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# **One Year Incidence of Infection in Pediatric Intestine Transplantation**

Jared W. Clouse, MD, Chandrashekhar A. Kubal, MD, PhD, Jonathan A. Fridell, MD, and

Richard S. Mangus, MD, MS, FACS

From the Department of Surgery, Transplant Division, Indiana University School of Medicine

and the Riley Hospital for Children, Indianapolis, Indiana, USA

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Corresponding author contact information: Dr. Richard S. Mangus, MD MS FACS

Director of Intestine Transplantation, Riley Hospital for Children

Transplant Division, Department of Surgery, Indiana University School of Medicine

550 N University Blvd, Room 4601, Indianapolis, Indiana 46202-5250

Phone: (317) 944-4370 Fax: (317) 948-3268 E-mail: <u>rmangus@iupui.edu</u>

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### Abstract

## Background

This study reports the infection rate, location of infection, and pathogen causing bacterial, fungal, or viral infections in intestine transplant recipients at a pediatric transplant center.

### Methods

Records from a pediatric center were reviewed for patients receiving an intestine transplant. Positive cultures and pathology reports were used to diagnose bacterial, fungal, and viral infections and also to determine location and infectious agent. Risk for infection was assessed based on liver or colon inclusion, and immunosuppression induction, as part of the intestine transplant.

## Results

During the study period 52 intestine transplants were performed on 46 patients. Bacterial, fungal, and viral infection rates were 90%, 25%, and 75%, respectively. Enterococcus (non-vancomycin resistant enterococci (VRE)) species were the most common pathogens and were isolated from 52% of patients. VRE was present in 12% of transplant recipients. Candida species were the most common fungal pathogens (23% of patients). Respiratory viral infections were common (44%) and cytomegalovirus infection rate was 17%. Common sites of infection were bloodstream, urinary, and upper respiratory tract. Colon and liver inclusion in the transplant graft was not associated with increased risk of infection, nor was addition of rituximab to the immunosuppression induction protocol.

#### Conclusion

Post-intestine transplant infections are ubiquitious in the pediatric population, including high rates of infection from bacterial, viral and fungal sources. Inclusion of the liver and/or colon as a

component of the transplant graft did not appear to greatly impact the infectious risk. Adding rituximab to the immunosuppression induction protocol did not impact on infectious risk. **Key words**: Intestine transplant, multivisceral transplant, infection, complications, outcomes

# Introduction

Intestine transplantation has become a potentially curative procedure for patients with intestine failure who have failed total parenteral nutrition (TPN) or who are poor candidates for long-term TPN.[1] It is also a therapeutic option for patients who have diffuse portomesenteric thrombosis, unresectable benign intraabdominal tumors, and other intraabdominal pathologies (frozen abdomen, long segment Hirschsprung's).[2, 3] Transplant options for pediatric patients include isolated intestine (IT) and multivisceral (MVT; liver, stomach, pancreas, intestine) transplant.[4, 5] The proximal large intestine may also be included in the composite graft to preserve the ileocecal valve, reduce the risk of dehydration by increasing water absorption, and to minimize stooling. Intestine transplant can be a lifesaving procedure, and in recent years outcomes have improved. However, intestine transplant continues to be associated with serious and life-threatening post-transplant complications.[6]

Infection is the most prevalent post-transplant complication for all solid organ transplants and is a leading cause of death and graft loss in intestine transplant recipients.[4] Patients are at the highest risk for bacterial, fungal, and viral infections in the first year post-transplant due to the invasive nature of the procedure and the requirement for high doses of immunosuppressive agents. The bloodstream is the most worrisome site of infection because it is associated with high morbidity and can lead to sepsis which is the primary cause of death in intestine transplant patients.[7] The incidence of bloodstream infections (BSI, bacterial and fungal) has been reported to be as high as 69% in the pediatric intestine transplant population. Reported risk factors for the development of BSI include liver inclusion, age less than 18 years, and bilirubin >5 mg/dL at the time of transplant.[8, 9]

