

Evaluation of Oral Antiseptic Rinsing before Sputum Collection To Reduce Contamination of Mycobacterial Cultures[∇]

Renata L. Peres,¹ Moisés Palaci,¹ Rafaela B. Loureiro,¹ Reynaldo Dietze,¹ John L. Johnson,² Jonathan E. Golub,^{3,4} A. Ruffino-Netto,⁵ and Ethel L. Maciel^{1*}

Center for Infectious Diseases, Federal University of Espírito Santo, Vitória, Brazil¹; University Hospitals Case Medical Center, Case Western Reserve University School of Medicine, Cleveland, Ohio²; Center for TB Research, Johns Hopkins University School of Medicine, Baltimore, Maryland³; Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland⁴; and University of São Paulo at Ribeirão Preto School of Medicine, Ribeirão Preto, Brazil⁵

Received 17 March 2011/Returned for modification 26 April 2011/Accepted 3 June 2011

To assess whether rinsing with oral antiseptics before sputum collection would reduce contamination of mycobacterial cultures, 120 patients with suspected tuberculosis were randomly assigned to rinse with chlorhexidine or cetylpyridinium mouthwash before collection. The culture contamination rate was significantly lower after rinsing with chlorhexidine before collection, especially for cultures grown in MGIT medium.

One of the main limitations of sputum culture for mycobacteria is contamination and overgrowth with organisms from the oral flora. Antiseptics have been used to supplement oral hygiene to minimize the proliferation of pathogenic organisms (13). Due to their glycolipid-containing cell wall, mycobacteria are relatively resistant to many common disinfectants (14, 15). Chlorhexidine (CHX) and cetylpyridinium chloride (CPC), two of the most widely used antiseptic compounds in mouthwashes, are active against common oral bacteria and fungi at low concentrations (2, 3, 4, 9, 11, 16) and are relatively non-toxic to human cells. Mycobacteria are highly resistant to CHX (16). CPC has been used at a 0.5% final concentration in the decontamination of sputum for mycobacterial culture (17).

Better methods are needed to decrease the contamination rate of sputum specimens for mycobacterial culture. We conducted a randomized trial to assess whether oral rinsing with mouthwashes containing CHX and CPC before sputum collection would decrease the rate of culture contamination. The protocol was approved by the institutional review board. All patients gave informed consent for participation. Eighteen-

60-year-old adults with suspected pulmonary tuberculosis (TB) evaluated at two public clinics from December 2007 to December 2008 were eligible. One sputum sample was collected from each patient on two consecutive days. On the first day, a first morning sputum sample was collected at the clinic using routine procedures (control group) in which patients washed their hands with soap and water and rinsed their mouths with tap water before collecting the specimens. Patients returned to the clinic the next morning, when they were randomly assigned using a computer-generated sequence to rinse their mouths with 10 ml of a commercially available mouthwash solution for 1 min before sample collection. The solutions used were (i) Periogard solution (Colgate-Palmolive, São Paulo, Brazil), containing a 0.12% final concentration of CHX gluconate, and (ii) Colgate Plus solution (Colgate-Palmolive, São Paulo, Brazil), containing a final concentration of 0.05% of CPC. Specimens were refrigerated at 4°C until processing within 2 h after collection. Decontamination of specimens was done as previously described (12). The sediment was resuspended in phosphate-buffered saline (PBS) and inoculated on Ogawa slants

TABLE 1. Recovery of *M. tuberculosis* from sputum specimens cultured on Ogawa or MGIT medium after routine oral rinsing with water or an antiseptic mouthwash before collection

Study group ^a	No. (%) of cultures that were:					Time to detection (days) using:	
	Positive for <i>M. tuberculosis</i>			Contaminated		Ogawa	MGIT
	Ogawa	MGIT ^b	Both	Ogawa	MGIT		
Control	28/120 (23)	35/120 (29)	40/120 (33)	11/120 (9)	22/120 (18)	22	9
Intervention	28/120 (23)	40/120 (33)	41/120 (34)	8/120 (7)	13/120 (11)	22	12

^a The control group rinsed with water, and the intervention groups rinsed with a commercial mouthwash containing chlorhexidine or cetylpyridinium chloride.

^b MGIT, Bactec MGIT 960.

* Corresponding author. Mailing address: Núcleo de Doenças Infecciosas, Centro de Ciências da Saúde, Universidade Federal do Espírito Santo, Av. Marechal Campos 1468, Maruípe, Vitória, ES CEP 29040-091, Brazil. Phone: 55 27 3335-7205. Fax: 55 27 3335-7210. E-mail: emaciel@ndi.ufes.br.

