EVALUATION OF SPONGE IMPREGNATED WITH PRESERVATIVE FOR PROTECTION OF URINARY TRACT PATHOGENS DURING TRANSPORT

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ABSTRACT (REVISED)

Background: Delays in transport of urine specimens may result in overgrowth of some species or die-off of others even when preservatives are used. The objective of this study was to evaluate a sponge (patent pending) impregnated with different concentrations and combinations of preservatives to measure viability of bacteria without inhibition or overgrowth over extended periods of time.

Methods: Seven ATCC strains of urinary pathogens were tested: Staphylococcus aureus 6538, Staphylococcus saprophyticus 15305, Pseudomonas aeruginosa 27853, Escherichia coli 25922, Enterococcus faecalis 29212, Klebsiella pneumoniae 700603 and Proteus mirabilis 7002. A 0.5 McFarland standard of each organism was prepared and diluted to contain 103 to 105 CFU/mL. Standard length sponges were impregnated with various concentrations and combinations of boric acid (BA) and sodium formate (SF), dried and sterilized by gamma irradiation. 2 mL of filter-sterilized (0.22 µM) urine was inoculated with each species and added to tubes containing the sponges. The sponge was squeezed several times, and excess urine was poured off. Each sponge nominally took up 1 mL of urine. All tubes were held at room temperature and sampled at 0, 1, 2 and 3 days. At each test time 1 µL of urine was plated in triplicate to appropriate media, incubated overnight at 35oC and colony counts were performed. As a control the same procedure was followed using the BD Vacutainer® Urine Preservative tube containing BA and SF.

Results: Colony counts for all species tested remained stable for up to 3 days in the impregnated sponges when the concentrations of BA and SF were adjusted to the appropriate concentration of sodium borate and sodium formate in a 2:1 ratio. By comparison, P. aeruginosa colony counts decreased significantly (by 2 logs) and E. faecalis increased significantly (by 1-2 logs) in the BD system held for the same period of time. Higher concentrations of BA in the sponges caused inhibition of both P. aeruginosa and E. coli, and lower concentrations resulted in overgrowth, particularly by E. faecalis and P. mirabilis.

Conclusions: Colony counts of urinary tract pathogens can be maintained static on a sponge impregnated with specific concentrations of BA and SF for protracted periods of time.

INTRODUCTION

Quantitation of bacterial urinary pathogens in samples submitted to the laboratory has long been recognized as essential for determination of the likelihood that the patient indeed has a urinary infection. For most situations, the colony counts that are considered significant are in the range of 10 – 100 x 106 / litre. When a urine sample arrives in the laboratory it is important that the numbers of organisms identified reflect the clinical condition.

It is well known in clinical medicine that bacteria prefer to adhere to and thereby survive on surfaces rather than in fluid media. For example, binding to catheters and to bladder endothelium is the norm. Collection of urine from patients (particularly midstream samples) is often difficult, and transport of samples to the laboratory becomes problematic because delays may result in changes in organism numbers. Several methods are now used to preserve organisms during transit. These include pouring urine onto a culture media dip-slide, sending the sample refrigerated in a tube or other sterile container, or as fluid in tubes containing preservatives (if delays longer than 2 hours are anticipated). All these methods are subject to variability. In fact, it is not well known how many organisms survive in transit, or in some cases if some species actually overgrow, thus masking the true pathogen in the infection.

Using the principle described of organism association with surfaces and incorporating a preservative, we have developed a method using an inert sponge impregnated with preservative to permit urinary pathogens to survive for prolonged periods of time in transit to the laboratory without either destruction or overgrowth. This novel method was compared to a commercial fluid based system containing a pellet of preservative.

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MATERIALS AND METHODS

Preparation of sponge: Pieces of sponge (2 cm long x 1 cm wide) from a single stock were impregnated with varying concentrations of sodium borate (6 – 18%) and sodium formate (3 - 9%). The borate:formate ratio was maintained at 2:1 for all assays. The sponges were cut so that the volume of fluid absorbed in the sponge was 1 – 1.2 mL. Sponges were sterilized by ethylene oxide sterilization in 5 mL tubes, with appropriate leeching times for the ethylene oxide.

Inoculation of Sponges: The sponges were inoculated with concentrations of 1 – 100 x 106 CFU's / L of ATCC strains of bacterial species commonly found in urinary infections. Urine was obtained from 24 hour urine collections from patients known not to have a urine infection and not on antibiotics, and was sterilized by filtration through a 0.22 µM membrane. ATCC strains of E. coli 25922, Klebsiella pneumoniae 700603, Enterococcus faecalis 29212, Pseudomonas aeruginosa 27853, Proteus mirabilis 7002, Staphylococcus aureus 6538, Staphylococcus saprophyticus 15305, Salmonella typhimurium 14028 and a clinical strain of Candida albicans were prepared from overnight cultures and inoculated to yield final concentrations as described above. The cultures were tested after 0, 24, 48 and 72 hours of storage on the sponges at 4° C and room temperature. At each test time, the sponge in the tube was squeezed so the fluid would be expressed from the sponge and the urine was inoculated with a 1 µL loop onto appropriate culture medium to mimic how a normal fluid urine sample would be inoculated in the laboratory. The cultures were incubated for 18 –20 hours at 35° C. To ensure that the sterilized urine was not inhibitory to the bacterial species, a control culture of each species in urine without sponge was run to establish baseline counts. Colonies were counted in triplicate and from two separate samples for each species at each time period.

