Disinfectant-susceptibility of multi-drug-resistant *Mycobacterium tuberculosis* isolated in Japan

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**Abstract**

**Background:** Multi-drug-resistant *Mycobacterium tuberculosis* has been an important problem in public health around the world. However, limited information about disinfectant-susceptibility of multi-drug-resistant strain of *M. tuberculosis* was available.

**Findings:** We studied susceptibility of several Japanese isolates of multi-drug-resistant *M. tuberculosis* against disinfectants, which are commonly used in clinical and research laboratories. We selected a laboratory reference strain (H37Rv) and eight Japanese isolates, containing five drug-susceptible strains and three multi-drug-resistant strains, and determined profiles of susceptibility against eight disinfectants. The *M. tuberculosis* strains were distinguished into two groups by the susceptibility profile. There was no relationship between multi-drug-resistance and disinfectant-susceptibility in the *M. tuberculosis* strains. Cresol soap and oxydol were effective against all strains we tested, regardless of drug resistance.

**Conclusions:** Disinfectant-resistance is independent from multi-drug-resistance in *M. tuberculosis*. Cresol soap and oxydol were effective against all strains we tested, regardless of drug resistance.

**Keywords:** Multi-drug-resistant *Mycobacterium tuberculosis*, Disinfectant, Microbicide

**Findings**

Tuberculosis is still a major cause of death in low to middle-income countries and areas [1]. Furthermore, drug resistant and multi-drug-resistant tuberculosis has been reported worldwide [2]. Tuberculosis has remained as a major public health threat even in this century [1, 2].

Disinfectants are widely used to eliminate infectious agents from possibly contaminated equipment and specimens. Effectiveness of disinfectants against *M. tuberculosis* was reported previously [3–5]. However, poor information about disinfectant effectiveness against multi-drug-resistant (MDR) strain of *M. tuberculosis* is available. In this study, we first evaluated effectiveness of disinfectants against MDR-*M. tuberculosis* in just short time (1-min), on the supposition that the disinfectants were used for routine environmental cleaning by spray, wipe, or wash in relatively short time to *M. tuberculosis*. Then we discussed relationship between drug resistance and disinfectant resistance in *M. tuberculosis*.

*M. tuberculosis* strains used in this study are listed in Table 1. *M. tuberculosis* strain H37Rv is the reference strain isolated in US in 1934. Other eight strains were fresh clinical isolates from Japanese patients. The strains were cultured in Middlebrook 7H9 broth (BD Biosciences, Sparks, MD) supplemented with 10 % OADC Enrichment (BD Biosciences) and 0.05 % (w/v) Tween 80 (this medium was referred as MB broth).

We selected commonly used and easily available eight disinfectants, including 0.2 % (W/V) alkylidiamoethylglycine-HCl (ADEG), 0.1 % (W/V) chlorhexidine gluconate (CG), 10 mg/ml povidine iodine (PI), 0.1 % (W/V) benzalkonium-HCl (BK), 3 % (W/V) oxydol (OX), 2 % (V/V) cresol soap (CS), 70 % (V/V) ethanol (EtOH), and 0.1 % (W/V) benzalkonium-HCl + 70 % (V/V) ethanol (BK + EtOH). The disinfectants were diluted with 2% Tween 80 to make 1% solutions (final concentration) before use. The solutions were sprayed or wiped on the culture plates, or poured into the culture broth, and the plates were incubated at 37 °C for 1 min. The plates were then washed twice with 2% Tween 80 (1 ml) and the bacteria were cultured in 1 ml of fresh MB broth. The bacteria were cultured for 1-2 weeks before they were confirmed as dead by growth in fresh MB broth. The disinfectants were diluted with 2% Tween 80 to make 1% solutions (final concentration) before use.
distilled water according to the instructions of the manufacturers. *M. tuberculosis* strains were cultured in MB broth for 14 days at 37 °C. Optical density at 650 nm (OD650) of each bacterial culture was adjusted to 1.0. The adjusted bacterial culture was diluted to 0.1 of OD650 with fresh MB broth, and then the suspension was cultured in a sterilized tube with screw cap. After 30 s incubation at room temperature, the tube was centrifuged for 30 s at 9600 × g at room temperature and the supernatants were removed. The pellet was immediately resuspended in 1 ml of diluted disinfectant in a sterilized tube with screw cap. After 30 s incubation at room temperature, the tube was centrifuged for 14 days at 37 °C. The 14 days culture was sufficient to detect growth of *M. tuberculosis* strain H37Rv, which is a widely used laboratory strain, was susceptible to all the disinfectants tested in this study, even MDR *M. tuberculosis* strains in this study, the strain was isolated in 1934 [7], it could be adapted to laboratory propagations. The strain might lose resistance against bactericidal compounds.

