collection

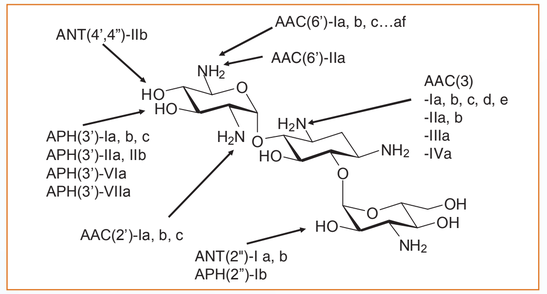
	Acinetobacter (n=50)		*Citrobacter* (n=24)		*E. coli* (n=50)	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
AMK	64	>128	8	64	8	32
APR	8	16	4	8	4	8
ARB	2	8	4	32	2	32
FOR	64	>128	4	8	4	8
GEN	128	>128	128	>128	64	>128
KAN	>128	>128	128	>128	64	>128
NEO	16	64	1	64	2	64
NET	8	>128	16	>128	16	128
TOB	16	64	32	128	32	128
	Enterobacter (n=50)		*Klebsiella* (n=60)		*P. mirabilis* (n=32)	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
AMK	16	>128	32	128	4	>128
APR	4	4	2	8	8	16
ARB	8	>128	8	32	4	16
FOR	4	>128	2	8	8	64
GEN	128	>128	32	>128	32	128
KAN	>128	>128	>128	>128	>128	>128
NEO	2	32	2	32	32	>128
NET	64	>128	64	>128	16	>128
TOB	32	>128	32	>128	16	64
	Proteae, Indole+ (n=23)		*Pseudomonas* (n=95)		*Serratia* (n=22)	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
AMK	8	16	8	32	32	>128
APR	4	8	16	32	4	8
ARB	8	16	4	32	16	>128
FOR	4	64	64	128	4	>128
GEN	64	>128	>128	>128	128	>128
KAN	128	>128	>128	>128	>128	>128
NEO	16	128	16	>128	2	16
NET	128	>128	16	>128	64	>128
TOB	16	64	64	>128	64	>128

Table 2: AG susceptibility of MDR subset of 2007 SENTRY collection

	Acinetobacter (n=58)		*Citrobacter* (n=11)		*E. coli* (n=48)	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
AMK	>64	>64	8	8	8	64
APR	8	16	4	4	4	8
ARB	8	>64	2	8	4	32
FOR	>64	>64	2	4	4	8
GEN	>32	>32	>32	>32	>32	>32
ISE	>64	>64	1	2	2	8
NEO	64	>64	2	>64	1	8
NET	>64	>64	16	64	16	64
TOB	>32	>32	32	32	32	>32
	Enterobacter (n=25)		*Klebsiella* (n=49)		*P. mirabilis* (n=10)	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
AMK	16	>64	16	>64	8	>64
APR	2	4	2	4	4	8
ARB	16	>64	8	>64	4	>64
FOR	2	>64	2	>64	8	>64
GEN	>32	>32	>32	>32	16	>32
ISE	2	>64	2	>64	8	>64
NEO	1	64	1	32	64	>64
NET	64	>64	64	>64	64	>64
TOB	>32	>32	32	>32	16	>32
	Proteae, Indole+ (n=23)		*Pseudomonas* (n=95)		*Serratia* (n=22)	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
AMK	48	8	64	>64	32	>64
APR	4	8	16	32	4	8
ARB	2	8	32	>64	16	>64
FOR	4	>64	64	>64	2	>64
GEN	>32	>32	>32	>32	>32	>32
ISE	2	8	64	>64	8	>64
NEO	8	64	>64	>64	2	168
NET	32	64	>64	>64	>64	>64
TOB	16	16	>32	>32	>32	>32

AMEs include acetyltransferases (AAC) which inactivate AGs by *N*-acetylation, adenylyltransferases (ANT) which inactivate AGs by *O*-adenylylation, and phosphoryltransferases (APH) which inactivate AGs by *O*-phosphorylation (Figure 1). Most enzymes are encoded by >1 gene. In the case of AAC(6')-I, 32 different genes have been reported.

