BIOMEDICAL AND ENVIRONMENTAL SCIENCES **18**, 124-127 (2005)

Comparison of Susceptibilities of *M. tuberculosis* H37Ra and *M. chelonei subsp. Abscessus* to Disinfectants[1]

GUO-QING WANG[*], CHAO-WU ZHANG[*], HENG-CHUAN LIU[*], AND ZHAO-BIN CHEN[#]

[*]*Department for Medical Technology, Huaxi School of Public Health, Sichuan University, Chengdu 610041, Sichuan, China;*
[#]*Nanshan CDC, Shenzhen 518052, Guangdong, China*

Objective To determine the susceptibilities of *M. tuberculosis* H37Ra and *M. chelonei subsp. absecessus* to several frequently-used disinfectants and to evaluate the practicability of surrogating *M. tuberculosis* by the latter. **Methods** A suspension quantitative bactericidal test was set up in accordance with Chinese Technique Standard for Disinfection to evaluate the susceptibility of each mycobacteria strain to each selected disinfectant. Killing log value was used as criterion in comparing the susceptibility to disinfectants between the two strains. **Results** *M. chelonei subsp. abscessus* was more resistant to chlorine disinfectant than *M. tuberculosis* while the two strains were similarly resistant to iodophor disinfectant, peracetic acid, alcohol and glutaraldehyde disinfectant. **Conclusion** *M. chelonei subsp. abscessus* has the potential to surrogate *M. tuberculosis* in evaluating mycobactericidal efficacies of disinfectants.

Key words: *Mycobacterium chelonei subsp. Abscessus*; *Mycobacterium tuberculosis*; Susceptibility

INTRODUCTION

Tuberculosis is still a major killer worldwide while nontuberculosis mycobacterial infection is more and more frequently reported, especially in immune-compromised individuals such as AIDS sufferers. Since mycobacterial infections are difficult to treat, prevention is of great importance. Mycobacteria are well known for their resistance to disinfectants, so disinfectants as weapons against mycobacteria should be selected and used properly. Many countries have their own authorized mycobactericidal tests, but they are different from one another either in test strain or test procedure. China has released a mycobactericidal test in 2002. In this test, *M. chelonei subsp. Abscessus* CMCC(B) 93326 is selected as the test strain. We studied several frequently-used disinfectants against *M. chelonei subsp. abscessus* and *M. tuberculosis* H37Ra.

MATERIALS AND METHODS

Strains and Agents

Test strains *M. tuberculosis* H37Ra CMCC(B) 93020 *and M. chelonei subsp. abscessus* CMCC(B) 93326, were obtained from the Chinese Medical Culture Collection of Bacteria.

Disinfectants used were *ERIC* (an iodophor based disinfectant, produced by Chengdu Yongan Pharmacia CO), GA-50 (a glutaraldehyde-based disinfectant, produced by Huaxi Center of Health Care Science and Technology), TC-101disinfection tablet (a chlorine-based disinfectant, produced by Chinese PLA 7018 Factory), peracetic acid and alcohol. Peracetic acid (PAA) was prepared by mixing hydrogen peroxide and acetic acid in our laboratory as described previously[1]. Alcohol was prepared by diluting dehydrated ethanol with sterile distilled water.

Neutralizers Neutralizers to each disinfectant were testified to ensure that they could inactivate its effects.

Mycobacteria dehydrated media (Shanghai Golden Bridge International Biotech Communication Center) were prepared according to the manufacturer's instructions. L-J media were prepared as previously discribed[2].

Methods

Concentration of disinfectants Before mycobactericidal test, each disinfectant solution was tested for its actual concentration in accordance with

[1] This work was supported by NSFC (No. 30070685).

Biographical note of the first author: Guo-Qing WANG, male, born in 1975, lecturer, M. D. candidate, majoring in hygienic microbiological research and teaching.

0895-3988/2005
CN 11-2816/Q

Chinese Technique Standard for Disinfection, then diluted in sterile distilled water to the selected concentrations.

Suspension of test mycobacteria Mycobacterial strains were incubated on a slant of mycobacteria dehydrated media at 37℃, H37Ra for 4 wk and *M. chelonei subsp. abscessus* for 4 d, respectively. Three to five mL sterile PBS solution (0.03 mol/L pH7.2 phosphorous buffer solution containing 0.1% Tween 80) was added to the slant and the culture was scraped to the PBS by a sterile incubation loop. Then the PBS mixture was transferred to a sterile flask containing glass beads and shaken vigorously for 5 to 10 minutes. Then it was diluted in sterile PBS to 10^7 cfu per mL - 10^8 cfu per mL.

Mycobactericidal test Five mL of each concentration of disinfectant was transferred to a tube at 20℃ for 5 min before 0.1 mL of mycobacterial suspension was added to each tube, then 0.5 mL mixture of the bacteria and disinfectant was transferred to 4.5 mL neutralizer solution to inactivate the disinfectant's effect. After10 min, 0.2 mL solution was inoculated to the surface of L-J medium plate, clone-forming units were counted after incubation at 37℃, then 0.1 mL mycobacterial suspension was added to 5.0 mL sterile PBS to serve as the mycobacterial viable control. Each test was carried out at least 3 times and average killing log of exposed time was calculated to evaluate the bactericidal efficacy of disinfectants against each mycobacterium.

