THE ANTIMICROBIAL ACTIVITY OF EMBALMING CHEMICALS AND TOPICAL DISINFECTANTS ON THE MICROBIAL FLORA OF HUMAN REMAINS

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(This manuscript has been accepted for publication in the October, 1976 issue of Health Laboratory Science, journal of the American Public Health Association.)

Abstract

The antimicrobial activity of embalming chemicals and topical disinfectants was evaluated to determine the degree of disinfection achieved during the embalming of human remains.

The administration of arterial and cavity embalming chemicals resulted in a 99% reduction of the postmortem microbial population after 2 hours of contact. This level of disinfection was maintained for the 24 hour test period. Topical disinfection of the body orifices was also observed. Therefore, it is probable that pre-sent embalming practices reduce the hazard from transmission of potentially infectious microbial agents within the immediate environment of embalmed human remains.

Introduction

For many years embalming chemicals have been utilized to preserve and disinfect biological tissues for anatomical studies, environmental storage, and hygienic safety. The majority of these embalming chemicals are formaldehyde-based products, whose disinfectant properties have been described by Walker (6) Lawrence and Block (2) and Spaulding (5).

It has been reported that pathogenic organisms, such as K. pneumoniae, H. influenzae, M. tuberculosis, and H. capsulatum are recoverable from embalmed human remains (3,7,8). Thus conceivably infection could occur from contact with post-embalming microbial survivors.

The objective of this study was to examine biological fluids and swabs of areas around orifices from cadavers to determine if commercially available embalming chemicals produce a significant reduction in the microbial flora.

Materials and Methods

Samples of biological fluids and swabs of the area around orifices were taken from eight cadavers to determine the antimicrobial activity of embalming fluids in vivo as a function of time. Four of the bodies were embalmed while the other four cadavers were not and served as controls. The primary cause of death of the subjects was diagnosed to be other than an infectious disease (i.e., coronary thrombosis, cerebrovascular accident, arteriosclerosis).

A. Embalming Procedure

The bodies were embalmed by a professional licensed mortician using the following procedure:

> The body was washed with an antiseptic soap containing 0.75% hexachlorophene and thoroughly rinsed. The cadaver was sprayed with a topical embalming disinfectant and the orifices swabbed with the same disinfectant. This disinfectant was a solution of 1.0% (w/v) formaldehyde and 0.5% (w/v) quarternary ammonium compounds in a base of isopropanol and ethylene dichloride.

Prior to dilution of the arterial embalming chemical, the tap water was treated with a water conditioning mixture formulated specifically for embalming use. This conditioner is a complexing agent that removes chemical consitituents found in municipal water supplies which could interfere with the preservative and disinfecting properties of the arterial solutions. This conditioner is basically a mixture of trisodium ethylene diamine tetraacetate and polyvinylpyrrolidone in a base of various glycols.

The arterial embalming chemical consisted of 29.8% (w/v) formaldehyde, 3.8% (w/v) anionic detergents, 4.0% (w/v) borate buffers, 3.75% (w/v) other preservatives and germicides, 9.6% (w/v) alcohol, and various inert ingredients in a water base. The arterial embalming chemical was then diluted 6 ounces to the half gallon of water. An equal amount of coinjection chemical for the purpose of stimulating drainage and inducing penetration was added to the solution. This chemical consisted of 8.9% (w/v) of chelating agents, 0.1% (w/v) reducing agents, 0.7% (w/v) preservatives, 2.0% (w/v) plasticizers, 9.9% (w/v) humectants, and various inert ingredients in a water base.

The total amount of solution injected into the body was approximately 2 to 3½ gallons, depending on the body size and weight. Two pints of cavity embalming chemical (24-28% (w/v) formaldehyde) were injected into each subject, one into the thoracic cavity and the other into the abdominal cavity. All products used in the study were commercially available embalming chemicals.

B. Sample Collection

Samples were drawn from superior and inferior anatomical sites, with sterile 18 gauge needles and 30 ml syringes. Needle puncture sites were topically disinfected with 95% ethanol. Swab samples were taken with sterile polyester swabs which just prior to swabbing were immersed in sterile phosphate buffered saline*. Biological fluids and swab cultures were taken from the following areas:

Fluid Samples

Lung - The needle was inserted three inches to the right of the midline, through the fifth intercostal space. Pulmonary fluids or aspirates were extracted from the middle lobe of the right lung.

Heart Blood - The needle was inserted one inch to the left of the midline, through the fifth intercostal space.

Descending Colon - A longitudinal incision three inches long was made midway between the tip of the twelfth rib and the anterior iliac spine; the descending colon was identified and the fecal matter extracted from the lumen of the colon.

Urinary Bladder - The needle was inserted through the external urethral orifice and the urine extracted from the lumen of the bladder.

Dehydrated or coagulated sample sites were injected with sterile phosphate buffered saline*.

* Phosphate buffered saline prepared by diluting 0.25M phosphate buffer 1/1000 in physiological saline (0.9% NaCl).

Swabs of Orifices

Oral Cavity - Samples were taken from the buccal furrow between the mucous membrane of the upper lip and the gingiva.

Nasal Cavity - Swabs were made from the vestibule of the left half of the nasal cavity.

Anus - Swabs were taken from the terminal inch of anal canal.

Immediately after taking samples, the swabs were placed in Stuarts Transport Media.

Samples were taken prior to embalming and then 2, 4, 8 and 24 hours after embalming. All bodies were covered with plastic sheeting while sampling procedure was not in process. All samples were packed in dry ice during transportation to the laboratories of Foster D. Snell, Inc., where they were immediately plated.

