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Aminoglycoside Resistance Among Gram Negative Bacilli in Sofia, Bulgaria

Emma E. Kevleyan, Higher Medical Institute, Sofia, Bulgaria

Increased frequency of resistance to the aminoglycoside antibiotics tobramycin and sisomycin in association with gentamicin resistance has been observed in Bulgaria. Gentamicin (Pharmachim) was introduced in Bulgaria in 1972. Tobramycin and amikacin were occasionally used at the end of the same decade. They took their place in the treatment of seriously ill patients in the middle of the 1980's. In a study of aminoglycoside resistance among Gram negative bacilli, undertaken at the Medical Academy in 1980, resistance to gentamicin was found in 29.3% of cases; resistance to amikacin was found 1.8% of the time. Percentages increased alarmingly during 1983-1984, when resistances to these drugs went up by 5.3% and 5.7% respectively.

For the present study, 372 gentamicin resistant strains were selected from the Medical Academy, Emergency Institute Priogov and Hygienic - Epidemiological Institute - Sofia, between 1984 and 1986: *E. coli* (34), *Klebsiella spp.* (77), *Enterobacter spp.* (60), *S. marcescens* (44), *H. alvei* (1), *C. freundii* (8), *Salmonella spp.* (5), *P. mirabilis* (74), *Proteus I+* (44), *P. aeruginosa* (6), and *Acinetobacter spp.* Most came from urinary tract cultures.

Susceptibility determinations were performed by twofold serial dilution method according to NCCLS, 1988. The interpretation of aminoglycoside resistance was by the criteria of the FSM, 1991. Among the gentamicin resistant isolates, resistance to tobramycin (tob) appeared in 97.1% of *E. coli*, 94.8% of *Klebsiella*, 91.7% of *Enterobacter*, 90.0% of *S. marcescens*, 100% of *C. freundii*, 80% of *Salmonella*, 98.6% of *P. mirabilis*, 94.4% of *Proteus I+* and 88% of Gram negative nonfermentative bacilli (GNNFB). Resistance to sisomicin (Sis) was also very commonly associated with these other aminoglycoside resistances: 93.9% of *E. coli*, 95.9% of *Klebsiella*, 98.2% of *Enterobacter*, 93.1% of *S. marcescens*, 100% of *C. freundii*, 75% of *Salmonella*, 100% of *P. mirabilis*, 96.3% of *Proteus I+*, and 95.6% of GNNFB.

In some species, netilmicin (Net) susceptibility was conserved. Resistance, however, was noted: 35.5 % of *E. coli*, 75.7% of *Klebsiella*, 76.9% of *Enterbacter*, 68.2% of *S. marcescens*, 75% of *C. freundii*, 0% of *Salmonella*, 34% of *P. mirabilis*, 39.2% of *Proteus I+*, and 60% of GNNFB.

Amikacin (Akn) was the most active aminoglycoside since the resistance frequency was low; 3% in *E. coli*, 1.3% in *Klebsiella*, none in *Enterobacter*, *C. freundii* or *Salmonella*, 1.3% in *P. mirabilis*, 2.3% in *Proteus I+*, but 36.4% in *S. marcescens* and 40% in GNNFB. The most frequent phenotypes of resistance are listed in Table 1.

In an attempt to study the genetic mechanisms of resistance, conjugational transfer to recipient *E. coli* K12 strains

(Continued on page 2)

Trimethoprim-Sulfamethoxazole Resistance in *Shigella* Causing Diarrhea in Children in Guatemala: Transfer *in vivo* from *Escherichia coli*

Olga Torres[1], Jose B. Cruz[1], Floridalma Cano[1], & Alfred Bartlett[1,2] Institute of Nutrition of Central America and Panama, Guatemala, Guatemala[1] and School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland 21205[2]

A prospective study of persistent diarrhea was conducted among 321 preschool children of Santa Maria de Jesus, a rural community of Guatemala, from February 1987 to December 1989 (1). During this period 380 strains of *Shigella* sp. were isolated. In the first months of study, all the *Shigellae* isolates were susceptible to trimethoprim- sulfamethoxazole (TxS), the drug of choice for shigella dysentery; therefore, all the cases were treated for five days with this drug. On November 27, 1987, the first isolate resistant to TxS (TxS^r) was isolated and by the second semester of 1989, 29% of the shigellae were TxS^r, with an average of 12% of resistant strains during the three years of surveillance (Table 1). This resistance was efficiently transferred *in vitro* by membrane filter crosses (2) to *Escherichia coli* C600.

