Microbial Contamination of Neonatal Injectable Lipid Emulsions at 12 and 24 Hours' Infusion Time With Evaluation of Infection Control Measures

Eman A. Omran, PhD; Faten F. Eisa, MSc; and Wafaa M.K. Bakr, PhD

OBJECTIVES This study aimed to assess the microbial contamination rate of injectable lipid emulsion (ILE) repackaged syringes at 12 and 24 hours of their infusion time. Probable risk factors associated with contamination of the ILEs were also assessed. In addition, the antimicrobial resistance pattern of the bacterial isolates was also determined.

METHODS Samples of ILE were collected from 152 repackaged syringes and their infusion lines after 12 hours and 24 hours of infusion time (73 and 79 samples, respectively). Samples were cultured, the isolates were identified, and the antimicrobial resistance pattern of the bacterial isolates was identified. A checklist was completed throughout the study to observe the compliance to infection control measures by pharmacists (who prepare) and nurses (who administer) the ILE infusions. Results of septic neonatal cultures were taken from records.

RESULTS Microbial contamination was found in 15.8% of ILE samples. The 2 most common pathogens found among positive samples were *Klebsiella pneumoniae* (29.2%) and *Candida albicans* (20.8%). Microbial contamination of repackaged syringes increased from 9.6% at 12 hours to 21.5% at 24 hours. This difference was found to be statistically significant ($p = 0.044$). A similar trend of predominance of those 2 pathogens, in both ILE and neonatal cultures, was observed. There was a statistically significant better performance of infection control measures of pharmacists rather than nurses. The *K pneumoniae* isolates ($n = 7$) showed antibiotic resistance in the following pattern: gentamicin (71.4%), cefazolin (85.7%), and cefoxitin (85.7%).

CONCLUSIONS The rate of ILE contamination was less at 12 hours' than at 24 hours' infusion time. However, contamination rates at 12 hours were unacceptably high. *Klebsiella pneumoniae* and *C albicans* were the most common pathogens isolated from ILE. Compliance with infection control measures was significantly worse among nurses compared with pharmacists.

ABBREVIATIONS ASPEN, American Society for Parenteral and Enteral Nutrition; CDC, Centers for Disease Control and Prevention; CoNS, coagulase-negative staphylococci; ILE, injectable lipid emulsion; MDR, multidrug resistant; NICU, neonatal intensive care unit; PN, parenteral nutrition; PPE, personal protective equipment

KEYWORDS antimicrobial resistance; infection control; injectable lipid emulsions; neonates; parenteral nutrition

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Introduction

Injectable lipid emulsions (ILEs) provide a condensed form of energy for neonates admitted in NICUs. They also prevent and treat essential fatty acid deficiency among neonates. The administration of ILEs could either be in a separate intravenous line, or combined as a constituent of parenteral nutrition (PN).¹ The fourth (and latest) generation of ILE is fish oil based. These newer lipid emulsions containing less soybean oil may be proven to be more beneficial, with less proinflammatory effects. This is attributed to the higher concentrations of omega-3 fatty acids rather than omega-6.²

Compounding PN, either manually or with automated

compounding devices, requires aseptic environmental conditions and trained staff. Microbial contamination of lipid emulsions may arise at different stages of ILE manipulation. Repackaging of ILEs from their original bottles into smaller-volume syringes is a possible portal of microbial entry. Hands of health care workers may pose a further threat of microbial contamination. This includes hands of pharmacists who prepare an ILE and nursing staff who administer it. Strict aseptic conditions in all manipulations of ILE should always be followed to prevent health care–associated infections in neonates, specifically bacteremia and septicemia.^{3,4}

As opposed to PN given as a 3-in-1 preparation, ILEs

provide a more ideal medium for microbial growth because of their relatively neutral pH (pH = 8) and high lipid content. Common contaminants include *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Acinetobacter baumannii*, in addition to fungi, such as *Candida albicans* and *Malassezia furfur*.⁵ Some studies reported 3.3% and 6.6% contamination rates in ILEs.^{6,7} There are no published data from Egypt on the rate of microbial contamination of ILEs used at NICUs.

