Discrepancies between *Aedes aegypti* identification in the field and in the laboratory after collection with a sticky trap

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Currently, sticky traps are regularly employed to assist in the surveillance of Aedes aegypti *infestation. We tested two alternative procedures for specimen identification performed by local health agents: directly in the field, as recommended by certain manufacturers, or after transportation to the laboratory. A total of 384 sticky traps (MosquiTRAP) were monitored monthly during one year in four geographically representative Brazilian municipalities. When the same samples were inspected in the field and in the laboratory, large differences were noted in the total number of mosquitoes recorded and in the number of specimens identified as* Ae. aegypti *by both procedures. Although field identification has the potential to speed vector surveillance, these results point to uncertainties in the evaluated protocol.*

Key words: *Aedes aegypti* - MosquiTRAP - entomological surveillance - dengue vector

One of the most important challenges faced by field entomologists is to develop a reliable and effective technique to sample the target species. Such a tool should provide significant information about several aspects of insect biology, including population density, dispersal and survival estimates. If we focus specifically on disease vectors, efficient and unbiased sampling tools are required to provide relevant insights into the effectiveness of vector control strategies and the risk of disease transmission (Service 1993).

On the American continent, the mosquito *Aedes aegypti* is the primary vector of dengue fever and is distributed from the United States of America to the Southern Cone of South America (Powell & Tabachnick 2013). This species is highly anthropophilic, living in close association with human dwellings: mosquitoes are more abundant in highly urbanised areas, feed preferentially on human blood, and lay eggs in man-made containers (Clements 1999). Although there is an extensive literature on *Ae. aegypti* sampling, the lack of a "gold standard" means that there is a need to continue the development of new surveillance techniques.

In the past decade, special attention has been focused on the design of mosquito traps. Collection of adult mosquitoes should provide better infestation indices than alternative techniques based on surveys of immature insects because the adult population is responsible for disease transmission. Sticky traps are a popular type of adult trap and many versions have been developed worldwide. One of these devices is the MosquiTRAP, which was developed in Brazil and has been subjected to exhaustive tests to determine its efficiency in collecting *Ae. aegypti* mosquitoes (Fávaro et al. 2006, Maciel-de-Freitas et al. 2008, de Resende et al. 2010, 2012, 2013). The MosquiTRAP consists of a one-litre matte black plastic cylindrical container filled with approximately 300 mL of 10% grass infusion substrate; alternatively, a synthetic oviposition attractant is employed. A sticky card is placed on the inner wall of the trap to capture gravid adult female mosquitoes attracted to the trap (Fávaro et al. 2006). One advantage of sticky traps over alternative sampling techniques is the opportunity to accelerate the surveillance procedure by counting and identifying captured mosquitoes in the field rather than in the laboratory under a microscope. In theory, this approach is possible because the specimens are fastened to an adhesive card, whereas they remain free and flying in other trap designs. This approach is included in the original MosquiTRAP protocol, which recommends that health agents perform species identification in the field to accelerate *Ae. aegypti* surveillance (Resende et al. 2010).

However, there is no consensus regarding where the identification procedure should be performed for various types of sticky traps. The site of identification has been reported to be in the field (Facchinelli et al. 2008), in the laboratory (Williams et al. 2006, Chadee & Ritchie 2008) or has even not been mentioned (Santos et al. 2012).

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In this study, we tested the hypothesis that misidentification of mosquitoes may be an important source of uncertainty and measurement error, undermining the potential gain from vector control policies based on field identification alone. This problem would be especially significant if a surveillance system based on the adult sticky traps were applied in a routine large-scale program. The motivation for the present evaluation is the observation that in routine surveillance programs, working conditions are generally not favourable for the accurate identification of mosquito species in the field. We present results from a MosquiTRAP surveillance study conducted in four cities where *Ae. aegypti* identification in the field was compared with further identification of the same samples under laboratory conditions using a stereomicroscope.

The field work, performed within the scope of the Brazilian dengue control program, was conducted in Parnamirim, state of Rio Grande do Norte (December 2010-November 2011), Santarém, state of Pará (March 2011-February 2012), Nova Iguaçu, state of Rio de Janeiro (July 2011-June 2012) and Campo Grande, state of Mato Grosso do Sul (December 2011-November 2012), all municipalities representing Brazilian regions with a high incidence of dengue. All of these cities have adequate routine mosquito surveillance programs, which include laboratory teams trained in the identification of *Ae. aegypti*, *Aedes albopictus* and *Culex quinquefasciatus*. In addition to their previous experience, a specific two-day training program was conducted for all field workers before initiating the study. Each municipality had 12 health agents responsible for MosquiTRAP installation, mosquito identification in the field, trap deployment and storage of sticky cards for laboratory team identification of the same samples. The complete training lasted two days (16 working hours) and was conducted before field surveillance began. The ability of field workers to identify mosquitoes under field conditions was evaluated during every round of MosquiTRAP monitoring, in which a consultant of the Brazilian Health Ministry or one of the co-authors supervised health agents during one week. In each municipality, two or three additional field workers were trained to guarantee the quality of mosquito identification if it was necessary to replace health agents.