The more common serotypes of *Shigella* found carrying TxS^r were *Shigella flexneri* 1, followed by *S. flexneri* 2 and

(Continued on page 6)

GUATEMALA (Continued from page 1)

S. sonnei (Table 2). By comparing the distribution of serotypes of all *Shigella* isolates from that community, it was clear that the reservoirs of TxS^r were not those serotypes which were more prevalent in the community, *S. flexneri* 6 and *S. sonnei*. Furthermore, strains resistant to more than three antimicrobial agents were, in general, more prevalent among the TxS^r *Shigella* than among the serotypes of strains commonly found susceptible to TxS (Table 3).

There were two cases of shigella dysentery in which the causative strain of *Shigella* was susceptible *in vitro* to TxS. When TxS treatment was given to the patients, the symptoms subsided for one or two days, but they reappeared despite medication compliance. In one case, a patient with *S. flexneri* 2 (#706602) with MIC of 2/64 µg/ml for TxS, later excreted *S. flexneri* 2 (#706640) with MIC ≥1000/2000 µg/ml. Four strains of *E. coli* isolated concurrently with 706602 showed MIC's of ≥1000/2000 g/ml for TxS. Another patient excreted *S. flexneri* 4 (#706631) with MIC of 2/64 and later *S. flexneri* 4 (#706699) with MIC ≥1000/2000 µg/ml. In this case, *E. coli* isolated concurrently with *Shigella* 706631 also showed MIC ≥1000/2000 µg/ml for TxS. Both patients were treated with nalidixic acid and symptoms were relieved.

There are several possible explanations for this phenomenon. TxS^r was the result of reinfection with another strain of *Shigella* resistant to TxS. TxS^r was expressed from silent genes in *Shigella* sp. 706631 and 706602. Under selective pressure exerted by the administration of TxS, *S. flexneri* which received genes from *E. coli* in the intestine were selected.

We do not support the first explanation. Epidemiological evidence (Table 2) shows that the most commonly isolated serotypes of *Shigella* in Santa Maria de Jesus were *S. flexneri* 6 and *S. sonnei*. In the cases described, shigellosis was caused by *S. flexneri* 2 and *S. flexneri* 4, which are not as frequently isolated. Moreover, there were only 1.8% of the total strains isolated belonging to *S. flexneri* 4, one of the least frequent types found. Average MIC levels for TxS among the shigellae of Santa Maria de Jesus during the period of study were ≥128/2432 µg/ml, while in these two cases the levels exceeded 1000/2000 µg/ml.

If the second explanation were true and TxS^r genes were expressed from silent genes, their prevalence would be much higher than the 29% observed for 1989. Also, the average MIC levels would be nearer the levels of 1000/2000 µg/ml than 128/2432 µg/ml observed.

We favor the third explanation because in both cases the non-pathogenic *E. coli* collected for further analyses showed very high levels of resistance to both T and S, identical to those of the second isolate of *Shigella*. In this cohort of children, TxS^r was present in 28% of the *E. coli* fecal isolates from children before antibiotherapy and in 55% after antimicrobial treatment. Therefore, if a child had received the drug before, the probability of being colonized with intestinal *E. coli* resistant to TxS would be higher. Epidemiological evidence of this situation was previously reported from an outbreak of *S. dysenteriae* 1 in Rabinal, Baja Verapaz, Guatemala (4). One of the TxS^r strains came from a boy who had taken TxS prophylaxis for respiratory illness one month before. Besides being used for treatment of shigella dysentery, TxS is being widely recommended for acute respiratory and urinary tract infections by medical, paramedical and pharmacy personnel of Santa Maria de Jesus. The two cases presented are not unique; similar observations have been made at the Hospital General San Juan de Dios in Guatemala City, but unfortunately the strains were not available for testing and *E. coli* were not collected (David Prado, personal communication).