As a means of reducing infectious risks, it is recommended by the American Society for Parenteral and Enteral Nutrition (ASPEN) to use a 1.2- μ m pore-sized filter in ILE line infusion sets. Those filters protect against the transmission of large molecules, precipitates, and *Candida* species, but they unfortunately do not filter smaller bacteria, such as *Staphylococcus epidermidis* and *E. coli*, or bacterial endotoxins. In addition, their expensive price is a further disadvantage, especially in resource-limited settings.⁸

Because of all the above-mentioned factors, ASPEN and the Centers for Disease Control and Prevention (CDC) recommend that ILEs should be infused within 12 hours, in order to minimize the risk of infection. Some institutions divide the total daily dose of ILEs into 2 separate syringes for infusion, to be completed during a 24-hour period, and keep syringes refrigerated to prevent microbial growth.^{8,9} The 24-hour period is often used because of the fact that neonates require longer infusion times and slower infusion rates of ILE. The AAP recommends that the ILE daily dose should infuse continuously during the course of 18 to 24 hours to facilitate metabolic clearance.¹⁰ Few studies compared the sterility of repackaged ILE syringes at 12 versus 24 hours.^{11–13} To the best of our knowledge, no published data from NICUs in Egypt are available in this respect.

This study aimed to determine the microbiologic safety of prepackaged ILE infusions for an extended time of 24 hours, as done routinely in this NICU department, versus 12 hours, as recommended in other guidelines. The antibiotic susceptibility of bacterial isolates was determined. Moreover, the performance of infection control measures by pharmacists (who prepare ILEs) and nurses (who administer ILEs) was reported and compared. Results of blood and bronchoalveolar lavage cultures for infected neonates who received ILEs were obtained from hospital records.

Methods

The present cross-sectional study was conducted during a period of 3 months, from October 2017 to December 2017. The study was carried out at the NICU of El Shatby University Hospital and the Microbiology Laboratory of the High Institute of Public Health, Alexandria, Egypt. The sample size was calculated based on a study by Crill et al⁵ to evaluate the microbial contamination rate associated with neonatal ILE repackaged

syringes. They reported a contamination rate of 3.3%. In the present study, according to the hospital records, the number of children admitted to NICU was estimated to be 2200 per year. When anticipating the prevalence of microbial contamination of ILE increased to 10%, the minimal required sample size was calculated to be 152 (using a power of 80% to detect the prevalence, precision of 5%, $p = 0.05$). Consecutive sampling was done until the required sample size was reached.

A total of 152 repackaged ILE samples after 12 and 24 infusion hours (73 and 79 samples, respectively) were examined. In addition, samples from 19 ILE bottles were examined upon their opening. A fourth-type generation ILE was examined in this study, because it is the type used in the NICU department. Each 100 mL of it contains 6 g of soybean oil, 6 g of medium-chain triglycerides, 5 g of olive oil, 3 g of fish oil, 1.2 g of phospholipids, 2.5 g of glycerin, 16.3 mg of α -tocopherol, and 0.3 g of sodium oleate, adjusted to a pH of 6 to 9. In this NICU department, regular ILE line infusion sets were used rather than those fitted with 1.2- μ m pore-sized filters.

Sample Collection Technique, Transport, and Processing. An extra 10 mL of ILE was added to each neonatal original dose, so that after a period of 12 or 24 hours the remaining 10 mL was withdrawn from the administration line connected to the repackaged syringe. The rate of infusion was adjusted by the infusion pump according to each neonatal requirement. The pump stops automatically after delivering the required volume of ILE to each neonate. The ILE was infused through a central catheter with low-volume tubing and a syringe pump. It was y-site connected to the dextrose/amino acid admixture solution.

Repackaged syringes and their infusion lines were immediately transported to the laboratory in an icebox. Samples of ILE were withdrawn from the line aseptically using another syringe, where they were aseptically introduced into brain heart infusion medium and incubated at 37°C. Blind subculture was done daily on blood and MacConkey agar plates at 37°C for up to 7 days before being discarded as negative. Moreover, 2 plates of Sabouraud dextrose agar (SDA) supplemented with chloramphenicol were inoculated using a cotton swab. One of the SDA plates was overlaid with sterile olive oil and incubated at 35°C for 5 days for isolation of *M. furfur*. The other plate was incubated aerobically at 28°C for 5 days.

