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Polymerase Chain Reaction of Nasal Swabs from Tuberculosis Patients and Their Contacts[1]

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Several studies using the polymerase chain reaction (PCR) technique have identified leprosy bacilli in nasal swabs from leprosy patients, their contacts, and other persons living in endemic areas ([3, 5, 9]). The nose is a site of carriage and shedding of *Mycobacterium leprae,* and a possible portal of entry. Nasal tuberculous lesions are rare in humans ([9, 10]) but *M. bovis* has been isolated from the nasal secretions of infected cattle ([2, 8]). Given the large numbers of bacilli coughed up from the lungs of patients with pulmonary tuberculosis, one might expect that some bacilli would be present in the mucosa of the upper respiratory tract, including the nasal cavity. The nose acts as an air filter, and so inhaled bacilli may concentrate there and be detectable, whether or not they multiply in the nasal cavity. Detection of *M. tuberculosis* in the noses of patients and contacts could give us clues to the transmission of tubercle bacilli in different environments.

In this pilot study in northern Malawi, nasal swabs were taken from tuberculosis patients, their contacts and various controls, and tested for both *M. tuberculosis* and *M. leprae* in laboratories in London and Amsterdam.

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MATERIALS AND METHODS

Nasal swabs were taken from each nostril of newly diagnosed, pulmonary tuberculous inpatients at Karonga District Hospital, northern Malawi. The sterile, cotton wool swabs were dipped in sterile saline before use and afterward were stored at –20°C. They were transported to Europe in cold boxes with ice. Nasal swabs were also taken from household contacts of three of the smear-positive patients, from healthy volunteers in London ("negative controls"), from the medical officer after a tuberculosis ward round in Malawi, and from leprosy patients. "Positive control" swabs were dipped in the sputum of smear-positive tuberculosis patients. Half of the swabs from each patient were sent to each of the two laboratories. The laboratories were blinded as to the source of the swabs.

Laboratory methods

Laboratory A. The target for the tuberculosis polymerase chain reaction (PCR) was the IS*6110* insertion sequence; for *M. leprae,* it was the *pra* gene sequence. Treatment of the swab specimens with lysis buffer ([5]) and PCR ([5, 7]) was carried out as described previously. Six tubes of lysis buffer were used as negative controls in each run. Positive amplification, as judged by agarose gel electrophoresis, was confirmed by hybridization. Negative samples were screened for inhibition of the PCR by spiking with a modified template for the *M. leprae* PCR ([3]) and with *M. tuberculosis* DNA for the *M. tuberculosis* PCR. When inhibition was found the sample was purified and retested as previously described ([3, 7]). Each extract was amplified twice to confirm the results, and the amplifications were repeated if they were not concordant.

TABLE 1. *PCR results for the two laboratories by specimen.*[a]

Source of swab	No. swabs	*M. tuberculosis* positive [undetermined]		*M. leprae* positive [undetermined]	
		Lab A	Lab B	Lab A	Lab B
Pulmonary TB patients					
Smear +	26 (27[b])	8	7 [4]	2	0 [4]
X-ray only	6	0	0 [3]	2	0 [3]
Extrapulmonary TB patients	4	0	0 [3]	0	0 [3]
TB contacts	13	1 [1]	0 [1]	0 [1]	0 [1]
Smear + sputa	8	8	7 [1]	1	0 [1]
Leprosy patients					
Multibacillary	10	0	0 [2]	6	6 [2]
Paucibacillary	6	0	0	1	0
London controls	14	0	0	0	0
Medical officer	1	0	0	0	0

[a] Results were undetermined due to the presence of inhibitors in one specimen tested by Laboratory A and in 14 tested by Laboratory B.
[b] Laboratory B received one additional swab.

Laboratory B. The target for the tuberculosis PCR was the IS*6110* insertion sequence; for *M. leprae,* it was the RLEP sequence. DNA extraction and PCR were carried out as described previously ([4]) except that individual swabs were resuspended in 100 μl PCR buffer containing 100 μg/ml proteinase K and 0.5% Tween 20, and incubated at 60°C for 1 hr prior to freeze-boiling. For each batch of samples five control specimens were prepared and treated identically to the test specimens except for the addition of swabs. PCR was carried out using a hot-start protocol. Samples were screened for the presence of inhibitors using PCR with amplifiable concentrations of *M. tuberculosis* DNA and primers specific for *M. tuberculosis* DNA in the extracts. Ten further negative controls (reaction mixture) were used in each run. PCR products were detected using colorimetric methods. Each extract was amplified twice to confirm the results, and this was repeated if they were not concordant.

RESULTS

Eighty nasal swabs for each laboratory were taken from 44 subjects. Eight additional sets of swabs were dipped in sputum specimens from smear-positive tuberculosis patients. The number of swabs from each source and the results of the PCR studies are shown Table 1. Results were undetermined in 14 specimens in Laboratory B and for one in Laboratory A due to the presence of inhibitors.