It is important to emphasize that in this community the selective pressure did not come singly from the study's use of TxS. TxS has become an increasingly popular drug in this and other communities where it is used, as mentioned before, by physicians and pharmacists to treat acute respiratory infections, diarrhea, otitis media and other common illnesses besides dysentery.

Our results support those of others who state that in area of the world where sanitation is low, where non-restricted use of antibiotics is prevalent and where the incidence of diarrhea is high, the use of TxS results in resistance to this drug (5-9). Untreated dysentery may have severe impact on health and nutritional status, and in occasional cases may lead to death. Moreover, the detrimental effect of dysentery is greater in children with malnutrition. Efforts to identify and control the factors leading to the emergence and spread of antibiotic resistance are of public health importance in developing countries where dysentery is common.

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1. Cruz JR, Bartlett A, Mendez H, Sibrian R: Acta Pediatr Supl 381, 1992.
2. Jacob A, Hobbs S: J. Bacteriol 117:360-372, 1974.
3. Sham D, Washington II: J. Manual of Clin. Micro. (5th ed.):1105-1116, 1991.
4. Cruz JR, Cano F, Rodriguez L, Rios CA, Guerra P, Leonardo Z: MMWR 40:421, 1991.
5. Smith H.G: J. Med Microbiol. 40:421, 1977.
6. Murray B, Rensimer K: J. Inf Dis 147:724-728, 1983.
7. Rudy P, Murray B: J. Inf Dis 150:25-29, 1983.
8. Farrar E: J. Inf Dis 152:1103-1106, 1985.
9. Freese K: APUA Newsletter (1):6-7, 1983.
10. Siegel S, Castellan J Jr: Non Parametric Stats for Beh Sci (2nd ed.):291-298, 1988.

Book Available

Through an agreement with Plenum Publishing Corp., APUA members can receive the recently published book, *The Antibiotic Paradox: How Miracle Drugs Are Detroying the Miracle*, by APUA President Stuart B,. Levy at 20% off the cover price, or $20 plus shipping and handling. (S&H: $2 for US and Canada; $5 for all others). Canadians must also include 7% GST. Foreign checks should be drawn on a US bank. Prepaid orders should be sent to: Michael Messina, Plenum Publishing Corp., 233 Spring Street, NY, NY 10013.

TABLE 1
ISOLATES OF *SHIGELLA* sp. RESISTANT TO TRIMETHOPRIM SULFAMETHOXAZOLE BY SEMESTER OF STUDY

Shigella sp.	1987 Semester		1988 Semester		1989 Semester		TOTAL	
	First	*Second*	*First*	*Second*	*First*	*Second*		
	# *TxS*^{r}	# *TxS*^{r}	# *TxS*^{r}	# *TxS*^{r}	# *TxS*^{r}	# *TxS*^{r}	#	*TxS*^{r}
S. dysenteriae	15 0	13 0	11 2	4 2	4 0	3 1	50	5
S. flexneri	47 0	55 5	59 10	16 5	22 3	20 7	219	30
S. boydii	7 0	1 0	6 0	6 1	11 1	4 0	35	2
S. sonnei	11 0	16 0	23 3	7 1	11 4	8 2	76	10
TOTAL	80 0	85 5	99 15	33 9	48 8	35 10	380	47
%	-	6	15	27	17	29	12	

