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Transparency Declaration

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Location of 47 oligonucleotide probes designed to cover *Mycobacterium tuberculosis pncA*.

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Speciation and susceptibility of *Nocardia* isolated from ocular infections

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Abstract

Twenty *Nocardia* spp. isolated from ocular infections were identified by 16S rRNA gene sequencing and susceptibility was determined using the E-test (AB Biodisk, Sweden). Species distribution among the 20 isolates was as follows: *Nocardia levis* (*n* = 7), *Nocardia farcinica* (*n* = 3), *Nocardia abscessus* (*n* = 2), *Nocardia brasiliensis* (*n* = 2), *Nocardia amamiensis* (*n* = 2), *Nocardia puris* (*n* = 1), *Nocardia beijingensis* (*n* = 1), *Nocardia otitidiscaviarum* (*n* = 1) and *Nocardia thailandica* (*n* = 1). All isolates were sensitive to amikacin. Eighteen (90%) isolates were sensitive to tobramycin, 11 (55%) to ciprofloxacin and gatifloxacin, and seven (35%) to azithromycin and clarithromycin. Molecular methods are useful for the identification and for the detection of *Nocardia* species that have not so far been reported in human infections.

Keywords: *N. amamiensis*, *N. thailandica*, *N. levis*, *N. puris*, ocular infections

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Identification of *katG* Mutations Associated with High-Level Isoniazid Resistance in *Mycobacterium tuberculosis*▽†

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Isoniazid (INH) is an effective first-line antituberculosis drug. KatG, a catalase-peroxidase, converts INH to an active form in *Mycobacterium tuberculosis*, and *katG* mutations are major causes of INH resistance. In the present study, we sequenced *katG* of 108 INH-resistant *M. tuberculosis* clinical isolates. Consequently, 9 novel KatG mutants with a single-amino-acid substitution were found. All of these mutants had significantly lower INH oxidase activities than the wild type, and each mutant showed various levels of activity. Isolates having mutations with relatively low activities showed high-level INH resistance. On the basis of our results and known mutations associated with INH resistance, we developed a new hybridization-based line probe assay for rapid detection of INH-resistant *M. tuberculosis* isolates.

Isoniazid (INH) is an effective drug used in the treatment of tuberculosis and has been in common use to treat tuberculosis since its introduction in 1952 (4). However, the emergence of INH-resistant (Inh^r) *Mycobacterium tuberculosis* is jeopardizing the continued utility of INH (10).

Drug resistance in *M. tuberculosis* is caused by mutations in restricted regions of the genome (36). Mutations in *katG*, the upstream region of the *fabG1-inhA* operon ($P_{fabG1\text{-}inhA}$), and *inhA* are responsible for INH resistance (36). The *katG* gene encodes the bifunctional catalase-peroxidase enzyme that converts INH to an active form (35).

Previously, we developed a DNA sequencing-based method to detect mutations in regions associated with INH resistance in *M. tuberculosis*, including *katG* and $P_{fabG1\text{-}inhA}$ (28). Consequently, five novel mutations in *katG* associated with INH resistance were found (28). In the present study, we cloned 21 *katG* mutants, including 15 novel mutants, and compared their INH oxidase activities. Certain *katG* mutations were shown to cause high-level INH resistance, which suggests the possibility of determining the degree of INH resistance, such as high- or low-level resistance, by detecting these *katG* mutations. Furthermore, to detect these mutations in ordinary-scale clinical laboratories without sequencing, we developed a new hybridization-based line probe assay (LiPA) for INH resistance in *M. tuberculosis* isolates, which can be applied easily in clinical use.

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MATERIALS AND METHODS

Bacterial strains and plasmids. One hundred eight Inh^r *M. tuberculosis* isolates were obtained from single patients at the International Medical Center of Japan and National Hospital Organization Tokyo National Hospital from 2003 to 2008. INH-susceptible (Inh^s) *M. tuberculosis* strains H37Rv and IMCJ 2751 were used. The IMCJ 2751 isolate has a *katG*(*G1388T*) [KatG(R463L)] neutral mutation. The *Escherichia coli* strains and plasmids used in this study are listed in Table 1. *E. coli* TOP10F′ (Invitrogen, Carlsbad, CA) was used as the host for cloning. *E. coli* UM262 (17) was used as the host for expression of *katG* derived from clinical isolates and H37Rv.

Drug susceptibility testing. All clinical isolates, H37Rv, and IMCJ 2751 were tested for drug susceptibility. Strains were analyzed by an agar proportion method with egg-based Ogawa medium (Vit Spectrum-SR [Kyokuto Pharmaceutical Industrial Co., Tokyo, Japan] or Wellpack [Japan BCG Laboratory, Tokyo, Japan]), which is based on a slightly modified WHO protocol (3) and is recommended by the Japanese Society of Tuberculosis (3, 12). The medium contained INH (0.2 μg/ml and 1.0 μg/ml), rifampin (RIF) (40 μg/ml), ethambutol (EB) (2.5 μg/ml), kanamycin (KM) (20 μg/ml), *p*-aminosalicylic acid (PAS) (0.5 μg/ml), streptomycin (SM) (10 μg/ml), ethionamide (TH) (20 μg/ml), enviomycin (EVM) (20 μg/ml), cycloserine (CS) (30 μg/ml), and levofloxacin (LVFX) (1.0 μg/ml). The results of drug susceptibility testing are shown in Table S1 in the supplemental material.

Isolation of genomic DNA. Genomic DNA from *M. tuberculosis* was extracted as described previously (22).

DNA sequencing of INH resistance-related genes. The *furA-katG* operon and its upstream region were amplified by PCR with primers −129*furA* (5′-GCTCA TCGGAACATACGAAG-3′) and *katG*+50 (5′-GTGCTGCGGCGGGTTGTG GTTGATCGGCGG-3′). The *fabG1-inhA* operon and $P_{fabG1\text{-}inhA}$ were also amplified, using primers −200*fabG1* (5′-TTCGTAGGGCGTCAATACAC-3′) and *inhA*+40 (5′-CCGAACGACAGCAGCAGGAC-3′). PCR products were used as templates for direct DNA sequencing. DNA sequences were compared with the H37Rv sequence using Genetyx-Mac, version 14.0.2 (Genetyx Corporation, Tokyo, Japan).

Construction of plasmids. The coding regions of *katG* from H37Rv, IMCJ 2751, and Inh^r clinical isolates with *katG* mutations were amplified by PCR with the primers *katG*-F-ccc (5′-CCCGAGCAACACCCACCCATTACAGAAAC-3′) and *katG*-R (5′-TCAGCGCACGTCGAACC-3′) and cloned into pTrcHis2-TOPO (Invitrogen) using the TA cloning method. The pTrcHis2-TOPO vector encodes a C-terminal peptide containing a c-*myc* epitope and a 6×His tag. However, the expressed recombinant KatG protein did not have any additional amino acid residues, such as the c-*myc* epitope and the 6×His tag, because the

TABLE 1. *E. coli* strains and plasmids used in this study

Strain or plasmid	Genotype or description	Source or reference
E. coli strains		
TOP10F′	F′ [*lacI*^{q} Tn*10* (Tet^r)] *mcrA* Δ(*mrr-hsdRMS-mcrBC*) ϕ80*lacZ*ΔM15 Δ*lacX74 recA1 araD139* Δ(*ara-leu*)*7697 galU galK rpsL* (Str^r) *endA1 nupG*	Invitrogen
UM262	*katG*::Tn*10 recA pro leu rpsL hsdM hsdR endl lacY*	17
Plasmids		
pTrcHis2-TOPO	TA cloning and expression vector; Ap^r Km^r	Invitrogen
p*katG*-wt	pTrcHis2-TOPO carrying *katG*	This study
p*katG*-1	p*katG*-wt carrying G1388T (neutral mutation)	This study
p*katG*-2	p*katG*-1 carrying C379G	This study
p*katG*-3	p*katG*-1 carrying C694T	This study
p*katG*-4	p*katG*-wt carrying A398C	This study
p*katG*-5	p*katG*-1 carrying T1147C	This study
p*katG*-6	p*katG*-1 carrying 1297::C, Δ1305C	This study
p*katG*-7	p*katG*-1 carrying a290g	This study
p*katG*-8	p*katG*-1 carrying C1465A	This study
p*katG*-9	p*katG*-wt carrying G944C	This study
p*katG*-10	p*katG*-1 carrying T1259C	This study
p*katG*-11	p*katG*-wt carrying G944C, G1159C	This study
p*katG*-12	p*katG*-1 carrying G368A, G895A	This study
p*katG*-13	p*katG*-1 carrying G1255C	This study
p*katG*-14	p*katG*-1 carrying C195T (silent mutation), T527C	This study
p*katG*-15	p*katG*-wt carrying Δ(478–479)	This study
p*katG*-16	p*katG*-1 carrying G944C	This study
p*katG*-17	p*katG*-wt carrying Δ371G	This study
p*katG*-18	p*katG*-1 carrying C1894T	This study
p*katG*-19	p*katG*-wt carrying C945A	This study
p*katG*-20	p*katG*-1 carrying Δ(571–576)	This study
p*katG*-21	p*katG*-1 carrying G1624C	This study

katG-R reverse primer included the native stop codon. The DNA sequences of all clones were confirmed by sequencing.

RFLP. IS*6110*-probed restriction fragment length polymorphism (RFLP) was performed as described previously (22). Patterns with more than 70% similarity were postulated to form a cluster.

Immunoblotting. Proteins separated by SDS-PAGE were transferred onto Immun-Blot polyvinylidene difluoride (PVDF) membranes (Bio-Rad, Hercules, CA). The proteins on the membranes were detected using primary antibodies specific for KatG (28). KatG was visualized with horseradish peroxidase-conjugated secondary antibodies.

Enzyme assays. KatG mediates free-radical formation from INH oxidation in the presence of H_2O_2. The activities of KatG were detected spectrophotometrically by following the reduction of nitroblue tetrazolium (NBT) at A_{560} (28, 32). Peroxidase activity was monitored spectrophotometrically by following the oxidation of 2,2′-azino-*bis*(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) at A_{405} (21). Catalase activity was measured spectrophotometrically by following the degradation of H_2O_2 at A_{240} (21). The catalase activity is shown as values subtracted from that of the vector control. All assays were carried out at 25°C. The absorbance was read 200 s after the initiation of the reaction.