Monthly, during one year, three areas of 1 km^2 in each of the four studied municipalities received 96 MosquiTRAPs loaded with a synthetic attractant (32 traps per 1 km^2 area, 384 traps in the study). After informed oral consent had been received from the householder, sticky traps were installed on the premises to be sampled. After seven days, a health agent collected the trap. While still in the house, the agent used a hand magnifying glass to inspect and identify mosquitoes stuck to the card. The traps were then carefully stored and brought to the entomological laboratory where mosquitoes were identified again, this time by laboratory technicians, with the help of a stereomicroscope and identification keys (Consoli & Lourenço-de-Oliveira 1994).

The Jaccard index was used to test the degree of similarity among mosquitoes identified as *Ae. aegypti* in the field or in the laboratory (Legendre & Legendre 1998). This index quantifies the similarity between two finite sample sets. It is defined as the size of their intersection relative to the size of the sum of the sample sets. The Jaccard index varies between 0-1; in the present work, 0 means no agreement, whereas 1 means total agreement between the field and laboratory measurements. Because the data take the form of number of mosquitoes per trap, it is not possible to assess the individual-level identification status of each specimen. To circumvent this problem, maximal agreement between field and laboratory identifications was assumed. Thus, for example, suppose that a trap contained 10 mosquito specimens, of which four and five specimens were identified as *Ae. aegypti* in the field and in the laboratory, respectively. In this case, we assumed that the four mosquitoes identified in the field belonged to the same group identified in the laboratory. This is a conservative assumption that favours field-laboratory agreement.

In general, the total numbers of mosquitoes recorded in the field were higher than those detected by the laboratory personnel (Table). This difference varied considerably among sites. A difference of < 15% between field and laboratory measurements was detected in Parnamirim and Nova Iguaçu, whereas a 70% difference was observed in Campo Grande. In Santarém, the opposite pattern was observed: laboratory measurements exceeded field records by 13%. Differences between laboratory and field measurements can be related to misidentification and to counting errors under field conditions as well as to losses or damage of insects during transportation to the laboratory that would interfere with identification. Also contributing to these differences are the difficulties involved in cleaning the sticky cards between two collection events. Because MosquiTRAPs were installed monthly and the sticky cards do not expire for 60 days, they were used twice, following advice that was intended to reduce costs. Further studies should investigate whether card reuse decreases the correctness of mosquito identification.

Overall, the amount of material not identified as *Aedes* (Table) (group "Non-*Aedes*", see also the "Non-*Aedes* total captured" column) varied between 35-70%, confirming the low to moderate specificity of MosquiTRAP for catching *Aedes* mosquitoes (Maciel-de-Freitas et al. 2008, Resende et al. 2013). In all municipalities, the proportion of these mosquitoes classified as non-*Aedes* was higher in field than in laboratory measurements. The difference between the field and the laboratory varied from 3% in Parnamirim and Nova Iguaçu to nearly 40% in Campo Grande. In Campo Grande, in addition to the difference of 70% in the number of specimens between the field and the laboratory, the contribution of the non-*Aedes* group to the total in the field assessment was almost 90%. These findings indicate specific difficulties with the Campo Grande results related to the work of the health agents in the field.

If only mosquitoes belonging to the genus *Aedes* are considered, except for Santarém, more than 80% were identified to the species level under both field and laboratory conditions (Table) [column "(aeg + alb)/*Aedes*)"].

TABLE

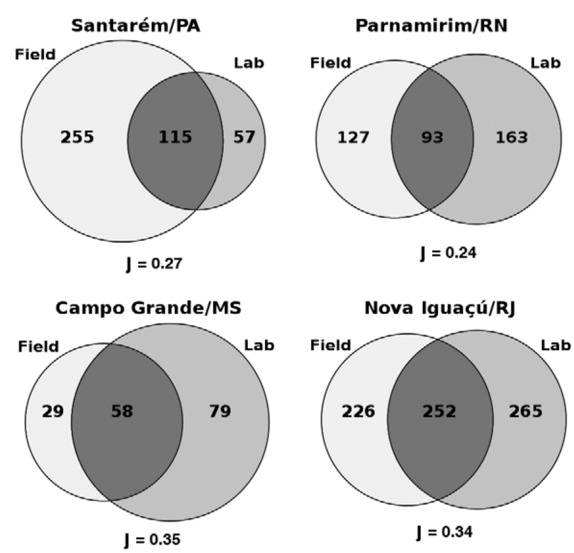
Total numbers and percentages of mosquitoes registered and identified in the field or in the laboratory in each locality

