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Experimental Study

Multiple Sterile Withdrawals from Iohexol Bottles Does Not Increase Contamination Risk

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Background: There is a global shortage of iohexol contrast media, commonly used in epidural injections, as a result of lockdown and decreased production due to COVID-19. Iohexol bottles are designated for single use, which, depending on the vials available, often leads to wasting up to 95% of this limited resource. However, avoiding multiple withdrawals may be unnecessary if withdrawing multiple times using sterile technique does not increase the risk for contamination.

Objectives: The purpose of our study is to determine whether multiple withdrawals from iohexol injection bottles using a sterile technique poses a greater risk of introducing contaminants than a single withdrawal. Furthermore, we wish to determine the extent to which bacteria can survive and grow in the contrast media.

Study Design: Experimental.

Setting: Outpatient fluoroscopic suite and laboratory.

Methods: Twenty-one 100 mL 300 mg(iodine)/mL iohexol injection bottles, after one clinical use, were tested after the first and last withdrawals (withdrawal one and withdrawal 9 or 10) for bacterial and fungal specimens using culture media and 3M™ Petrifilms™. To determine the ability of methicillin-susceptible Staphylococcus aureus (MSSA) to survive or grow in the media, MSSA was added to different concentrations (0, 25, 50, 75, and 100%) of iohexol contrast media.

Results: There was no growth observed in cultures or on Petrifilms among the first and last draws of any of the samples. When bacteria were grown in different dilutions of the media, there was a significant, approximately one log decrease in counts from 0% contrast media to 100% contrast media (8.4×10^8 vs 5.6×10^7, $P < 0.01$).

Limitations: Our study is limited in the number of samples tested and would benefit from additional investigation before consideration of clinical application.

Conclusions: Our results suggest that single-use 300 iohexol bottles may be reusable and that the contrast media is mildly antimicrobial, but not enough to retard contamination. In setting of shortages, contrast media bottles can safely be reused. This is valuable for conserving resources and limiting unnecessary health care-associated costs.

Key words: Contrast media, antimicrobial, infection, antibacterial

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There has been a global shortage in iohexol as a result of a government-instituted lockdown in Shanghai, People's Republic of China in March 2022. Iohexol is a commonly used contrast media in x-ray imaging tests, such as computed tomography (CT) scans, that exhibits low osmolarity and minimum adverse effects (1). It is a popular choice due to its lower toxicity than ionic and other nonionic contrast media (2). Thus, when a spike in COVID-19 cases left the primary GE Healthcare pharmaceutical manufacturing facility for iohexol functioning at 25% production capacity, the effects have been long-standing (3).

In May 13, 2022, the American Society of Health-System Pharmacists published a fact sheet (4) summarizing the iohexol shortage with considerations for managing it. Its recommendations include conserving contrast media through using alternative imaging techniques, performing scans without using a contrast medium, limiting nonurgent scans, and using alternative contrast agents (4).

Many of these agents are supplied in large vials, containing multiple doses, although the vials are designated as single use. The US Centers for Disease Control and Prevention (CDC) states that the safest practice is to enter vials labeled as single dose or single use only once for a single patient during a single case, procedure, or injection in order to prevent inadvertent contamination (5). According to its rules and regulations, if a single-dose vial must be entered more than once for a single procedure, a new needle and syringe should be used and the bottle should not be stored for future use (5).

Only a small amount of contrast medium, typically one-5 mL aliquot, is used clinically in an epidural and other fluoroscopically guided spinal injections. This amount is commonly withdrawn in clinical settings from single-use bottles containing a volume of 5-100 mL. Since the bottles are intended to be used once, roughly 95% of the contrast medium is then discarded, depending on available vial size. Determining whether contrast medium bottles can safely be reused is valuable for conserving resources and limiting unnecessary health care-associated costs. This study aimed 1) to evaluate if any contamination resulted from multiple withdrawals from a single-use iohexol contrast medium bottle and 2) to elucidate the ability of bacteria to grow or survive in the contrast medium.

Methods

Sample Selection and Storage

As seen in Fig. 1 and Fig. 2, we used Omnipaque™ 300 in our study.

Twenty-one iohexol 300 injection bottles (100 mL) were collected after being used a single time for a fluoroscopy-guided injection. Between 5-15 mL of contrast medium was used from each vial during the single clinical procedure. The bottles were individually labeled, noting the date and time they were used in a procedure; none of the bottles were expired on the day of the collection. The vials were stored in a medicine cabinet in the fluoroscopy suite.

