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Clinical Investigation

Microbial Growth in Neonatal Intravenous Fat Emulsion Administered Over 12 Versus 24 Hours

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OBJECTIVES To determine whether an extended infusion time (24 hours) of intravenous fat emulsion is associated with an increase in microbial growth, versus a shorter infusion time (12 hours).
METHODS Samples were collected from intravenous fat emulsions (n=132), from intravenous fat emulsions prepared in the current 24-hour infusion method (n=55), and from intravenous fat emulsions prepared in the twice-daily (12-hour infusion) method (n=55). In addition, samples were collected from pharmacy (n=22) to test for possible contamination.
RESULTS No growth was observed in either arm of the study.
CONCLUSIONS Current Kern Medical Center policy of preparation and administration of neonatal intensive care unit intravenous fat emulsion is safe and effective in regard to microbial growth.

INDEX TERMS infection, intravenous fat emulsion, lipid, microbial, neonatal

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INTRODUCTION

The ability of intravenous fat emulsion (IVFE) to support microbial growth has been studied for decades. Intravenous fat emulsion provides an ideal environment for microbial growth due to the relatively neutral pH of 8 and high fat content.[1] Multiple studies have been conducted to observe microbial growth patterns under conditions simulating "touch contamination" of the IVFE. Substantial growth of *Escherichia coli, Candida albicans, Pseudomonas aeruginosa* , and coagulase-negative *Staphylococcus* spp. flourished within 48 hours after contamination.[2,3] In fact, *E coli* has been observed within 12 hours of preparation when the sample was kept at room temperature.[2] Additional studies evaluated for microbial growth within the IVFE after repackaging into smaller-volume syringes.[4,5] Because of manipulations by both pharmacy staff in preparation and by nursing staff in administration of IVFE, there is an increased risk of touch contamination among these intravenous preparations.

The objective of this study was to determine whether an extended hanging time of IVFE was associated with an increase in microbial growth in the neonatal intensive care unit (NICU). This study was a prospective, open-label design investigating microbial growth in IVFE at different intervals. The NICU at Kern Medical Center (KMC) is American Academy of Pediatrics-certified level IIIA unit licensed for 28 beds. During 2009-2011, the NICU neonatologist noticed an increase in IV line infections; the increase was purely speculative but did warrant further investigation. The organism most commonly seen at KMC was *Staphylococcus epidermidis*. Other common organisms included *P aeruginosa* and *Acinetobacter baumannii*. As shown by numerous studies, IVFE is a major cause of coagulase-negative staphylococcal bacteremia in the NICU.[6-8] Because of conclusions from studies such as these, it was hypothesized by the neonatologist that the increased occurrence of IV line infections at KMC might have been attributed to the IVFE preparations. The US Centers for Disease Control and Prevention (CDC) recommends, "…complet(ion) of infusion of IVFEs alone within 12 hours of hanging the emulsion. If volume considerations require more time, the infusion should be completed within 24 hours."[9] The American Society of Parenteral and Enteral Nutrition, in agreement with the recom-

mendations of the CDC, recommends an infusion time of no more than 12 hours.[10] The maximum rate of administration in neonates, however, is 0.15 g/kg/hr due to the increased risk of fat accumulation in the lungs.[1,11] Because of the indistinct recommendation and rate limitations for neonates, many facilities, including KMC, administer neonate IVFE over a 24-hour period.

Studies have evaluated microbial growth when IVFE is added to parenteral nutrition (PN) to create a total nutrient admixture (3-in-1). To this end, Otsuka Pharmaceutical (Japan) performed 2 studies in 2010 that evaluated the growth of microorganisms in PN solutions, both with and without IVFE. Based on the results from these studies, the lower pH of the PN prevented bacterial growth compared to IVFE alone. *C albicans* was able to grow regardless of pH.[12,13] Generally, the addition of IVFE to the PN was recommended to help prevent bacterial growth. In neonates, however, addition of IVFE is generally not recommended. This patient population has increased phosphorus and calcium requirements and, subsequently, a higher risk of precipitation within the PN. If IVFE were also added to the PN, the milky white nature of the IVFE product would make it impossible to visually detect precipitate.[14] Therefore, a separate infusion of IVFE is preferred.

