EVALUATION OF THE STERILITY OF SINGLE-DOSE MEDICATIONS USED IN A MULTIPLE-DOSE FASHION; A PILOT STUDY AND LITERATURE REVIEW

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Introduction
Many medications manufactured for human patients are used in an off-label manner in the veterinary field. Additionally, medications labeled as single dose vials (SDV) are commonly used in a multiple dose fashion in veterinary hospitals. The reason for this is likely multifactorial including packaging sizes, financial constraints at the institutional and patient level, and to reduce waste.1 Single dose vials lack a preservative and are manufactured to be used only as a single dose administered to a single patient.2, 3, 4 Multiple dose vials (MDV) are labeled as such because they contain an antimicrobial preservative and are designed to withstand contamination that could occur with multiple punctures.2, 5 Because it is against the safe injection practices of the Centers for Disease Control and Prevention (CDC) and World Health Organization (WHO) to use SDVs in a multiple dose fashion in human beings, no prospective studies exist evaluating whether this common veterinary practice has the potential to place patients at risk for infection.2, 3, 4

Multiple case reports and case series in the human literature demonstrate the transmission of bacteria and viruses through improper injection practices using single and multiple dose vials, with the majority of infections occurring at pain clinics or with contrast agents used in imaging studies.2, 5, 6, 7, 8, 9, 10 Several point prevalence studies evaluating hospital injectable medication contamination rates revealed a range of contamination rates (0.9-5.6%) in the human hospitals involved.5, 6 A point prevalence study at a veterinary teaching hospital revealed an 18% contamination rate, with the majority of contamination noted in preservative-free, single-use saline vials being used in a multi-dose fashion to dilute medications before administration.11 Given the dearth of information in the literature about the potential for commonly administered SDV medications to become contaminated when used in a multiple dose fashion the authors undertook a prospective, in vitro pilot study.

The study described herein was designed to evaluate certain SDV medications that are commonly used in the veterinary emergency room setting in an off-label, multi-dose fashion. By mimicking clinical conditions in an emergency room setting, the goal was to prospectively evaluate whether these SDV drugs are likely to be inoculated under experimental conditions and, once contaminated, whether they were capable of supporting bacterial proliferation over time. An additional objective was to evaluate nurse hand-hygiene practices when performing withdrawals from multi-dose vials. Our first hypothesis was that for certain SDV medications, the inherent properties of the solution would be hostile to microbial colonization despite the lack of a preservative, while others would support microbial growth. Our second hypothesis was that nurse hand-hygiene practices would be inadequate and inconsistent when performing withdrawals from multi-dose vials, when compared to recommendations in human medicine.1

Materials and Methods
The following medications were evaluated: 6% hydroxyethylstarch in 0.9% NaCl (denoted Hetastarch)9, 20% mannitol in water9, 50% dextrose in water9, 7% hypertonic saline9 and 10U/mL heparinized 0.9% saline.6, 9 These drugs were chosen because they are SDVs commonly used in a MDV fashion and they represent a wide spectrum of pH and tonicity (Table 1), which may impact each drug’s ability to sustain microbial growth.

Table 1: Characteristics of the fluids evaluated in this study relative to canine plasma

<table>
<thead>
<tr>
<th>Fluid type</th>
<th>Fluid characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH</td>
</tr>
<tr>
<td>Canine Plasma</td>
<td>7.4</td>
</tr>
<tr>
<td>6% Hydroxyethylstarch (HESpan ®)</td>
<td>5.9</td>
</tr>
<tr>
<td>20% Mannitol</td>
<td>6.3</td>
</tr>
</tbody>
</table>
50% Dextrose | 3.5-6.5 | 2525 | NaOH
---|---|---|---
Heparin | 7.0 | 287 | Present | Present | HCl, NaOH
0.9% NaCl | 5.5 | 308 | 154 | 154
Hypertonic Saline 7.2% | 5.0 | 2464 | 1,232 | 1,232

Figure 1 illustrates the basic design of our study. Additional information will be provided in the lecture.