Infections caused by fungi are not as common as bacterial infections in intestine transplant recipients but are responsible for 31% of all infection-related deaths.[4] *Aspergillus* and *Candida* are the most prominent fungal species infecting pediatric intestine transplant patients. The majority of fungal infections in this population are caused by *Candida* species with *Candida albicans* being the most commonly isolated species.[10] Viral infections are also frequently found in pediatric intestine transplant patients and can be caused by reactivation of a latent infection or by de novo infection. Additionally, viral infections have been associated with serious complications such as post-transplant lymphoproliferative disorder (PTLD) and acute cellular rejection, among others.[11, 12]

This study reports bacterial, fungal, and viral infection rates at an active pediatric intestine transplant center in the first year post-transplant. Incidence of infection is reported by anatomic site and individual pathogenic species. Risk for infection is assessed based on liver and large intestine inclusion, as well as an examination of potential risk factors for the development of BSI.

#### Methods

A retrospective chart review was conducted for all patients receiving an intestine transplant at a single pediatric transplant center from 2003 to 2015. Intestine transplantation occurred as either an IT or MVT. Additionally, a subset of patients received the proximal large intestine from the donor as part of the intestine graft. All patients were included in the final analysis regardless of outcome.

MVT and IT protocols employed at our center have been previously described.[3, 13, 14] Neither the donor nor the recipient received mechanical or pharmacologic decontamination of the intestine as part of intestine transplant process.[15] To ensure an isolated intestine graft was

not exposed to an intestine decontamination solution when the pancreas was procured by another center, the duodenal-jejunal junction was stapled before the use of a bowel decontamination solution.[16] Each patient received similar induction therapy with 3 to 5 alternating doses of rabbit antithymocyte globulin (total 5-6mg/kg) and solumedrol (3 doses:10mg/kg, 5mg/kg, 2.5mg/kg). Additionally, a single dose of rituximab was also administered to 75% of the patients (2007 to 2015) as part of the immunosuppression induction ( $150 \text{mg/m}^2$  (body surface area)). Maintenance of immunosuppression involved the use of tacrolimus and low dose steroids.[17] Serum target levels for tacrolimus were 10-12ng/dL in the first 6 months post-transplant and then were decreased to 7-8ng/dL thereafter. Patients with any history of rejection were maintained on the higher target level during 1-year post-transplant, before the target was lowered. Also, those patients experiencing any episode of rejection were maintained on long-term low dose prednisone (5-10mg/day). Tacrolimus target levels were consistently maintained at higher target values, for a longer period, for patients receiving isolated intestine transplant. Multivisceral transplant recipients have a much lower rate of rejection and are more aggressively weaned to lower targets. In the majority of cases, prophylactic empiric antibiotic use (piperacillin/tazobactam or cefepime) was continued for the first 48 hours post-transplant. If there was considerable gross contamination at the time of surgery, antibiotic prophylaxis was continued for up to seven days. Antifungal prophylaxis consisted of a one-time dose of 100-400mg fluconazole prior to surgery with 25-100mg taken daily for the first year post-transplant (dosing per patient age and size). Daily trimethoprim-sulfamethoxazole is used for Pneuomocystis jirovecii pneumonia prophylaxis and is taken indefinitely. Prophylactic treatment for viral infections included intravenous ganciclovir and cytomegalovirus immune globulin (cytogam) in the early post-transplant period. When the patient was tolerating enteral

medications, the ganciclovir was changed to enteral valganciclovir (usually within 2-3 weeks of the intestine transplant). Enteral valganciclovir and monthly cytogam were continued up to 1year post-transplant. At that time, they were both withdrawn if there had been no episodes of cytomegalovirus infection. Monitoring for signs of infection was performed at least daily in the early post-transplant period, and included measurement of the white blood cell count and routine vital signs. If leukocytosis was present, or a fever detected, blood and urine samples were sent for culture. Sputum cultures were not routinely assessed, but were sent in cases of respiratory symptoms such as cough, shortness of breath, infiltrate on chest radiograph, or need for supplemental oxygen. Additionally, all fluid drained from identified abnormal fluid collections or body cavities was sent for culture. Treatment of infections consisted of antibiotic therapy for 7-14 days, or until patients were asymptomatic, with individual pathogen sensitivities governing antibiotic selection.

Positive cultures and pathology reports were reviewed to determine the presence of an infection, the organism causing the infection, and the anatomic location of infection. All data collected in the first year post transplant was utilized for the analysis presented in this paper. All postoperative positive cultures were included regardless of location or infectious organism. Bloodstream infections were not limited to those caused by an indwelling catheter. Active infections at the time of transplant were excluded from this study.