We found that there were two groups in the Japanese isolates of *M. tuberculosis* based on susceptibility against disinfectants. Strain 2E-1-9, 2U-11-2, LV-15, and LV-79 were susceptible to most disinfectants used in this study, whereas the strain 2A-3-6, 2U-5-12, 2Z-1-3, and LV-36 showed resistance against most disinfectants except oxydol and cresol soap (Fig. 1, Table 1). There was no relationship between multi-drug-resistance and disinfectant-resistance in the selected *M. tuberculosis* strains. The tick and waxy cell wall of *M. tuberculosis* is assumed to act as a major barrier to penetration of antibiotics and disinfectants [8], and could affect to resistance against both antibiotics and disinfectants. Since there was no correlation between drug resistance and disinfectant resistance to the *M. tuberculosis* strains in this study, the drug resistance and the disinfectant resistance might be based on different mechanism in the strains.

Oxydol and cresol soap were effective against all *M. tuberculosis* strains tested in this study, even MDR strains. Our results suggested that more than 1 min

### Table 1 *M. tuberculosis* strains used in this study

| Strain | Place | Year | Multidrug resistance | Disinfectant |
|--------|-------|------|-----------------------|--------------|
| H37Rv  | US    | 1934 | Type strain          | +            |
| 2A-3-6 | Japan | 2002 | –                     | +            |
| 2E-1-9 | Japan | 2002 | –                     | +            |
| 2U-5-12| Japan | 2002 | –                     | +            |
| 2U-11-2| Japan | 2002 | –                     | +            |
| 2Z-1-3 | Japan | 2002 | –                     | +            |
| LV-15  | Japan | 2008 | INH, RFP, SM, EB, LVFX| –            |
| LV-36  | Japan | 2010 | INH, RFP, SM          | –            |
| LV-79  | Japan | 2009 | INH, RFP, SM, LVFX    | +            |

INH, isoniazid; RFP, rifampicin; SM, streptomycin; EB, ethambutol; and LVFX, levofloxacin, ADEG alkylidiaminoethylglycine-HCl (0.2 % W/V); PI, povidone iodine (10 mg/ml as active iodine); BK benzalkonium-HCl (0.1 % W/V); OX oxydol (3 % W/V); CS cresol soap (2 % V/V); EtOH ethanol (70 % V/V); and BK + EtOH benzalkonium-HCl (0.1 % W/V) + ethanol (70 % V/V)

+: the disinfectant inhibited growth of the bacteria (effective disinfectants)

−: the disinfectant did not inhibit growth of the bacteria (not effective)
treatments with oxydol or cresol soap were promising to eliminate contamination of *M. tuberculosis*, regardless of drug resistance. However, it is known that disinfectants could decrease their efficacy by organic compounds, for example, blood, sputum, and other dirt. It should be noticed that disinfectant could be used carefully against *M. tuberculosis* in clinical specimens and things that contain organic compounds.

**Competing interests**
The authors declare that they have no competing interests.

**Authors’ contributions**
NS performed study design, data analyses and manuscript preparation. SM assisted with the microbiological test. ES assisted with study design and data analyses. MW all assisted with study design, data analysis and manuscript preparation. All authors read and approved the final manuscript.

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