Withdrawal Technique

In the fluoroscopy suite, a sterile nonfenestrated drape was placed on a flat, clean surface. Using a sterile process, 9 10-mL syringes and 9 18G 1 ½ inch needles were opened on top of the drape. A resident physician in Physical Medicine and Rehabilitation and a board-certified interventional physiatrist withdrew the contrast medium using the sterile process described in the steps below:

1. One bottle of iohexol was selected; both physicians verbally confirmed that the iohexol was not expired
2. The bottle of iohexol was cleaned using a new alcohol swab as shown in Fig. 1
3. The bottle was held upside down and stabilized while the attending physician, wearing sterile gloves, used the 18G needle and the 10 mL syringe to sterilely withdraw 10 mL of contrast medium from the selected bottle (Fig. 2)
4. 10 mL were dispensed into a conical test tube and set aside, and the previous needle and syringe were discarded appropriately
5. The test tube was labeled with the letter F, as well as the number of the vial it was drawn from
6. Steps 1-3 were repeated with the interventional physiatrist maintaining sterility; the contrast medium was discarded until there was approximately 10 mL left in the bottle
7. The last 10 mL of the contrast medium was dispensed into a conical test tube which was then labeled with the letter L as well as the number of the vial it was drawn from
8. The two test tubes were placed in a bag which was labeled with the date that the vial was used, the vial's number, the bottle's lot number and the expiration date
9. On a separate piece of paper, it was noted if 8 or 9 samples were taken.

There were no restrictions where the needle was placed or a need to identify the previous puncture sites. The test tubes were stored at room temperature for several weeks before being tested for contamination.

Testing for Contamination

One mL from each sterilely collected contrast medium sample was added to a cell culture tube with 4 mL Trypticase Soy Broth (TSB; Becton-Dickinson) and incubated overnight at 37°C and 180 rpm. Another one mL from each sample was plated onto 3M Petrifilms and

incubated standing at 37°C overnight. Each method included 2 trials.

Scoring System

Specimen growth was measured using a 0- to 2-point system. Specifically, 0 = no growth; 1 = a clear medium but a few colonies on a plate; 2 = a cloudy medium indicating overnight growth and colonies on a plate. Scoring was performed by one of the authors (CP), and digital images of the cultures and plates were reviewed by 2 additional observers (MD, ND).

Bacterial Growth in Contrast

Methicillin-susceptible Staphylococcus aureus (MSSA) ATCC® 25923™ (American Type Culture Collection) was grown from a single colony in TSB overnight at 37°C and 180 rpm. Overnight cultures were diluted by comparison to a 0.5 McFarland standard which is approximately 107 colony-forming unit (CFU)/mL for MSSA. 106 CFU of bacteria were inoculated into 0, 25, 50, 75, and 100% contrast medium in TSB in 96-well plates and incubated overnight at 37°C, 180 rpm. Bacteria were retrieved and plated on 3M Petrifilms. All conditions included 4 trials from the contrast medium samples with 3 replicates.

Statistical Analyses

Statistical significance was determined using the Mann–Whitney U test for simple comparisons. A *P* value of 0.05 represented statistical significance., The statistical analyses were performed using Prism GraphPad 9.0.1 (GraphPad Software).

Results

Bacteria and fungi were not present in any of the 42 samples— the first and last draws taken from the 21 contrast medium bottles (Fig. 3). All observing authors agreed with these findings.

In a separate study evaluating the ability of bacteria to survive or grow in the contrast medium (Fig. 4), there was a significant, approximately one log decrease in counts from 0% contrast medium to 100% contrast medium (8.4×10^8 vs 5.6×10^7, $P < 0.01$); a nonsignificant increase in counts from 0% contrast medium to 25% contrast medium (8.4×10^8 vs 9.25×10^8, $P > 0.05$); a nonsignificant decrease in 50% contrast medium (8.4×10^8 vs 7.4×10^8, $P > 0.05$); and a nonsignificant increase in 75% contrast medium (8.4×10^8 vs 1.18×10^9, $P > 0.05$). The significant decrease in counts from 0% contrast medium to 100% contrast medium suggests that

