ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 2011, p. 4930–4932 Vol. 55, No. 10
0066-4804/11/$12.00 doi:10.1128/AAC.00242-11

Genetic Diversity of Tn*916*-Related Transposons among Drug-Resistant *Streptococcus pneumoniae* Isolates Colonizing Healthy Children in Venezuela▽

Beatríz Quintero,[1,2,3] María Araque,[2] Christa van der Gaast-de Jongh,[3] and Peter W. M. Hermans[3]*

Department of Microbiology and Parasitology, Faculty of Medicine, Los Andes University, Mérida, Venezuela[1]; Department of Microbiology and Parasitology, Faculty of Pharmacy and Bioanalysis, Laboratory of Molecular Microbiology, Los Andes University, Mérida, Venezuela[2]; and Laboratory of Pediatric Infectious Diseases, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands[3]

Received 22 February 2011/Returned for modification 26 April 2011/Accepted 16 July 2011

Among a collection of 48 multidrug-resistant pneumococcal strains colonizing healthy children in a small municipality of Mérida, Venezuela, we identified sequence types (STs) related to a variety of internationally spreading drug-resistant clones, as well as ST135, thus far isolated only in Europe. The clones invariably harbored one or more of the Tn*916*-related transposons Tn*3872*, Tn*5253*, Tn*6002*, Tn*2009*, and Tn*2010*. Finally, our data suggest both structural rearrangements in certain transposons and occurrence of novel transposable elements.

In the past decade, the emergence and spread of resistant *Streptococcus pneumoniae* strains has caused major concern, and elucidation of the genetic mechanisms involved in the dissemination of drug resistance has become an important subject for study (11). The non-beta-lactam multidrug resistance in *S. pneumoniae* is related to the Tn*916* family of transposons; however, not much is known about their prevalence in different geographical regions.

The city of Mérida is one of the main centers for education and tourism in Western Venezuela, and the Spinetti Dini municipality is located in this metropolitan area. In 2007, its population consisted of 32,630 inhabitants, of which 2,871 were children under the age of 5 years. We investigated *S. pneumoniae* colonization in the noses and nasopharynges of 475 healthy children, aged 0 to 5 years and living in this municipality, using a sample size well above the calculated size for representative sampling. The prevalence of *S. pneumoniae* colonization was 27% ($n = 126$ strains), of which 38% ($n = 48$ strains) were found to be multidrug-resistant (MDR) strains.

The MDR strains belonged to serotypes 6B ($n = 23$), 14 ($n = 11$), 23F ($n = 6$), 23A ($n = 3$), 19A ($n = 1$), and 19F ($n = 1$); 3 strains were nontypeable. Among all MDR strains ($n = 48$) we investigated the presence of the Tn*916* family of transposons by PCR using the following genes as markers: *tetM* (14), *ermB* (13), *mefA* (15), *mefE* (15), cat_{pC194} (12), *aphA-3* (4), *int*-Tn*916* (8), *xis*-Tn*916* (1), *tnpA*-Tn*917* (5), *tnpR*-Tn*917* (5), and *int*-Tn*5252* (15). According to their serotype and genetic profile, 21 representative strains were selected for multilocus sequence typing (MLST) (9) (Table 1).

We identified nine previously described STs, as well as seven novel STs (4835, 4836, 4837, 5165, 5166, 5167, and 5168). We found strains displaying STs identical to those of Spain^{6B}-2 (ST90), Spain^{9V}-3 (ST156), Spain^{23F}-1 (ST81), and Taiwan^{23F}-15 (ST242) clones, as well as strains having single-locus variant (SLV) or double-locus variant (DLV) types of England^{14}-9 (ST15 and ST2563), Spain^{23F}-1 (ST5165), Tennessee^{23F}-4 (ST42), Taiwan^{19F}-14 (ST320 and ST5167), and Colombia^{23F}-26 clones (ST5166).

In addition to the presence of several international clones, we observed a group of serotype 6B MDR strains showing ST135/SLV135 displaying the same genetic profile (Table 1). Interestingly, at least 20 other ST135/SLV135 strains have been isolated in several European countries, such as Spain, Germany, Austria, United Kingdom, Poland, and Turkey, from healthy carriers as well as from patients suffering from both invasive and noninvasive pneumococcal diseases. These European strains display serotype 6B or 23F, and some of them are MDR (www.mlst.net). Whether these strains have the same genetic characteristics as the serotype 6B ST135/SLV135 clonal group we describe here and, consequently, constitute a pandemic clone remains to be investigated.