The results are shown by patient in Table 2. All patients contributed one or two nasal

TABLE 2. *PCR results for the two laboratories by patient.*[a]

Source of swab	No. people	*M. tuberculosis* positive [undetermined]		*M. leprae* positive [undetermined]	
		Lab A	Lab B	Lab A	Lab B
Pulmonary TB patients					
Smear +	16	6	5 [1]	2	0 [1]
X-ray only	3	0	0 [1]	2	0 [1]
Extrapulmonary TB patients	2	0	0 [1]	0	0 [1]
TB contacts	10	1	0	0	0
Smear + sputa	8	8	7 [1]	1	0 [1]
Leprosy patients					
Multibacillary	3	0	0	1	1
Paucibacillary	2	0	0	1	0
London controls	7	0	0	0	0
Medical officer	1	0	0	0	0

[a] The most positive result is shown for patients from whom more than one specimen was taken.

swabs for each laboratory, except for two of the leprosy cases. One multibacillary (MB) leprosy case had six specimens taken, all of which were positive for *M. leprae* and negative for *M. tuberculosis* in both laboratories; one paucibacillary (PB) leprosy case had four specimens taken, all of which were negative for both tests in both laboratories. Nine of the 16 smear-positive tuberculosis cases were confirmed by culture.

Thirty patients contributed duplicate swabs for each laboratory (including pairs from each of the leprosy patients). Excluding those with undetermined results, Laboratory A found agreement in 25/29 pairs (86%) for *M. tuberculosis,* and in 24/29 (83%) for *M. leprae.* Laboratory B found agreement for all 20 pairs for both species of mycobacteria when both results were determined, but only two of the tuberculosis patients with positive results had paired results, and the only positive leprosy results came from the MB patient with six specimens.

Both laboratories detected *M. tuberculosis* in all of the (positive control) smear-positive sputa (although Laboratory B was unable to obtain results from one of these swabs because of inhibitors); neither laboratory found any positive results among the London (negative) controls.

Laboratory A detected *M. tuberculosis* by PCR in 8/32 (25%) specimens from pulmonary cases and in 1 of 13 (8%) from tuberculosis contacts (one not determined due to the presence of inhibitors); whereas Laboratory B identified *M. tuberculosis* in 7/33 (21%) specimens from pulmonary tuberculosis cases (seven not determined) and in none of the 13 specimens from tuberculosis contacts (one not determined). Among the pulmonary tuberculosis cases detected, three were detected by both laboratories.

M. leprae were found by each laboratory in all six nasal swabs obtained from one MB (slit-skin smear-positive) leprosy patient. Of the other 4 leprosy patients (2 MB and 2 PB), only one PB patient had a positive specimen (Laboratory A). No household contacts of leprosy patients were tested, but *M. leprae* were reported in nasal swabs from 4 of 21 tuberculosis patients and one tuberculosis sputum by Laboratory A, but not by Laboratory B.

DISCUSSION

Since PCR techniques are very sensitive and capable of detecting even nonviable bacilli, and since smear-positive tuberculosis patients may produce 10^5 to 10^7 bacilli per ml of sputum, some of which get into airborne droplets which can be exhaled or inhaled, we had expected to detect *M. tuberculosis* in the noses of the majority of these patients. In practice, bacilli were detected in about one third of the smear-positive patients and in only one specimen from the contacts of tuberculosis patients.

The techniques used by Laboratory B have been shown to be able to detect one leprosy bacillus or 5–20 tuberculosis bacilli using purified genomic DNA ([4, 12]). Equivalent figures for Laboratory A are 20 *M. leprae* and 2 tuberculosis bacilli ([3, 7]). In general, however, larger numbers of organisms appear to be necessary to yield a positive result by PCR when using clinical samples ([1, 6]). It is possible that some degradation of nucleic acid occurred during the transport from Malawi, although efforts were made to keep the specimens cold. Among the sputum specimens, in which larger numbers of bacilli were present, sensitivity was 100%.

Where the results from the two laboratories differed, it is difficult to know whether this reflects lack of sensitivity by one or of specificity by the other. *M. leprae* have previously been identified in the noses of persons without leprosy from endemic areas ([5]) and the "failure" of Laboratory B to detect *M. leprae* in any specimens except those from one MB leprosy patient may be attributable to the lack of a purification step in this laboratory when inhibitors were found: of the eight nasal swabs from the four tuberculosis patients found positive for *M. leprae* by Laboratory A, Laboratory B could only determine results for three. Alternatively, the five specimens from tuberculosis patients found positive for *M. leprae* by Laboratory A may be false-positives. This is unlikely to be due to crossreaction since, if crossreaction did occur, it would be expected preferentially in the sputum specimens since they contain the most *M. tuberculosis,* and this was not the case. Laboratory contamination is a possible explanation for these results, and could have occurred at the DNA purification step since all five of

these swabs showed inhibition initially and underwent purification during *M. leprae* tests (compared to 26 specimens overall).

These results show that *M. tuberculosis* can be found in the nose of some pulmonary tuberculosis patients, albeit in fewer than might be expected. The results from the positive and negative controls support the accuracy of the results of the assays, but the comparisons with *M. leprae* and between laboratories suggest that the technique is less reliable and probably less sensitive than might be hoped. Given the poor yield of positive PCR results from nasal swabs from tuberculosis cases, the finding of only one positive result among their contacts is not surprising. The results to date come only from a small number of patients, and it would be useful to expand this investigation as an adjunct to studies of transmission of mycobacterial infections in different settings.