LiPA. The line probe assay (LiPA) was performed as described previously (1, 29). In brief, 41 oligonucleotide probes were designed to cover mutations in the *furA-katG* (35 probes for *katG* and 2 for *furA*), $P_{fabG1\text{-}inhA}$ (2 probes), and *fabG1* (2 probes) regions (Table 2). These probes were immobilized on two strips. Six regions, located within $P_{fabG1\text{-}inhA}$ (477 bp), *fabG1* (209 bp), *furA* (256 bp), and *katG* (612 bp, 698 bp, and 907 bp), were amplified by nested PCR. Immobilized probes on the two strips were hybridized with the biotinylated PCR products and then incubated with streptavidin labeled with alkaline phosphatase. The color development was performed by incubation with 5-bromo-4-chloro-3′-indolylphosphatase *p*-toluidine and NBT.

RESULTS

Drug susceptibility profiles. As shown in Table S1 in the supplemental material, among 108 Inh^r isolates, 65 (60%) were resistant to INH at 0.2 μg/ml but susceptible to INH at 1.0 μg/ml. The remaining 43 (40%) were resistant to INH at 1.0 μg/ml. Among the 108 isolates, 44 (41%) were resistant to INH but susceptible to other antituberculosis drugs. Thirteen (12%) were multidrug-resistant (MDR) isolates and five (5%) were extensively drug resistant (XDR).

IS*6110*-probed RFLP. The results of IS*6110*-probed fingerprinting of the 108 Inh^r isolates are shown in Fig. S1 in the supplemental material. Five clusters were detected, consisting of a total of 63 isolates (58%), including 12 (11%) in cluster I, 22 (20%) in cluster II, 12 (11%) in cluster III, 12 (11%) in cluster IV, and 5 (5%) in cluster V. These observations suggested that the majority of Inh^r isolates in Japan expanded in a clonal manner.

Correlation between drug susceptibility and IS*6110*-probed RFLP. With regard to the degree of INH resistance, the proportions of high-level Inh^r isolates, i.e., isolates resistant to INH (1.0 μg/ml), were 1 (8%) in cluster I, 8 (36%) in cluster II, 4 (33%) in cluster III, 4 (33%) in cluster IV, and 5 (100%) in cluster V. These results indicated that the majority of isolates belonging to cluster I were resistant to INH (0.2 μg/ml) and susceptible to INH (1.0 μg/ml) and that those belonging to cluster V were highly resistant to INH. Six of 13 MDR isolates (46%) and 1 of 5 XDR isolates (20%) belonged to the clusters, but other MDR and XDR isolates did not belong to any clusters, indicating that they emerged sporadically in Japan.

Mutations in *furA-katG*, *fabG1-inhA*, and their upstream regions. We sequenced the *furA-katG* operon, the *fabG1-inhA* operon, and their upstream regions in all Inh^r isolates tested. Of the 108 isolates, 105 had at least one mutation (see Table S1

TABLE 2. Locations of 41 oligonucleotide probes designed to cover a mutation(s) associated with INH resistance

Probe	Amino acid (nucleotide) region covered by probe
inhA-1	(−17 to −3)[a]
inhA-2	95–100
fabG1-1	202–206
fabG1-2	230–235
furA-1	12–17
furA-2	6–12
katG-1	45–51
katG-2	63–68
katG-3	92–97
katG-4	94–99
katG-5	105–111
katG-6	123–127
katG-7	132–137
katG-8	135–140
katG-9	140–145
katG-10	157–163
katG-11	170–174
katG-12	174–179
katG-13	178–183
katG-14	190–194
katG-15	228–236
katG-16	247–252
katG-17	256–261
katG-18	271–277
katG-19	294–299
katG-20	313–318
katG-21	323–327
katG-22	326–330
katG-23	383–387
katG-24	389–391
katG-25	417–422
katG-26	457–462
katG-27	479–482
katG-28	486–490
katG-29	522–528
katG-30	539–543
katG-31	553–558
katG-32	565–569
katG-33	591–596
katG-34	631–635
katG-35	707–712

[a] Nucleotide position relative to the initiation codon of *fabG1*.

in the supplemental material), while the remaining 3 had no mutations in the regions sequenced. Of the 105 isolates with mutations, 64 had mutations in the *furA-katG* operon, 62 had mutations in *fabG1-inhA* operon, and 21 had mutations in both regions. Of the 64 with mutations in the *furA-katG* operon, six had a large-scale deletion adjacent to the *furA-katG* operon (Fig. 1; see also Table S1 in the supplemental material). As shown by genetic maps (Fig. 1), these isolates had large-scale deletions, ranging in size from 2.3 to 34.4 kb. The remaining 58 isolates did not have large-scale deletions.

Twenty-eight different mutations were found among the 58 isolates with mutations in the *furA-katG* operon (see Table S1 in the supplemental material). Twenty-three were in *katG*, two were in *furA*, and three were in the intergenic region. Seven different mutations were found among the 62 isolates with mutations in the *fabG1-inhA* operon (see Table S1 in the supplemental material). Three were in the upstream region, two were in *fabG1*, and two were in *inhA*. Of the 28 different mutations found in the *furA-katG* operon, 22 were novel (2 in *furA*, 3 in the intergenic region of the *furA-katG* operon, and 17 in *katG*). Of the seven different mutations found in the *fabG1-inhA* operon, four were novel: one in the upstream region of the *fabG1-inhA* operon, two in *fabG1*, and one in *inhA* (see Table S1 in the supplemental material).

Correlation between INH resistance and mutations. We recently reported 5 novel mutations in *katG* (28). Including these mutations, 280 different mutations in *katG* were found in PubMed (http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed) when articles were searched by the keywords "*katG*," "mutation," and "tuberculosis." In addition, six mutations in the upstream region of the *fabG1-inhA* operon, including C−15T, and seven in *inhA* cause INH resistance (27, 28, 36). In this study, we found an additional 17 novel mutations in *katG*. One was a silent mutation (C195T [A65A]), while the other 16 caused amino acid substitutions. These mutations and amino acid substitutions are shown in Table 3. Furthermore, several novel mutations were detected in the present study: one in *fabG1* (G609A [L203L]), one in *furA* (C41T [A14V]), and three in the intergenic region of the *furA-katG* operon (G−7A, A−10C, and G−12A).

We will report elsewhere that these mutations in *furA* and the intergenic region are associated with INH resistance induced by downregulation of *katG* expression (H. Ando and T. Kirikae, unpublished results), and those in *fabG1* are also associated with INH resistance induced by upregulation of *inhA* expression (Ando et al., unpublished). In the present study, we examined whether novel mutations in *katG* are associated with INH resistance.

Correlation between mutations and IS*6110*-probed RFLP. As shown in Fig. S1 and Table S1 in the supplemental material, all isolates belonging to cluster I detected in the IS*6110*-probed RFLP analysis, 11 (50%) in cluster II, and 8 (67%) in cluster III had a C−15T mutation in the *inhA* promoter region. All isolates in cluster IV had a C41T mutation in *furA*. All isolates in cluster V had a G944C/G945A (S315T/R) mutation. Isolates harboring *katG* mutations, except those with the G944C/G945A (S315T/R) mutation, did not cluster in the IS*6110*-probed RFLP.

Enzymatic activity of the novel KatG mutants. We cloned a wild-type (WT) *katG* gene (p*katG*-wt) from H37Rv, a *katG* gene carrying a G1388T neutral mutation (p*katG*-1) from IMCJ 2751, and 20 *katG* genes harboring mutations causing amino acid substitutions (p*katG*-2 to -21) from Inh^r isolates (Tables 1 and 3). Among the mutants, 15 were novel and 6 had been reported previously (the *katG*-1, -7, -9, -13, -16, and -19 mutants) (Table 3). These *katG* genes were expressed in *katG*-deficient *E. coli* UM262. As shown in Fig. 2, *E. coli* isolates with *katG*-wt expressed KatG (lanes 1 and 15), whereas *E. coli* isolates with an empty vector did not (lanes 2 and 16). *E. coli* isolates carrying *katG* mutants other than the *katG*-15 (lane 8) and *katG*-17 (lane 24) mutants expressed KatG proteins at levels similar to those observed for *E. coli* isolates carrying p*katG*-wt. *E. coli* isolates with *katG*-15 (lane 8) and *katG*-17 (lane 24), which had a frame shift mutation (Table 3), did not express *katG*.

INH oxidase, peroxidase, and catalase activities were assessed using these clones (Table 4). Of the cloned mutants, one with KatG(R463L) from IMCJ 2751 showed levels of these activities similar to those observed for the wild type, and the KatG(R463L) mutation was not associated with INH resis-