Different Tn*916*-related elements, including Tn*1545*, Tn*3872*, Tn*6002*, and Tn*6003*, have previously been detected in *S. pneumoniae* isolates with *ermB*-mediated erythromycin resistance, with geographical as well as temporal variations in their presence (4). Of these, the first *ermB*-carrying transposon described in *S. pneumoniae* (4), Tn*1545*, has been detected among Denmark^{14}-32, England^{14}-9, Spain^{14}-5, Spain^{9V}-3, and Poland^{6B}-20 clones (3). However, it was not found among our MDR strains.

On the other hand, 10% of the strains in our study carried the combination of *ermB*, *tetM*, *int*-Tn*916*, and *xis*-Tn*916* genes but not the *tnpA*-Tn*917* and *tnpR*-Tn*917* genes, which suggests the presence of the Tn*6002* element. This transposon was detected among strains related to the England^{14}-9 clone as previously described (3), as well as in strains related to the Tennessee^{23F}-4 clone. The Tn*6002*

* Corresponding author. Mailing address: Laboratory of Pediatric Infectious Diseases, Radboud University Nijmegen Medical Centre, P.O. Box 9101, 6500 HB Nijmegen, Netherlands. Phone: (31) 24 3666406. Fax: (31) 24 3666352. E-mail: P.Hermans@cukz.umcn.nl.

▽ Published ahead of print on 25 July 2011.

TABLE 1. Genetic characteristics, transposons, and STs among MDR strains[a]

Serotype	No. of strains with same characteristics	Presence of gene											Transposon(s)	Code(s) of representative strain(s) selected for MLST	MLST type[b]	Related international clone[c]
		ermB	*mefA*	*mefE*	*tetM*	*cat*	*int*-Tn*916*	*xis*-Tn*916*	*tnpR*-Tn*917*	*tnpA*-Tn*917*	*int*-Tn*5252*	*Aph3′-III*				
6B	7	+	−	−	+	+	+	+	+	+	+	−	Tn*5253* plus Tn*917*	I-121	90	Spain^{6B}-2
	6	+	−	−	+	−	+	+	+	+	+	−	Tn*5253* plus Tn*917*	I-15	90	Spain^{6B}-2
6B	8	+	−	−	+	+	+	+	+	+	−	−	Tn*3872*	I-163	135	None
														I-13	**4835** (SLV135)	
	1	+	−	−	+	−	+	+	+	+	−	−	Tn*3872*	H-183	135	None
	1	−	−	−	−	+	−	−	−	−	−	−	None	H-222	135	None
14	7	+	−	−	−	−	+	+	+	+	−	−	Tn*3872*-related	I-206	15	SLV England^{14}-9
	1	+	−	−	−	−	+	+	+	+	−	+	SpnRi3*erm*(B) or SpnAp3*erm*(B)	H-215	15	SLV England^{14}-9
	2	+	−	−	+	−	+	+	−	−	−	−	Tn*6002*	H-12	2563	DLV England^{14}-9
14	1	−	−	+	−	−	−	−	−	−	−	−	None	I-177	156	Spain^{9V}-3
23F	1	+	−	−	+	+	+	+	+	+	−	−	Tn*3872*	I-196	**5165** (SLV81)	SLV Spain^{23F}-1
	1	−	−	+	+	+	+	+	−	−	−	−	Tn*2009*	I-246	81	Spain^{23F}-1
23F	2	+	−	−	+	−	+	+	+	+	−	−	Tn*3872*	I-176	242	Taiwan^{23F}-15
	1	−	−	+	+	−	+	+	−	−	−	−	Tn*2009*	H-59	242	Taiwan^{23F}-15
23F	1	+	−	−	+	−	+	+	+	+	−	−	Tn*3872*	I-210	**5166**	DLV Colombia^{23F}-26
23A	3	+	−	−	+	−	+	+	−	−	−	−	Tn*6002*	I-120	42	DLV Tennessee^{23F}-4
19F	1	+	−	+	+	−	+	+	−	−	−	−	Tn*2010*	I-248	**5167** (SLV320)	DLV Taiwan^{19F}-14
19A	1	+	−	+	+	−	+	+	−	−	−	−	Tn*2010*	H-123	320	DLV Taiwan^{19F}-14
NT[d]	1	−	−	+	+	−	+	+	−	−	−	−	Tn*2009*	I-29	**5168** (SLV1106)	None
	1	−	−	+	+	−	+	+	−	−	−	−	Tn*2009*	I-34	**4837** (DLV1106)	None
	1	−	−	+	+	−	+	+	−	−	−	−	Tn*2009*	H-22	**4836** (SLV1106)	None