RESULTS

The efficacy of TC-101 solution against mycobacteria is showen in Table 1. TC-101 solution with available chlorine 40 mg/L could get a 4.15 log killing of *M. tuberculosis* H37Ra after exposure for 5 min while available chlorine 80 mg/L could only get a 3.10 log killing of *M. chelonei subsp. abscessus*. If more than 6 log reduction was taken as criterion of qualified disinfection, available chlorine 40 mg/L for 10 min exposure was required for H37Ra while available chlorine 80 mg/L for 20 min exposure was required for *M. chelonei subsp. abscessus*.

Tables 2 to 5 show the comparative efficacy of the other 4 disinfectants against *M. chelonei subsp. abscessus* and H37Ra. The 4 disinfectants had a lower killing log value of *M. chelonei subsp. abscessus* than H37Ra, but the difference was very slight, indicating that these two strains had similar resistance to iodophor, glutaraldehyde, alcohol and peracetic acid.

TABLE 1

Efficacy of TC-101 Solution Against *M. chelonei subsp. abscessus* and *M. tuberculosis* H37Ra

Mycobacterial Strain	Concentration of TC-101 Solution (mg/L Available Chlorine)	Average killing Log Value of Each Exposure Time (min)			
		1	5	10	20
M. tuberculosis H37Ra	40	2.61	4.15	⩾6	⩾6
	20	1.71	2.61	3.85	4.93
	10	0.98	1.84	2.64	4.02
M.chelonei subsp. abscessus	80	1.70	3.10	4.55	⩾6
	40	0.97	2.00	2.87	4.24
	20	0.52	0.97	1.95	2.79

TABLE 2

Efficacy of ERIC against *M. chelonei subsp. abscessus* and *M. tuberculosis* H37Ra

Mycobacterial Strain	Concentration of ERIC (mg/L Available Iodine)	Average Killing Log Value of Each Exposure Time (min)			
		1	5	10	20
M. tuberculosis H37Ra	40	2.46	3.85	⩾6	⩾6
	20	1.37	2.18	2.91	4.09
	10	1.05	1.48	2.20	2.86
M. chelonei subsp. abscessus	40	2.22	3.35	5.23	⩾6
	20	0.96	1.77	2.47	3.48
	10	0.61	1.03	1.79	2.48

TABLE 3

Efficacy of GA-50 against *M. chelonei subsp. abscessus* and *M. tuberculosis* H37Ra

Mycobacterial Strain	Concentration of GA-50 (% GTA)	Average Killing Value Log of Each Exposure Time (min)			
		1	5	10	20
M. tuberculosis H37Ra	1	3.14	4.33	5.23	⩾6
	0.5	2.18	3.13	4.33	⩾6
	0.25	1.03	1.73	2.50	3.24
M. chelonei subsp.abscessus	1	3.20	4.53	⩾6	⩾6
	0.5	2.12	3.23	4.63	5.23
	0.25	0.77	1.57	2.26	3.21

TABLE 4

Efficacy of PAA against *M. chelonei subsp. abscessus* and *M. tuberculosis* H37Ra

Mycobacterial Strain	Concentration of PAA (% PAA)	Average Killing Log Value of Each Exposure Time (min)			
		1	5	10	20
M. tuberculosis H37Ra	0.2	3.35	4.45	4.93	⩾6
	0.1	1.83	3.19	4.45	⩾6
	0.05	0.89	2.17	3.13	4.45
M.chelonei subsp.abscessus	0.2	3.60	⩾6	⩾6	⩾6
	0.1	1.71	2.62	3.71	5.03
	0.05	0.89	1.77	2.65	3.77

TABLE 5

Efficacy of Alcohol against *M. chelonei subsp. abscessus* and *M. tuberculosis* H37Ra

Mycobacterial Strain	Concentration of Alcohol (% Ethanol)	Average Killing Log Value of Each Exposure Time (min)			
		1	5	10	20
M. tuberculosis H37Ra	60	4.21	⩾6	⩾6	⩾6
	45	2.61	4.00	5.11	⩾6
	30	0.43	0.86	1.44	1.81
M. chelonei subsp. abscessus	60	4.13	⩾6	⩾6	⩾6
	45	2.19	3.33	5.08	⩾6
	30	0.39	0.70	1.04	1.65

Exposed to 40 mg/L available chlorine for 10 min, *M. tuberculosis* H37Ra could be reduced by more than 6 log. *M. chelonei subsp. abscessus* exposed to 80 mg/L available chlorine for 20 min, the killing log value also could reach 6 or more, indicating that *M. chelonei subsp. abscessus* was more resistant to chlorine disinfectant than *M. tuberculosis* H37Ra. The differences in killing log value of iodophor, glutaraldehyde, alcohol and peracetic acid between *M. chelonei subsp. abscessus* and *M. tuberculosis* H37Ra, were very slight, thus leading to a conclusion that these two strains of mycobacteria have similar resistance to the 4 selected disinfectants.