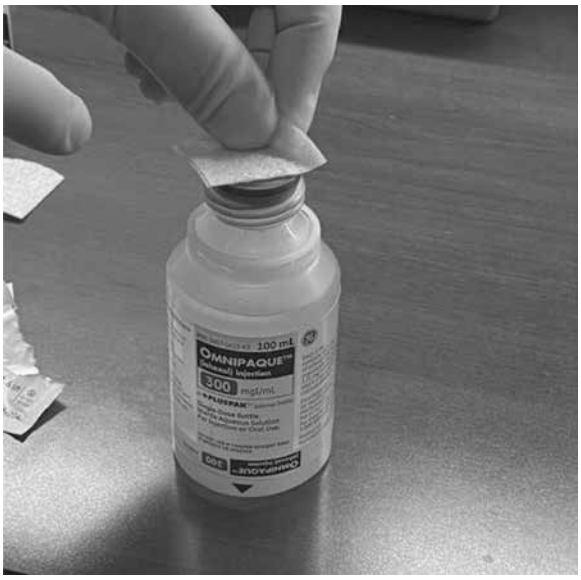

Fig. 1. *Wiping the iohexol bottle.*

Fig. 2. *Sterilely withdrawing iohexol.*

Observer	Test	Sample																																			
		1		2		3		4		5		6		7		8		9		10		11		12		13		14		15		16		17		18		19		20		21	
		F	L	F	L	F	L	F	L	F	L	F	L	F	L	F	L	F	L	F	L	F	L	F	L	F	L	F	L	F	L	F	L	F	L	F	L	F	L	F	L
CP	Cultures	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Cultures	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Plates	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Plates	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MD	Cultures	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Cultures	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Plates	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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ND	Cultures	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Cultures	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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Fig. 3. *Scoring of bacterial growth after overnight culture. No samples showed growth in liquid culture.*

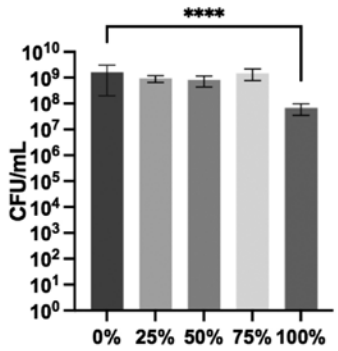

Fig. 4. *Effect of increasing doses of iohexol on S. aureus survival. Bacteria were counted after an overnight incubation.*
n = 12, **** $P \leq 0.0005$ by Mann-Whitney U test.

the contrast medium is mildly antimicrobial, but not enough to assume that it will retard bacterial growth sufficiently to cause it to be uncontaminated.

Discussion

Our results suggest that taking multiple withdrawals using our described sterile technique, that involves using separate syringes on a sterile drape, and alcohol swabs to clean the contrast medium vial in between each withdrawal, did not result in an additional introduction of contaminants. However, our study is limited in the number of samples tested and would benefit from additional investigation before considering our technique for clinical application. A CDC Morbidity and Mortality Weekly Report in 2012 (6) described the dangers associated with nonadherence to standard precautions, citing 2 outbreaks of bacterial infections following multiple uses of a single-dose container for more than one patient. In addition, there have been cases of hepatitis C transmission from multidose saline vials used before contrast medium-enhanced CT scans following breaches in safe injection practices (7,8).

If single-dose vials must be used for more than one patient, the CDC report highlights the importance of full adherence to US Pharmacopeia standards (9). These standards permit withdrawal from multiple-use containers as long as the needle's removal does not result in the destruction of the closure and that the closure immediately closes upon the needle's withdrawal (10).

A prospective study on the reutilization of single-use vials containing 600 initial samples found that contents transferred from single-use vials into disposable syringes maintained sterility after 4 withdrawals over 3 days (11). Another study found that liposomal bupivacaine remained sterile for 5 days with one aliquot withdrawn daily (12). Our results showing no growth in the first and last draws from iohexol bottles and the mildly antimicrobial properties of the contrast medium itself hold potential for conserving this limited material as well as other single-use vials in clinical practice pending further vigorous testing. Other contrast agents such as iopamidol deserve future study as well.

Conclusions

No bacterial or fungal growth was observed after multiple withdraws of iohexol using our sterile tech-

nique. In situations where contrast medium is in short supply, it may be reasonable to reuse vials with our sterile technique. The contrast medium also demonstrated mild antimicrobial properties.

Acknowledgments

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