[a] All genetic characteristics except for MLST were explored in all 48 MDR strains. MLST was performed in 21 representative strains.
[b] New STs are depicted in bold numbers.
[c] SLV, single-locus variant; DLV, double-locus variant.
[d] NT, nontypeable strains.

element was ranked first among *ermB*-carrying strains in two previous studies as well (2, 4).

Tn*3872* is a composite mobile genetic element resulting from the insertion of the *ermB*-containing Tn*917* transposon into Tn*916* (4). In our collection, Tn*3872*-related elements were the most frequent elements associated with *ermB*-carrying MDR *S. pneumoniae* strains (44%) and were distributed across different serotypes, i.e., 6B, 23F, and 14. Further, Tn*3872*-related elements were found in strains related to different international clones, i.e., England^{14}-9, Spain^{23F}-1, and Taiwan^{23F}-15, as previously described (3), as well as in one strain related to the Colombia^{23F}-26 clone. In strains showing serotype 6B or 23F this transposon contained the expected *tetM* gene. However, a Tn*3872*-related element was observed in a group of serotype 14 strains in which the *tetM* gene was not detected. Interestingly, one of those strains carried the *aphA-3* gene. Similarly, Cochetti and coworkers in 2007 described two new Tn*3872*-related elements, SpnRi3*erm*(B) and SpnAp3*erm*(B), which carried the *aphA-3* gene but lacked the *tetM* gene (5).

Another transposon frequently observed among our MDR *S. pneumoniae* strains, particularly among serotype 6B strains, was Tn*5253* (13% of strains). Tn*5253* consists of a Tn*916*-like *tetM*-carrying element designated Tn*5251*, inserted within the Tn*5252* element, which carries the *cat* gene (10); this transposon has been described in a variety of international clones (10). In our study, the *cat* gene could not be detected in some Spain^{6B}-2 strains, suggestive of modifications of the transposon. As hypothesized previously (10), the high degree of variability described within the Tn*5253* transposon might explain the absence of the *cat* gene in those strains. Interestingly, in our strains the *tnpA* and *tnpR* genes, which are usually not present on Tn*5253* elements, were detected. We hypothesize that an additional Tn*917* transposon or a new composite element might be carried by the Spain^{6B}-2 strains circulating in our community.

Among the macrolide-resistant *mefE*-carrying strains, the presence of genetic elements carrying a mega-element such as Tn*2009* and Tn*2010* was expected (6). Indeed, the Tn*2009* transposon was found among *mefE*-carrying strains related to the Taiwan^{23F}-15 clone, as previously described (3), as well as in one strain of the Spain^{23F}-1 clone. However, the Tn*2010* transposon was detected only in the two *ermB*$^+$ *mefE*-carrying strains showing DLV types of the Taiwan^{19F}-14 clone. This Tn*2010* transposon has been described in MDR strains of ST320 and other STs belonging to clonal complex 271 that are currently emerging (7).

Interestingly, our results are indicative of horizontal gene transfer processes involving transposable elements within some *S. pneumoniae* clones. For instance, in the strains related to Spain^{6B}-2, Spain^{23F}-1, Taiwan^{23F}-15, and England^{14}-9 clones, more than one transposon was detected.

Variability in the structure of the Tn*5253* and Tn*3872* elements was observed among strains belonging to the same clone. The presence of different transposons or of similar transposons with different structures within a clone supports at least two hypotheses. First, it is likely that those clones have entered the population more than once. Second, horizontal gene transfer processes might have occurred within these clones after they entered this community. Mérida is a tourist city, frequently visited by people from all over the world. Consequently, it is plausible that the spread of these clones and transposons has been facilitated by international travel (16).

In summary, our study describes a variety of international resistant clones and a potentially new pandemic clone among MDR *S. pneumoniae* strains colonizing healthy children in our community. In addition to the demonstration of the presence of a plethora of transposons, our PCR analyses suggest structural changes in certain transposons, as well as the occurrence of new transposable elements in some MDR clones. Our study further demonstrates that nasopharyngeal carriage in children is an important reservoir for perpetuation and dissemination of MDR pneumococcal clones in the community.