Standard statistical techniques, which included chi-square analysis for categorical variables and analysis of variance (ANOVA) testing for continuous variables, were performed using the Statistical Package for the Social Sciences (IBM SPSS Statistics 24, IBM Corporation, Armonk, New York, USA). For subgroup analysis, patients were grouped based upon liver or large intestine inclusion as part of the small intestine inclusive graft. Evaluation of potential risk

factors for BSI included transplant of the liver or large intestine with the intestine allograft, age ( $\leq 2$  years or 2 to 18 years), gender, and race (white or non-white). Although a p-value of 0.05 is a standard often used to determine statistical significance, there were a large number of individual tests performed in analysis of data for this cohort. Therefore, given the high number of tests performed and the possible interrelatedness of the tests, an accurate p-value for determining significance may be as low as 0.01. However, this is primarily a descriptive clinical paper. The p-values will be reported as calculated and the reader may determine the clinical importance of these findings. The institutional review board at the Indiana University School of Medicine reviewed and approved retrospective analysis of this data from the transplant database.

### Results

There were 46 pediatric patients who underwent 52 intestine transplants during the study period. A total of 34 patients (65%) received a MVT while 18 (35%) received an IT. The proximal large intestine was transplanted with the small bowel in six (12%) patients. Median age of recipients was 1.6 years (range of 0.3-18 years). Donors had a median age of 1.5 years with trauma being the leading cause of death among them. For immunosuppression induction, 75% of patients received both antithymocyte globulin and rituximab. **[Table 1]** 

Bacterial infections were the most common type of infection and occurred in 90% of patients. The most common sites of bacterial infection were the urinary tract (73%) and bloodstream (75%). Pulmonary and wound infections were not as prevalent and occurred in less than 30% of patients, while abscesses developed in 4% of intestine recipients. *Clostridium difficile* infections were present in 23% of patients and were less common in MVT (17%) than IT (27%) (p=0.43). **[Table 2]** Gram-positive infections were primarily caused by non-vancomycin-resistant *Enterococci* (VRE) species (52%), coagulase-negative *Staphylococcus* (CoNS; 48%),

and *Streptococcus* species (23%). VRE and methicillin-resistant *Staphylococcus aureus* (MRSA) were isolated from 12% and 4% of patients, respectively. Other Gram-positive pathogens including methicillin-susceptible *Staphylococcus aureus* (MSSA), *Bacillus* species,

*Lactobacillus* species, and *Corynebacterium* species were all isolated in 10% or less of patients. There were no *Clostridium* infections outside of the *C. difficile* infections previously noted. The most frequently isolated Gram-negative bacteria were *Enterobacter* species (48%), *Pseudomonas* species (37%), *Klebsiella* species (37%), and *Escherichia coli* (37%). Infection rates of other Gram-negative bacteria (*Bacteroides, Citrobacter, Stenotrophomonas, Prevotella, Serratia,* and *Proteus*) are also noted. [**Table 3**] Liver inclusion had no significant effect on bacterial strain or site of infection, transplant of the large intestine was observed to be protective against CoNS (pvalue=0.01) and bloodstream infections (p-value=0.03). [**Tables 2 and 3**]

Fungal pathogens were isolated from 25% of patients. Similar to bacterial infections, the urinary tract (10%) and bloodstream (10%) were the most common sites of fungal infection. Only two patients (4%) developed a pulmonary fungal infection, one patient (2%) developed a fungal abscess, and a single patient (2%) had a fungal wound infection. *Candida* species were the most commonly isolated fungal pathogen and were found in 23% of patients. *Candida albicans* was isolated from 12% of patients and found in half of all *Candida* infected patients. Infections caused by other *Candida* species (*C. krusei, C. glabrata*, and *C. parapsilosis*) were less common. *Aspergillus*-related infections were rare and only seen in a single patient (2%). **[Tables 2 and 4]** Although not statistically significant, only one patient who received a large intestine as part of the intestine transplant developed a fungal infection.