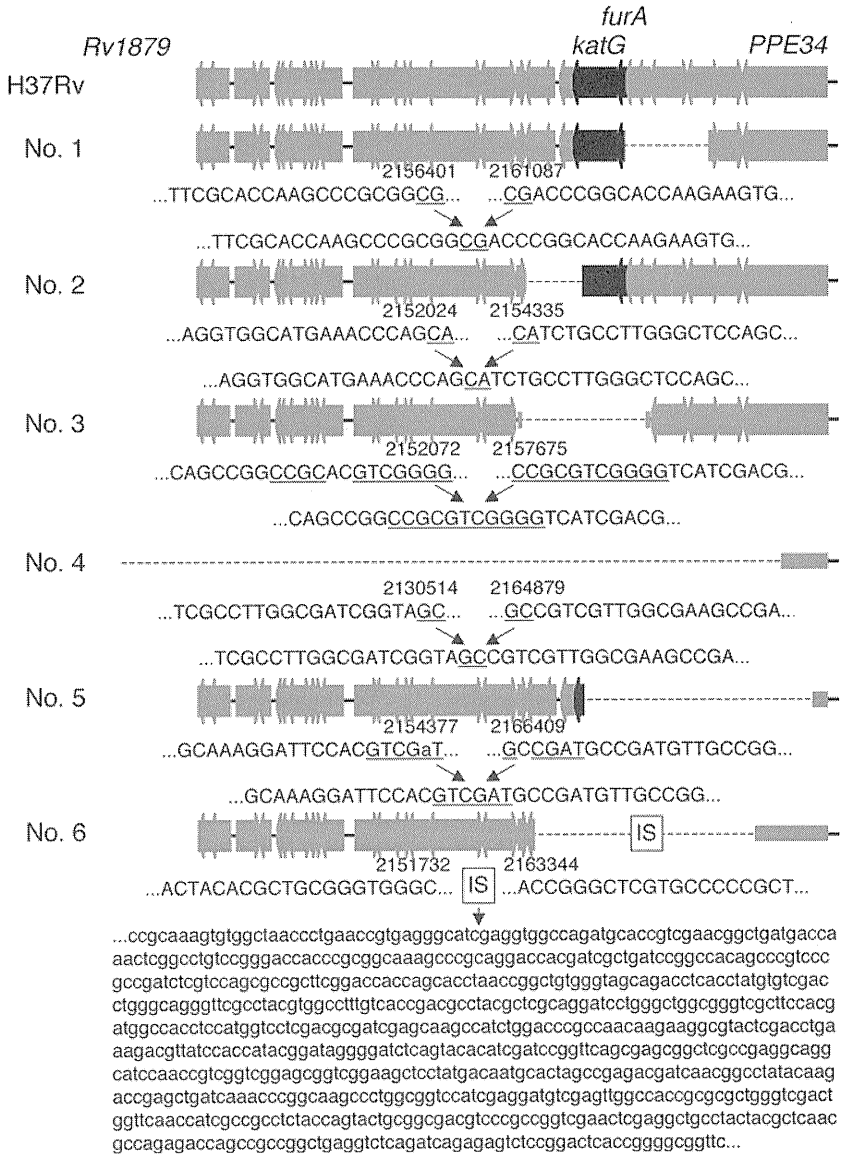

FIG. 1. Maps of large-scale deleted regions adjacent to *katG* in six Inh^r *M. tuberculosis* isolates. Bold arrows indicate the open reading frames annotated in the H37Rv genome sequence (http://genolist.pasteur.fr/TubercuList/). The dotted lines correspond to the deleted regions, with the end sequences and H37Rv genome coordinates given below. Underlined sequences are possible substrates for recombination. The box labeled "IS" represents the 750-bp fragment of IS*6110*. Numbers 1 to 6 represent the names of the isolates and correspond to the numbers shown in Table S1 in the supplemental material. A nucleotide shown in lowercase in region 5 indicates a mutation.

tance (Table 4). With regard to INH oxidase activity, *E. coli* isolates with *katG*-2 to -8 showed 1/3 to 1/17 less activity than those with *katG*-wt. *E. coli* isolates carrying *katG*-9 to -13 showed reduced activity compared to those carrying *katG*-2 to -8. *E. coli* isolates carrying *katG*-14 to -21 showed no activity (i.e., levels similar to those observed for vector controls). These results indicated that the degree of INH oxidase activity is correlated with that of INH resistance. *E. coli* isolates with *katG*-wt and *katG*-1 showed the highest levels of INH oxidase activity, and *M. tuberculosis* isolates with these genes were sensitive to INH. *E. coli* isolates carrying *katG*-2 to -8 showed slightly weaker activities, and *M. tuberculosis* isolates with these genes were resistant to INH at 0.2 μg/ml but susceptible to INH at 1.0 μg/ml. *E. coli* isolates with *katG*-9 to -21 showed weak or no activity, and *M. tuberculosis* isolates with these genes were resistant to INH at 1.0 μg/ml.

The peroxidase and catalase activities of *E. coli* isolates with mutations were correlated well with each other and also with INH oxidase activity (Table 4). However, in *E. coli* isolates carrying some clones, peroxidase/catalase activities were different from INH oxidase activity, i.e., *E. coli* isolates with *katG*-16 and -9 showed weak activity.

Development of a LiPA for detection of INH resistance. To detect novel mutations associated with INH resistance, we developed a new LiPA based on the reverse hybridization principle (25). Forty-one oligonucleotide probes were designed for the LiPA to detect mutations containing the *furA-katG* operon, the *fabG1-inhA* operon, $P_{fabG1\text{-}inhA}$, and *fabG1* (Table

TABLE 3. *katG* mutations found in Inh^r isolates

Clone	Mutation(s)	
	Nucleotide	Amino acid
katG-1[a]	G1388T	R463L
katG-2[c]	C379G[b]	Q127E[b]
katG-3[c]	C694T[b]	P232S[b]
katG-4	A398C[b]	N133T[b]
katG-5[c]	T1147C[b]	S383P[b]
katG-6[c]	1297::C[b], Δ1305C[b]	KQT433-435QAD[b]
katG-7[c]	A290G	H97R
katG-8[c]	C1465A[b]	R489S[b]
katG-9	G944C	S315T
katG-10[c]	T1259C[b]	M420T[b]
katG-11	G944C, G1159C[b]	S315T, D387H[b]
katG-12[c]	G368A[b], G895A	G123E[b], G299S
katG-13[c]	G1255C	D419H
katG-14[c]	C195T[b], T527C[b]	A65A[b], M176T[b]
katG-15	Δ(478–479)[b]	Frame shift[b]
katG-16[c]	G944C	S315T
katG-17	Δ371G[b]	Frame shift[b]
katG-18[c]	C1894t[b]	R632C[b]
katG-19	C945A	S315R
katG-20[c]	Δ(571–576)[b]	Δ(191W-192E)[b]
katG-21[c]	G1624C[b]	D542H[b]

[a] *katG*-1 carrying a G1388T (R463L) neutral mutation was cloned from the Inh^s strain IMCJ 2751.

[b] These mutations have not previously been reported. Other mutations were previously reported in references 36 (G1388T), 7 (A290G), 36 (G944C), 7 (G895A), 6 (G1255C), and 36 (C945A).

[c] This clone also had a G1388T neutral mutation.

2). As shown in Fig. S2 in the supplemental material, the LiPA could detect all mutations found in this study.

DISCUSSION

The results of RFLP and sequence analysis in the present study indicated that there are several predominant strains of Inh^r *M. tuberculosis* with different genetic backgrounds in Japan (see Fig. S1 and Table S1 in the supplemental material). These strains had *katG*(*G944C*) (S315T), an *inhA* promoter mutation, *fabG1*(*G609A*) (L203L), and *furA*(*C41T*) (A14V) (see Table S1 in the supplemental material). Inh^r isolates were reported to expand clonally in several regions, including northwestern Russia (20), the Netherlands (30), San Francisco, CA (13), Venezuela (2), and Sierra Leone (15). These clonal Inh^r strains had a KatG(S315T) or *inhA* promoter mutation. Gagneux et al. (13) reported that the strains carrying the KatG(S315T) or *inhA* promoter mutation were more likely to spread than those carrying other mutations; our results were consistent with these previous findings. In addition, strains with *fabG1*(*G609A*) (L203L) and *furA*(*C41T*) (A14V) mutations were also more likely to spread in Japan.

Of Inh^r isolates, a smaller number (22%) had S315T/R mutations in Japan (Table S1). The prevalences of the KatG(S315T) mutation in *M. tuberculosis* strains from around the world differ, especially with regard to the prevalence of tuberculosis. In regions where the prevalence of tuberculosis is low or intermediate, the mutation has been reported relatively infrequently: it occurred in 26% to 30% of 95 isolates from Singapore (16) and Madrid (23) and rarely in isolates from Scotland (11) and Finland (19). In contrast, the S315T mutation accounted for INH resistance in 52% to 64% of strains in Africa (8, 14, 31), 79% in Peru (9), 91% in Russia (18), and 58% in New York, NY. (23).

We found four KatG mutations (D419H, M420T, D542H, and R632C) that are associated with high-level INH resistance, and we also found three KatG mutations (H97R, N133T, and P232S) that are associated with low-level INH resistance (Table 4). The S315 mutation is known to confer high-level INH resistance (24, 26, 33). KatG is a functional homodimer, and each monomer is composed of two domains that are mainly α-helical. The N-terminal domain contains a heme binding site, whereas the C-terminal domain lacks this feature (34). The high-level INH resistance-associated mutations D419H and M420T are located in the region connecting the N-terminal and C-terminal domains (5). The interdomain interactions between the N-terminal and C-terminal domains of the two monomers are essential for forming the functional homodimer (5). The changes in the interdomain interactions due to the D419H and M420T mutations may result in loss of enzymatic activities of KatG. D542H and R632C are located in the 16th

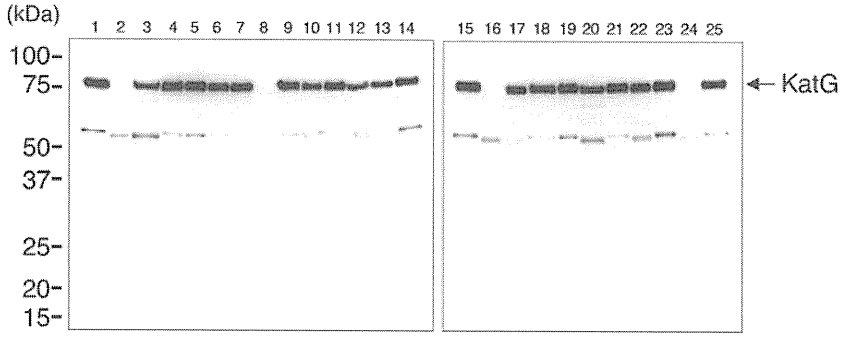

FIG. 2. Western blot of whole-cell extracts from *katG*-deficient *E. coli* strain UM262 transformed with the empty vector, pTrcHis2-TOPO, or recombinant plasmids expressing various KatG mutations as follows: lanes 1 and 15, WT; lanes 2 and 16, empty vector; lane 3, R463L and D542H; lane 4, S315T and R463L; lane 5, Q127E and R463L; lane 6, P232S and R463L; lane 7, G123E, G299S, and R463L; lane 8, frame shift mutation from position 160; lane 9, S315T and D387H; lane 10, R463L and R489S; lane 11, S315R; lane 12, M420T and R463L; lane 13, A65A, M176T, and R463L; lane 14, H97R and R463L; lane 17, Δ(191W-192E) and R463L; lane 18, N133T; lane 19, R463L; lane 20, R463L and R632C; lane 21, S315T; lane 22, D419H and R463L; lane 23, S383P and R463L; lane 24, frame shift mutation from position 124; lane 25, in-frame insertion and deletion and R463L. The positions of molecular mass markers are shown on the left.