[∇] Published ahead of print on 15 June 2011.

TABLE 2. Contamination of solid and liquid medium cultures of sputum samples collected after oral rinsing with chlorhexidine gluconate and cetylpyridinium chloride

Culture medium	No. of patients	No. (%) of contaminated cultures in CHX group ^a		OR ^b	95% CI ^c	P value	No. of patients	No. (%) of contaminated cultures in CPC group ^d		OR	95% CI	P value
		Control	Intervention					Control	Intervention			
Ogawa	59	6 (10.2)	3 (5.1)	0.25	0.005–2.532	0.38	61	5 (8.2)	5 (8.2)	1.0	0.724–13.795	1.0
MGIT	59	11 (18.6)	4 (6.8)	0.12	0.002–0.932	0.04	61	11 (18.0)	9 (14.8)	0.6	0.138–2.811	0.75

^a The control group rinsed with water, and the intervention group rinsed with a commercial mouthwash containing chlorhexidine.
^b OR, odds ratio.
^c 95% CI, 95% confidence interval.
^d The control group rinsed with water, and the intervention group rinsed with a commercial mouthwash containing cetylpyridinium chloride.

(7) (200 µl of inoculum per slant) and MGIT (*Mycobacteria* growth indicator tube; Becton-Dickinson, Sparks, MD) medium supplemented with PANTA (contains polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin; Becton-Dickinson) (500 µl of inoculum per tube). Cultures were incubated at 37°C and examined daily for up to 6 weeks. Time until detection in MGIT was defined as the number of days until a positive growth index was obtained. After 42 days, tubes that showed no growth were read as negative. Cultures were determined to be contaminated with fungi or other bacteria according to standard procedures. Mycobacterial cultures were identified to species level as *Mycobacteria tuberculosis* complex based on morphology and ability to grow in paranitrobenzoic acid and thiophen-2-carboxylic acid hydrazine (5). Differences between groups were assessed using McNemar’s test (Stata 9.0, College Station, TX). *P* values of less than 0.05 were considered significant.

One hundred thirty-six patients participated in the study. Sixteen (13.3%) did not return for collection of the second sputum sample and were excluded from analysis. Of the 120 patients who came back to the clinic for collection of the second sputum sample, 59 were randomly assigned to the CHX rinse group and 61 were assigned to the CPC rinse group.

The median volumes of sputum submitted from the first and second sputum collections from each patient were 5.0 ml (range, 25 to 75 ml; interquartile range [IQR], 2.5 ml) and 7.5 ml (range, 25 to 75 ml; IQR, 5.0 ml), respectively, and did not differ. Most samples were mucopurulent (97% for the CHX group versus 98% for the CPC group), while a minority were classified as saliva (3.4% for the CHX group and 1.7% for the CPC group). For the 6 sputum samples obtained from 3 patients that were classified as saliva, contamination was present in 1 culture inoculated in MGIT; the other cultures were negative.

The recovery rate for *M. tuberculosis* and time to detection for cultures on Ogawa and MGIT media after routine oral rinsing with water (control group) or commercial chlorhexidine or cetylpyridinium mouthwash (intervention group) before sputum collection are detailed in Table 1. The overall recovery rates did not differ between the control and intervention groups.

Fourteen patients had cultures positive for *M. tuberculosis* only on specimens collected after oral rinsing with one of the antiseptic rinses. When we compared the rates of culture contamination by type of oral rinse (CHX or CPC) and culture medium, we found that rinsing with CHX before sputum collection decreased culture contamination in MGIT medium (19% after tap water rinsing versus 7% after CHX rinsing;

odds ratio [OR] of 0.125, *P* = 0.04); a smaller, 5%, nonsignificant reduction was found for samples cultured on Ogawa medium. Rinsing with CPC mouthwash before sputum collection did not decrease contamination of cultures on either medium compared to rinsing with tap water before sputum collection (Table 2).

No nontuberculous mycobacteria were recovered from any culture. Most contaminants were bacteria or fungi. In this randomized trial, we found that rinsing with commercial mouthwashes containing chlorhexidine but not cetylpyridinium chloride before sputum collection decreased culture contamination, particularly for MGIT culture, where contamination rates are frequently higher. Rinsing with these mouthwashes before sputum collection did not appear to adversely affect culture positivity and time until detection. Fourteen of the 40 patients confirmed to have TB had positive cultures only on specimens collected after rinsing with an antiseptic mouthwash. Our results add to the growing body of knowledge showing that attention to sputum collection methods and oral hygiene may enhance the yield of sputum examination (1, 6, 8, 10).