At this time, commercially available volumes of IVFEs are not appropriate to be hung as manufactured for neonates. Because of the limited volumes available, pharmacies often repackage IVFE into syringes to be administered via a syringe pump.[11] As with any manipulation of a manufacturer's sealed package, when the pharmacy prepares these syringes, there is a risk of contamination.

Because IVFE is an ideal environment for microbial growth, and the CDC recommends a 12-hour infusion time, some institutions divide the total daily dose of IVFE into 2 separate syringes for infusion, to be completed over a 24-hour period. The purpose of this study was to evaluate whether there was an association between the length of time an IVFE was administered and an increase in microbial growth.

METHODS

The study was divided into three phases: Phase I, which evaluated the current methods for testing microbial growth; Phase II, which tested for microbial growth with a 24-hour infusion of IVFE; and Phase III, which tested for microbial growth with a 12-hour infusion of IVFE.

Phase I

Due to the nature of IVFE (e.g., color and pH), we wanted to evaluate the current methods used for detecting microbial growth. According United States Pharmacopeia (USP), sterility testing of all pharmaceutical products use 2 different types of agar, incubated for 14 days.[14] Newer technology, however, has emerged. The Bact/Alert (Biomerieux, Inc., Durham, NC) is a machine used to test for microbial growth specifically in blood. Research, has been conducted using pharmaceutical products in the Bact/Alert machine. Results from these studies showed that the Bact/Alert machine was faster and more accurate at detecting microbial growth compared to traditional methods.[15-18] The Bact/Alert machine is continually evaluating for microbial growth based on pH changes within the sample, potentially detecting microbial growth much faster than the human eye can. In our study, we aimed to recreate these studies in order to validate the Bact/Alert machine's ability to detect microbial growth in IVFE at our facility. IVFE samples were purposely inoculated with microbes (*P aeruginosa*, *A baumannii*, *S epidermidis*, and *C albicans*) at 20 colony-forming units (CFUs) per milliliter to simulate touch contamination. The concentrations of microbes within any medium typically must rise to approximately 10^8 CFU/mL before they can be detected. These specific microbes were chosen based on laboratory statistics of the organisms most commonly found in our facility, as well as the those most commonly found in this patient population. Coagulase-negative *Staphylococcus* sp. is historically the organism most commonly detected in neonates. The purposely inoculated vials (10 mL each) were placed in the Bact/Alert machine and samples were cultured on agar plates as recommend by USP. Samples were incubated for 14 days (Table 1).

Phase II

Current practice for preparation and administration of IVFE at KMC is as follows: IVFE doses are prepared in individual syringes at approximately 2:00 PM daily. These syringes are sent to the NICU and placed in the medication

Table 1. Time to Detectable Microbial Growth

Organism	Time to Detectable Growth (hr)
Phase 1	
Pseudomonas aeruginosa	11.6
Acinetobacter baumannii	12.5
Staphylococcus epidermidis	20
Candida albicans	30.1

room (stored at room temperature) until ready for use. IVFE syringes are changed at approximately 10:00 PM daily. As current practice dictates, IVFE syringes are kept at room temperature for approximately 32 hours (from time of preparation to time of removal from the patient's IV line). During this phase of the study, we did not change our current practice for preparation of IVFE syringes except for the addition of an appropriate volume of overfill (10 mL) for testing in the laboratory. At the conclusion of each infusion, nursing staff was instructed to remove all patient information from the IVFE syringe, cap the end of the syringe, and place it in the refrigerator. Nursing staff labeled the syringes with the time administered and removed from the patient. These syringes were then collected by pharmacy on the following morning and sent to the laboratory for culturing. In addition, for each batch of IVFE syringes sent to NICU, an extra syringe was prepared by pharmacy. This syringe was left at room temperature for approximately the same time as if it was infusing into a patient. This syringe served as a control for contamination by pharmacy staff. Phase II samples were collected over approximately a 3-week period. Similar to Phase I, all samples were incubated for a 14-day period (Table 2).

Phase III

After completion of Phase II of the study, in an effort to be compliant with CDC recommendations, we sent the daily dose of IVFEs divided into two 12-hour infusions. Each syringe was infused for 12 hours, and IV lines were changed with each new syringe as well. IVFEs were prepared twice daily by the pharmacy (approximately 2 hours prior to administration) and sent with a pharmacy control syringe twice daily as well. Nurses were to follow the same protocol at the conclusion of each IVFE infusion as in Phase II. Syringes were also tested in the same fashion for microbial growth. The purpose of this phase was to assess whether there was a change in the incidence of contamination compared with Phase II. Phase III was conducted over a 2-week period, and all samples were incubated for 14 days (Table 3).