		n					%			
Municipality (state)	Identification protocol	Total captured	*Aedes aegypti*	*Aedes albopictus*	*Aedes* sp.	Non-*Aedes*	Non-*Aedes*/ total captured	(aeg + alb)/ *Aedes*[a]	aeg/ *Aedes*[a]	aeg/ (aeg + alb)
Santarém	Field	3,976	370	94	779	2,733	68.7	37.3	29.8	79.7
(Pará)	Laboratory	4,494	172	0	1,691	2,631	58.5	9.2	9.2	100
Parnamirim	Field	786	220	79	68	419	53.3	81.5	59.9	73.6
(Rio Grande do Norte)	Laboratory	684	256	79	1	348	50.9	99.7	76.2	76.4
Nova Iguaçu	Field	1,066	478	48	121	419	39.3	81.3	73.9	90.9
(Rio de Janeiro)	Laboratory	949	517	84	0	348	36.7	100	86	86
Campo Grande	Field	1,050	87	16	15	932	88.8	87.3	73.7	84.5
(Mato Grosso do Sul)	Laboratory	305	137	11	5	152	49.8	96.7	89.5	92.6

a: *Aedes* accounts for the sum of *Ae. aegypti*, *Ae. albopictus* and *Aedes* sp.; *Aedes* sp.: specimens identified only up to the genus level; non-*Aedes*: mosquitoes belonging to other genera or that could not be identified as *Aedes* ones. The columns "percent" exhibit ratios of non-*Aedes* mosquitoes relative to the total of caught specimens (non-*Aedes*/total captured), of *Aedes* mosquitoes identified up to the species level [*Ae. aegypti* (aeg) or *Ae. albopictus* (alb)] among those identified as *Aedes* [(aeg + alb)/*Aedes*] and of identified *Ae. aegypti* mosquitoes, both considering all specimens identified as *Aedes* (aeg/*Aedes*) and those identified up to species level, *Ae. aegypti* and *Ae. albopictus* [aeg/(aeg + alb)].

The percentage of identification to the species level was higher in the laboratory (96-100%) than in the field (81-88%). The same was true for the specimens identified as *Ae. aegypti* (Table) (column "aeg/*Aedes*"): this proportion was higher in the laboratory (75-90%) than in the field (60-75%). In all cases, a high proportion of *Ae. aegypti* mosquitoes among those identified to the species level was observed (Table) [column "aeg/(aeg + alb)"]. Lastly, the degree of similarity between *Ae. aegypti* identification in the field and in the laboratory based on the Jaccard index was low, ranging from 0.24-0.35 in the various municipalities (Figure).

The significant differences observed between *Ae. aegypti* identification in the field and in the laboratory suggest that these procedures provide discordant measurements of mosquito infestation and raise questions regarding the most appropriate protocol. Although Resende et al. (2010) reported a high level of agreement between field and laboratory identifications, the authors did not explain how the problem of potentially unidentifiable specimens was circumvented. Laboratory identification may be more accurate if health agents working in the field are subjected to multiple biotic and environmental stressors. In addition, mosquitoes adhere to the adhesive card in many different positions, a characteristic that may hamper identification that is primarily performed under field conditions. Moreover, loss or damage of material during transportation to the laboratory may be a potential problem for laboratory identification. Note that either under field or laboratory conditions, the loss or damage of identifiable specimens is inherent to sticky traps, a characteristic that introduces unforeseen uncertainties in the population estimators and might result in biased entomological indicators. Future studies should determine to what extent these differences could impact routine entomological surveillance.



Conformity of identification in the field and in the laboratory of *Aedes aegypti* specimens caught with MosquiTRAP. For each municipality mosquitoes identified only in the field are at the left side, while those identified only at the laboratory, at the right side. The intersection represents specimens identified by both procedures, considering maximal conformity after inspection of each individual field bulletin. The Jaccard index (j), that reflects similarity between both mosquito sets, varies from 0 (completely distinct sets) to 1 (total identity). The Brazilian states: Mato Grosso do Sul (MS), Pará (PA), Rio de Janeiro (RJ) and Rio Grande do Norte (RN).