DISCUSSION

Tuberculosis (TB) is threatening human's health in both developing and developed countries. It kills approximately 2 million people each year[4]. China is one of the highest burdened regions, about 44.5% of citizens are infected with *M. tuberculosis* in China[5]. Infection of nontuberculous mycobacteria has increased rapidly since 1980s[6-8]. Disinfection is an

effective way to control mycobacterial infection. Because of their high content of lipid in cell wall, mycobacteria are more resistant to disinfectants than vegetatives of other bacteria, hence vegetatives of general bacteria can not be used to evaluate mycobactericical efficacies of disinfectants.

In order to evaluate the microbiocidal efficacy of a disinfectant, a specific means is needed to inactivate the lasting effect of disinfectant after the interaction between microbes and disinfectant, or else, the result can only reflect the inhibitory ability of disinfectant. Frequently-used methods to inactivate the disinfectant's effect can be classified as chemical and physical methods. Chemical methods function by chemical reaction with disinfectants while physical ones by physical processes such as dilution and centrifugation. Chemical methods can give a more accurate evaluation of disinfectants' microbiocidal efficacy because they react with disinfectants rapidly, the compounds used to inactivate the disinfectant is called neutralizers. The ability of both chemical and physical methods inactivate the disinfectants must be verified so that the inactivation processes can really inactivate the disinfectant's effect and the processes themselves do no harm to the test strains. Neutralizers used in this study have been verified in accordance with Chinese Technique Standard for Disinfection. The results (not listed in this paper) prove that ①0.03 mol/L pH 7.2 PBS containing 0.5% Tween 80 and 2% $Na_2S_2O_3$ can be used as a neutralizer to *ERIC*, TC-101 and peracetic acid; ② 0.03 mol·Ll pH 7.2 PBS containing 1% Tween 80 and 1% glycin and 1% lecithin can be used as a neutralizer to GA-50; and ③0.03 mol/L pH 7.2 PBS containing 0.5% lecithin and 1% Tween 80 can be used as a neutralizer to alcohol.

Mycobacterium, the pathogen of tuberculosis, should be used as the most direct subject. However, its high pathogenesis and slow growth limit its use. Selecting a rapidly growing and low-pathogenic mycobacterium is an important item of mycobacterial disinfection study. We compared the susceptibility of *M. tuberculosis* and a rapid growing mycobacterium, *M. chelonei subsp. Abscessus*, to disinfectants and found that *M. chelonei subsp. abscessus* was as resistant as, *M. tuberculosis* to chlorine, iodopher, glutaraldehyde, alcohol and peracetic acid disinfectants, suggesting that it can surrogate *M. tuberculosis* in mycobactericidal evaluation tests.

CONCLUSION

In conclusion, *M. chelonei subsp. abscessus* CMCC(B) 93326 is more resistant to chlorine disinfectant than *M. tuberculosis* H37Ra and is similarly resistant to other 4 disinfectants with *M. tuberculosis* H37Ra. Since it grows rapidly and is less pathogenic to human beings, *M. chelonei subsp. abscessus* may be used as a standard strain in mycobactericidal tests.

REFERENCES

1. Wang, F. and Yang, H. M. (2002). Peroxide disinfectants. pp.79-86. *In* Yang, H. M., Yi, B. (Ed.). Modern hospital disinfection. Peoples Military Press, Beijing. (In Chinese)
2. Chinese Antituberculosis Association. (1996). Standard for tuberculosis diagnostic bacterial test. *Chin. J. Anti-Tuberculosis* **18**(1), 28-31. (In Chinese)
3. Ministry of Health of P. R. China (1999). Chinese technique standard for disinfection. 1st part. 3rd Edition. (In Chinese)
4. WHO (2002). Tuberculosis. http://www.who.int/mediacentre/factsheets/fs104/en/
5. National Technic Steering Group of the Epidemiological Sampling Survey for Tuberculosis. (2002). Report on fourth national epidemiological sampling survey for tuberculosis. *Chin. J. Tuberc. Respir. Dis*. **25**(1), 3-7. (In Chinese)
6. Zhang, Y. R., Gui, X. H., and Li, J. (2000). Analysis of the nosocomial infection caused by atypical mycobacteria. *Chin. J. Nosocomiol*. **10**(6), 425-426. (In Chinese)
7. Ramaswamy, A. A., Biswas, J., Bhaskar, V., Gopal, L., Rajagopal, R., and Madhavan, H. N. (2000). Postoperative *Mycobacterium chelonae* endophthalmitis after extracapsular cataract extraction and posterior chamber intraocular lens implantation. *Ophthalmology* **107**(7), 1283-1286.
8. Massimo, B., Diego, P., and Robert, C. A. (2003). *Mycobacterium chelonae* interface infection after endokeratoplasty. *Am. J. Ophthalmol.* **135**(3), 393-395.

(*Received May* 26, 2004 *Accepted November* 26, 2004)