TABLE 4. Enzymatic activities of KatG mutants detected in this study

Plasmid	Amino acid mutation(s)		Mean activity ± SD[a]			Additional mutation associated with INH resistance	INH resistance level[b]
	Not previously reported	Previously reported	INH oxidase (10^3 A_{560} units)	Peroxidase (10^2 A_{405} units)	Catalase (10^2 A_{240} units)		
pTrcHis2-TOPO[c]			4.84 ± 0.17	6.89 ± 0.70	0.00 ± 0.24		
p*katG*-wt			177.16 ± 18.50	286.08 ± 0.43	142.26 ± 0.16		S
p*katG*-1		R463L	162.00 ± 11.31	289.62 ± 1.40	141.85 ± 0.13		S
p*katG*-2	Q127E	R463L	60.18 ± 0.95	256.07 ± 7.80	143.21 ± 0.35	$P_{fabG1\text{-}inhA}$C−15T	0.2
p*katG*-3	P232S	R463L	54.47 ± 0.36	62.25 ± 0.05	76.63 ± 0.52		0.2
p*katG*-4	N133T		40.67 ± 6.31	36.00 ± 0.26	100.61 ± 5.55		0.2
p*katG*-5	S383P	R463L	38.49 ± 0.04	42.24 ± 3.64	107.65 ± 4.13	$P_{fabG1\text{-}inhA}$C−15T	0.2
p*katG*-6	KQT433-435QAD[d]	R463L	20.02 ± 0.48	106.47 ± 1.17	142.81 ± 0.11	$P_{fabG1\text{-}inhA}$C−15T	0.2
p*katG*-7		H97R, R463L	17.42 ± 0.35	26.80 ± 0.44	27.86 ± 2.01		0.2
p*katG*-8	R489S	R463L	10.40 ± 0.16	27.34 ± 0.27	14.83 ± 0.93	$P_{fabG1\text{-}inhA}$C−15T	0.2
p*katG*-9		S315T	8.83 ± 0.04	102.00 ± 2.54	71.26 ± 1.71		1.0
p*katG*-10	M420T	R463L	8.42 ± 0.14	21.02 ± 0.37	46.45 ± 0.20		1.0
p*katG*-11	D387H	S315T	7.93 ± 0.08	34.75 ± 0.61	35.71 ± 0.41		1.0
p*katG*-12	G123E	G299S, R463L	6.87 ± 0.66	6.02 ± 0.17	−0.70 ± 1.42	$P_{fabG1\text{-}inhA}$T−8C	1.0
p*katG*-13		D419H, R463L	6.30 ± 0.52	7.67 ± 0.01	4.49 ± 0.39		1.0
p*katG*-14	M176T[e]	R463L	5.14 ± 0.01	4.67 ± 0.07	1.06 ± 0.30	$P_{fabG1\text{-}inhA}$C−15T	1.0
p*katG*-15	Frame shift[f]		5.02 ± 0.24	4.01 ± 0.57	−1.75 ± 1.16		1.0
p*katG*-16		S315T, R463L	3.83 ± 0.18	84.41 ± 0.17	117.07 ± 7.56		1.0
p*katG*-17	Frame shift[g]		3.30 ± 0.69	4.59 ± 0.09	2.07 ± 1.51		1.0
p*katG*-18	R632C	R463L	3.26 ± 0.13	1.56 ± 0.08	−7.41 ± 0.76		1.0
p*katG*-19		S315R	3.19 ± 0.76	3.24 ± 0.02	−2.36 ± 0.71		1.0
p*katG*-20	Δ(191W-192E)[h]	R463L	2.78 ± 0.09	2.09 ± 0.04	2.61 ± 1.86		1.0
p*katG*-21	D542H	R463L	1.63 ± 0.49	0.32 ± 0.17	−7.00 ± 0.69		1.0

[a] Mean (n = 3) ± SD.
[b] The INH susceptibility levels for clinical isolates with *katG* mutations are shown, as follows: S, INH sensitive; 0.2, resistant to INH (0.2 μg/ml) and susceptible to INH (1.0 μg/ml); and 1.0, resistant to INH (1.0 μg/ml).
[c] A vector control.
[d] 1297::C and Δ1305C.
[e] This isolate had an additional A65A silent mutation.
[f] Δ(478–479).
[g] Δ371G.
[h] Δ(571–576).

and 19th α-helices in the C-terminal domain, respectively, and showed no enzymatic activities, although the functional role of the C-terminal domain in KatG remains unclear (5, 34). The mutations associated with low-level INH resistance, H97R, N133T, and P232S, are located adjacent to the INH binding pocket (5). They may weakly affect the binding affinity of INH. The S315T mutation located at the INH binding pocket could block binding of INH without interfering with catalysis (5).

The new LiPA was able to distinguish high-level INH resistance (resistant to 1.0 μg/ml) from low-level INH resistance (resistant to 0.2 μg/ml and sensitive to 1.0 μg/ml) in clinical isolates without sequencing. Thus, we were able to determine the degree of INH resistance using this LiPA. This assay would be useful in clinical application in combination with culture-based drug susceptibility tests. We have recently developed a LiPA to detect a *pncA* mutation(s) for rapid detection of pyrazinamide-resistant *M. tuberculosis* (29), which was shown to be readily usable in clinical applications (1). The whole procedure takes only 9 h, and the estimated cost per sample is $35. The clinical trials for *in vitro* diagnosis are in progress (from April 2009 to March 2010) in Japan. The trials will reveal the specificity of the LiPA. It will be beneficial especially in developing countries where the laboratories are scarcely equipped because of the high cost of setting them up.

Assessment of INH oxidase activities of *M. tuberculosis* isolates may provide useful information about INH resistance. The INH oxidase activities of KatG mutants showed good correlations with the degree of INH resistance (Table 4). Other enzymatic activities of KatG mutants, i.e., peroxidase and catalase activities, were also correlated with the degree of INH resistance (Table 4). However, the activities of the S315T mutant were not, i.e., this mutant showed catalase-peroxidase activities but no INH oxidase activity (Table 4). Other S315 mutants, such as the S315R (Table 4) and S315N (32) mutants, have lost all three kinds of enzymatic activity. Thus, the Inh^r isolates with KatG(S315T), retaining catalase-peroxidase activities, may have a survival advantage, and this may explain the global spread of strains with the KatG(S315T) mutation.

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日本のコッホ現象報告の分析

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要旨：〔目的〕コッホ現象の実態を把握し，課題を明らかにする。〔方法〕平成17年4月から4年間の厚生労働省へのコッホ現象報告814例を集計・分析した。〔結果〕接種部位の反応は概ね接種後3日までに気づかれていた。コッホ現象による重篤な副反応は見られなかった。判定結果は非特異的反応578（71.0%），経過観察34（4.2%），コッホ現象104（12.8%）（発病3例含む），他院紹介54（6.6%），不明44（5.4%）であった。非特異的反応は経年的に減少した。〔考察〕報告数の地域差は接種時の保護者への説明，相談後の対応など人為的な要因の関与が考えられた。非特異的反応は局所反応の経過等によって判断できることが普及したため，減少したと考えられる。「真のコッホ現象」は推定年間感染危険率よりも少なかった。これは，感染危険があった児は通常のBCG接種を受けないこと，結果不明例があること，局所反応が見逃された可能性，局所反応判明後に適切な感染診断がなされなかった可能性，対象月齢の感染危険は推定年間感染危険率より低い可能性などが考えられた。〔結論〕医療従事者，保護者への正しい知識の普及が必要である。

キーワーズ：BCG直接接種，コッホ現象，管針法，感染危険

はじめに

わが国のBCG接種は事前にツベルクリン反応検査（以下，ツ反）を実施して感染していないことを確認してから行われてきたが，平成17年4月結核予防法改正後は，ツ反を行わない直接接種に変更になった。結核感染者にBCG接種を行った場合，接種局所に早期に起こる皮膚反応はコッホ現象として古くから知られていたが[1]，管針法によるBCG接種はわが国以外では韓国，南アフリカなど限られた国でのみ実施されているため，十分な知見が集められていなかった。法改正に伴って，コッホ現象を診察した医師は保健所，都道府県を通して厚生労働省に報告することとなっている。本研究は日本におけるコッホ現象の実態を明らかにして，発生時の対応，報告のあり方の検討を行うため，厚生労働省結核感染症課の依頼によって，厚生労働科学研究補助金新型インフルエンザ等新興・再興感染症研究事業「結核対策の評価と新たな診断・治療技術の開発・実用化に関する研究」（研究代表者：加藤誠也）の一環として実施した。

方　法

対象は平成17年4月から21年3月までの4年間に厚生労働省に「コッホ現象」として報告された814例で，性別，接種月齢，気づくまでの期間，都道府県別の報告数，ツ反，結果判定の集計・分析を行った。都道府県ごとの出生対報告数の算出に際しては平成17年から19年の都道府県別の出生数の平均を計算して用いた。判定について，調査票では「要観察」「化学予防」「要治療」「その他」となっているが，基準が明確でなかったため，真の感染であるかどうかについて調査票に記載された担当者の判断に加えて，「局所所見の状況・経過」およびツ反結果より，次のように判断しなおした。

非特異的反応：結核に感染していない（真のコッホ現象でない）と判定された者。ツ反の結果が陰性で，調査票の分類で「経過観察」に区分された者を含めた。

経過観察：ツ反陽性で結核感染の疑い濃厚であるが，未治療のままで経過観察された者。潜在性結核感染症の治療を勧められたが家族が治療を拒否した者を含む。

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コッホ現象：結核に感染していると判断された者で，発病および潜在性結核感染症として治療を行った者。なお，「発病」はコッホ現象例の中で，その後の画像検査・菌検査による発病と判断され治療対象となった者とした。

他院紹介：検査または治療のために他院に紹介された者で最終判定結果が不明の者。

不明：検査等の情報が不足で最終判定が不明な者。

結　　果

（1）性別および月齢

男児370例（45.5%），女児383例（47.1%），報告書中に性別，生年月日等の個人情報が隠されていた，あるいは記載がなかったため，不明であった者は61例（7.5%）であった。接種月齢は3カ月未満3例（0.4%），3～4カ月302例（37.1%），4～5カ月264例（32.4%），5～6カ月58例（7.1%），6カ月以降8例（1.0%），上記と同様の不明179例（22.0%）となっている。不明を除いた接種月齢の平均は4カ月（日齢で124日）であった。