The proper use of mosquito traps requires an adequate work environment and dedicated worker teams. In this sense, it is important to invest time and resources on the training and qualification of health agents. Although traps decrease the bias in sampling resulting from variation in the motivation of health agents, these tools are still dependent on human operation and skills.

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TABLE I

Codes of clusters, cluster-groups and orphan cases identified by spoligotyping, mycobacterial interspersed repetitive units-variable number tandem repeats (MIRU-VNTR) and restriction fragment length polymorphism (RFLP)-IS*6110*

Cluster	Case number	Code S[a]	Code M[b]	Code R[c]
Group 1: *Mycobacterium tuberculosis* (MTB)-isolates clustered by spoligotyping, MIRU-VNTR and RFLP-IS*6110* (SMR)				
SMR1	803	777777777760771	234332242426222	09.228268288392472492548732920
	967	777777777760771	234332242426222	10.228268284384464476492548732916
SMR2	929	767777777720771	233532233536433	08.052152244332492600732748
	1003	767777777720771	233532233536433	08.052152244332492600732748
SMR3	852	777737777760731	223631333527423	10.140216256368468636696732744940
	918	777737777760731	223631333527423	10.140216252368464636700732744940
Group 2: MTB-isolates clustered by spoligotyping and RFLP-IS*6110* (SR)				
SR1	982	777777777760731	232533223226222	11.100168220256376480496532572608772
	867	777777777760731	232533223226232	10.184216256376480492532572632776
SM1	910	777777777760771	233332142425232	11.212256272412440476492532576604800
	856	777777777760771	233332142425232	10.212256412476492532576600800856
SM2	880	777776777760771	233533433549453	03.208392740
	823	777776777760771	233533433549453	03.212392740
SM3	997	777776777760771	233533433549433	02.216744
	932	777776777760771	233533433549433	02.212744
SM4	1068	777776777760771	243434433338233	04.188380492740
	936	777776777760771	243434433338233	04.196384496740
SM5	939	777776777760771	243524433338233	04.212384496744
	835	777776777760771	243524433338233	04.208380492740
Group 3: MTB-isolates clustered by spoligotyping and MIRU-VNTR (SM)				
SM6	1061	700076777760771	243534433233433	04.208376488740
	879	700076777760771	243534433233433	04.208376488740
	810	700076777760771	243534433233433	05.100208380492740
	1020	700076777760771	243534433233433	04.208376488740
SM6	1040	700076777760771	243534433233433	04.204372484736
Group 4: MTB-isolates clustered by spoligotyping (S)				
S1	1044	777776777760771	243523433338233	3.420488740
	1057	777776777760771	243535433238233	5.192384492600744
	1034	777776777760771	233533433539433	04.196216328748
	1011	777776777760771	23353343343(8,6)43	03.216424740
S2	878	777776777760771	233533433644433	04.212524740812
	878	777776777760771	233533433644433	04.212524740812
	836	777776777760771	2335334335,455433	02.216740
	1021	777776777760771	23253113344(8,5)(5,4,2)33	03.216280744
	930	777776777760771	243534433236233	05.208384448496740
	829	777776777760731	243534233548433	03.216424740
	899	777776777760731	2335324335412430	02.216492
	1051	777777777760771	234432242424232	10.208244264368444452464524536712768
	1035	777777777760771	233431243425242	11.176216228332428544608632692700748940
	1024	777777777760771	234532242425232	9.156184228268388468492544732
	986	777777777760771	234332242325232	9.204228272288388464492544732

