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Distribution of Strain Families of *Mycobacterium tuberculosis* Causing Pulmonary and Extrapulmonary Disease in Hospitalized Children in Cape Town, South Africa

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We studied the association between strain family and extrapulmonary tuberculosis among 285 children presenting to a pediatric hospital. Extrapulmonary disease occurred in 56% of children without known human immunodeficiency virus infection, with meningitis accounting for 22% of the cases. Two strain families, LAM3/F11 and W-Beijing, predominated; but there was no overall association with extrapulmonary disease.

South Africa faces one of the worst tuberculosis epidemics in the world, with disease rates 60 times higher than those in the United States or Western Europe. The Western Cape region is especially affected, with an annual incidence of 917/100,000 population in 2002 and high rates of childhood tuberculosis and tuberculous meningitis (1, 2).

Childhood tuberculosis is characterized by a wide range of presentations, from limited pulmonary or nodal disease to severe extrapulmonary or disseminated disease (12). The determinants of these outcomes remain largely unknown. Hitherto, most attention has focused on the role of host factors. There is, however, increasing interest in the bacterial determinants that influence the outcome, with clear indications that clinical strains of *Mycobacterium tuberculosis* differ in their behaviors in vitro and in vivo (7).

Animal models demonstrate the increased virulence of selected strains (9, 10), but there is no compelling evidence from human studies that particular strain families are more virulent. An important manner in which strains may differ is their ability to disseminate and cause extrapulmonary tuberculosis.

We reviewed the records and typed all strains of *M. tuberculosis* from 285 children (<14 years of age) presenting to Red Cross War Memorial Children's Hospital, a pediatric referral hospital in Cape Town, South Africa, from December 2000 to December 2003 and compared the strain family with the site of disease.

Ethical approval for this study was granted by the Research Ethics Committee of the University of Cape Town (reference no. 320/2002).

Genotyping was performed by two PCR-based techniques, spoligotyping and 12-locus mycobacterial interspersed repetitive unit (MIRU)-variable number tandem repeat (VNTR) analysis. Spoligotyping was performed as described previously (6). MIRU-VNTR analysis was performed by the semiautomated method described by Supply et al. (13), modified for use on an ABI 3100 analyzer (Applied Biosystems, Foster City, CA). The shared type (ST) designation in the international spoligotype database (the SpolDB3 database) (5) has been renamed the spoligo-international type (SIT). The types determined from the typing data received a spoligo-international type and MIRU-VNTR international type (VIT) designation according to the cluster assignment after the sequences were processed by use of the SpolDB4 database (this is an updated database due to be released in 2005, when it will be available for public interrogation [K. Brudley et al., unpublished data]). SpolDB3 is available for public interrogation at www.pasteur-guadeloupe.fr/tb/spoldb3.

For analysis, cases with both pulmonary and extrapulmonary disease were classified as having extrapulmonary disease. Cases presenting with pleural effusion were categorized as having pulmonary disease. The chi-square test was used for contingency analysis.

Strains from 285 children were isolated over 3 years. In all 40 cases where more than one positive culture was obtained for a child, the infecting strain was identical by typing and was included once. The median age at the time of diagnosis was 2 years, with 75% of the children being less than 5 years of age. The age and sex distributions of the cases are shown in Table 1. Children with isolated lymph node or bone and joint tuberculosis were significantly older than those with pulmonary disease ($P = 0.0128$ and 0.0187, respectively). Extrapulmonary tuberculosis accounted for 47% of the cases, isolated pulmonary disease accounted for 46% of the cases, and isolated extrapulmonary lymph node accounted for 7% of the cases. The most common extrapulmonary manifestations were meningitis ($n = 58$), bone and joint tuberculosis ($n = 18$), pericardial tuberculosis ($n = 12$), peritoneal tuberculosis ($n = 5$), and miliary disease (with or without localized manifestations; $n = 39$). Testing for human immunodeficiency virus (HIV; by enzyme-linked immunosorbent assay and/or PCR for children

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TABLE 1. Sex and age distribution of cases

Site of disease and type of tuberculosis	Sex (% female)	Median age (mo)
Pulmonary	41.6	22
Extrapulmonary		
Meningitis	37.0	31
Lymph node	33.3	49
Bone and joint	57.9	43
Miliary	57.5	25
All cases	43.7	28

<18 months of age) was performed when it was clinically suspected (130 children). Among those tested for HIV infection, HIV infection was documented in 13% of the children with isolated involvement of lymph nodes, 20% of the children with pulmonary tuberculosis, and 19% of the children with extrapulmonary tuberculosis. There was no difference in the geographic distribution (based on postal code) of pulmonary and extrapulmonary cases (data not shown).

A total of 122 different MIRU alleles and 69 different spoligotypes were identified. Spoligotyping identified 11 genotype families, namely, LAM3/F11 ($n = 86$), W-Beijing ($n = 70$), X (European low-copy-number family by IS*6110*-based restriction fragment length polymorphism analysis; $n = 30$), S/F28 ($n = 21$), Zimbabwean ($n = 7$), other LAM ($n = 8$), Tuscany and Russian ($n = 12$) (Brudley et al., unpublished), Haarlem ($n = 11$), T clade ($n = 20$), CAS ($n = 5$) and "unknown" clades ($n = 15$) (4, 8).