（2）気づくまでの期間（Fig. 1）

接種翌日に気づいた例が66.1%と最も多く，接種後3日までに95.6%が気づかれていた。一方で，接種後1カ月以降に3種混合の予防接種時に担当者から指摘された例もあった。

（3）報告例数，出生10万対報告例数

4年間の報告例数は愛知123例に続いて，静岡54例，愛媛51例，岡山および千葉44例の順になっている。一方，新潟，宮崎は2例，高知が1例，富山，鳥取，佐賀では報告がなかった。出生数10万対では愛媛109.2，大分74.8，岡山64.6，山形64.3，愛知44.5の順で，全国では18.8となっている（Table 1左）。

（4）ツベルクリン反応検査結果

ツ反の結果は陰性555例（68.2%），陽性144例（17.7%），記載なし113例（13.9%），未実施2例（0.2%）であったが，陽性例の中で接種後14日未満にツ反をされた者が80例（55.6%），14日以上は18例（陽性者中の12.5%），接種からツ反までの期間が記載されていない者が46例（同31.9%）あった。

（5）判定結果およびその年度別推移

判定結果をTable 2に示す。非特異的反応の中にBCG接種後14日以降にツ反が実施されていたツ反陽性例2例が含まれている。ツ反陽性で治療されずに経過観察となった34例中，14日未満のツ反実施が確認できたのは17例（50.0%）で，14日以降6例（17.6%），実施時期不明は11例（32.4%）であった。潜在性結核感染症の治療が必要と診断されたが，保護者の理解が得られず治療を拒否した例が2例あった。コッホ現象として潜在性結核感染症の治療を受けた104例中，ツ反陰性例が7例あった。その理由として，3例は局所の皮膚反応が著しい，または減弱しないこと，1例は菌陽性の結核患者との接触が挙げられていた。他院への紹介および不明はいずれも最終的な判定が把握できなかった者で，98例（12.0%）であったが，その中の12例はツ反陽性で精査または潜在性結核感染症治療を目的とした紹介であった。年度別に見ると，非特異的反応は毎年減少し，4年間で半減した。一方，コッホ現象と判定された例数は明らかな変化はなかった（Fig. 2）。

（6）「真のコッホ現象」と考えられた数（Table 1右欄）

コッホ現象として潜在性結核感染症または結核発病の治療を受けた例，コッホ現象として治療を勧められたが家族が拒否した例を「真のコッホ現象」として集計した。都道府県別で最も人数が多かったのは大阪府15人，続いて愛知12人，東京10人，和歌山8人，千葉7人の順であった。出生10万対では全国平均は2.5で，都道府県別の上位は和歌山県25.6，愛媛県10.7，鹿児島県8.3，山

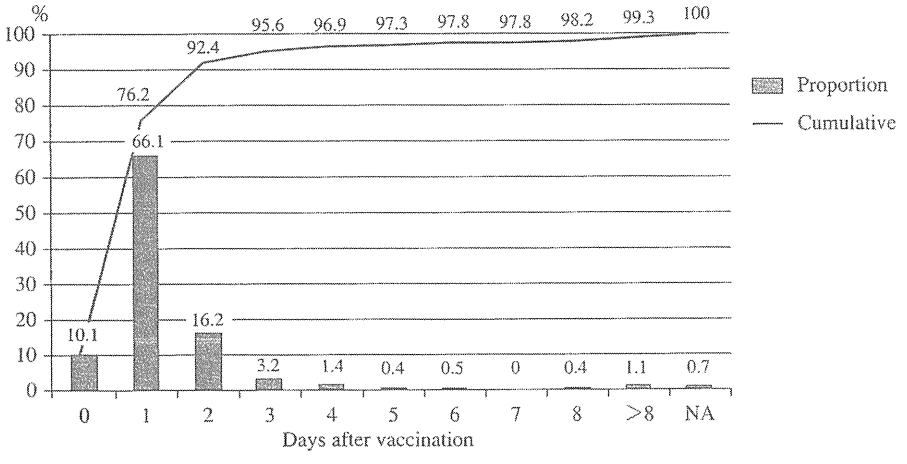

Fig. 1 Time to the awareness of skin reaction

形県8.0，奈良県6.6であった。

（7）コッホ現象局所の重篤な障害

今回の報告および予防接種副反応報告では，コッホ現象に伴う重篤な障害は認められなかった。

考　察

管針法でも皮内接種法と同様，コッホ現象による局所反応はほとんどの場合3日以内に出現することは平成17年度の報告の集計結果から報告されていたが[2]，今回

Table 1　Number of reported cases and cases with "true" Koch's phenomenon

Prefecture	Total number of reported cases		Cases per 100,000 births		Cases with "true" Koch's phenomenon		Cases per 100,000 births	
	No.	Rank	Rate	Rank	No.	Rank	Rate	Rank
Hokkaido	7	30	4.2	43	2	14	1.8	21
Aomori	6	32	14.4	25	0	30	0.0	30
Iwate	4	34	9.5	32	0	30	0.0	30
Miyagi	12	20	15.3	23	2	14	2.5	17
Akita	3	36	9.8	31	0	30	0.0	30
Yamagata	24	12	64.3	4	3	10	8.0	4
Fukushima	18	15	25.9	12	1	21	1.4	26
Ibaraki	10	23	10.1	30	1	21	1.0	28
Tochigi	11	21	15.8	22	0	30	0.0	30
Gunma	16	18	23.5	15	1	21	1.5	25
Saitama	41	6	16.9	20	4	9	1.7	22
Chiba	44	4	21.4	16	7	5	3.4	11
Tokyo	30	9	7.4	36	10	3	2.5	18
Kanagawa	19	14	6.1	37	3	10	1.0	29
Niigata	2	42	2.7	44	0	30	0.0	30
Toyama	0	45	0.0	45	0	30	0.0	30
Ishikawa	10	23	24.5	14	0	30	0.0	30
Fukui	9	25	31.2	10	1	21	3.5	10
Yamanashi	3	36	10.6	28	0	30	0.0	30
Nagano	4	34	5.4	38	2	14	2.7	16
Gifu	13	19	18.2	17	2	14	2.8	14
Shizuoka	54	2	41.3	6	0	30	0.0	30
Aichi	123	1	44.5	5	12	2	4.3	8
Mie	11	21	17.6	19	2	14	3.2	12
Shiga	7	30	13.2	26	0	30	0.0	30
Kyoto	8	27	9.2	34	2	14	2.3	19
Osaka	32	8	10.4	29	15	1	4.9	6
Hyogo	28	11	14.5	24	2	14	1.0	27
Nara	8	27	17.7	18	3	10	6.6	5
Wakayama	9	25	28.8	11	8	4	25.6	1
Tottori	0	45	0.0	45	0	30	0.0	30
Shimane	6	32	25.5	13	0	30	0.0	30
Okayama	44	4	64.6	3	3	10	4.4	7
Hiroshima	38	7	37.5	7	0	30	0.0	30
Yamaguchi	17	16	36.5	9	0	30	0.0	30
Tokushima	3	36	12.4	27	1	21	4.1	9
Kagawa	3	36	8.6	35	1	21	2.9	13
Ehime	51	3	109.2	1	5	6	10.7	2
Kochi	1	44	4.2	42	0	30	0.0	30
Fukuoka	17	16	9.4	33	5	6	2.8	15
Saga	0	45	0.0	45	0	30	0.0	30
Nagasaki	8	27	16.3	21	1	21	2.0	20
Kumamoto	3	36	4.7	40	1	21	1.6	23
Oita	30	9	74.8	2	0	30	0.0	30
Miyazaki	2	42	5.0	39	0	30	0.0	30
Kagoshima	22	13	36.7	8	5	6	8.3	3
Okinawa	3	36	4.6	41	1	21	1.5	24
Total	814				106			
Average	17.3		18.8		2.3		2.5	

Table 2 Outcomes of reported cases

Classification	N	%	Remark
Non-specific reaction	578	71.0	
Follow-up	34	4.2	In 2 cases family rejected LTBI treatment
Koch's phenomenon	104*	12.8	including 3 cases with active disease
Referred to other hospital	54	6.6	PPD (+) ; 11
Unknown	44	5.4	PPD (+) ; 1
Total	814	100.0	

*For the difference from 106 as in Table 1 see text.

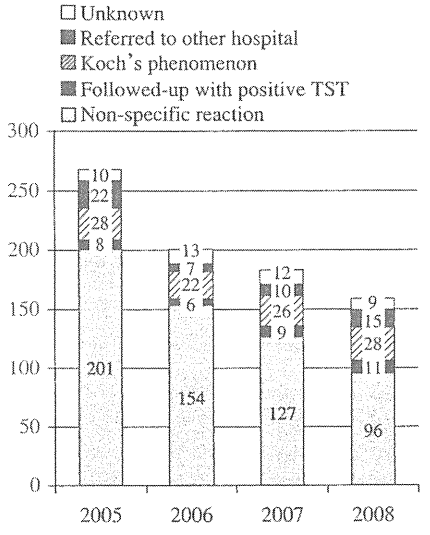

Fig. 2 Trends of reported number of cases and their outcomes

の集計結果からも確かめられた。

「コッホ現象」の報告数は都道府県で実数および出生数当たりでも大きな地域差が認められた。これは都道府県によって感染危険度に違いがあったが，それ以上に人為的な要因が関連していたものと考えられる。BCG接種の際に保護者等に対して，写真等を見せながら，「接種後にコッホ現象が生ずることがあるので，接種部位を注意深く観察し，2～3日以内に皮膚反応が生じた場合に接種医もしくは市町村に連絡すること」を十分に説明する必要がある。事実，報告例の中で保護者が気づかずに3種混合予防接種の際に保健センターで指摘された例が含まれていた。一方，これまでBCG接種直後に局所に発赤を生ずる例が一定数存在することが医療従事者に意識されていなかったため，直接接種の開始当初は接種後，局所に発赤が生じたと相談を受けたすべての事例をコッホ現象として報告していた地域もあった。特に，保護者への説明の際に比較的軽度の発赤の写真を示して説明した場合には，接種後に「コッホ現象」または「コッホ現象の疑い」としてきわめて多くの相談が寄せられた。このように保護者へのコッホ現象に関する説明，保護者から相談を受けた後の対応による違い，さらに，相談を受けた施設から市町村，都道府県，厚生労働省への報告システムが十分に機能していない可能性も考えられる。高松らの検討によって，接種直後の軽微な局所所見（化膿疹や痂皮形成を伴わない発赤のみなど）が1～2日程度の短い経過で消退するケースにツ反陽性を呈する例はなく結核感染が否定できることが明らかとなり[3]，その知見が普及するとともに「非特異的反応」の厚生労働省への報告数が経年的に減少したものと考えられる。報告数がきわめて高かった，あるいは，少なかった道府県は原因を検討して，保健所を介して市町村に対して必要な改善策を指導することが望まれる。

