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Original Research

Risk of Microbiological Contamination when Preparing Total Parenteral Nutrition for Pediatric Patients: A Pilot Study at a Regional Hospital in Southern Vietnam

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ABSTRACT

Background: Total parenteral nutrition (TPN) presents a risk of contamination because it is a mixture of multiple additives and an excellent growth medium for microbes. In Vietnam, no official standard for TPN preparation has been established, thereby causing inconsistencies in implementation in healthcare facilities. We investigated the risk of microbiological contamination during the preparation of TPN for pediatric patients at a regional hospital in southern Vietnam.

Methods: This cross-sectional study was conducted from July to September 2019 at Children Hospital No. 2 in Ho Chi Minh City, southern Vietnam. Vaminolact 6.5% was the chosen TPN product for analysis. Collected bacterial samples were isolated in petri dishes containing nutrient agar as a solid growth medium. Each colony grown in the dish was subjected to Gram staining, after which species-specific identification was conducted on the basis of 16S ribosomal deoxyribonucleotide acid sequences.

Results: The results showed that after TPN preparation, ambient air in the laboratory contained contaminative agents. The bacteria isolated from medical devices and medical employees' hands corresponded to colonies grown in two petri dishes. The bacterial species found included *Moraxella osloensis, Micrococcus endophyticus, Micrococcus luteus, Macrococcus canis, Staphylococcus epidermidis, Brachybacterium muris, Micrococcus yunnanensis, Rothia amarae, Bacillus jeotgali, and Staphylococcus haemolyticus*.

Conclusion: Disease-causing bacteria were found to proliferate during TPN preparation, highlighting the necessity of considering the risk of external contamination from sampling and testing.

Keywords: Bacteria, Gram staining, intravenous nutrition, Staphylococci, Vietnam.

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INTRODUCTION

Total parenteral nutrition (TPN) is a life-saving source of intravenous sustenance for patients suffering from diseases or conditions that impair food intake or nutrient digestion or absorption. In 2014 in the US, TPN was administered to more than 290,000 patients, among whom 40% were children and newborns.[1] parenteral nutrition for pediatric patients can be combined into two-in-one or three-in-one admixtures. The former contains amino acids, carbohydrates, and electrolytes in a single container, with lipid emulsion kept in a separate container, whereas the latter has all components, including lipid emulsion, in a single vessel.[2]

TPN is especially at risk of contamination because it is a mixture of multiple additives and an excellent growth medium for microbes.[3] The production of fluids for intravenous use is optimally performed in an aseptic environment, and the finished product should be free of microbes, spores, endotoxins, chemical contaminants, and physical matter. Compounding such a product therefore requires trained personnel, an adequate preparation environment, and effective techniques.[4] In preparations laden with bacteria, fungi, or viruses, these contaminants are directly transmitted to patients-an outcome that will likely result in infection or, potentially, death.[5] In 2012, 70 deaths and 750 meningitis cases stemming from fungal contamination during TPN co-pounding in pharmacies occurred in 20 states in the US.[6] In 2014, 19 patients who received TPN from pharmacies suffered from septicemia caused by *Serratia marcessens*, resulting in nine deaths.[7] *S. marcessens* was identified from a water-containing area, admixture containers, and unsealed amino acid ingredient powder.[7] That same year, a broader scale of 89 deaths and 1,049 adverse medication errors were caused by biological contamination during TPN preparation.[8]

The above-mentioned problems drove the implementation of measures intended to combat contamination during TPN preparation. In 2016, for example, about half of US states initiated compliance with quality assurance standards for sterile compounding, and 18 other states continued to update and adjust regulations to minimize harm to patients.[9] In Vietnam, however, no official standard for TPN preparation has been established, leading to inconsistencies in implementation in healthcare facilities. The majority of hospitals in the country use available TPN products supplied by manufacturers, whereas the rest prepare these supplements themselves, thereby exposing products to multiple sources of contamination. With these issues in mind, we investigated the risk of microbiological contamination during the preparation of TPN for pediatric patients at a regional hospital in southern Vietnam.

METHODS

Study design

This is a cross-sectional study conducted between July and September 2019 at Children Hospital No. 2 in Ho Chi Minh City, Southern Vietnam. From a list of self-compounded TPN

products in the hospital, we chose the most ingredient complex, Vaminolact 6.5%, for analysis. The formula for 1 L of Vaminolact is presented in Table 1.