RESULTS

Phase I consisted of testing the efficacy of the Bact/Alert machine and its ability to detect microbes in IVFE. Table 1 shows the time to detectable growth for each microbe as demonstrated by the Bact/Alert machine. Samples were inspected at rates similar to results found in other studies.[2,3,12] *P aeruginosa* and *A baumannii* were detected at approximately 12 hours, followed by *S epidermidis* and *C albicans*. All samples were detected within 32 hours, the approximate time IVFE syringes are at room temperature with current practice. In Phase II, the 24-hour infusion yielded no contamination from either the patient syringes or pharmacy test syringes (Table 2). Table 3 shows the number of samples contaminated following a 12-hour infusion (Phase III), including the test syringes sent from the pharmacy. Similar to Phase II, contamination was not detected in any sample.

Because of the lack of contamination from either once- or twice-daily dosing of IVFE, it can be concluded that either of the practices is appropriate for our facility. There are pros and cons to both administrations. The once-daily infusion will have a decreased cost of materials, work load, and manipulations by pharmacy and nursing. However, because it does not comply with the CDC recommendation of a 12-hour infusion time, the syringes have potential for greater microbial growth if contaminated, due to extended administration. In addition, there is a higher risk of serious harm to the patient should a rate programming error occur, allowing the volume of a 24-hour dose of IVFE to infuse too rapidly versus the smaller volume in the 12-hour IVFE syringe. In comparison, the benefits of twice-daily infusion include complying with CDC recommendations and less opportunity for serious harm to the patient due to volume of IVFE. The possible problems with this administration include increased cost of materials, pharmacy and nurse staff work load, and number of manipulations that could introduce contamination.

Table 2. Results From the 24-Hour Infusion

24-Hour Infusion	Samples from NICU (n=55)	Samples from Pharmacy (n=11)
Phase II		
Number contaminated	0	0
Percentage of contamination	0	0

NICU, neonatal intensive care unit

CONCLUSIONS

Current KMC policy for preparation and administration of NICU IVFE is safe and effective in regard to microbial growth. Reiter[5] conducted a study similar to our Phase II study: the pharmacy prepared samples, and IVFE syringes were chosen at random after a 24-hour infusion to test for microbial contamination. In that study, all samples were negative for bacterial growth at 48 hours; however, in three of the 90 samples (3.3%), Gram-positive rods were detected at 7 days. One of these contaminated samples was a pharmacy test syringe.[5] In comparison, a study by Crill et al[4] also showed a 3.3% contamination rate among repackaged syringes. Similar to our Phase III study, that study prepared IVFE syringes twice daily for 12-hour infusions. Unlike the studies by Reiter[5] and Crill et al,[4] where syringes were incubated for up to 7 days, our study incubated samples for a full 14 days to ensure ample time for any contaminant to grow. Our sample size for each phase of the study was smaller than those in both of the above-mentioned studies. Having a smaller sample size may have contributed to our lack of contaminant findings. After discussing this with the neonatologist and comparing practices with other children's hospitals in California, it was decided that KMC would continue the once-daily infusion of IVFE. There were 2 minor changes made to current practice at our facility as a result of this study. IVFE syringes are now prepared approximately 2 hours prior to expected administration and are stored in the refrigerator until used for the patient. The change in storage from room temperature to refrigeration is supported by the results of a 1983 study showing that Gram-negative bacteria were not able to proliferate while under refrigeration but have potential to reach significant levels when left at room temperature.[19] Refrigeration limits time and ability of microbes to grow if touch contamination occurs. This was a valuable study that helped reinforce the current aseptic practice of both the pharmacy (following USP requirements) and nursing, in addition to validating the Bact/Alert machine as a viable way to detect microbial growth in IVFE. If we were to switch to 12-hour infusions of IVFE, KMC would be confident that there would not be a greater occurrence of contamination. This study was limited by its small sample size and open-label design, which may have caused a heightened adherence to aseptic technique by those involved. Known as the "Hawthorne" effect, staff may have altered normal aseptic technique as a precaution for the study. Staff was educated prior to the start of the study. This bias could not be avoided due to the methods of the study and required participation by nursing and pharmacy staff.