C. Quantitative Measurement of Microbiological Flora

Immediately following receipt in the laboratory, the biological fluids were serially diluted in thiotone peptone water blanks and subsequently plated on MacConkey and Heart Infusion Agar with 5% defribrinated sheep blood. Into both agar preparations, 0.5% Tween-80 and 0.1% lecithin were incorporated to neutralize residual microbial activity from the embalming fluids.

The plates were incubated at 35-37 ^{0}C. for 48 hours following which a colony count was performed.

D. Microbial Identification

Isolates were identified using standard morphological and biochemical tests with the general outline utilized for identification as defined in "Bergey's Manual of Determinative Bacteriology", Eighth edition (1), and V. Skerman's "Identification of the Genera of Bacteria", Second edition (4).

Results and Discussion

In vitro, germicidal activity of formaldehyde has been established and documented for many years (2). However, the amount of microbiological data concerning the in vivo efficacy of embalming chemicals on human remains is scant. In the present study, formaldehyde base embalming fluids were found to be highly active in reducing the microbial flora in human remains. The microbial population was reduced greater than 99 percent at every site two hours after embalming: with control bodies, as anticipated, a continuous microbial growth pattern was observed (Table 1). The antimicrobial action of formaldehyde based embalming chemicals was apparently not limited or adversely affected by the proteinaceous material or other macromolecules present in the biological fluids and tissues.

Following topical disinfection of the areas around the orifices, no growth or limited growth could be detected after 24 hours of exposure. Disinfection of the orifices occurred within 2 hours of contact. Random positive results after disinfection, however, were seen because the bodies were not protected from the environment with the exception of a non-sterile plastic cloth.

Differential monitoring of the microbial population revealed that micro-organisms translocate across anatomical barriers which during the life prevent penetration and translocation. These body defenses are as follows:

epithelial and mucous membrane coverings
reticuloendothelial system
blood drain barrier

The organisms which were isolated from the human remains are listed in Table 2.

Comparison of the microbial flora prior to and after embalming produced no pattern or general trend. Specific microbial resistance to the antimicrobial action of formaldehyde based embalming chemicals was not observed. No pathogenic bacterial were found following embalming.

In conclusion, it was found that the use of formaldehyde based embalming chemicals is a satisfactory disinfectant when applied as a public health measure to reduce microbial hazards when human remains are handled.

Acknowledgement

We thank J. B. Christensen* of the George Washington School of Medicine, Department of Anatomy, Washington, D.C. and R. Swaminathan, Foster D. Snell, Inc., for their professional assistance. This study was supported by the Embalming Chemical Manufacturers Association.

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Table 1. Antimicrobial Activity of Embalming Chemicals and Topical Disinfectants on Microflora of Human Remains

Treatment	Anatomical Site	Mean Microbial Populations[1] Pre-embalming	Treatment Period (hours) 2	4	8	24	% Reduction After 24 Hours
Embalmed	Heart	$8.0x10^5$	$1.8x10^2$	$1.6x10^2$	$2.0x10^2$	70	>99%
Embalmed	Lung	$2.5x10^4$	<10	<10	<10	<10	>99%
Embalmed	Colon	$7.4x10^4$	80	<10	<10	<10	>99%
Embalmed	Bladder	$1.3x10^5$	$3.8x10^2$	<10	<10	<10	>99%
Embalmed	Oral Cavity	++[2]	0	0	0	0	--
Embalmed	Nasal Cavity	+	0	0	+	0	--
Embalmed	Anus	+++	0	+	0	0	--
Unembalmed	Heart	$2.8x10^5$	$7.2x10^5$	$7.8x10^5$	$7.5x10^5$	$9.0x10^5$	--
Unembalmed	Lung	$2.5x10^5$	$3.2x10^5$	$3.0x10^5$	$2.4x10^5$	$3.8x10^5$	--
Unembalmed	Colon	$1.5x10^6$	$1.6x10^6$	$1.7x10^6$	$1.5x10^6$	$2.2x10^6$	--
Unembalmed	Bladder	$2.9x10^6$	$2.3x10^6$	$2.3x10^6$	$2.4x10^6$	$2.5x10^6$	--
Unembalmed	Oral Cavity	+	+	+	+	+	--
Unembalmed	Nasal Cavity	+	+	+	+	+	--
Unembalmed	Anus	+++	+++	++	++	+++	--

[1]organism/ml (mean of 4 subjects per group)

[2]scale of growth from swab cultures: 0 = none, + = slight, ++ = moderate, +++ = heavy

Table 2. Isolation and Distribution of Microflora Associated with Human Remains

Anatomical Sites			
Heart	**Lung**	**Colon**	**Bladder**
Proteus Mirabilis	Escherichia coli	Escherichia coli	Escherichia coli
Pseudomonas sp.	Pseudomonas aeruginosa	Micrococcus sp.	Klebsiella aerogenes
Staphylococcus aureus	Staphylococcus aureus	Proteus Mirabilis	Proteus vulgaris
Staphylococcus epidermidis	Staphylococcus epidermidis	Proteus vulgaris	Proteus morganii
Streptococcus	Streptococcus sp.	Pseudomonas aeruginosa	Pseudomonas aeruginosa
Bacillus sp.	Alcaligenes faecalis	Staphylococcus aureus	Staphylococcus aureus
Escherichia coli		Staphylococcus epidermis	Staphylococcus epidermidis
		Streptococcus sp.	Bacillus sp.
		Bacillus sp.	
		Klebsiella aerogenes	