A data sheet was filled in, including all relevant information for sample lot numbers, as well as date and time of preparation of ILE. Moreover, results of positive neonatal cultures (blood and bronchoalveolar lavage) were taken from patients' records. A checklist was filled in, to evaluate the compliance of infection control measures by pharmacists and nurses during preparation and administration of ILEs. Pharmacists and nurses were not aware of the observation being done. It was performed early in the morning shift, 3 times weekly

Table 1. Microbiologic Contamination of Intralipid Emulsion (ILE) Administered Over the Course of 12 Versus 24 Hours in the NICU of El Shatby University Hospital, Alexandria, 2017

	Contamination of ILE, n (%)			χ^2	p value
	12 hr (n = 73)	24 hr (n = 79)	Total (N = 152)		
Sterile	66 (90.4)	62 (78.5)	128 (84.4)	4.061	0.044
Positive	7 (9.6)	17 (21.5)	24 (15.8)		
<i>Klebsiella pneumoniae</i>	2 (2.7)	5 (6.3)	7 (4.6)		
<i>Staphylococcus aureus</i>	0	3 (3.8)	3 (2)		
<i>Enterococcus faecalis</i>	0	1 (1.3)	1 (0.7)		
<i>Acinetobacter baumannii</i>	0	3 (3.8)	3 (2)		
<i>Candida albicans</i>	2 (2.7)	3 (3.8)	5 (3.3)		
<i>Pseudomonas aeruginosa</i>	2 (2.7)	0	2 (1.3)		
<i>Escherichia coli</i>	1 (1.4)	0	1 (0.7)		
Coagulase-negative staphylococci	0	2 (2.5)	2 (1.3)		

for 6 weeks. All pharmacists (n = 3) and nurses (n = 15) who were present during each shift were observed.

Identification of Isolates. Isolated colonies were identified by characteristic colony morphology, Gram staining, and biochemical tests according to standard methods described by Forbes et al.¹⁴ Isolates with variable biochemical profiles were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.¹⁵ All identified bacterial isolates were subjected to antimicrobial susceptibility testing using the Kirby-Bauer disc diffusion method, described by the Clinical and Laboratory Standards Institute.¹⁶

It is the policy of the NICU department to routinely prescribe cefoperazone, and amikain or gentamicin, as well as fluconazole, to neonates upon admission until the results of cultures appear. In case of a microbiologically proven infection, physicians prescribe 3 types of antibiotics according to culture results as follows: if the bacterial isolate was sensitive to cefoperazone and amikain or gentamicin, then they are used, and a third one is added based on culture results. If either or both them were ineffective, they are changed as well according to culture results.

Routine Protocol in the NICU. Repackaging of ILE syringes is done by pharmacists routinely in the pharmacy of the NICU department. In the anteroom, before any manipulations with the ILE, pharmacists perform handwashing and then alcohol application. Next, they move to the compounding room, where they wear gloves and gowns and wash their supplies. Repackaging of syringes is then done in the compounding room in a biologic safety cabinet type 2 under complete aseptic techniques. Volumes of ILE are repackaged from their original bottles into sterile 20-mL syringes. The ILE samples are kept refrigerated until they are administered to neonates. It is the routine in this NICU department to infuse ILE continuously over the course of 24 hours in a single syringe.

Statistical Analysis of the Data. Data were analyzed using IBM SPSS software package version 20.0 (Armonk, NY).¹⁷ Qualitative data were described using number and percentage. Significance of the obtained results was judged at the 5% level.

Results

Microbial growth was found in none of the 19 ILE bottles, but it was found in 15.8% of all repackaged syringe samples. Positive samples comprised: *K pneumoniae* (29.8%) and *C albicans* (20.8%), followed by *A baumannii* and *S aureus* (12.5% each), *P aeruginosa*, and coagulase-negative staphylococci (CoNS; 8.3% each), and lastly, *E coli* and *Enterococcus faecalis* (4.2% each). Microbial contamination increased from 9.6% at 12 hours to 21.5% at 24 hours. This difference was found to be statistically significant ($p = 0.044$). *Pseudomonas aeruginosa* (n = 2) and *E coli* (n = 1) appeared only in samples collected at 12 hours and not at 24 hours. On the other hand, *A baumannii* (n = 3), *E faecalis* (n = 1), and *S aureus* (n = 3) appeared only in samples at 24 hours (Table 1).