	917	777777777760771	234532242426232	12.0962122242723683924564725327247776904
	806	777777777760771	234532232426232	11.212252272372392456472532720776908
	1053	777777777760771	234432142325232	12.208252412436472488528572600756800876
	1022	777777777760771	22453224242(5,2)232	12.228268288384464488544600632696732784
	969	777777777760771	234532142426232	09.204260348368448464524712900
	921	777777777760771	323631333427423	10.148228264380476644704740752948
S2	874	777777777760771	234532232(3,2)25232	08.380388440456480536624728
	983	777777777720771	233533233436433	13.052092244316380508568596624720748824916
	832	777777777720771	235532222225232	12.120240252272372452476532608672720772
	837	777777777720771	233533233535423	08.052244332380496600732748
S3	831	777777777760731	234632241426232	09.068220256276376460476536724
	1015	777777777760731	231434242125252	10.220232252372476532572688712772
S4	809	777776777760601	243531443449432	04.112232748772
	1042	777776777760601	243533433347273	03.236748792
S5	840	777777774020771	234432242326232	11.248256276360376396464480536728776
	853	777777774020771	253533233627433	9.060248324336384492732752884
S6	862	677737607760771	241631342336122	12.200216236264424432444472536612680948
	1047	677737607760771	241631342436122	10.204220240268372428472540812948
	855	777737607560771	233525342239123	12.216236248264376420444492556784832868
	900	777737607560771	233521342236120	13.088220236268320372416440492680708776856
S7	941	777777607760771	242331242439122	9.220236268420440472488648892
	877	777777607760771	242531422438122	13.132212232264392420440472484516528648892
	943	777777607760771	143323352136123	13.176216236268332444472596632716780824860
S8	804	776177604360771	243433242434212	10.228264280420440472520624640940
	1017	776177604360771	243433242334212	11.228260280416440472488520620636940
S9	992	776137607760771	2425334223310212	13.152212232260276432440468496516624796900
	801	776137607760771	242(5,4)31422434212	11.208224256272428464512712896
	1037	776137607760771	3625334224310212	11.212232264280432440468516624784900
S10	811	677777477413771	5432421448105220	11.192392468496544628640668716772784
	813	677777477413771	5432421449105220	13.104168264388496544580620644672720772784
S11	816	777776777760771	233533433439433	02.212740
	949	777776777760771	233533433447433	02.212744
	888	777776777760771	233534433549433	02.212740
	859	777776777760771	233543433349413	02.212740
	828	777776777760771	223533433539231	02.212740
	887	777776777760771	243533433338233	04.196384496740
	980	777776777760771	243432333338233	04.192384496744
	903	777776777760771	243534433337233	04.192384496740
	1059	777776777760771	2325344333382(6,5,4)3	04.212380492740
	998	777776777760771	243534333338233	04.216384496744
S12	850	777776777760601	243533443246433	02.236752
	814	777776777760601	243533443248433	02.236752
S13	982	777777777760731	232533223226222	10.0168220256376
	867	777777777760731	232533223226232	09.42162563764804
Group 5: MTB-isolates clustered by MIRU-VNTR (M)				
M1	1027	777777777760771	234532242325232	08.224264384424464488540728
	1041	000000177760771	234532242325232	11.250366417446479538563579604742792
M2	1033	777777777720771	253533133638433	09.052244312504572596628724744
	1048	000000007720771	253533133638433	10.196241313379504571596713738867
M3	851	777777777720771	253533133536433	12.052068244332380512600732748808900908
	870	777777774720771	253533133536433	7.312359581

↳

Group 6: orphan cases. MTB-isolates not clustered by any genotyping method			
1008	777777577760771	233332142525232	10.2002454044334674885215635927 79
962	777776477760771	233533433448433	01.721
923	763777777760371	232534243425232	08.35538943345951
926	777777763660771	234432232435232	10.154191225246342429450508742788
822	576377777760771	333534344519232	10.303311346377455476745797827
1060	776377777760771	333335343518232	11.19220834637941348851375879 2825904
925	776357777760571	343534344518232	09.30336339447249
1025	566377777760771	333534244519232	10.333375408425483513792817842875
841	777731777760731	226611433448423	09.23827334660266
845	777761777760771	225522242326232	05.37744545951168
805	777777777760031	233321242424252	12.225290380450485545563628671749784840
812	617776777760601	243434443435433	01.675
800	677735607760771	221631342335122	09.238 337 380 393 4
1013	677737601760771	241631341334122	11.192225254363413425438458467521842
854	777777607560771	225566342237122	07.40742447248562
957	777777606760771	233631143425272	11.183254300396517575600654667713892
864	777777207760771	225555342237122	09.19921624735139
904	777777205760771	343415342226122	08.34638040341647
1026	777777607420771	231531441438132	10.204225258417442471646763846875
1007	776137607760731	242532322338212	11.204216250271408429458508592733875
863	776177607760731	224422442345212	14.208220247268316403420459472511528589645878
1014	777700017560771	242522342247122	12.213229267342375421442483642663729758
808	777777760000171	232533241323232	6.233376415
868	777777760000131	232533241323232	07.39844146351953
961	777777760000611	243433433343423	06.32543761370472
819	477576770000000	252323433224433	05.2854195066848
919	676573777077600	321322257333421	01.632
839	000000000003771	227744444658432	17.27730732935538144248549854164968471974075777979 2874
826	777777777760771	234332242426222	09.21625627237645647653272 4904
827	777737777760731	333631333528423	10.140172216252368636696732744932
819	477576770000000	252323433224433	05.2854195066848
919	676573777077600	321322257333421	01.632
839	000000000003771	227744444658432	17.27730732935538144248549854164968471974075777979 2874
826	777777777760771	234332242426222	09.21625627237645647653272 4904
827	777737777760731	333631333528423	10.140172216252368636696732744932
1039	677776777760771	243545433337233	05.17537945472986
1019	777776777560771	233533233648433	04.375725800871
948	777736777760771	233533333548433	05.188712
906	777777777720671	253531333435433	09.294 359 390 489 58
942	777777777760711	234432232422232	11.171208233250354375433454513742858