**Figure 1:** Schematic of study design

### Results

Of all of the test medications, only a single vial of 50% dextrose, from the once weekly puncture group, grew a microorganism. Specifically, this SDV grew <100 CFU/mL of *Micrococcus luteus* on culture day seven. At the next culture time point (day 14), the culture was negative. After reviewing the nurse puncture records, two contamination events (the technician’s hand contacted the rubber stopper of this dextrose) had been documented on day zero and one of the study for this SDV. No other test medications were positive for any bacterial growth throughout the study.

Of the intentionally inoculated group, only hydroxyethylstarch and heparinized saline grew bacteria beginning on day seven of the study. Both had positive growth for *P. aeruginosa* starting on day seven (Figure 2). On day 14, both showed an increase in the CFU/mL suggesting proliferation in each of these two vials, followed by a marked decrease in CFU/mL by day 28. There was no growth of *P. aeruginosa* in dextrose, manniitol, or hypertonic saline. *Staphylococcus aureus* failed to grow in any of the intentionally inoculated vials. The physical appearance of the intentionally inoculated containers did not change at any time point throughout the investigation.

Observations regarding technician hand hygiene and injection safety practices are reported in Table 2. Less than 10% of technicians performed hand hygiene, defined as hand washing with soap and water or with an alcohol based hand sanitizer. A greater proportion of technicians wore gloves (39%) than washed their hands, though the same percentage of technicians didn’t wear gloves at all (39%). In addition, roughly 30% cleaned the rubber stoppers of the medications with alcohol swabs, however, in the majority of these instances, the alcohol wasn’t allowed to dry prior to performing punctures. There was a high rate (43%) of witnessed contamination events, defined as hand-to-stopper or hand-to-needle contact, most of which occurred when the bags or vials of medication were picked up from the counter.

**Table 2:** Summary of observed, technician, hand hygiene practices when handling medication vials

<table>
<thead>
<tr>
<th>Hand Hygiene Practice</th>
<th>Technicians performing % (n=28)</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Washed hands OR used alcohol based rub</td>
<td>7%</td>
<td>2-23%</td>
</tr>
<tr>
<td>Wore Gloves: 100% of the punctures</td>
<td>39%</td>
<td>24-58%</td>
</tr>
<tr>
<td>Wore Gloves: only when puncturing intentionally inoculated</td>
<td>21%</td>
<td>10-40%</td>
</tr>
</tbody>
</table>
An investigation of the ability of hyperosmolar medications such as these to support bacterial growth is warranted. Dextrose, hypertonic saline, and mannitol were able to support growth of *Pseudomonas aeruginosa* when intentionally inoculated. In addition, technician hand hygiene practices were poor and inconsistent.

### Test medication results

Of the 20 test medication vials for each of 5 different medication types, only a single dextrose vial from the once weekly puncture group yielded a positive aerobic bacterial culture, of *Micrococcus luteus*, at a single time point (day 7). *Micrococcus* is typically a non-pathogenic, environmental commensal bacteria that can colonize human skin, and in this case hand contamination of the vial stopper was observed on two instances (day 0 and 1). Interestingly, by day 14, this vial cultured negative for aerobic growth and remained negative for the remainder of the study. Some many find it interesting that this contaminant failed to proliferate in the dextrose it may provide a substrate for bacterial energy production. However, 50% dextrose has actually previously been found to have antimicrobial properties in other contexts and it is possible that the high osmolarity of the solution (Table 1) contributes to bacteriostatic properties.\(^2\)