As seen from Table 2, *K pneumoniae* was the most common microorganism isolated from ILE samples (29.2% of all positive ILE samples). It was followed by *C albicans* (20.8%), and then *S aureus* and *A baumannii* (12.5% of all positive ILE samples each). On the other hand, the most common microorganism isolated from neonatal cultures was CoNS, accounting for 36.0% of all positive neonatal cultures. It was followed by *C albicans*, then *K pneumoniae* (20% and 18%, respectively). *Pseudomonas aeruginosa* and *A baumannii* accounted for 10% each, of all positive neonatal cultures. It is noted that nearly the same pattern of microorganism isolation was obtained from both ILEs and neonatal infections except for CoNS, which was significantly higher in neonatal cultures compared with ILEs ($p = 0.012$; Tables 2 and 3).

Table 2. Distribution of Isolated Microorganisms From Contaminated Intralipid Emulsion (ILE) and Infected Neonatal Samples, in the NICU of El Shatby University Hospital, Alexandria, 2017

	ILE Samples (n = 152)	Neonatal Cultures (n = 152)	χ^2	p value
Sterile	128 (84.2)	102 (67.1)	27.644*	<0.001
Positive	24 (15.8)	50 (32.9)		
<i>Klebsiella pneumoniae</i>	7	29.2	9	18.0
<i>Staphylococcus aureus</i>	3	12.5	3	6.0
<i>Enterococcus faecalis</i>	1	4.2	0	0.0
<i>Acinetobacter baumannii</i>	3	12.5	5	10.0
<i>Candida albicans</i>	5	20.8	10	20.0
<i>Pseudomonas aeruginosa</i>	2	8.3	5	10.0
<i>Escherichia coli</i>	1	4.2	0	0.0
Coagulase-negative staphylococci	2	8.3	18	36.0

Concerning the compliance with infection control measures, there was a statistically significant better performance of infection control measures of pharmacists compared with nurses ($p < 0.001$, data not shown). These measures were in the form of: handwashing, proper hand hygiene, application of skin antiseptic and allowing it to dry, the wearing of personal protective equipment (PPE; mask, gown, and gloves) during administration of ILE, and the removal of gloves after use. All the examined infection control measures were performed by all pharmacists. Unfortunately, nurses who were in direct contact with neonates did not all follow infection control measures, because only 63.2% of them washed their hands with water and soap, whereas 36.8% did not apply antiseptic and none of them followed proper hand hygiene. Also, 63.2% did not perform hand hygiene at the end of ILE handling and did not use PPE, rarely disinfected the y-connector, and rarely isolated the hub. Each of the observed infection control practices was given a score, and the relationship between infection control score and ILE culture positivity was recorded (Table 4). The mean score of culture-negative samples was 5.0 ± 1.41 , whereas positive ILE cultures had a lower infection control score (3.0 ± 0.89). This difference was statistically significant ($p = 0.012$).

When antibiotic resistance patterns were determined (Table 5), high levels of multidrug-resistant (MDR) isolates were found. *Klebsiella pneumoniae* isolates ($n = 7$) showed antibiotic resistance in the following pattern: gentamicin (71.4%), cefazolin (85.7%), and ceftazidime (85.7%). *Acinetobacter baumannii* isolates ($n = 3$) showed 66.7% resistance to each of the following antibiotics: gentamicin, tobramycin, piperacillin-tazobactam, and ceftazidime. One of the 2 isolates of *P. aeruginosa* isolates was identified as being MDR because of resistance to gentamicin, tobramycin, and ceftazidime. There was a single isolate of each of *E. faecalis* and *E. coli*, both of which were MDR.

Also, of the 3 isolates of *S. aureus*, 2 were identified

as being methicillin-resistant *Staphylococcus aureus* based on ceftazidime resistance. The overall resistance of all the isolates was: gentamicin (66.7%), tetracycline (33.3%), chloramphenicol (33.3%), tobramycin (33.3%), azithromycin (66.7%), sulfamethoxazole-trimethoprim (66.7%), ceftazidime (66.7%), and clindamycin (66.7%). It is also noted that a considerably high resistance (50%–100%) was seen among most isolates to gentamicin, which is among the antimicrobial protocol for this NICU department.

