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Aminoglycoside resistance patterns of *Serratia marcescens* strains of clinical origin

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SUMMARY

Aminoglycoside resistance patterns of 147 *Serratia marcescens* strains of clinical origin were studied. All strains analysed belonged to three different bacterial populations. The periods of study and the institutions the strains were isolated from correlated significantly with the resistance patterns shown by the strains. The most frequent resistance patterns found were the following: ACC (6′)-I at the Hospital Infantil de México (Children's Hospital of México), and ANT (2″)+ AAC(6′)-I at the Instituto Nacional de Pediatría (INPed or National Institute of Pediatrics) in Mexico City. Furthermore, the isolation frequency of aminoglycoside-sensitive strains decreased remarkably at the INPed over a 12-year period. These results suggest that there has been a selection of *Serratia marcescens* strains that are very resistant to aminoglycosides.

INTRODUCTION

Formerly, *Serratia marcescens* was considered as a non-pathogenic bacterium. However since 1960, increasing numbers of studies have been reported where it actively participates in infectious processes, particularly in patients with immunosuppression, or those undergoing invasive, diagnostic or therapeutical procedures [1–3].

Currently, *S. marcescens* is a frequent cause of nosocomial infections, presenting a serious problem mainly in epidemic outbreaks with high lethality rates [3, 4]. This bacterium has several pathogenic mechanisms, and the expression of some of these *in vitro* is subject to the temperature of the medium [3, 5–7]; among these its capability of resisting a great number of antibiotics is outstanding [8–10].

Although aminoglycosides have traditionally been used for the treatment of *S. marcescens* infections, aminoglycoside-resistant strains are being isolated at increasing frequencies; many of the strains are also resistant to many other antibiotics [10–12].

S. marcescens can show resistance to aminoglycoside activity because of the presence of acetylating, adenylating or phosphorylating enzymes, and because of its capability of modifying its permeability barrier. The relation between aminoglycoside resistance and changes in the bacterial phenotype has been

described previously [9, 13–15]. It has also been reported that the use of aminoglycosides in hospital environments exerts a selective pressure that allows the proliferation of multi-resistant *S. marcescens* strains, and that when this selective pressure disappears the population changes again, favouring the presence of sensitive strains [16–18].

In the present study, we established the frequency of aminoglycoside resistance patterns (AGRP) in 147 *S. marcescens* strains of clinical origin, belonging to three different bacterial populations. We found that the site and period of isolation correlated significantly with the distribution of AGRPs of the bacterial populations.

MATERIAL AND METHODS

Bacterial strains

One hundred and forty-seven *S. marcescens* strains isolated from infected patients were studied. Eleven strains were isolated from the Instituto Nacional de Pediatría (INPed) in Mexico City in 1977; 31 strains were isolated from the Hospital Infantil de México (HIM) in 1978, and 105 strains were isolated between 1988 and 1989 from the INPed [19]. For the purpose of this study, strains were recovered from conservation media. Every strain was re-identified according to the American Society for Microbiology (ASM) criteria [20] and was stored at 4 °C.

Aminoglycoside resistance patterns (AGRP)

The method described by Hare and colleagues [21, 22] was used to study the bacterial contents of aminoglycoside-modifying enzymes, and to establish aminoglycoside resistance patterns. The following 12 different antibiotics were used: netilmicin, gentamicin, tobramycin, amikacin, fortimicin, apramycin, kanamycin, schizomycin-22591, neomycin, schizomycin-21420, 2′-*N*-ethyl-netilmicin and 6′-*N*-ethyl-netilmicin (Sensidisks impregnated with each antibiotic were provided by Schering-Plough Corp.).

Each strain was recovered from special gelose vials kept at 4 °C, and grown at 37 °C for 18 h in Mueller–Hinton broth. The following day, the bacteria were reseeded in the same medium and incubated at 37 °C until the culture reached the exponential growth phase (approximately 4 h). Each culture's turbidity was then adjusted to that of the 0·5 McFarland nephelometer tube. Bacteria were massively seeded in Petri-dishes with a swab, in unsupplemented Mueller–Hinton agar. Inoculated plates were left to dry at room temperature, and sensidisks for each antibiotic placed. Cultures were then incubated at 37 °C for 24–48 h, and inhibition halos were read with the help of a Vernier calliper. (The base of the Mueller–Hinton medium was provided by Schering-Plough Corp., lot C1DT1J BBL.)