Table 1. Vaminolact formula

Ingredient	Mass	Ingredient	Mass
L-Alanine	6.3	L-Threonine	3.6
L-Histidine	2.1	L-Serine	3.8
L-Arginine	4.1	L-Tryptophan	1.4
L-Isoleucine	3.1	L-Tyrosine	0.5
L-Aspartic acid	4.1	L-Valine	3.6
L-Leucine	7.0	L-Lysine	5.6
L-Cysteine	1.0	L-Methionine	1.3
L-Phenylalanine	2.7	L-Glutamic acid	7.1
L-Proline	5.6	Taurine	0.3
Glycine	2.1		
Total amino acid (g/L)	65.3		
Nitrogen (g/L)	9.3		
Total energy (kcal/L)	240		
Osmotic pressure (mili-osmole/kg)	510		
pH	5.2		

Ethical considerations

The study protocol was approved by the Ethical Council of Children Hospital No. 2. No animals or human participants were involved in experiments in this work.

Subjects and materials

All TPN products used in Children Hospital No. 2 are prepared in accordance with a process wherein each step is illustrated through a standard operating procedure (SOP). On the basis of this process, we collected bacterial samples from six potential sources: ambient air, trays, trolleys, lockers, needled, and the palms of employees. Samples were collected before and after Vaminolact preparation four times on four different days.

The collected samples were isolated in Petri dishes containing nutrient agar as a solid growth medium. Each colony grown in the dish was subjected to Gram staining, after which species-specific identification was performed on the basis of 16S ribosomal deoxyribonucleotide acid (DNA) sequences.

RESULTS

Table 2 shows the results of microbiological isolation from ambient air in the laboratory, trays, trolleys, lockers, needles, and employees' hands. After TPN preparation, ambient room was found containing contaminative agents. The samples isolated from medical devices and employees' hands corresponded to two Petri dishes of bacterial colonies.

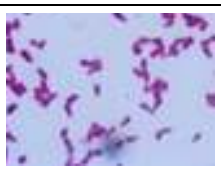
Each colony from the two Petri dishes was divided into two groups: The first was subjected to Gram staining, and the second was cultured in a tryptic soy broth environment for 16S rDNA identification. From Petri dish 1, we found five species (coded 1.1 to 1.50, and from Petri dish 2, we found seven species (coded 2.1 to 2.7) (Table 3).

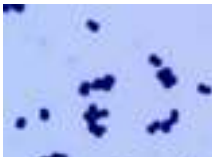
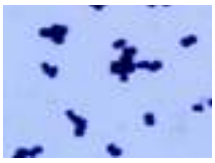
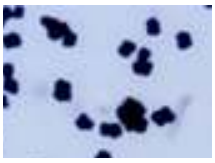
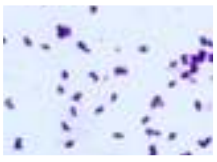
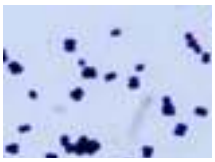
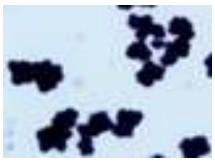
Table 2. Microbiological isolation

		Day 1		Day 2		Day 3		Day 4	
		Before	After	Before	After	Before	After	Before	After
Air	Biological contamination (CFU/m³)	NA	373	66	189	28	372	24	286
	Particular matter ≥ 0.5µm	NA	NA	NA	154,336	12,364	137,789	94,779	152,310
	Particular matter ≥ 5µm	NA	NA	NA	46	36	777	20	90
Tray		NA	7 colonies	No growth	No growth	No growth	No growth	No growth	No growth
Trolley		NA	20 colonies	No growth	No growth	No growth	No growth	No growth	No growth
Locker		NA	No growth	NA	No growth	No growth	No growth	No growth	No growth
Needle		NA	No growth	NA	No growth	NA	No growth	NA	No growth
Staff's hand		NA	NA	NA	NA	NA	No growth	NA	No growth

Acronyms: CFU, colony-forming unit; NA, not applicable

Table 3. Gram staining and species-specific identification

Code	Description	Colony count	Gram staining		Species-specific identification
1.1	Evenly round, smooth surface, convex cross- section with crown, brownish pink	01		Gram-negative coccobacilli	*Moraxella osloensis*

Code	Description	Colony count	Gram staining		Species-specific identification
1.2	Evenly rounded, smooth surface, high convex cross-section, pale yellow	05		Gram-positive cocci	*Micrococcus endophyticus*
1.3	Evenly rounded, smooth surface, high convex cross-section, dark yellow	13		Gram-positive cocci	*Micrococcus luteus*
1.4	Evenly rounded, non-glossy surface, low convex cross-section, milky color	01		Gram-positive bacilli	*Macrococcus canis*
1.5	Evenly rounded, non-glossy surface, high convex cross-section, pale yellow	02		Gram-positive cocci	*Micrococcus luteus*
2.1	Evenly rounded, smooth surface, low convex cross-section, milky white	05		Gram-positive cocci	*Staphylococcus epidermidis*
2.2	Evenly rounded, smooth surface, high convex cross-section, pale yellow	14		Gram-positive cocci	*Brachybacterium muris*
2.3	Evenly rounded, non-glossy surface, high convex cross-section, pale yellow	05		Gram-positive cocci	*Micrococcus yunnanensis*
2.4	Evenly rounded, non-wrinkled surface, not shiny, milky cross-section, milky white	01		Gram-negative coccobacilli	*Rothia amarae*
2.5	Round, glossy surface, convex cross-section with white tip	01		Gram-positive bacilli	*Bacillus jeotgali*
2.6	Evenly rounded, non-glossy surface, high convex cross-section, milky white, slightly yellowish	02		Gram-positive cocci	*Micrococcus luteus*