Figure 1: Major AMEs found in Gram-negative bacteria as illustrated with kanamycin B



AGRM were assigned for each strain from the observed antibiogram and changes in MIC of the R strain versus its wild-type parent (Table 3).[4] When enzymes are present as combinations, AGRM assignment becomes complex. In these cases, susceptibility data from AGs that are not commercially available is informative.[4]

Table 3: AGRM phenotype assignment

	APR	FOR	GEN	TOB	AMK	ISE	NET	KAN	NEO
Perm/Efflux	+	+	+	+	+	+	+	+	+
AAC(2')-I			+	+			+		+
AAC(3)-I		+	+						
AAC(3)-II			+	+			+	±	
AAC(3)-III			+	+				+	
AAC(3)-IV	+		+	+			+		
AAC(3)-VI			+	±			±	±	
AAC(6')-I			±	+	+	±	+	+	
AAC(6')-II			+	+			+	+	
ANT(2")-I			+	+				+	
ANT(4')-II				+	+	+		+	
APH(3')-VI					+	+		+	+

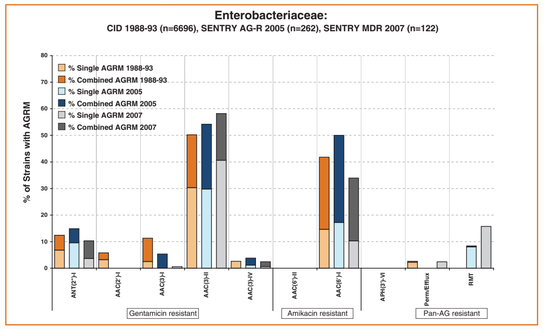
+: significant MIC change vs wild-type; ±: MIC change observed if enzyme is expressed at high level.

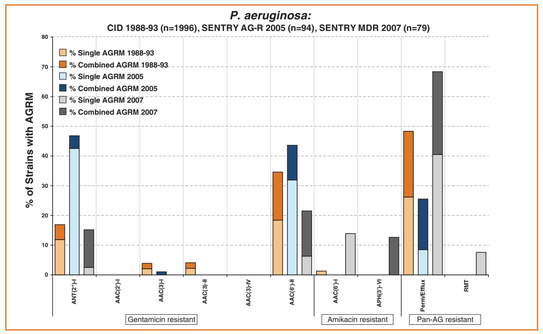
Polymerase chain reaction (PCR) was used to confirm the phenotypes assigned to 89 strains from the 2005 SENTRY collection for 6 AME genes [AAC(6')-Ib/II, AAC(3)-Ia, AAC(3)-IIa, AAC(3)-IVa, ANT(2")-Ia, and APH(3')-VIa]. Of the 119 mechanisms assigned, 16 were false positives and 14 were false negatives. Errors were most frequent in *P. aeruginosa*, where combinations of AMEs and permeability/efflux changes are common. Where phenotypes were not confirmed by PCR (false positives) it is likely that an alternate gene is responsible for the phenotype (see Figure 1). False negatives may occur if a gene is present but the corresponding enzyme is expressed at low levels.

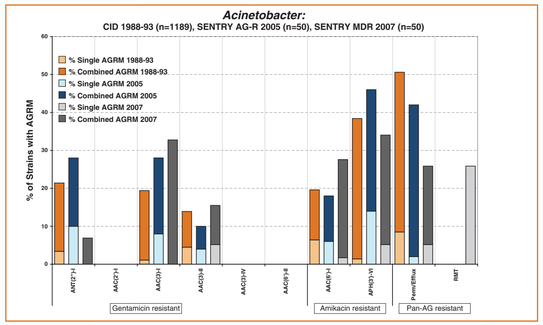
AGRM findings were compared to a published survey (1988-93, Figure 2).[3] The incidence of combined AGRM remained high due to GEN and TOB modifying enzymes [AAC(3)-I, AAC(3)-II, and ANT(2")-I] with the TOB, NET, and AMK modifying enzyme AAC(6')-I. R to all of the current clinically used AGs results from these combinations of AMEs. This broad spectrum AG-R continues to occur as a result of permeability/efflux and now due to RMT.

The type of AGRM observed varies between Enterobacteriaceae, *P. aeruginosa* and *Acinetobacter*. Low-level R to most AGs results from permeability/efflux. This occurs in approximately half of all AG-R *P. aeruginosa* and *Acinetobacter*, while it is rarely observed amongst Enterobacteriaceae. RMT are new to the AG-R landscape, but their presence among non-MDR strains remains very low. In the initial AME surveillance study,[3] half of *P. stuartii* isolates screened were found to express the chromosomal AAC(2')-I. This phenotype was not observed in either of the more recent studies. In all 3 studies the KAN-/NEO-R phenotype caused by APH(3')-I/II was not evaluated.

Figure 2: Occurrence of AGRM in Gram-negative bacteria surveyed at 3 different times shows similar distributions within Enterobacteriaceae, *P. aeruginosa*, and *Acinetobacter*







CONCLUSIONS

These results demonstrate that while the overall prevalence may be increasing (companion abstract by Biedenbach et al[5]), the distribution of AGRM amongst AG-R isolates worldwide remained stable during the past 20 years. This knowledge has served as a useful tool in the design of neoglycosides that evade the majority of these AGRM.

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