a: spoligotyping octal codes were formed according to Dale et al. (2001); *b*: MIRU-VNTR-codes. These indicate the number of repetitions of MIRU 4, 10, 16, 26, 31, 40, Mtub 04, ETRC, ETRA, QUB11b, Mtub21, QUB26, Mtub30, Mtub39 and QUB4156 (Supply et al. 2006) identified in each MTB-isolate; *c*: RFLP-IS*6110* codes were composed of two arms of numbers, which were separated by a dot. The left arm is constituted by one or two figures. This arm includes information about the quantity of bands found in each MTB isolate. The right arm contains the R_f of every DNA band having an IS*6110* element. Each R_f was represented by three numbers. From left to right, the R_f values were placed in descending order of DNA-molecular weight. The R_f values were calculated by dividing the distance between the origin and front of the electrophoresis by the distance traveled by each DNA fragment containing a IS*6110*-element multiplied by 1×10^3.

TABLE II

Groups of clusters and risk factors under each participant was exposed

Cluster	Case number	Medical facility: First level[b]	Medical facility: Second level[c]	Municipality	Comorbidity	Occupation	Profile of drug resistance	Drug consumption
Group 1: SMR[a]								
SMR1	803	29	4	Guadalupe	None	Car painter	SRRSR	Occasional drinker
	967	6	6	San Nicolás	None	Miner	No data	Alcoholic
SMR2	929	27	4	Guadalupe	Diabetes mellitus 2	Housewife	RRRSS	Occasional smoker
	1003	32	4	Guadalupe	None	Unskilled worker	RRSSS	No data
SMR3	852	35	17	Monterrey	Diabetes mellitus 2	Baker	RRRSR	None
	918	35	17	Monterrey	Diabetes mellitus 2	Production supervisor	RRRSR	Occasional drinker/smoker
Group 2: SR[d]								
SR1	982	29	4	Guadalupe	Unknown	Unskilled worker	RRSRR	No data
SR1	867	37	17	Monterrey	None	Mason	SRSSS	None
Group 3: SM[e]								
SM1	910	30	4	Monterrey	Diabetes mellitus 2	Merchant	SSSSR	None
	856	43	6	Monterrey	Bronchitis	Fork lift operator	SSSSS	Alcoholic
SM2	880	5	21	Monterrey	Diabetes mellitus 2	Plasterer	SSSSS	Smoker
	823	26	17	Monterrey	Diabetes mellitus 2	Merchant	SSSSS	Smoker/alcoholic
SM3	997	36	17	Monterrey	Diabetes mellitus 2	Taxi driver	SSSSS	Alcoholic
SM3	932	58	21	Santa Catarina	None	Painter	SRRSS	Alcoholic
SM4	1068	7	21	Santa Catarina	Diabetes mellitus 2	Truck driver	RRRSS	Smoker
	936	58	21	Santa Catarina	Diabetes mellitus 2	Unskilled worker	RRRSR	Smoker/alcoholic
SM5	939	36	17	Monterrey	None	Housewife	SRSSS	Occasional drinker
	835	35	17	Monterrey	Diabetes mellitus 2	Machine operator	SSSSS	Smoker/alcoholic
SM6	1061	26	17	Monterrey	None	Maid	SSSSS	Occasional drinker
	879	7	21	Santa Catarina	None	Housewife	SSSSS	None
	810	28	17	Monterrey	None	Mechanic	SSSSS	Smoker/alcoholic
	1020	7	21	Santa Catarina	Diabetes mellitus 2	Mason	SSSSS	Alcoholic