Only diagnostic specimens were examined in this study. The rates of culture contamination are higher when examining sputum specimens collected during treatment (unpublished data), and antiseptic rinsing before sputum collection might be even more beneficial in this setting. Nevertheless, diagnostic specimens constitute most of the workload for clinics and TB programs in countries with a high TB burden.

Antiseptic mouth washes are widely available in many countries. Their use would add some additional cost to sputum collection, although this expense would likely be much less than the cost of repeated cultures and other examinations.

This study was supported by the Fundo de Apoio à Ciência e Tecnologia do Município de Vitória (FACITEC) and an International Clinical Operational and Health Services Research and Training Award, Edital ICHORTA 5U2R TW006883-02.

REFERENCES

- Alisjahbana, B., et al. 2005. Better patient instruction for sputum sampling can improve microscopic tuberculosis diagnosis. *Int. J. Tuberc. Lung Dis.* 9:814–817.
- Barros, V. M. R., I. Y. Ito, R. V. P. Azevedo, D. Morello, and P. A. Rosatelli. 1998. Estudo comparativo da eficiência de três métodos de anti-sepsia intrabucal na redução do número de estreptococos do sulco gengival. *Rev. Odontol. Univ. Sao Paulo* 12:201–206.
- Denton, G. W. 1991. Chlorhexidine, p. 274–289. In S. S. Block (ed.), *Disinfection, sterilization and preservation*, 4th ed. Lea and Fibiger, Philadelphia, PA.
- Grossman, E., G. Reiter, and O. P. Styrzenberg. 1986. Six-month study of effects of a chlorhexidine mouthrinse on gingivitis in adults. *J. Periodontal Res.* 343:33–43.

5. **Kent, P. T., and G. P. Kubica.** 1985. Public health mycobacteriology: a guide for the level III laboratory. Department of Health and Human Services, Centers for Disease Control, Atlanta, GA.
6. **Khan, M. S., O. Dar, C. Sismanidis, K. Shah, and P. Godfrey-Faussett.** 2007. Improvement of tuberculosis case detection and reduction of discrepancies between men and women by simple sputum-submission instructions: a pragmatic randomised controlled trial. *Lancet* **369**:1955–1960.
7. **Kudoh, S., and T. Kudoh.** 1974. A simple technique for culturing tubercle bacilli. *Bull. World Health Organ.* **51**:71–82.
8. **Maciel, E. L. N., et al.** 2009. Guided sputum sample collection and culture contamination rates in the diagnosis of pulmonary TB. *J. Bras. Pneumol.* **35**:460–463.
9. **McDonnell, G., and A. D. Russell.** 1999. Antiseptics and disinfectants: activity, action, and resistance. *Clin. Microbiol. Rev.* **12**:147–179.
10. **Muzanye, G., K. Morgan, J. Johnson, and H. Mayanja-Kizza.** 2009. Impact of mouth rinsing before sputum collection on culture contamination. *Afr. Health Sci.* **9**:200.
11. **Pardini, M., et al.** 2005. Cetylpyridinium chloride is useful for isolation of *Mycobacterium tuberculosis* from sputa subjected to long-term storage. *J. Clin. Microbiol.* **43**:442–444.
12. **Peres, R. L., et al.** 2009. Comparison of two concentrations of NALC-NaOH for decontamination of sputum for mycobacterial culture. *Int. J. Tuberc. Lung Dis.* **13**:1572–1575.
13. **Roldán, S., et al.** 2004. Comparative effects of different chlorhexidine mouth-rinse formulations on volatile sulphur compounds and salivary bacterial counts. *J. Clin. Periodontol.* **31**:1128–1134.
14. **Russell, A. D., and I. Ahonkhai.** 1982. Antibacterial activity of a new thia-tetracycline antibiotic, thiacycline, in comparison with tetracycline, doxycycline and minocycline. *J. Antimicrob. Chemother.* **9**:445–449.
15. **Russell, A. D., S. A. Hammond, and J. R. Morgan.** 1986. Bacterial resistance to antiseptics and disinfectants. *J. Hosp. Infect. Rev.* **7**:213–225.
16. **Russell, A. D.** 1996. Activity of biocides against mycobacteria. *J. Appl. Bacteriol.* **81**:87S–101S.
17. **Smithwick, R. W., C. B. Stratigos, and H. L. David.** 1975. Use of cetylpyridinium chloride and sodium chloride for the decontamination of sputum specimens that are transported to the laboratory for the isolation of *Mycobacterium tuberculosis*. *J. Clin. Microbiol.* **1**:411–413.