### Inoculated vials that cultured positive

Of the intentionally inoculated medications, both 6% hydroxyethylstarch in 0.9% NaCl and heparinized 0.9% NaCl supported proliferation of *P. aeruginosa* as documented by a 10⁻⁵ increase in the number of CFU/mL in the containers. Despite not becoming contaminated as a test fluid, hydroxyethylstarch and heparinized saline were both able to support growth and allow for proliferation of bacteria when intentionally inoculated with *P. aeruginosa*. This suggests that if contamination occurs, bacterial proliferation (at least for *P. aeruginosa*) can occur. While the study reported here did not evaluate the potential for microbial proliferation in 0.9% NaCl alone, these data suggest that 0.9% NaCl is not inherently bacteriostatic. These findings are consistent with a previous veterinary study demonstrating that contamination of 0.9% NaCl SDVs used in a multi-dose fashion is common in veterinary hospitals.\(^1\) Although this study was an experimental pilot study only, the potential application of these findings to clinical practice can be preliminarily considered. Based on the ability of 6% hydroxyethylstarch in 0.9% NaCl and heparinized saline flush syringes to support growth of *P. aeruginosa* the authors’ caution against the use of these fluids in a multi-dose fashion. Additionally, the practice of making in-house heparinized saline flushes may need to be reconsidered. Of interest, single-use 0.9% NaCl pre-dosed syringes are available, and a recent experimental veterinary study showed 0.9% NaCl to be equally effective at preventing catheter occlusion as heparinized saline.\(^1\)

*Why did Pseudomonas but not S.aureus grow?*

It is unclear why hydroxyethylstarch and heparinized saline were able to support the growth of *Pseudomonas aeruginosa* and not *Staphylococcus aureus*. *S. aureus* was chosen as one of the intentional inoculants because it has previously been reported as a common contaminant in MDVs at a veterinary teaching hospital.\(^1\) Despite intentional inoculation, however, it failed to grow in any of our five samples. This is likely related to different growth characteristics of the bacteria (species and strain). Although *Staphylococcus* are facultative anaerobes, it is possible that the *Pseudomonas* strain used was just more successful under the growth conditions available in these medications.

### Inoculated vials that cultured negative

Of the intentionally inoculated medications, dextrose, hypertonic saline and mannitol did not support growth of either *P. aeruginosa* or *S. aureus*. Possible reasons for the lack of bacterial growth despite intentional bacterial inoculation include an inadequate sample size to detect growth in some vials, characteristics of the fluids / medications themselves relative to growth requirements of the bacteria, inadequate oxygen tension in the vials, a very small inoculum, bacterial growth as a biofilm on the sides of the medication vials rather than in a planktonic state, and/or inherent limitations of aerobic bacterial culture. Although the small sample size here is more likely to affect the results of the test medication portion of the study (given a presumed lower rate of contamination), it is still possible that a small sample size influenced the likelihood of getting a positive culture in the intentionally inoculated medications. The authors believe that it would inappropriate to infer from this study that use of preservative-free dextrose, hypertonic saline or mannitol in a multi-dose fashion is safe. Nonetheless it is interesting to note that these medications that did not support growth in this small study are all hyperosmolar (>1100mOsm/L). Further investigation of the ability of hyperosmolar medications such as these to support bacterial growth is warranted.

<table>
<thead>
<tr>
<th>Vials</th>
<th>Did not wear gloves at any point</th>
<th>39%</th>
<th>25-58%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Swabbed stopper with alcohol</td>
<td>29%</td>
<td>15-47%</td>
</tr>
<tr>
<td></td>
<td>Skin to stopper OR skin to needle contact during punctures</td>
<td>43%</td>
<td>27-61%</td>
</tr>
</tbody>
</table>