REFERENCES

1. **Amezaga, M. R., P. E. Carter, P. Cash, and H. McKenzie.** 2002. Molecular epidemiology of erythromycin resistance in *Streptococcus pneumoniae* isolates from blood and noninvasive sites. J. Clin. Microbiol. **40:**3313–3318.
2. **Calatayud, L., et al.** 2007. Serotypes, clones, and mechanisms of resistance of erythromycin-resistant *Streptococcus pneumoniae* isolates collected in Spain. Antimicrob. Agents Chemother. **51:**3240–3246.
3. **Calatayud, L., et al.** 2010. Serotype and genotype replacement among macrolide-resistant invasive pneumococci in adults: mechanisms of resistance and association with different transposons. J. Clin. Microbiol. **48:**1310–1316.
4. **Cochetti, I., E. Tili, M. Mingoia, P. E. Varaldo, and M. P. Montanari.** 2008. *erm*(B)-carrying elements in tetracycline-resistant pneumococci and correspondence between Tn*1545* and Tn*6003*. Antimicrob. Agents Chemother. **52:**1285–1290.
5. **Cochetti, I., et al.** 2007. New Tn916-related elements causing *erm*(B)-mediated erythromycin resistance in tetracycline-susceptible pneumococci. J. Antimicrob. Chemother. **60:**127–131.
6. **Del Grosso, M., R. Camilli, F. Iannelli, G. Pozzi, and A. Pantosti.** 2006. The *mef*(E)-carrying genetic element (mega) of *Streptococcus pneumoniae*: insertion sites and association with other genetic elements. Antimicrob. Agents Chemother. **50:**3361–3366.
7. **Del Grosso, M., J. G. Northwood, D. J. Farrell, and A. Pantosti.** 2007. The macrolide resistance genes *erm*(B) and *mef*(E) are carried by Tn*2010* in dual-gene *Streptococcus pneumoniae* isolates belonging to clonal complex CC271. Antimicrob. Agents Chemother. **51:**4184–4186.
8. **Doherty, N., K. Trzcinski, P. Pickerill, P. Zawadzki, and C. G. Dowson.** 2000. Genetic diversity of the *tet*(M) gene in tetracycline-resistant clonal lineages of *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **44:**2979–2984.
9. **Enright, M. C., and B. G. Spratt.** 1998. A multilocus sequence typing scheme for *Streptococcus pneumoniae*: identification of clones associated with serious invasive disease. Microbiology **144:**3049–3060.
10. **Henderson-Begg, S. K., A. P. Roberts, and L. M. Hall.** 2009. Diversity of putative Tn*5253*-like elements in *Streptococcus pneumoniae*. Int. J. Antimicrob. Agents. **33:**364–367.
11. **Lynch, J. P., III, and G. G. Zhanel.** 2009. *Streptococcus pneumoniae*: does antimicrobial resistance matter? Semin. Respir. Crit. Care Med. **30:**210–238.
12. **Marchese, A., M. Ramirez, G. C. Schito, and A. Tomasz.** 1998. Molecular epidemiology of penicillin-resistant *Streptococcus pneumoniae* isolates recovered in Italy from 1993 to 1996. J. Clin. Microbiol. **36:**2944–2949.
13. **Nagai, K., et al.** 2001. Evaluation of PCR primers to screen for *Streptococcus pneumoniae* isolates and beta-lactam resistance, and to detect common macrolide resistance determinants. J. Antimicrob. Chemother. **48:**915–918.
14. **Olsvik, B., I. Olsen, and F. C. Tenover.** 1995. Detection of *tet*(M) and *tet*(O) using the PCR in bacteria isolated from patients with periodontal disease. Oral Microbiol. Immunol. **10:**87–92.
15. **Shiojima, T., Y. Fujiki, H. Sagai, S. Iyobe, and A. Morikawa.** 2005. Prevalence of *Streptococcus pneumoniae* isolates bearing macrolide resistance genes in association with integrase genes of conjugative transposons in Japan. Clin. Microbiol. Infect. **11:**808–813.
16. **Tomasz, A., et al.** 1998. Molecular epidemiologic characterization of penicillin-resistant *Streptococcus pneumoniae* invasive pediatric isolates recovered in six Latin-American countries: an overview. PAHO/Rockefeller University Workshop. Pan American Health Organization. Microb. Drug Resist. **4:**195–207.