

The effectiveness of three procedures in decontaminating surgical instruments used in veterinary field necropsy

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Summary

Mycobacterium spp. other than *Mycobacterium bovis*, *Pseudomonas aeruginosa*, *Escherichia coli* and a *Bacillus* sp. were used to contaminate surgical instruments. They were then treated by each of three methods commonly used to decontaminate such instruments used in veterinary field necropsies. Flaming for 15 s and boiling for 5 min were equally effective in destroying the mycobacterial and non-mycobacterial organisms tested. Alcohol flaming did not decontaminate the instruments satisfactorily.

INTRODUCTION

When tissue samples submitted from field necropsies are cultured, bacteria, commonly classed as contaminants, are often isolated. These bacteria may be within the tissue or may originate from such external sources as non-sterile instruments (Mackey and Derrick 1979; Smeltzer and Thomas 1981) post-mortem invasion of the carcass (Correy 1978) or contamination of samples by soil, dust, water or airborne bacteria.

The presence of contaminating bacteria may confuse the interpretation of results. These bacteria may also overgrow cultures and mask the presence of pathogenic bacteria. This problem is relevant to the Australian National Tuberculosis and Brucellosis Eradication Scheme. When mycobacteria other than *Mycobacterium bovis* (OM), are isolated from the tissues of cattle that have reacted to the bovine tuberculin test, it is uncertain whether these mycobacteria were the source of the tuberculin sensitivity or were accidentally introduced during the necropsy.

This investigation was performed to compare the effectiveness of three common techniques used to decontaminate surgical instruments of OM and bacteria commonly regarded as contaminants.

MATERIALS AND METHODS

Bacteria selected for use as experimental contaminants had been isolated during routine diagnosis. A few OM, two gram negative rods and one spore forming gram positive bacillus were studied. Staphylococci, fungi and viruses were not included. All mycobacteria used were isolated from tissues of cattle reacting to the bovine tuberculin test.

Bacteria employed were *M. terrae*, *M. phlei*, *M. flavescens*, (strains 1,2 and 3), *Pseudomonas aeruginosa*, *Bacillus* sp. and *Escherichia coli*.

P. aeruginosa, *Bacillus* sp. and *E. coli* were each grown on sheep blood agar plates at 37°C for 24 h. Colonies (3 or 4) of each bacterium were then inoculated into separate 25 mL bottles containing 20 mL of sterile physiological saline, and dispersed by shaking. Similar suspensions were made of the mycobacteria. Each was grown on Stonebrink agar slopes at 37°C until heavy growth appeared. With a sterile spatula, approximately 0.06 g of each culture was dispensed into separate 25 mL bottles containing 20 mL of sterile 0.01% Tween 80 in physiological saline.

Sets of instruments, each comprising one pair of 15 cm surgical scissors and one pair of 15 cm surgical rat-tooth forceps, were sterilised separately in Sterilope bags (Reed Paper and Packaging Ltd) by autoclaving at 103 kPa for 15 min. Each set of instruments was artificially contaminated by immersing the blades of the scissors and the teeth of the forceps for 10 s into one of the cultures suspensions. The instruments were then immediately subjected to one of the following procedures:

1. No treatment (control).
2. Instruments flamed over a Bunsen burner for 15 s then cooled in sterile saline.
3. Instruments momentarily dipped in alcohol then drawn rapidly through a Bunsen burner flame and the alcohol allowed to burn off.
4. Instruments immersed in boiling water for 5 min.

Each treatment was carried out in duplicate for each organism tested. After treatment, the blades of the scissors and teeth of the forceps were immersed in either 20 mL sterile nutrient broth or 20 mL Dubos broth to test for non-mycobacterial and mycobacterial organisms, respectively. This technique was repeated with a new set of sterilised instruments for each procedure and for each organism.

All broths were incubated for 24 h at 37°C. Each broth was then inoculated using a sterile swab onto 1 blood agar, 1 Stonebrink's agar, 1 Lowenstein Jensen's agar slope, and 1 Herrold's egg yolk agar slope. All culture media were incubated at 37°C and checked for growth over 21 days.

RESULTS

The effects of the treatments on the artificially contaminated instruments are given in Table 1. Untreated (control) instruments all yielded the contaminating organisms on culture.

Table 1. Effect of sterilisation techniques on recovery of bacteria from artificially contaminated instruments

Treatment	Media#	<i>M. terrae</i>	<i>M. phlei</i>	<i>M. flavescens</i> (1)	<i>M. flavescens</i> (2)	<i>M. flavescens</i> (3)	<i>P. aeruginosa</i>	<i>Bacillus</i> sp.	<i>E. coli</i>
Untreated	BA	—*	+	+	+	—	+	+	+
	S	+	+	+	+	+	+	+	+
	LJ	+	+	+	+	+	+	+	+
	H	+	+	+	+	—	+	+	+
Alcohol	BA	—	+	+	+	+	+	+	+
	S	—	+	+	+	+	+	+	+
Flamed	LJ	—	—	+	—	+	+	+	+
	H	+	—	—	—	—	+	+	+
	Flamed	No bacteria recovered on any of the four media							
15 s	No bacteria recovered on any of the four media								
Boiled	No bacteria recovered on any of the four media								
5 min	No bacteria recovered on any of the four media								

* Recovery: +colonies present; —no colonies.

#BA, blood agar; S, Stonebrink's media; LJ, Lowenstein Jensen's media; H, Herrold egg yolk media.

P. aeruginosa, *Bacillus* sp. and *E. coli* did not grow when instruments had been flamed for 15 s or boiled before being placed into the nutrient broths. All three species

were recovered from instruments treated by alcohol flaming. No mycobacteria grew when the artificially contaminated instruments were subjected to flaming or boiling prior to immersion in broths. All mycobacteria did grow on one or more of the different culture media after instruments had been alcohol flamed.

DISCUSSION

This investigation showed that instruments that are not suitably decontaminated may be potential sources of contamination. Bacteria such as *P. aeruginosa*, *Bacillus* sp. and *E. coli* which are often regarded as contaminants, were transferred to culture media if instruments were not sterilised. This also occurred for *M. phlei*, the three strains of *M. flavescens* and *M. terrae*.

Sharp (1963), in an investigation of aseptic autolysis in tissues, reported that a single alcohol flaming of the surface of tissues plus surface painting with an alcoholic solution of crystal violet and brilliant green gave sterile tissue samples approximately 70% of the time. The species or quantity of surface contaminants was not stated. Nielsen (1976), in an investigation of the effect of alcohol flaming on surface contamination of biopsy samples by *E. coli*, found such flaming of tissues once or twice was ineffective in eliminating surface contamination. In our investigation, alcohol flaming was not effective in sterilising artificially contaminated instruments.

Flaming for 15 s and boiling for 5 min were equally effective in decontaminating instruments. Bacteria adhering to the surface of instruments would probably be subjected to more prolonged heat through these two procedures than through surface flaming of residual alcohol on the instruments. Less than 5 min boiling may be sufficient to decontaminate instruments. Peel and Simmons (1978) found that although momentarily immersing knives in water at 82°C was ineffective, a minimum of 10 s immersion at 82°C was sufficient to destroy all contaminant salmonellas.

Gross contamination of tissues from instruments coming in contact with faecal material and dirt is possible during necropsies (Mackey and Derrick 1979). It is apparent from the present investigation that precautions should be taken to decontaminate instruments effectively prior to taking tissue samples which are intended for microbiological examination.

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