For comparative purposes, the BD Vacutainer® Urine Preservative tube was tested under the same conditions. Urine containing the appropriate dilution of organisms was inoculated using the Vacutainer hub and needle into the tubes containing the pellet of boric acid (9.5%) and sodium formate (4.5%). The urine samples were mixed in the tubes, held for the same periods of time and then plated with a 1 µL loop onto the same media. Colonies were counted after 18 – 20 hours of incubation at 35° C.

In addition, mixtures of these species that are often observed in mixed urinary infections were inoculated as described above into the two systems, stored for up to 72 hours and plated onto differential media to permit identification of the specific species.

RESULTS

Table 1. Stability of Bacterial Species in Sponge with Preservative and BD Vacutainer® Urine Transport System

Species	Time	Sponge (CFU)**	Vacutainer® (CFU)**
E. coli	0	126	116
	24	27	50
	48	10	26
	72	8	21
K. pneumoniae	0	243	248
	24	169	174
	48	172	163
	72	131	147
E. faecalis	0	125	124
	24	142	245
	48	383	TNTC
	72	599	TNTC
P. aeruginosa	0	121	126
	24	77	71
	48	58	43
	72	14	12
S. aureus	0	143	134
	24	112	122
	48	93	113
	72	87	108
S. saprophyticus	0	70	77
	24	47	74
	48	40	73
	72	25	57
P. mirabilis	0	365	399
	24	270	302
	48	295	317
	72	176	173
C. albicans	0	143	133
	24	64	100
	48	56	78
	72	66	81

TNTC: Too numerous to count (> 2000 colonies)
****Average of triplicate counts on 2 samples.**

RESULTS (CONT'D)

Table 2. Colony Counts of Mixtures of Bacteria Preserved on Sponge with Preservative vs. BD Vacutainer® Urine Transport System

Species Mixture	Time	Sponge (CFU)**	Vacutainer (CFU)**
E. coli	0	48	60
	72	55	74
E. faecalis	0	60	87
	72	274	TNTC
P. aeruginosa	0	55	60
	72	4	8
E. faecalis	0	77	53
	72	535	TNTC
P.aeruginosa	0	62	62
	72	6	14
E. coli	0	63	76
	72	37	26
E. coli	0	105	20
	72	45	19
S. aureus	0	64	12
	72	31	8

**** Average of triplicate counts on 2 samples.**

Sponge: The sponge performed as expected. From pilot experiments a specific sodium borate / sodium formate concentration in a 2:1 ratio provided the best long term preservation of bacteria on the sponge (proprietary information). The control data showed that the sponges were not inhibitory to any species tested. All species of bacteria (and C. albicans) tested were maintained in the sponge with the 2:1 ratio of sodium borate / sodium formate for up to 72 hours without significant loss of viability or overgrowth. E. coli and P. aeruginosa were the most susceptible to prolonged storage, but there was only a one log loss of organisms and only after 72 hours of storage (Table 1). The control cultures of the these species in urine alone showed the same trend. E. faecalis, an organism that is known to overgrow in some systems remained stable over the course of the storage period, both at 4° C and room temperature. S. typhimurium (data not shown) remained stable.

BD Vacutainer®: In the BD Vacutainer® transport system, E. coli and P. aeruginosa also deteriorated on storage, and E. faecalis overgrew in these experiments by 48 hours of storage. All other organisms were comparable between the two compared transport methods. Of interest, the original 510k submitted by Becton Dickinson for their product in 1979 showed similar degradation and overgrowth for these organisms.

When mixtures of organisms were compared the same observations were made as for the individual species (Table 2). In the E. coli / S. aureus mixture, S. aureus counts were lower in the BD Vacutainer® system.

CONCLUSIONS

The adherence of urinary pathogens to a sponge impregnated with appropriate concentrations of preservatives provides a stable support matrix for prolonged and stable viability of these organisms during transport from collection at the bedside to the microbiology laboratory. Upon testing, the organisms are readily released from the sponge so that concentrations that accurately reflect the clinical situation in the patient can be enumerated and identified. This system works well for both pure and mixed cultures of the most common bacterial urinary tract pathogens and for Candida albicans.

This patented method is now being incorporated into a device that will facilitate collection of urine samples without leakage, will survive delayed transport to the microbiology laboratory, and yet still provide an accurate estimation of the microbial load in the infected patient.