Discussion

Neonates require small quantities of ILE. Some hospitals repackage the ILEs to a more convenient size, but this potentially could lead to emulsion contamination during its preparation or administration.^{5,18} Other potential sources of its contamination include repeated central venous line access, and the inability to maintain an aseptic catheter insertion site. Proper aseptic techniques are mandatory during administration of ILE to neonates by the nursing staff to avoid transmission of infections, particularly skin flora, such as CoNS and *Candida* spp. Any of these variables combined with contaminated infusate increases the risk of intravenous-related infection and/or sepsis.¹⁹

In the present study, microbial growth was found in 15.8% of ILE repackaged samples (Table 1). Positive samples comprised: *K. pneumoniae* (29.2%), *C. albicans* (20.8%), *A. baumannii* and *S. aureus* (12.5% each), *P. aeruginosa* and CoNS (8.3% each), and lastly, *E. coli* and *E. faecalis* (4.2% each). Kuwahara et al²⁰ reported that the most commonly isolated organisms were *Serratia marcescens* and *Bacillus cereus*. Mershon et al²¹ reported that their most common organisms were *S. epidermidis*, *E. coli*, and *C. albicans*, in descending order.

In the present study, no *Malassezia* spp were isolated, and this is in accordance with the absence of recent publications on *Malassezia* spp in ILE. All previous studies that isolated this fungus in ILE were in the 1980s

Table 3. Distribution of the 24 Positive Intralipid Emulsion (ILE) Samples and Their Corresponding Neonatal Cultures, in the NICU of El Shatby University Hospital, Alexandria, 2017

Neonatal ID Number	Results of 24 Positive ILE Samples	Corresponding Neonatal Culture
1	<i>Klebsiella pneumoniae</i>	<i>K pneumoniae</i>
3	<i>K pneumoniae</i>	<i>K pneumoniae</i>
6	<i>Staphylococcus aureus</i>	<i>S aureus</i>
8	<i>K pneumoniae</i>	<i>K pneumoniae</i>
16	<i>Enterococcus faecalis</i>	<i>Pseudomonas</i>
19	<i>K pneumoniae</i>	<i>K pneumoniae</i>
22	<i>Acinetobacter baumannii</i>	<i>A baumannii</i>
28	<i>A baumannii</i>	<i>A baumannii</i>
39	<i>K pneumoniae</i>	<i>K pneumoniae</i>
44	<i>S aureus</i>	<i>S aureus</i>
50	Coagulase-negative staphylococci	Sterile
60	<i>Candida albicans</i>	<i>C albicans</i>
73	Coagulase-negative staphylococci	Sterile
85	<i>K pneumoniae</i>	<i>K pneumoniae</i>
90	<i>Pseudomonas aeruginosa</i>	Sterile
92	<i>C albicans</i>	<i>C albicans</i>
102	<i>C albicans</i>	<i>C albicans</i>
122	<i>Escherichia coli</i>	Sterile
126	<i>K pneumoniae</i>	<i>K pneumoniae</i>
127	<i>S aureus</i>	<i>S aureus</i>
129	<i>A baumannii</i>	<i>A baumannii</i>
130	<i>C albicans</i>	<i>C albicans</i>
131	<i>C albicans</i>	<i>C albicans</i>
132	<i>P aeruginosa</i>	<i>P aeruginosa</i>

and 1990s, before the addition of medium-chain fatty acids to newer generations of ILE. A study by Papavasiliou et al²² in 1999 reported that medium-chain fatty acids markedly inhibited the growth of *Malassezia* spp.

In the present study, microbial contamination increased from 9.6% at 12 hours to 21.5% at 24 hours of infusion time. This difference was found to be statistically significant ($p = 0.044$). Crill et al⁵ found that 3.3% of the samples from syringes had growth of *Klebsiella oxytoca* and *Citrobacter freundii* after 24 hours of infusion.

Reiter et al¹¹ tested for microbial contamination after a 24-hour ILE infusion. In their study, only 2.27% of samples showed growth. Similarly, Matlow et al²³ reported a 1.35% contamination rate of neonatal ILE at 24 hours of administration. Dedonato et al¹³ kept syringes of ILE unrefrigerated before administration, and yet no microbial growth was detected in any of their samples at both 12 and 24 hours. They concluded that the policy of preparation and administration of their ILE for the NICU patients was effective regarding microbial growth.