Code	Description	Colony count	Gram staining		Species-specific identification
2.7	Evenly round, smooth surface, high convex cross-section, milky white, slightly yellowish	02		Gram-positive cocci	*Staphylococcus haemolyticus*

DISCUSSION

Despite the implementation of sanitation and preventive measures in the case hospital, we found a risk of biological contamination during TPN preparation for pediatric patients. According to the ISO 14644-1:2016, ambient air in a preparation room/laboratory should comply with level 7 of allowable bacterial concentrations.[4,10] The 2012 standards of the World Health Organization requires compliance in this regard with level C or D before preparation; concentrations at level D and higher are beyond effective control or management.[10,11]

Among 12 bacterial species isolated from trays and trolleys, certain disease-causing agents that require considerable attention are discussed as follows. *M. osloensis* presents in many tissues in the human body and sometimes infect bodily parts such as the eyes, bone marrow, and blood.[12-14] *M. osloensis* and *M. nonliquefaciens* are among the important Moraxella strains implicated in eye infections, but the former is more resistant to vancomycin than the latter.[13] *Micrococcus* spp. often exist harmlessly on the skin, mucous membranes, and pharynx but sometimes cause illness, especially in immunocompromised patients.[15] In such cases, most occurring strains are identified as *Micrococcus luteus*.[15] Micrococci have also been occasionally reported as a cause of pneumonia, ventricular shunt-associated meningitis, septic arthritis, septicemia, peritonitis, intraocular inflammation, catheter-related bloodstream infection, and endocarditis.[15,16] *Staphylococcus epidermidis* and *S. haemolyticus* are non-aureus species, which are part of skin microflora and mostly cause disease in immunocompromised patients or hospitalized patients undergoing invasive interventions that cause local or systemic infections.[17-23] *S. haemolyticus* can create biofilms and resistant phenotypes, thereby presenting considerable difficulties in treatment.[24] *Macrococcus canis* and *Brachybacterium muris* are bacteria found in animals. *Macrococcus canis* is a strain of bacteria that appears on healthy skin or dog infections, and its presence in locations where infections show signs of antibiotic resistance raises concerns about this new bacterium.[25,26] *Brachybacterium muris* was first found in rat liver in 2003.[27] *Bacillus jeotgali* is found in Korean jeotgal cuisine or fermented seafood.[28]

The existence of typically harmless non-aureus staphylococcus strains on the skin and strains found on animals or food during the sample identification indicate the necessity of considering the risk of external contamination from sampling and testing. Direct preparation should be carried out within an environment that complies with level 5 standards of Primary Engineering Control (PEC), according to ISO 14644-1:2015.[4,10,11] Two or more preparations should not be created at the same time. In case of non-compliance with aseptic techniques, a requirement is to remove an inoculant after preparation and disinfect raw materials, equipment, and PEC before starting a new phase.[4,10,11] Preparation procedures should comply with a reference (required) and manufacturers' instructions (if any) listed in an SOP.[29] Gloves must be disinfected using 70% isopropyl alcohol every time they are placed into PEC or after contact with contaminated surfaces.[4,10,11,30] If gloves are torn, they must be removed, and hands and forearms must be sanitized before new gloves are worn. Gloves should be used only once.[4,10,11] After mixing, raw materials and preparations must be intact, dry, and free of moisture.[4,10,11] All containers (e.g., solution bags, vials, and tubes) should be checked prior to use to ensure that they are clean with no debris or dust on them.[4,10,11] All equipment surfaces should be disinfected using 70% isopropyl alcohol before placement into PEC;[4,10,11] ethanol is an ineffective sterilizing substance.[31] Employees should prevent direct contact between the body and a work surface and should refrain from removing liquid solutions from packaging via the direct insertion of a syringe.[30,32] Aseptic dispensing procedures need to be tested at least every six months.[32]

CONCLUSION

We found disease-causing bacteria during the TPN preparation procedure at Children Hospital No. 2 in Ho Chi Minh City. This finding underscores the need to examine the risk of external contamination from sampling and testing.

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CONFLICT OF INTEREST: The authors have no conflicts of interest to declare.

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