コッホ現象が疑われる場合にはBCGによる影響を回避するため接種後14日までにツベルクリン反応を行うことが推奨されているが[4]，ツ反結果の記載がない者が多数あり，適切な時期にツ反が実施されていない懸念がある。

結核感染（真のコッホ現象）として治療を受けた104例中，ツ反陰性例が7例あり，多数の針痕に一致して化膿疹や痂皮形成を認めるなど局所反応が強い例，局所反応が早期に減弱しない例，結核患者との接触があった例が含まれていた。感染からツ反陽転までの一定の期間を必要とするため，ツ反陰性のみを根拠に結核感染を否定せず，局所所見の程度とその推移，感染源となりうる結核患者との接触歴等も参考に慎重な感染判断を行い，感染が疑われる事例に対しては潜在性結核感染症の治療が必要と考えられる。また，この月齢では感染後発症に至る頻度が高いとされており，コッホ現象と判断された例に対して慎重な発病診断を行うことも必要である。ツ反を用いた感染診断は偽陽性が生ずることが知られている。一方，QFTは，特異度は高いが，小児，特に乳幼児における潜在性結核感染症の診断における感度は必ずしも十分とは言えない[5]。今後，T-SPOT TBを含めた小児におけるIGRAを用いた感染診断の知見が集積され，潜在性結核感染症がより的確に診断されるようになることが期待される。

わが国の年間感染危険率は0.02～0.04%程度と考えられているが[1)4)6)]，結核菌への曝露からコッホ現象が成立するまでの期間は必ずしも明らかになっていない。接

Table 3 Estimated number of infections (per 100,000)

Required time from exposure to Koch's phenomenon (days)	Annual risk of infection		
	0.02%	0.03%	0.04%
60	3.9	5.8	7.8
45	4.7	7.1	9.4
30	5.5	8.3	11.1
15	6.4	9.5	12.7

種時の日齢を今回の報告で算出可能であった者の平均124日，BCG接種からツ反まで7日，乳児の年間感染危険率（annual risk of infection; r）を0.02～0.04%と仮定して，曝露からコッホ現象成立までの期間（T）が15，30，45，60日であった場合のコッホ現象発生率（N）を対象10万対で計算すると$N = 100{,}000 \times r \times (124 + 7 - T) / 365$はTable 3に示すように3.9から12.7の間に分布しており，今回の「真のコッホ現象」の報告率10万対2.5はそれよりも少なかった。この原因として，感染危険があった児は保健所から接触者として対応されるため通常のBCG接種を受けないこと，今回の報告での「真のコッホ現象」106例（13.0%）に対して，最終的な転帰が不明な他院に紹介された例54例（6.6%）および不明44例（5.4%）に「真のコッホ現象」が含まれていたと推定されること，保護者が局所反応を見過ごして報告されなかった可能性，局所反応判明後に適切な感染診断がなされなかった可能性，対象月齢が生後4カ月程度で推定されている年間感染危険率よりも感染が起こっていない可能性などが考えられる。

結　語

報告数には人為的な要因が関係している可能性があり，適切な措置の徹底を図るため，今後とも保護者および医療機関等に対してコッホ現象に関する正しい情報提供をする必要がある。コッホ現象にかかわる重篤な副反応の報告は見られず，BCG直接接種は安全であることが確認できた。

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Original Article

ANALYSIS OF KOCH'S PHENOMENON BY BCG VACCINATION WITH THE MULTI-PUNCTURE METHOD IN JAPAN

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Abstract [Purposes] In Japan, BCG vaccination without a prior tuberculin skin test was started in 2005. Koch's phenomenon is well known as a skin reaction that appears within a few days at the BCG vaccination site if the vaccination is given to a person infected with tuberculosis. However, little has been known regarding Koch's phenomenon in cases where BCG is administered by the multi-puncture method. All doctors who observe Koch's phenomenon are requested to provide a report to the local government, which then transfers the report to the Ministry of Health, Labour, and Welfare. The purpose of the present study was to clarify the issues and challenges regarding Koch's phenomenon in Japan.

[Methods] We analyzed a total of 814 reports of Koch's phenomenon submitted between April 2005 and March 2009. The results were redefined in this study as follows. Non-specific reaction: Cases that we judged to not be infected with *M. tuberculosis* (not true Koch's phenomenon). This category includes cases classified as "follow up" on the report with a negative PPD result. Follow-up with positive tuberculin test: Cases that were highly suspected to be infected from a positive tuberculin test but that were followed up without treatment. This category includes cases in which treatment was recommended but was refused by the guardians. Koch's phenomenon: Cases that were treated as latent tuberculosis infection or disease. Referred to other hospital: Cases that were referred to another hospital and their final outcomes are not known. Unknown: Cases for which the final outcomes are not known due to a lack of information.

[Results] The age at vaccination from 3 to 6 months in most cases, with an average age of 4 months (124 days). Skin reactions were noticed within 3 days in most (95.6%) of the cases. No serious reactions due to Koch's phenomenon were reported. The numbers of reported cases and the rates by the number of births were quite diverse among prefectures. The results for the reports were as follows: non-specific reaction: 578 (71.1%); follow-up with positive tuberculin test: 34 (4.2%); Koch's phenomenon: 106 (13.0%); referred to other hospital: 54 (6.6%); unknown: 44 (5.4%).

[Discussion] The differences in the number of reports by prefecture may partially be explained by differences in the risk of infection, but mostly by human factors such as: 1) explanation of Koch's phenomenon to guardian at the time of vaccination; 2) reaction to notification from guardian; 3) report system from doctor in charge to MHWL etc.

The results showed a trend toward a steady decrease in the non-specific reaction over the 4-year period. When BCG direct vaccination was started in 2005, health professionals were not aware that a mild skin reaction at the vaccinated site could appear and then fade out within a few days without any special reason. Almost all the noted skin reactions in the first year were reported. It is now known, however, that such non-specific reactions can appear together with a negative tuberculin skin test and then fade out within a few days. The incidence of a "true" Koch's phenomenon (cases treated as LTBI or disease as well as cases diagnosed as LTBI but for which treatment was refused by guardians) was less than estimated based on the annual risk of infection. This result is probably due to the following: 1) some cases with a risk of infection do not receive the BCG; 2) a final result was not obtained in 12.0% of the cases, which must include a certain number of cases with a "true" Koch's phenomenon; 3) skin reactions were sometimes missed by guardians; 4) a proper diagnosis was not made for a suspected case; 5) the actual risk of infection in infants aged less than 4 months is less than estimated.

[Conclusion] Accurate information regarding Koch's phenomenon should be provided to guardians as well as doctors and/or health workers in charge of BCG vaccinations.

Key words: BCG, Koch's phenomenon, Multi-puncture method, Risk of infection

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業　務

結核看護の質の向上をめざして…
「コホート観察」による治療・患者支援の評価

結核研究所 山内祐子（本稿作成），永田容子，小林典子，森　亨

はじめに

平成19年より国の結核サーベイランスの電算システムが新しくなり，名称も『結核発生動向調査システム』から『結核登録者情報システム』に変わりました。それに伴って従来のシステムでは他の関連項目を入力すると自動的に内容が決められるよう設定されていた「コホート観察」の入力項目や，転帰の区分決定の論理（アルゴリズム）も大幅に変更され，画面に表示されている名称も「コホート観察」から「治療成績」となりました。

結核研究所保健看護学科で研究活動の中で開発してきた『結核看護システム』〔保健師・看護師の結核展望No.89（2007.前期）に紹介〕における「コホート観察」は，国の旧システムの入力項目・転帰（治療成績）判定のアルゴリズムを基本に展開しています。国の新システムはより個別的・臨床的な治療成績評価に重点を置いているのに対して，『結核看護システム』では患者支援と治療成績の関連をみることを重視している点で，現場では両者が相補う関係にあります。

そこで，将来のよりよい治療評価や患者支援のための情報システムの向上を目指して試行を行っている『結核看護システム』について，国の「治療成績」との関係を検証し，今後のシステムの改善を検討しました。

方　法

『結核看護システム』の入力項目と国の「治療成績」の関連項目は完全な互換性はないので，厚生労働科学研究費新興再興感染症研究班（主任研究者結核研究所加藤誠也副所長）の分担課題の一つとして行っている「服薬支援看護ワークショップ」に参加している県市保健所の研究協力者の協力の下，各保健所の登録者情報をCSVファイルに変換したファイルを個人識別情報を消去したうえで提供してもらい，これらの登録者について従来のアルゴリズムによる「コホート観察」を再現し，これと「治療成績」とを比較しました。

作業の流れは図1のとおりで，〔取り込み→「コホート観察」の自動設定→帳票印刷〕をシステム化（『「コホート観察」サポートシステム』）しています。この作業の手順はこの研究の目的のために開発されたプログラムによるものです。

協力保健所

図2は，今回図1のようにして国の新システムから研究用のファイルを作成し，提供していただいた保健所です。13県42保健所から19年の新登録肺結核患者2,328人について新旧の関連情報を得て，これについて分析を行いました。