The AGRP assignation was made on the basis of the differential activities of the antibiotics and the differential resistance pattern of each tested strain as proposed by Hare and colleagues and Shimizu and co-workers [21, 22] (Table 1).

Unequal increases in resistance (i.e. Gm 16-fold; Amk-twofold), even if they involved all aminoglycosides, suggested the presence of combinations of modifying enzymes. Permeability resistance can be detected as an equal increase in MIC (decrease in diameters) with all aminoglycosides tested.

Table 1. *Aminoglycoside resistance mechanism Gram-negative bacteria substrate profiles (resistance patterns)*

Aminoglycoside	*AAC*(3)-I (*AAC*(3)-*a*)	*AAC*(3)-Ia (*AAC*(3)-*b*)	*ANT*(2″)	AAC(3)-III (AAC(3)-d)	AAC(3)-IV (AAC(3)-a)	AAC(3)-V or AAC(3)-II (AAC(3)-c	AAC(2′)	AAC(6′)-II (AAC(6′)-b)	ANT(2″)+ AAC(6″)	AAC(3)-V+ AAC(6′)	AAC(6′)-I or AAC(6′)-IV (AAC 6′-a)
Gentamicin	+	+	+	+	+	+	+	+	++	++	±
Tobramycin			+	+	+	+	+	+	++	++	+
Dibekacin			+	+	+	+	+	+	++	++	+
Netilmicin		+			+	+	+	+	+	++	+
Amikacin									±	+	+
Sch 21420									±	+	±
2′-Net		+			+	+		+	+	+++	+
6′-Net		+			+	+	+			+	
Sch 22591				+	±			+	±	+	+
Portimycin	+										
Apramycin					+						

Resistance caused by AAC(6′)-I is usually low level and *Sch 21420* is usually two- to fourfold more active than amikacin, therefore, *21420* is often active (i.e. MICs 16) vs. these strains. AAC(3)-b could be a subset of AAC(3)-c with low-level resistance to TOB and DKB. A plus (+) indicates a change (eightfold) normal values while a ± indicates that smaller changes or variable changes depending on host background or enzymic activity are usually seen (Hare *et al.* 1988).

Table 2. *Aminoglycoside resistance patterns of* Serratia marcescens *strains of clinical origin*

	INPed (1977) ($n = 11$)	HIM (1978) ($n = 31$)	INPed (1988–9) ($n = 105$)
ANT(2″)	1	4	4
AAC(6′)-I	2	10	3
APH(3′)-I	—	2	1
ANT(2″)+ AAC(6′)	2	2	70
ANT(2″)+ AAC(6′)-I+ APH(3′)-I	—	1	23
Permeability	2	2	1
Sensitive	3	5	—
N.D.*	1	5	3

* Not determined.

Controls

Two reference strains were tested for each assay (*Escherichia coli* ATCC-25992 and *Pseudomonas aeruginosa* ATCC-35218). The results of the assays were considered as satisfactory when the control strains showed a behaviour similar to that previously described for each one [23].

RESULTS

It was possible to assign the AGRP to 95·9% of the strains studied. Table 2 shows the strains grouped according to the period of study and the hospital from which they were isolated. As shown, the AGRPs characterized were significantly different at each institution.

Among the strains isolated from the INPed in 1977, the most frequent were aminoglycoside-sensitive, followed by those with AAC(6′)-I and ANT(2″) aminoglycoside resistance patterns. However, among the INPed strains isolated in 1988–9, the most frequent AGRP was ANT(2″)+AAC(6′)-I, followed by ANT(2″)+AAC(6′)-I+APH(3′)-I, and no aminoglycoside-sensitive strains were found. Among the bacteria isolated from the HIM, the most frequent AGRP found was AAC(6′)-I, while five strains were aminoglycoside sensitive.

In a previous study [19], we determined the biotypes of the bacteria analysed in this study. Table 3 shows the AGRPs found according to the biogroups of the strains. There was no clear correlation of AGRPs and biogroups, except for the case of biogroup A5/8, which correlated with AGRPs ANT(2″)+AAC(6′)-I and ANT(2″)+AAC(6′)-I+APH(3′)-I.