**Discussion:**

Overall, this small pilot study documented a relatively low rate of bacterial contamination and growth in single dose medication vials punctured under experimental conditions to simulate use in a multidose fashion. That being said, hetastarch and hypertonic saline were able to support growth of *Pseudomonas aeruginosa* when intentionally inoculated. In addition, technician hand hygiene practices were poor and inconsistent.
Lack of culture positive findings in our study may well be due to limitations of aerobic culture rather than the absence of bacteria in the medication vials. It is important to note that the first cultures made on day one after intentionally inoculating fluids with Pseudomonas (Hетасталч: 0.6CFU/mL, Hep saline: 0.3CFU/mL) were negative but the one week cultures were positive for the inoculated bacteria. This illustrates that while considered the “gold standard” in clinical medicine for identification of microbial contamination of a normally sterile site, low numbers of bacteria are not detected by this methodology. This is concerning because this magnitude of contamination may be clinically relevant if the contaminated fluids were injected IV into a patient. Future studies examining contamination of IV fluids and medications should consider using alternative, and more sensitive, methods of microbial detection. Methods such as PCR or assays that provide indirect evidence of microbial presence (such as assays for ATP and endotoxin) may be more likely to detect transient or low-level bacterial contamination. Additionally, using a larger inoculum may also be prudent in future studies to truly assess the ability of these fluids to support microbial growth.

**Technician hand hygiene practices**

Data collected involving the technicians’ hand hygiene practice offers some window into the potential for medication contamination in this study. It was interesting that only 7% of the nurses washed their hands prior and only 39% wore gloves for all medication vial punctures. Given that the participants knew the aim of the study was to evaluate contamination of medication vials and the likely observer effect of having an investigator watch the withdrawals, it was an unsettling that the frequency of hand hygiene was not higher than observed. It is also interesting to note that despite the high frequency of skin to injection stopper contact, the contamination rate was actually very low. Nonetheless there is plenty of evidence to support hand hygiene practices, and technician education regarding hand hygiene in this practice should be addressed.

Some limitations of this study have already been discussed, however specific mention of limitations is prudent. Firstly this was a small study, powered to detect high contamination rates for SDVs used in a multidose fashion. Secondly this study was experimental in nature, and thus did not address the potential clinical relevance of medication contamination. The limitations of aerobic C&S have been described, however it is also noteworthy that anaerobic culture was not performed, and thus we cannot rule out the presence of anaerobic bacterial growth in the test vials. Additionally, we did not investigate for potential viral or fungal contamination, which may be valuable since particularly fungal contamination of medications has contributed to significant morbidity and mortality in human medicine. This study was also limited by the small number of medications evaluated; likely many other SDVs are used in a multidose fashion in veterinary medicine. And finally, only two bacterial species were used in the experimental inoculation part of this study, and thus these findings cannot be extrapolated to other bacteria.

In conclusion, this study revealed that accidental contamination events can occur when SDVs are used in a multiple dose fashion in controlled, experimental conditions, and that microbial growth and proliferation was possible even with the introduction of a very small bacterial inoculum into certain medications. Based on the ability of 6% hydroxyethylstarch and heparinized saline to support growth and proliferation of P. aeruginosa when intentionally inoculated, the use of these medications in a multi-dose fashion should be considered a patient safety risk. Although the other fluids did not support bacterial proliferation it would be inappropriate to infer that the use of preservative-free 50% dextrose, hypertonic saline or mannitol in a multiple dose fashion is safe, given the pilot nature of this study. Further investigation of the use of SDVs in a multiple dose fashion is warranted in veterinary medicine.

**Footnotes**

a. Hespan, Braun, Irvine, CA.
b. Mannitol Injection 20%, NeoGenVet, Lexington KY
c. Dextrose 50% Injection, VetOne, MWI, Boise, ID
d. Equi-Phar Equine 7 HSS, VEDCO, St. Joseph, MO
e. 0.9% Sodium Chloride Injection, USP, Hospira, Lake Forest, IL
f. Heparin Sodium Injection, Sagent Pharmaceuticals, Schaumburg, IL
g. Nipro Medical Corporation, Miami, FL
h. Quanti-Cults Plus, Remel Microbiology Products, Lenexa, KS.
i. Idexx laboratories, North Grafton, MA.

**References**


http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6127a1.htm?s_cid=mm6127a1_w 