図1　『「コホート観察」サポートシステム』について

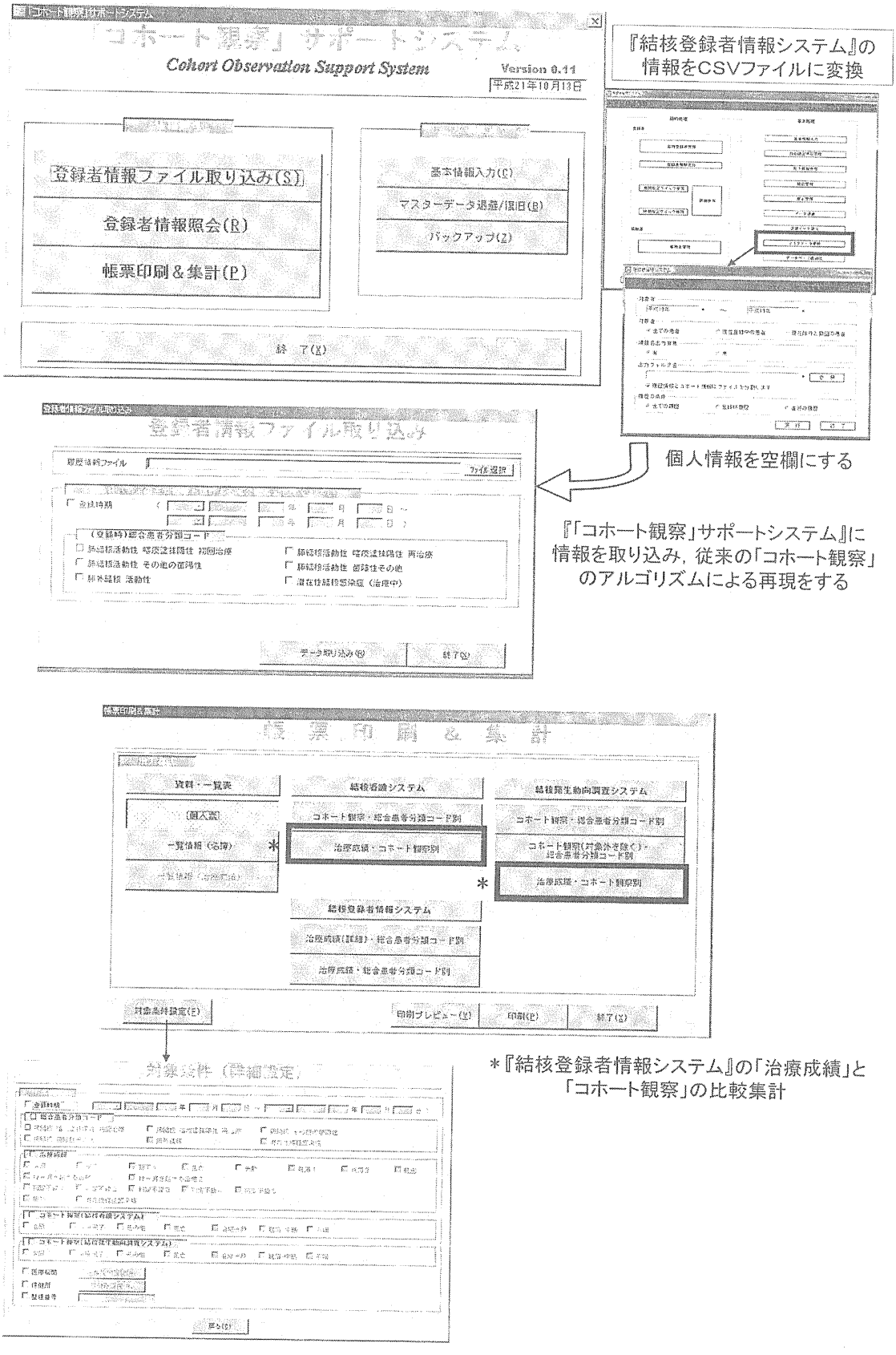


図２　協力保健所一覧

県名	保健所名
山形県	最上，置賜，庄内
茨城県	常陸大宮，日立，鉾田，竜ヶ崎，土浦，筑西，常総，ひたちなか，古河，つくば
群馬県	前橋市，富岡，中之条，館林，桐生
東京都	台東区，板橋区
神奈川県	横浜市
石川県	南加賀，石川中央，能登中部，能登北部
静岡県	御殿場
愛知県	一宮，豊川，西尾，師勝，衣浦東部
大阪府	堺市
和歌山県	和歌山市，海南，岩出，橋本，湯浅，田辺，新宮
岡山県	岡山市
熊本県	御船
大分県	日田玖珠

結核登録者情報システム（国の新システム）

対象者2,328人の登録時の患者区分は肺結核喀痰塗抹陽性初回治療43.8%，同再治療3.9%，その他の結核菌陽性26.8%，菌陰性・その他25.5%でした。これらの患者の『結核登録者情報システム』の治療成績の判定区分（判定コード）は**図３**に示したとおり①～⑮に分けられますが，集計の際には「治癒」「完了」「死亡」「失敗」「脱落」「転出」「12か月を超える治療」「判定不能」の八つの「判定区分」に再分類されます。

図４は，『結核登録者情報システム』内で自動的に決定される「治療成績」の「判定コード」別に件数を出力した集計表です。「治療成績」が空白となっているケースについては，判定不能として対応します。

図５は**図４**の表を，「治療成績」をより簡便な「判定区分」別に整理したものです。平成19年肺結核登録患者2,328人の国の新システムによる「治療成績」は，治療成功（治癒＋完了50.2%），死亡（11.8%），失敗（0.8%），脱落（8.4%），転出（2.0%），12か月を超える治療（9.3%），判定不能（17.5%）となっていました。

結核看護システム

『結核発生動向調査システム』（旧システム）と『結核看護システム』は，分析する集合客体（以下客体）が異なるだけで，「コホート観察」の判定区分（コード）および判定の決定論理（アルゴリズム）は同じです。つまり，「コホート観察」では治療開始後６／９カ月時点で治療結果を**図６**に示すような定義に基づいて「治癒」「治療完了」「その他」「治療失敗」「脱落中断」「不明」と電算機が自動的に判定します。

肺結核登録患者2,328人を，「コホート観察」の判定区分で判定すると，治療成功（79.8%），死亡（12.2%），治療失敗（2.2%），脱落中断（0.5%），対象外（5.3%）でした。

図3 『結核登録者情報システム』…治療成績コード

判定区分	判定コード	説明
1. 治癒	①治癒	1年以内で指示中止完遂の月を含む過去3ヶ月間とそれ以前の2回菌陰性を確認
2. 完了	②完了	1年以内で指示中止完遂の月を含む過去3ヶ月間かそれ以前どちらか1回菌陰性を確認
↓	③完了＊	菌陽性結果後菌陰性を確認せず指示中止完遂
3. 死亡	④死亡	1年以内で治療完遂前に死亡
4. 失敗	⑤失敗	5ヶ月目以降に培養陽性が1度でもあり
5. 脱落	⑥脱落1	連続60日以上あるいは2月以上中断
↓	⑦脱落2	指示中止完遂だが180日未満あるいは270日未満の治療
6. 転出	⑧転出	1年以内で治療完遂前に転出
7. 12か月を	⑨12か月超治療1	標準治療が途中から変更となり長期化の可能性
超える治療 ↓	⑩12か月超治療2	その他の理由で長期化
8. 判定不能	⑪判定不能1	治療開始時化療なし(治療開始前死亡，剖検診断等)
↓	⑫判定不能2	治療開始時化療内容不明(入力手技の誤り，未把握等)
↓	⑬判定不能3	治療開始時標準治療でない
↓	⑭判定不能4	1年以内で治療完遂したが，途中でINHあるいはRFP中止
↓	⑮判定不能5	判定の情報不十分，その他

図4 『結核登録者情報システム』集計表①

結核登録者情報システム　治療成績(詳細)・総合患者分類コード別

平成21年10月14日

総　数

		結核患者												肺外結核		潜在性結核感染症	
		総数		肺結核													
				総数		喀痰塗抹陽性				その他の結核菌陽性		菌陰性・その他					
						総数		初回治療		再治療							
総　数	(%)	2,372	(100.0%)	2,328	(100.0%)	1,111	(100.0%)	1,029	(100.0%)	91	(100.0%)	623	(100.0%)	594	(100.0%)	544	(100.0%)	325	(100.0%)
1.治癒	(%)	277	(9.8%)	277	(11.9%)	172	(15.5%)	157	(15.4%)	15	(16.5%)	74	(11.9%)	31	(5.2%)	-	-	-	-
2.完了	(%)	740	(25.3%)	740	(31.9%)	319	(28.7%)	302	(29.6%)	17	(18.7%)	153	(24.6%)	268	(45.1%)	-	-	-	-
3.完了＊	(%)	152	(5.3%)	152	(6.5%)	46	(4.1%)	45	(4.4%)	1	(1.1%)	99	(15.9%)	7	(1.2%)	-	-	-	-
4.死亡	(%)	275	(9.6%)	275	(11.8%)	173	(15.6%)	162	(15.9%)	16	(17.6%)	55	(8.8%)	42	(7.1%)	-	-	-	-
5.失敗	(%)	18	(0.6%)	18	(0.8%)	10	(0.9%)	9	(0.9%)	1	(1.1%)	8	(1.3%)	-	-	-	-	-	-
6.脱落1	(%)	14	(0.5%)	14	(0.6%)	7	(0.6%)	6	(0.6%)	1	(1.1%)	4	(0.6%)	3	(0.5%)	-	-	-	-
7.脱落2	(%)	182	(6.3%)	182	(7.8%)	36	(3.2%)	35	(3.4%)	1	(1.1%)	65	(10.4%)	81	(13.6%)	-	-	-	-
8.転出	(%)	46	(1.6%)	46	(2.0%)	31	(2.8%)	29	(2.8%)	2	(2.2%)	8	(1.4%)	6	(1.0%)	-	-	-	-
9.12か月を超える治療1	(%)	36	(1.3%)	36	(1.5%)	22	(2.0%)	18	(1.8%)	4	(4.4%)	9	(1.4%)	5	(0.8%)	-	-	-	-
10.12か月を超える治療2	(%)	181	(6.3%)	181	(7.8%)	94	(8.5%)	83	(8.1%)	11	(12.1%)	56	(9.0%)	31	(5.2%)	-	-	-	-
11.判定不能1	(%)	45	(1.6%)	45	(1.9%)	20	(1.8%)	19	(1.8%)	1	(1.1%)	13	(2.1%)	12	(2.0%)	-	-	-	-
12.判定不能2	(%)	139	(4.8%)	139	(6.0%)	82	(7.4%)	76	(7.5%)	6	(6.6%)	28	(4.5%)	28	(4.9%)	-	-	-	-
13.判定不能3	(%)	52	(1.8%)	52	(2.2%)	25	(2.3%)	16	(1.6%)	9	(9.9%)	13	(2.1%)	14	(2.4%)	-	-	-	-
14.判定不能4	(%)	14	(0.5%)	14	(0.6%)	5	(0.5%)	5	(0.5%)	-	-	8	(1.3%)	1	(0.2%)	-	-	-	-
15.判定不能5	(%)	155	(5.4%)	155	(6.7%)	63	(5.7%)	57	(5.6%)	6	(6.6%)	29	(4.7%)	63	(10.6%)	-	-	-	-
16.肺外	(%)	543	(18.9%)	-	-	-	-	-	-	-	-	-	-	-	-	543	(99.8%)	-	-
17.潜在性結核感染症	(%)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	325	(100.0%)
空白	(%)	3	(0.1%)	2	(0.1%)	1	(0.1%)	1	(0.1%)	-	-	-	-	1	(0.2%)	1	(0.2%)	-	-