DISCUSSION

Serratia marcescens is currently a serious hospital epidemiological problem, because it is associated with outbreaks that have high lethality rates [4].

Table 3. *Biotyping and aminoglycosides resistance patterns of* Serratia marcescens *strains of clinical origin*

					A5/8								
Biogroup	A1	A2/6	A3	A4	A5	A8a	A8b	A8d	Nt*	TCT	ND†	Aux‡	Nt*
AGRP													
ANT(2″)	—	—	1	1	2	1	2	1	—	—	1	—	—
AAC(6′)-I	—	—	—	1	3	—	1	4	3	—	—	—	1
APH(3′)-I	—	—	—	—	1	—	1	—	1	—	—	—	—
AAC(6′)-II	—	—	—	—	1	—	—	—	—	—	—	—	—
AAC(3)-V	—	—	1	—	—	—	—	—	—	—	—	—	—
AAC(3′)-III	—	—	—	—	—	—	1	—	—	—	—	—	—
AAC(6′)-I APH(3′)	—	—	—	—	—	—	1	—	—	—	—	1	—
ANT(2″) AAC(6′)-I	—	—	—	—	5	—	11	59	—	—	2	—	—
ANT(2″) AAC(6′)-I APH(3′)-I	—	—	—	—	3	—	6	13	—	—	—	—	—
Permeability	—	—	—	2	—	—	1	—	—	—	—	—	—
Sensitive	1	—	1	2	—	1	—	—	—	2	—	—	—
ND	—	1	—	—	2	—	1	—	1	1	—	—	—

* Nt, Non-typable.
† ND, Not-determined.
‡ Aux, Auxotrophs.

This microorganism is capable of acquiring and propagating antibiotic resistance genes, with the consequent development of multi-resistant strains in environments with selective pressure which inhibit the development of susceptible populations [24].

We studied this microorganism's resistance to aminoglycosides in three different bacterial populations, with the purpose of assessing its adaptability; also to study its evolution over a 12-year period at the INPed. We were able to assign AGRPs to most of the strains studied; it is important to mention that in certain cases, the assignment of AGRPs may have been imprecise, because the presence and expression of several aminoglycoside resistance genes may result in the same AGRP. However, the AGRP identification technique we used is simple to implement, and correlates well with the bacterial genotype and phenotype as regards antibiotic resistance [25].

AGRPs of bacteria isolated from the HIM showed a wide diversity. This particular population was also the most heterogeneous of those analysed. We found a low number of sensitive strains and of strains with aminoglycoside resistance due to barrier permeability changes.

We analysed two different bacterial populations from the INPed, isolated 12 years apart. Over this period, we observed a decrease in the percentage of strains with aminoglycoside resistance due to permeability changes, the disappearance of aminoglycoside-sensitive strains, an increase in the diversity of identified AGRPs, and a better capability of the bacteria to resist a wide spectrum of aminoglycosides. AGRPs of bacteria isolated in 1988–9 were more homogeneous, possibly because

most bacteria from this population were isolated from a previously reported nosocomial outbreak [26].

Currently, the AGRP most frequently associated with aminoglycoside-resistant *S. marcescens* is ANT(2″)+AAC(6′)-I [21, 22, 25]. According to our data, there seems to have occurred a selection of resistant strains from bacterial populations that were originally mostly aminoglycoside sensitive, and that AGRPs are evolving to ANT(2″)+AAC(6′)-I+APH(3′)-I.

The correlation of biogroup A5/8 with more effective antibiotic resistance has been previously reported [27]. Our results are in accordance with the latter, and biotype A8d showed the most efficient antibiotic resistance activity.

Considering our result and those of Sifuentes and co-workers [27], we can infer that in Mexico City, biogroup A5/8, aminoglycoside-modifying enzymes ANT(2″) and AAC(6′)-I, and AGRP ANT(2″)+AAC(6′)-I are the most important among *S. marcescens* populations. The virulence of these populations is apparently increasing, along with the importance of this microorganism as a causal agent of infectious processes in our country.

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