SUMMARY

Previous studies have found *Mycobacterium leprae* in nasal swabs from leprosy patients, their contacts, and persons living in endemic areas. It might be expected that *M. tuberculosis* would be present on nasal mucosa of pulmonary tuberculosis patients, but whether they can be detected in patients or contacts is unknown. We used the polymerase chain reaction (PCR) technique on nasal swabs from tuberculosis patients, contacts of tuberculosis patients, leprosy patients, and London controls to look for both *M. tuberculosis* and *M. leprae.* Swabs dipped in sputum specimens from smear-positive patients were used as positive controls. The PCRs were conducted in two independent laboratories. *M. tuberculosis* was detected in nasal swabs from 6/16 smear-positive tuberculosis patients and from 1/10 household contacts by one of the laboratories. All of the sputum swabs were positive for *M. tuberculosis,* and all of the London controls were negative. *M. leprae* were found in nasal swabs from 2/5 leprosy patients, but one laboratory also reported *M. leprae* in swabs from 4/21 tuberculosis patients and from one sputum specimen. The results show that *M. tuberculosis* can be found in the noses of some tuberculosis patients, and suggest that the bacilli also may be detected in some household contacts. The comparisons with *M. leprae* and between the two laboratories give further insights into the sensitivity and specificity of the technique.

RESUMEN

En estudios previos ya se ha reportado la presencia de *Mycobacterium leprae* en los exudados nasales de los pacientes con lepra, en los de sus contactos, y en personas que viven en áreas endémicas. Podría esperarse que *M. tuberculosis* estuviera presente en la mucosa nasal de los pacientes con tuberculosis pulmonar o en sus contactos, pero esto hasta ahora no se había estudiado. Nosotros usamos la reacción en cadena de la polimerasa (PCR) para buscar tanto *M. tuberculosis* como *M. leprae* en los exudados nasales de pacientes con tuberculosis, en sus contactos, en pacientes con lepra, y en controles londinenses. Como controles positivos se usaron hisopos sumergidos en el esputo de pacientes bacilíferos. Los PCRs se hicieron en 2 laboratorios independientes. Uno de los laboratorios detectó *M. tuberculosis* en los exudados de 6 de 16 pacientes con tuberculosis BAAR positivos y en 10 de sus contactos convivientes. Todas las muestras de esputo fueron positivas para *M. tuberculosis* y todos los controles londinenses fueron negativos. Dos de 5 pacientes con lepra tuvieron *M. leprae* en sus exudados pero un laboratorio también reportó *M. leprae* en los exudados de 4 de 21 pacientes con tuberculosis y en una muestra de esputo. Los resultados muestran que *M. tuberculosis* puede encontrarse en la naríz de algunos pacientes con tuberculosis y en algunos de sus contactos convivientes. La comparación de los hallazgos con *M. leprae* y de los resultados de los 2 laboratorios, proporciona información adicinal sobre la sensibilidad y la especificidad de la técnica.

RÉSUMÉ

Des études antérieures ont trouvé des *Mycobacterium leprae* dans les décharges nasales de malades de la lèpre, de leurs contacts, et de personnes vivant en régions endémiques. On pourrait s'attendre à ce que *M. tuberculosis* soit présent sur les muqueuses nasales de patients présentant une tuberculose pulmonaire, mais on ne sait pas s'il peut être détecté chez les patients ou des contacts. Nous avons utilisé la technique de la réaction de polymerase en chaîne (PCR) sur des produits de décharge nasale provenant de patients tuberculeux, des contacts de patients tuberculeux, des patients lépreux et des témoins de la ville de Londres pour rechercher *M. tuberculosis* et *M. Leprae.* Des cotons plongés dans des specimens de crachats provenant de patients dont les expectorations étaient positives à l'examen direct ont été utilisés comme contrôles positifs. Les PCR ont été réalisées dans deux laboratoires indépendants. *M. tuberculosis* a été détecté dans les décharges nasales de 6 patients tuberculeux sur 16 positifs à l'examen des expectorations, et chez un contact domiciliaire sur dix par l'un des lab-

oratoires. Tous les cotons imbibés d'expectorations étaient positifs pour *M. tuberculosis*, et tous les contrôles londoniens étaient négatifs. *M. leprae* a été trouvé dans les décharges nasales de deux patients lépreux sur cinq, mais un laboratoire a aussi rapporté du *M. leprae* dans les décharges de 4 patients tuberculeux sur 21 ainsi que d'un échantillon d'expectorations. Les résultats montrent que *M. tuberculosis* peut être trouvé dans le nez de certains patients tuberculeux, et suggèrent que les bacilles peuvent aussi être détectés chez certains contacts domiciliaires. Les comparaisons avec *M. leprae* et entre les deux laboratoires donnent des informations supplémentaires quant à la sensibilité et la spécificité de la technique.

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