Ybarra et al¹² recorded microbial growth in 7.9% of their preparations, despite using an automated compounding device. They attributed this to repackaging taking place at the end of the working day in their sterile products area, when the pharmacy staff completed compounding of 2-in-1 PN and other solutions with the automated compounding device.

Contamination rates recorded in the present study are the highest rates at both 12 and 24 hours' ILE infusion. Improper infection control measures have likely contributed to these high rates, because cultures of the original ILE bottles were sterile. Touch contamination, which had occurred within 12 hours of administration, had probably multiplied to reach even higher levels at the end of the 24-hour ILE infusion time. To the best of our knowledge, no similarly high rates at either 12 or 24 hours from repackaged ILE syringes were recorded in other published studies.

ASPEN, in agreement with the recommendations of the CDC, limits the "hang time" of ILEs to 12 hours after the manufacturer's container is spiked with the

Table 4. Relationship Between Infection Control Score and the Results Of Intralipid Emulsion (ILE) Cultures

ILE Culture Results	Infection Control Measures Score, Mean $\pm$ SD (Range)	Test of significance	p value
No growth	5.0 $\pm$ 1.41 (4–6)	Student <i>t</i> = 2.851	0.012*
Positive growth by culture	3.0 $\pm$ 0.89 (2–5)		

* p value significant at < 0.05

appropriate administration set. This increases the cost of materials, and the pharmacy and nurse workload, but it carries fewer chances for microbial contamination. If a slower infusion is desirable and the selected rate of administration exceeds 12 hours, then the ILEs shall be given in 2 separate bottles so as not to exceed a 12-hour hang time for any single container.^{8,9} The once-daily continuous infusion is recommended by the AAP, because it decreases material costs, workload, and manipulations by pharmacy and nursing. However, the syringes have potential for greater microbial growth if contaminated, because of extended administration.¹⁰

As observed in this present study, not only did the rates of contamination between 12 and 24 hours' ILE vary, but also the microbial profile showed discrepancy, possibly owing to variation in microbial growth rates among species (Table 2). The most frequently isolated

bacteria from ILE after 12 hours were *K pneumoniae*, *C albicans*, and *P aeruginosa*, each with a prevalence of 2.7%, followed by *E coli* (1.4%). *Pseudomonas aeruginosa* (n = 2) and *E coli* (n = 1) appeared only in samples collected at 12 hours and not at 24 hours. On the other hand, *A baumannii* (n = 3 [3.8%]), *E faecalis* (n = 1 [1.4%]), and *S aureus* (n = 3 [3.8%]) appeared only in samples at 24 hours. However, the small number of those isolates does not reach statistical significance to prove that any of them flourished in ILE samples earlier than the others. Keammerer et al²⁴ reported that the growth of the CoNS was minimal at 48 hours, whereas *E coli* and *P aeruginosa* showed marked growth at 12 hours. In 2013 Dedonato et al¹³ spiked ILE samples with various species and detected their rate of growth after incubating in a Bact/Alert machine. They reported that *P aeruginosa* and *A baumannii* were detected at ap-

Table 5. Antibiotic Resistance Profile of Bacteria Isolated From Contaminated Intralipid Emulsions in the NICU of El Shatby Hospital, Alexandria University, 2017

Antimicrobial Agent	Bacterial Isolates, % of Resistant Isolates					
	<i>Staphylococcus aureus</i> (n = 3)	<i>Enterococcus faecalis</i> (n = 1)	<i>Klebsiella pneumoniae</i> (n = 7)	<i>Escherichia coli</i> (n = 1)	<i>Acinetobacter baumannii</i> (n = 3)	<i>Pseudomonas aeruginosa</i> (n = 2)
Gentamicin	66.7	—	71.4	100.0	66.7	50.0
Meropenem	—	—	0.14	0.0	0.0	0.0
Levofloxacin	0.0	—	0.0	0.0	0.0	0.0
Cefepime	—	—	0.0	0.0	0.0	0.0
Piperacillin-tazobactam	—	—	0.0	0.0	66.7	0.0
Ceftazidime	—	—	—	—	66.7	50.0
Tetracycline	33.3	100.0	—	—	—	—
Vancomycin	0.0	0.0	—	—	—	—
Chloramphenicol	33.3	0.0	0.0	0.0	—	—
Tobramycin	33.3	—	—	—	66.7	50.0
Azithromycin	66.7	100.0	—	—	—	—
Sulfamethoxazole-trimethoprim	66.7	—	—	—	—	—
Clindamycin	66.7	—	—	—	—	—
Cefazolin	—	—	100	100.0	—	—
Aztreonam	—	—	0.0	0.0	0.0	0.0
Cefoxitin	66.7	—	85.7	100.0	—	—
Linezolid	0.0	0.0	—	—	—	—