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ABBREVIATIONS CDC, Centers for Disease Control; CFUs, colony-forming units; IV, intravenous; IVFE, intravenous

Table 3. Results From the 12-Hour Infusion

12-Hour Infusion	Samples from NICU (n=55)	Samples from Pharmacy (n=11)
Phase III		
Number contaminated	0	0
Percentage of contamination	0	0

NICU, neonatal intensive care unit.

fat emulsion; KMC, Kern Medical Center; NICU, neonatal intensive care unit; PN, parenteral nutrition; USP, United States Pharmacopeia

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REFERENCES

1. Intralipid 20% (20% I.V. Fat Emulsion) [package insert]. Silver Spring, MD: US Food and Drug Administration; 2007.
2. Keammerer D, Mayhall CG, Hall GO, et al. Microbial growth patterns in intravenous fat emulsions. *Am J Health Syst Pharm.* 1983;40(10):1650-1653.
3. Crocker KS, Noga R, Filibeck DJ, et al. Microbial growth comparisons of five commercial parenteral lipid emulsions. *JPEN J Parenter Enteral Nutr*. 1984;8(4):391-395.
4. Crill CM, Hak EB, Robinson LA, Helms RA. Evaluation of microbial contamination associated with different preparation methods for neonatal intravenous fat emulsion infusion. *Am J Health Syst Pharm*. 2010;67(11):914-918.
5. Reiter PD. Sterility of intravenous fat emulsion in plastic syringes. *Am J Health Syst Pharm.* 2002;598(19):1857-1859.
6. Jarvis WR, Highsmith AK, Allen JR. et al. Polymicrobial bacteremia associated with lipid emulsion in a neonatal intensive care unit. *Pediatr Infect Dis*. 1983;2(3):203-208.
7. Freeman J, Goldmann D, Smith N, et al. Association of intravenous lipid emulsion and coagulase-negative staphylococcal bacteremia in neonatal intensive care units. *N Engl J Med*. 1990;323(5):301-308.
8. Avila-Figueroa C, Goldmann D, Richardson D. et al. Intravenous lipid emulsions are the major determinant of coagulase-negative staphylococcal bacteremia in very low birth weight newborns. *Pediatr Infect Dis J.* 1998;17(1):10-17.
9. O'Grady NP, Alexander M, Burns LA, et al. Guidelines for the prevention of intravascular catheter-related infections. Am J Infect Control. 2011;39(Suppl 1):S1-34.
10. Mirtallo J, Canada T, Johnson D, et al. Safe practices for parenteral nutrition. *JPEN J Parenter Enteral Nutr*. 2004;28S(6):65-70.
11. Kerner JA, Poole RL. The use of IV fat in neonates. *Nutr Clin Pract.* 2006;21(4):374-380.
12. Kuwahara T, Shimono K, Kaneda S, et al. Growth of microorganisms in total parenteral nutrition solutions containing lipid. *Int J Med Sci.* 2010;7(3):101-109.
13. Kuwahara T, Kaneda S, Shimono K, and Inoue Y. Growth of microorganisms in total parenteral nutrition solutions without lipid. *Int J Med Sci*. 2010;7(1):43-47.
14. Kastango ES. Quality-control analytical methods: USP chapter <797> compounded sterile preparations sterility requirements and their relationship to beyond-use dating. *Int J Pharm Compound.* 2004;8(5):393-397.
15. Parveen S, Kaur S, Wilson David SA, et al. Evaluation of growth based rapid microbiological methods for sterility testing of vaccines and other biological products. *Vaccine*. 2011;29(45):8012-8023.
16. Khuu HM, Stock F, and McGann M. Comparison of automated culture systems with a CFR/USP-compliant method for sterility testing of cell-therapy products. *Cytotherapy.* 2004;6(3):183-195.
17. Khuu HM, Patel N, Carter CS, et al. Sterility testing of cell therapy products: parallel comparison of automated methods with a CFR-compliant method. *Transfusion.* 2006;46(12):2071-2082.
18. Kielpinski G, Prinzi S, Duguid J, du Moulin G. Roadmap to approval: use of an automated sterility test method as a lot release test for Carticel, autologous cultured chondrocytes. *Cytotherapy.* 2005;7(6):532-541.
19. Jarvis W, Highsmith A. Bacterial growth and endotoxin production in lipid emulsion. *J Clin Microbiol.* 1984;19(1):17-20.