If relevant clusters are defined on the basis of identical MIRU and spoligotyping alleles, a total of 27 clusters totaling 148 clinical isolates were found (52% of all the isolates studied) (Table 2).

Records were available for 282 cases. Of these, 53 were known to have HIV coinfection and were not included in the strain-clinical phenotype analysis. W-Beijing and LAM3/F11 did not appear to differ in their propensities to cause extrapulmonary disease in general or meningitis in particular (chi-square test value = 2.36; $P = 0.94$). The proportion of cases of extrapulmonary disease varied from 50 to 70% between strain families. The clinical presentations associated with the major strain families are detailed in Table 3.

Strains of *M. tuberculosis* from children in Cape Town are of interest for several reasons. First, the Western Cape region represents an area of particularly intense tuberculosis transmission, with incidence rates approaching 1% per annum (1). The reasons for this are as yet unclear and may relate to host or bacterial determinants. Second, since extrapulmonary disease is frequent among children hospitalized with tuberculosis in Cape Town, this represents an opportunity to study potential associations between strains and their ability to cause extrapulmonary disease.

Our results are in concordance with the work of others (15), suggesting that the LAM3/F11 and W-Beijing strains predominate among adults in the Western Cape. Taken together, these two clones represent half of all the clustered isolates.

TABLE 2. Clusters of isolates based on identical MIRU and spoligotyping alleles

Family designation[a]	Cluster no.	VIT no.	SIT no.	No. of isolates	% of clustered isolates
Central Europe 1	1	220	39	3	2
LAM3	2	236	33	6	4
LAM3	3	213	33	32	22
LAM4	4	249	130	2	1
LAM3	5	213	719	13	9
LAM3	6	213	1294	2	1
LAM3	7	213	2014	2	1
Russia 1	8	140	254	3	2
S	9	212	34	3	2
S	10	250	71	2	1
S	11	252	71	2	1
S	12	262	71	2	1
T3	13	257	73	2	1
Tuscany[b]	14	140	1737	2	1
W-Beijing	15	104	1	24	16
W-Beijing	16	223	1	5	3
W-Beijing	17	254	1	2	1
W-Beijing	18	83	1	3	2
W-Beijing	19	238	1	5	3
W-Beijing	20	17	1	9	6
W-Beijing	21	99	1	7	5
W-Beijing	22	245	1	2	1
X1	23	112	119	5	3
X1	24	117	119	2	1
X1	25	258	2019	2	1
X3	26	34	92	4	3
Z	27	237	811	2	1

[a] Family and VIT-SIT designations are from SpolDB4 (Brudley et al, unpublished).
[b] Data are from reference 8.

This dominance may be due to ecological or historical factors or, alternatively, may be because they are specifically virulent in these populations.

It has been suggested that the W-Beijing lineage of clinical isolates is more virulent than other clinical strains in animal models (3, 11). This virulence has been attributed to the ability of these strains to produce a phenolic glycolipid able to interfere with host immunity (11). In our study we did not find an association between W-Beijing strains and a propensity to cause extrapulmonary disease or meningitis. We chose extrapulmonary disease and meningitis as outcome measures, since these are common and easily defined and represent a clear spectrum of disease. It is possible that the use of alternative measures of severity might reflect more subtle differences between strains. In addition, these results should not be extrapolated to adult tuberculosis, where the strain distribution of extrapulmonary cases may be different.

There is considerable diversity within strain families based on MIRU-VNTR analysis and, more recently, deletion analysis (14). It is feasible that the genotypic diversity between strains within the same family may influence the clinical outcome of infection. Higher-resolution typing of a functionally important polymorphism is necessary to address this in detail.

The W-Beijing and LAM3/F11 strain families predominate among children presenting to hospital with tuberculosis in our setting. There was no evidence that these strain families dif-

TABLE 3. Site of disease among children without known HIV infection, classified according to strain family

Strain family	No. of strains (% of total strains)	No. of pulmonary strains only (% of family)	No. of extrapulmonary strains (% of family)	Major extrapulmonary disease sites (no. [%] of patients)			
				Meningitis	Lymph node	Bone and joint	Miliary[a]
All HIV negative	229 (100)	101 (44)	128 (56)	51 (22)	18 (8)	18 (8)	23
LAM3/F11	72 (31)	33 (46)	39 (54)	16 (22)	4 (6)	6	4
W-Beijing	52 (23)	26 (50)	26 (50)	12 (23)	4 (8)	6	5
X	24 (10)	9 (38)	15 (62)	4 (16)	3 (13)	0	4
S	19 (8)	7 (37)	12 (63)	6 (32)	3 (16)	0	4
T	15 (7)	5 (33)	10 (67)	6 (40)	0 (0)	1	2
Other	47 (21)	21 (45)	26 (55)	8 (17)	4 (9)	5	4

[a] Miliary disease frequently occurred together with localized disease at an extrapulmonary site.

fered in their propensity to cause extrapulmonary infection; however, further analysis of the strain diversity within families is needed to exclude such an association.

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