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				Group 4: S[f]				
S1	1040	27	4	Guadalupe	Allergies	Housewife	SSSSS	Alcoholic
	1044	20	4	Guadalupe	None	Manufacturer of electric coils	RSSSS	None
	1057	23	17	Monterrey	Unknown	Unskilled worker	SSSSS	No data
S1	1034	35	17	Monterrey	Diabetes mellitus 2	Housewife	SSSSS	None
	1011	32	4	Guadalupe	None	Unskilled worker	SSSSS	No data
S1	878	7	21	Santa Catarina	Asthma	Secretary	SRSSS	None
	836	37	17	Monterrey	None	Housewife	SRSSS	None
	1021	35	17	Monterrey	None	Assembler	SRSSS	None
	930	27	4	Guadalupe	None	Housewife	SSSSS	None
	829	35	17	Monterrey	None	Retired	SSSSS	None
	899	7	21	Santa Catarina	None	Unskilled worker	SSSSS	None
	1051	64	21	Santa Catarina	None	Unskilled worker	SSSSS	No data
	1035	35	17	Monterrey	None	Housewife	SSSSS	None
	1024	27	4	Guadalupe	None	Security guard	SSSSS	Smoker
	986	39	17	Monterrey	None	Unskilled worker	RRRRR	No data
	917	35	17	Monterrey	None	Driver	SSSSS	Smoker/alcoholic
	1053	31	6	Monterrey	Diabetes mellitus 2	Housewife	SSSSS	None
S1	1022	35	17	Monterrey	Diabetes mellitus 2	Housewife	SSSSS	Smoker
	969	29	4	Guadalupe	None	Retired	RRRSR	None
	921	43	6	Monterrey	Diabetes mellitus 2	Housewife	RRRSR	None
	874	35	17	Monterrey	Diabetes mellitus 2	Housewife	No data	None
S2	983	36	17	Monterrey	None	Painter		
	832	35	17	Monterrey	None	Cook	SSSSS	Smoker/alcoholic
	837	39	17	Monterrey	Left-stroke hemiplegic	House keeper	No data	None
S3	831	28	17	Monterrey	Psoriasis	Housewife	RRSSR	Smoker
	1015	7	21	Santa Catarina	Diabetes mellitus 2	School house keeper	SSSSS	None
S4	809	28	17	Monterrey	COPD[g]	Retired	RRSSS	Smoker
	1042	43	6	Monterrey	None	Unskilled worker	SSSSS	No data
S5	840	35	17	Monterrey	None	Unskilled worker	RRRSS	No data
	853	36	17	Monterrey	Diabetes mellitus 2	Retired	RRSSS	Alcoholic
S6	862	36	17	Monterrey	None	Retired	RSSSS	None
	1047	43	6	Monterrey	None	Unskilled worker	SSSSS	No data
	855	37	17	Monterrey	None	Quality inspector	SSSSS	None
	900	7	21	Santa Catarina	None	Security guard	SSSSS	Alcoholic

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S7	941	5	21	Monterrey	Diabetes mellitus 2	Secretary	SRSSR	None
	877	7	21	Santa Catarina	Anxiety	Financial adviser	SSSSS	None
	943	3	3	Monterrey	None	Operator	SRSSS	Occasional drinker
S8	804	29	4	Guadalupe	None	Welder	RRSSS	Occasional smoker
	1017	35	17	Monterrey	None	Welder	SSSSS	Occasional smoker/drinker
S9	992	43	6	Monterrey	None	Retired	RRRSR	None
	801	29	4	Guadalupe	None	Welder	SSSSS	Smoker
	1037	29	4	Guadalupe	None	Unskilled worker	SSSSS	No Data
S10	811	28	17	Monterrey	Diabetes mellitus 2	Housewife	SSSSS	None
	813	26	17	Monterrey	Diabetes mellitus 2	Housewife	RRRSR	None
S11	816	35	17	Monterrey	Diabetes mellitus 2	Housewife	SSSSS	No data
	949	35	17	Monterrey	Diabetes mellitus 2	Charger	SSSSS	Smoker/alcoholic
	888	6	6	Monterrey	None	Unskilled worker	No data	No data
	859	3	3	Monterrey	Diabetes mellitus 2	Urban bus driver	SSSSS	Smoker/alcoholic
	828	26	17	Monterrey	None	Unskilled worker	SRSRS	None
	887	36	17	Monterrey	None	Unskilled worker	SSSSS	None
	980	29	4	Guadalupe	None	Production supervisor	RRRSS	None
	903	3	3	Monterrey	Diabetes mellitus 2	Street vendor	RSSSS	Smoker/alcoholic
S11	1059	43	6	Monterrey	None	Unskilled worker	SSSSS	No data
	998	36	17	Monterrey	Diabetes mellitus 2	Retired	RRSSS	None
S12	850	43	6	Monterrey	Diabetes mellitus 2	Hair dresser	SSSSS	Smoker/alcoholic
	814	35	17	Monterrey	Asthma	Merchant	RSSSS	No data
S13	982	29	4	Guadalupe	None	Unskilled worker	RRSRR	No data
	867	35	17	Monterrey	None	mason	SRSSS	None
Group 5: M[h]								
M1	1027	35	17	Monterrey	None	Unskilled worker	SSSSS	No data
	1041	30	4	Monterrey	Sarcoidosis	Retired	SSSSS	No data
M2	1033	35	17	Monterrey	None	Housewife	SSSSS	No data
	1048	2	2	Monterrey	Diabetes mellitus 2	Trucking	SRRSS	Smoker/alcoholic
M3	851	64	33	Monterrey	Diabetes mellitus 2	Housewife	SSSSR	None
	870	15	6	San Nicolás	Diabetes mellitus 2	Private driver	SSSSS	Smoker/alcoholic
Group 6: orphan cases[i]								
1008		15	6	San Nicolás	None	Bus driver	SSSSS	Alcoholic
962		32	4	Guadalupe	None	Unskilled worker	RSSSS	No data