【集計条件】

●登録開始時期：平成19年1月1日 ～ 平成19年12月31日

「空白」は「判定不能」として対応

図５　『結核登録者情報システム』集計表②

結核登録者情報システム　治療成績・総合患者分類コード別

平成21年10月14日

総　数

		結核患者												潜在性結核感染症	
		総数	肺結核								肺外結核				
			総数	喀痰塗抹陽性				その他の結核菌陽性	菌陰性・その他						
				総数	初回治療		再治療								
総　数	(%)	2,372	(100.0%)	2,328	(100.0%)	1,111	(100.0%)	1,020	(100.0%)	91	(100.0%)	823	(100.0%)	594	(100.0%)	544	(100.0%)	325	(100.0%)
1.治癒	(%)	277	(9.6%)	276	(11.9%)	172	(15.5%)	157	(15.4%)	15	(16.5%)	74	(11.3%)	31	(5.2%)	-	-	-	-
2.完了+3.完了*	(%)	892	(31.1%)	892	(33.3%)	365	(32.9%)	347	(34.0%)	18	(19.8%)	252	(40.4%)	275	(46.3%)	-	-	-	-
4.死亡	(%)	275	(9.6%)	275	(11.2%)	178	(16.0%)	162	(15.9%)	16	(17.6%)	55	(8.5%)	42	(7.1%)	-	-	-	-
5.失敗	(%)	18	(0.6%)	18	(0.8%)	10	(0.9%)	9	(0.9%)	1	(1.1%)	8	(1.2%)	-	-	-	-	-	-
6.脱落+7.脱落2	(%)	198	(6.9%)	196	(8.4%)	43	(3.9%)	41	(4.0%)	2	(2.2%)	69	(11.1%)	84	(14.1%)	-	-	-	-
8.転出	(%)	46	(1.6%)	46	(2.0%)	31	(2.8%)	29	(2.8%)	2	(2.2%)	9	(1.4%)	6	(1.0%)	-	-	-	-
9.12か月…+10.12か月…	(%)	217	(7.6%)	217	(9.3%)	118	(10.6%)	101	(9.9%)	15	(16.5%)	65	(10.4%)	36	(6.1%)	-	-	-	-
11.判定不能1~15.判定不能5+空白	(%)	408	(14.2%)	407	(17.5%)	196	(17.6%)	174	(17.1%)	22	(24.2%)	91	(14.6%)	120	(20.2%)	1	(0.2%)	-	-
16.肺外+17.潜在性…	(%)	543	(18.9%)	-	-	-	-	-	-	-	-	-	-	-	-	543	(99.8%)	325	(100.0%)

【集計条件】
●登録開始時期：平成19年1月1日 ～ 平成19年12月31日

治療成功50.2%

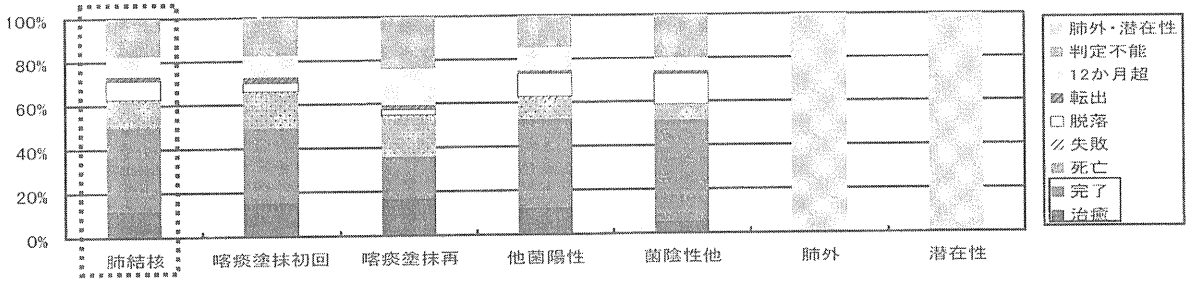


図６　『結核発生動向調査システム』・『結核看護システム』…「コホート観察」コード

「コホート観察」コード	説明
1. 治癒	所定治療期間の前半の最後の菌所見が陰性で，かつ後半の期間中に菌陽性所見がなく，かつ菌陰性の所見が1回以上あること
2. 治療完了	所定治療期間の最後の菌所見が陰性で，かつ「1. 治癒」，「治療失敗」に該当しない者
3. その他	他のどのコードにも該当しない者
4. 死亡	所定治療期間中に死亡した者
5. 治療失敗	所定治療期間の後半に一度でも菌陽性の所見のある者
6. 脱落・中断	所定治療期間中に2ヶ月以上治療を受けなかった者
7. 不明	所定治療期間中の情報が何一つ入力されていない者

図7 『結核看護システム』集計表

結核看護システム　コホート観察・総合患者分類コード別

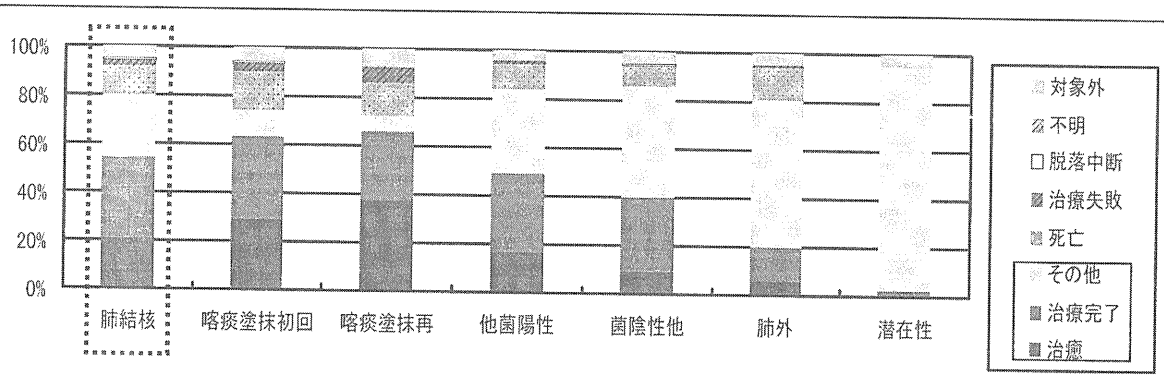
平成21年10月19日

総数

	結核患者 総数		肺結核 総数		喀痰塗抹陽性 総数		喀痰塗抹陽性 初回治療		喀痰塗抹陽性 再治療		その他の結核菌陽性		菌陰性 その他		肺外結核		潜在性結核感染症	
総数	2,972	(100.0%)	2,328	(100.0%)	1,111	(100.0%)	1,020	(100.0%)	91	(100.0%)	823	(100.0%)	594	(100.0%)	544	(100.0%)	325	(100.0%)
治癒 (%)	522	(18.2%)	491	(21.1%)	330	(29.7%)	296	(29.0%)	34	(37.4%)	106	(17.0%)	55	(9.3%)	31	(5.7%)	-	-
治療完了 (%)	842	(29.3%)	765	(32.9%)	380	(34.2%)	354	(34.7%)	26	(28.6%)	202	(32.4%)	183	(30.8%)	77	(14.2%)	7	(2.2%)
その他 (%)	932	(32.5%)	600	(25.8%)	109	(9.8%)	103	(10.1%)	6	(6.6%)	217	(34.8%)	274	(46.1%)	332	(61.0%)	303	(93.2%)
死亡 (%)	359	(12.5%)	285	(12.2%)	176	(15.8%)	164	(16.1%)	12	(13.2%)	61	(9.8%)	46	(7.7%)	74	(13.6%)	-	-
治療失敗 (%)	53	(1.8%)	52	(2.2%)	42	(3.8%)	36	(3.5%)	6	(6.6%)	9	(1.4%)	1	(0.2%)	1	(0.2%)	-	-
脱落中断 (%)	15	(0.5%)	11	(0.5%)	4	(0.4%)	4	(0.4%)	-	-	3	(0.5%)	4	(0.7%)	4	(0.7%)	-	-
不明 (%)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
対象外 (%)	149	(5.2%)	124	(5.3%)	70	(6.3%)	63	(6.2%)	7	(7.7%)	25	(4.0%)	29	(4.9%)	25	(4.6%)	15	(4.6%)

【集計条件】
●登録開始時期：平成19年1月1日 ～ 平成19年12月31日

治療成功79.8%



両システムの比較

図8はシステム間の違いのうち，まず判定の対象（客体）の定義の違いを表した図です。

現在の国のシステムでは，肺結核を対象としていますが，化療内容コード4～10，つまり非標準的な治療方式の患者は，「判定不能2」「判定不能3」と分類されます。そして実質の判定の対象は化療内容コード1～3に限定されます。

旧システムでは，肺結核で標準治療を行っている患者（化療内容コード1～4）を対象としています。両システムの違いとなる「化療内容コード4」（INHとRFPの2剤治療）は，旧システムが用いられていた時代は標準治療でしたが，現在ではもはや標準治療ではなくなったため，国の現行システムでは「判定不能」に一括されています。

試行中の『結核看護システム』では，「コホート観察」分析を標準治療患者に，ではなく，すべての患者に服薬支援をという視点で治療成績の評価を行おうとする点に重要な違いがあります。

図9は三つのシステムの入力項目や入力方式の違いです。

現行『結核登録者情報システム』では，治療経過に関連する履歴情報がコホート情報へも反映されて，「コホート情報」項目が機械内部で自動的に書き込まれます。この「コホート情報」画面からも直接入力できます。

旧『結核発生動向調査システム』では，独立した「コホート情報」画面から治療経過情報を