proximately 12 hours, followed by *S epidermidis* and *C albicans* (20 and 30 hours, respectively).

In this study a similar trend of predominance of *K pneumoniae* and *C albicans* in both ILE and neonatal cultures was observed. This might suggest that ILE was one possible source of sepsis for those neonates. However, it is impossible to prove this, because genotyping for microbial strains from both sources was not done. Jarvis et al⁷ in 1983 reported that *K pneumoniae* and *Enterobacter cloacae* were responsible for an outbreak of polymicrobial bacteremia associated with the receipt of ILE. In their study, 25% of neonates in the NICU developed sepsis as a result. The most recently published similar outbreak has been recorded in Korea in 2018. Four neonates died consecutively within a time span of 80 minutes, and *Citrobacter freundii* was isolated from the blood samples of them all. The sequences of the antibiotic resistance gene of *C freundii* were reported to be identical between the clinical isolates and the ILE isolates.²⁵

Freeman et al²⁶ found an association between the risk of CoNS bacteremia among infants in the NICU and the administration of ILE. In the present study neonatal cultures showed a higher prevalence of CoNS isolates (36%) compared with those isolated from ILE samples (8.3%). This difference is attributed to the higher prevalence of CoNS results in neonatal samples (n = 18) versus those isolated from ILE samples (n = 2). Higher CoNS isolation rates in neonatal cultures than ILE denote other possible routes of infection in neonates other than ILEs.

According to CDC guidelines, hand decontamination, skin antisepsis, and wearing PPE (e.g., masks, gowns, and gloves) prior to any procedure are all an integral step of the process that should be done by the team working in direct contact with patients.²⁷ In our study, there was a direct relationship between inefficient infection control measures and positive ILE culture results (Table 4). This might explain the high *Candida* contamination rate of ILE (20.8%).

An increase in infections by MDR organisms nowadays is due to the abuse of antibiotics. In the present study *K pneumoniae*, *E coli*, *A baumannii*, *P aeruginosa*, *S aureus*, and *S enterococcus* were all MDR. The mortality and morbidity of antimicrobial-resistant organisms may be related to increased virulence, delay in appropriate therapy, and fewer treatment options. Antimicrobial stewardship is recommended to limit antimicrobial-resistant organisms and improve quality of care. The Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America developed guidelines for developing an institutional program to enhance antimicrobial stewardship. It is based on timely antimicrobial management, appropriate selection, administration and de-escalation of antimicrobial agents, and access to infectious disease expertise.²⁸

This study evaluated the difference in contamination rates of ILEs at 12 and 24 hours, rather than identifying specific modes of microbial contamination of the ILEs. This limitation would have been of value if identified in further studies, because this might help in applying specific preventive measures. This, accordingly, might reduce contamination rates of ILEs even if the 24-hour protocol is used. Further microbiologic testing of ILEs after refrigeration and immediately before hanging to neonates might add more data on point-of-contamination sources of ILEs.

Conclusion

The rate of ILE contamination was less at 12 hours than at 24 hours of infusion time. Compliance with infection control measures was significantly worse among nurses compared with pharmacists. *Klebsiella pneumoniae* and *C albicans* were the most common pathogens isolated from ILE and neonates. Bacterial isolates were MDR on antibiotic susceptibility testing.

ARTICLE INFORMATION

Affiliations Department of Microbiology (EAO, WMKB), High Institute of Public Health, Alexandria University, Alexandria, Egypt, Neonatal Intensive Care Unit (FFE), El Shatby University Hospital, Alexandria, Egypt

Correspondence Eman A. Omran, PhD;
eman79omran@gmail.com

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