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923	36	17	Monterrey	Diabetes mellitus 2	Housewife	SSSSS	None
926	5	21	Monterrey	Diabetes mellitus 2	Guard	SSSSS	None
822	39	17	Monterrey	None	Taxi driver	SRRSR	Smoker/alcoholic/drug addict
1060	35	17	Monterrey	None	Drug store clerk	SSSSS	None
925	7	21	Santa Catarina	Diabetes mellitus 2	Retired	SRSSS	Smoker/alcoholic
1025	35	17	Monterrey	Diabetes mellitus 2	Retired	RRRRR	None
841	5	21	Monterrey	Diabetes mellitus 2	Presser	RRSSR	None
845	26	17	Monterrey	None	Unskilled worker	SSSRS	Alcoholic
805	12	37	Monterrey	None	Student	SSSSS	None
812	28	17	Monterrey	None	Street vendor	SSSRS	Smoker/alcoholic
800	37	17	Monterrey	None	Presser	SSSSS	Alcoholic
1013	3	3	Monterrey	HIV-AIDS	Retired	SRRRS	None
854	31	6	San Nicolás	Diabetes mellitus 2	Housewife	RRSRR	None
957	36	17	Monterrey	Infection of urinary tract	Housewife	SSSSS	None
864	27	4	Guadalupe	None	Unskilled worker	SSSSS	No data
904	31	6	San Nicolás	Diabetes mellitus 2	Housewife	RRSRR	None
1026	3	3	Monterrey	None	Operator	SSSSS	Occasional drinker
1007	35	17	Monterrey	None	Welder	No data	Alcoholic
863	31	6	Monterrey	None	Retired	RRRRR	Occasional drinker
1014	35	17	Monterrey	None	System operator	SRSRS	None
808	5	21	Monterrey	Diabetes mellitus 2	Electrician	SSSSS	Alcoholic
868	39	17	Monterrey	None	Student	SSSSS	None
961	5	21	Monterrey	Silicosis	Street vendor	No data	Alcoholic
819	32	4	Guadalupe	None	Unskilled worker	SRRRR	No data
919	26	17	Monterrey	None	Diaper packer	SSSSS	None
839	43	6	Monterrey	Diabetes mellitus 2	Housewife	SRSSR	None
826	26	17	Monterrey	None	Unskilled worker	RSSSS	No data
827	26	17	Monterrey	None	Retired	SRRRR	None
1039	26	17	Monterrey	Diabetes mellitus	Unskilled worker	SSSSS	None
1019	20	4	Monterrey	Diabetes mellitus	Street vendor	SSSSS	None
948	35	17	Monterrey	None	Mason	SSSSS	Smoker/alcoholic
906	30	4	Monterrey	Diabetes mellitus 2	Housewife	RRSSS	None
942	27	4	Guadalupe	None	Unskilled worker	RRSSP	None

a: group of clusters having equal spoligotyping, mycobacterial interspersed repetitive units-variable number tandem repeats (MIRU-VNTR) and restriction fragment length polymorphism (RFLP)-IS*6110* codes; *b*: clinics of familiar medicine which are giving medical services to residents of various neighbourhoods; *c*: zonal general hospitals. The metropolitan area of Monterrey has been divided by the National Institute of Social Security into zones. Each zone has a zonal general hospital and several clinics of familiar medicine. Familiar physicians refer their patients to the correspondent zonal hospital when they need a more specialised attention; *d*: clusters having equal spoligotyping, RFLP-IS*6110*, and different MIRU-VNTR-codes; *e*: clusters having equal spoligotyping, MIRU-VNTR and different RFLP-IS*6110*-codes; *f*: clusters having identical spoligotyping, different MIRU-VNTR and RFLP-IS*6110*-codes; *g*: chronic obstructive pulmonary disease; *h*: clusters having equal MIRU-VNTR and different spoligotyping and RFLP-IS6110-codes; *i*: cases that did not formed clusters with any of the three typing methods currently used; HIV-AIDS: human immunodeficiency virus-acquired immune deficiency syndrome.

Supplementary data 2