TABLE 2
SEROTYPES OF *SHIGELLA* sp. ASSOCIATED WITH RESISTANCE TO TRIMETHOPRIM/SULFAMETHOXAZOLE

Shigella serotype	Proportion of TxS^r strains by serotype		Frequency of each serotype in all isolates	
	#	%	#	%
S. flexneri 1	15	31.9	63	16.6
S. flexneri 2	11	25.5	43	11.3
S. sonnei	10	21.4	78	20.6
S. dysenteriae	5	10.7	48	12.6
S. flexneri 4	1	2.1	7	1.8
S. flexneri 6	2	4.2	88	23.2
S. boydii 1	1	2.1	10	2.6
S. boydii 2	1	2.1	10	2.6
Other serotype	0		33	8.7
TOTAL	47	100.0	380	100.0

TABLE 3
SUSCEPTIBILITY PATTERNS OF *SHIGELLA* sp. RESISTANT TO TRIMETHOPRIM/SULFAMETHOXAZOLE

		(# resistant isolates)						% resistant
Shigella	sp.	TxS	Amp	Cep	Cam	Gen	Nal	Tet	to ≥ 3 Drugs
S. flexneri	1	15	14	2	5	0	0	13	33.3
S. flexneri	2	12	9	2	0	0	0	11	16.7
S. sonnei		10	7	0	0	0	1	10	10.0
S. dysenteriae		5	0	0	0	0	0	3	0.0
S. flexneri	6	2	2	1	2	0	0	2	50.0
S. flexneri	4	1	0	0	0	0	0	1	0.0
S. boydii	1	1	1	0	0	0	0	1	0.0
S. boydii	2	1	1	0	1	0	0	1	100.0

Key:
Amp = ampicillin, Cep = cephalothin, Cam = chloramphenicol, Gen = gentamicin, Nal = Nalidixic acid, TxS = trimethoprim-sulfamethoxazole, Tet = tetracycline

BULGARIA (Continued from page 2)

GNNFB. Thirty percent of the resistance was transferable. Different aminoglycoside-modifying enzymes, or their combinations, were responsible for the resistance patterns. The finding of three different amikacin-modifying enzymes, belonging to three different classes, is notable.

The increasing frequency of gentamicin and amikacin resistance (36.4% and 6.9% in 1989 and 58.5% and 10.2% in 1991, respectively, at the Medical Academy) is a warning, and calls for a quick change in antibiotic policy and usage.

1. Acar J.F., Goldstein F.W., Menard R., Bleriot ZJ. - P.:Am J Med 80/Suppl 6B/:82, 1986.
2. Courvalin P., Philippon A. :332 In:L.le Minor, M. Veron/Eds/:Bacteriologie Medicale, 2 ed., Flammarion, Paris, 1989.
3. Hancock R.E.W., Farmer S.W., Susheng L., Poole K: Antimicrob Agents Chemother 35:1309, 1991.
4. Hopkins J.D., Flores A, del Pilar Pla M, Lester S, O'Brien T F: Antimicrob Agents Chemother 35:1605, 1991.
5. Miller G. and World Study Group: Aminoglycoside Resistance Surverys. Schering-Plought Res Inst. 1992.
6. Naber K.G., Grimm N., Rosenthal EJK, Shoh PN, Wiedemann B: J. Internat Med Res 18:6D, 1990.
7. Pancoast S: Med Clin N Am 72:581, 1988.
8. Phillips I, King A, Shannon I: Am J Med 80/Suppl 6B/:48, 1986.
9. Tran Van Nhieu G., Bordon F., Collatz E: J. Med Microbiol 3683, 1992.

Spanish Newsletter Soon Available

Through the auspices of the Spanish Society of Chemotherapy, the quarterly *APUA Newsletter* will be translated into Spanish and printed for mailings in Spain and other spanish-speaking countries. This project, spearheaded by Dr. Fernando Banquero, member of APUA's Scientific Advisory Board, and Beatriz Perez Gorricho, of the Nino Jesus Hospital in Madrid who will coordinate the project, was recently endorsed by the Spanish Society. Those interested in helping with this project should contact APUA. We thank our Spanish colleagues and members of the Spanish Society of Chemotherapy for supporting this project.