Viral infections were observed in 75% of pediatric intestine recipients. Gastrointestinal tract and upper respiratory tract viral infections were common and occurred in 52% and 39% of

patients, respectively. Only 12% of patients developed lower respiratory viral infections, while viremia was seen in 31% of recipients. Cytomegalovirus (CMV) infected nine patients (17%, active viremia) and was tissue invasive in four (8%). Adenovirus and Rhinovirus were the most frequently isolated viruses and were found in 29% and 27% of patients, respectively. All other cultured viruses (Epstein-Barr virus [EBV], rotavirus, norovirus, herpesvirus 6, and parainfluenza), with the exception of respiratory syncytial virus (RSV; 6%), were isolated from at least 10% of patients. Large intestine inclusion was associated with an increased risk for infection by herpesvirus 6 (p<0.01) and upper respiratory infections (p=0.03). [Tables 2 and 4]

Bloodstream infections (bacterial or fungal) developed in 77% of patients, with a median time of 38 days to the first infection. Age, race, gender, liver inclusion and rituximab induction had no significant impact on BSI development, whereas patients receiving a large intestine graft were at a lower risk for developing a BSI (p=0.02). Of the covariates evaluated, only rituximab induction had significant impact on median time to first BSI (43 days with rituximab vs. 16 days without, p=0.02). [**Table 5**]

### Discussion

Infection is the most common post-transplant complication, and the leading cause of morbidity and mortality, in pediatric intestine transplant recipients. The high incidence of infection could be due to the invasiveness and non-sterile nature of the transplant procedure, or due to the need for increased levels of immunosuppression in comparison with other solid organ transplants. Identification of commonly infecting pathogens and risk factors for the development of infections is needed to develop therapeutic plans to mitigate the harmful effects of infection in this high-risk population. Bacterial infections are the most common type of infection in intestine transplant recipients.[4] Infection rates have been reported to be as high as 93% in intestine transplantation which is similar to the 90% rate reported in this cohort.[18] In an attempt to prevent gramnegative and fungal infections, some centers use an intestine decontamination solution prior to, or during, transplantation of the small intestine graft. However, the efficacy of its use has yet to be definitively shown and may provide no significant benefit.[15, 19] Certainly, use of this therapy disrupts the native microbiome which may negatively impact on the function of the graft.

Antibiotic-resistant bacteria are increasing in prevalence and pose unique challenges in solid organ transplantation.[20] In adult patients, the incidence of VRE and MRSA has been reported to be as high as 75% and 100%, respectively.[21] While this rate is alarming, in pediatric patients the incidence is much lower, with 36% of *S. aureus* infection being methicillin-resistant and 21% of *Enterococci* infections being vancomycin-resistant.[8, 22] The present study finds similar results with 29% of *S. aureus* infection being MRSA and 18% of *Enterococci* infections being VRE. The overall incidence of MRSA and VRE was relatively low at 4% and 12%, respectively. Although the resistant rates of VRE and MRSA in the pediatric population are lower than that seen in adults, these pathogens are still associated with substantial morbidity and have limited treatment options. Prevention protocols and novel treatments need to be developed and implemented to mitigate the deleterious effects these pathogens.

Additionally, fungemia has been associated with antibiotic use in intestine transplantation, further highlighting the need for infection prevention.[23] In this study, the incidence of fungal infections was 25% and the two most prominent genera causing fungal infections were *Candida* and *Aspergillus* which is congruent with previously published data. Additionally, this study and previous data have shown *Candida albicans* to be the most common

fungal species isolated from pediatric intestine transplant recipients. *Aspergillus* infections are associated with significantly shorter survival times, and with higher morbidity, when compared to *Candida* infections.[10] Although Aspergillus infections are associated with a higher mortality, disseminated infections in intestine transplant patients have been treated successfully.[24]

Viral infections caused by CMV, adenovirus, and respiratory viruses were the most commonly isolated viruses in this cohort of patients. CMV is associated with increased mortality, infection, and rejection episodes in intestine transplantation.[12] Adenovirus is associated with respiratory or gastrointestinal infections, and can also be a contributing factor in graft rejection and patient mortality.[25] Respiratory tract viral infections are frequently seen in solid organ transplant recipients. Tran et al. reported that 83% of pediatric patients with respiratory symptomatology were positive for at least one virus. While respiratory viruses were common, only respiratory syncytial virus (RSV) and adenovirus were associated with morbidity.[26] EBV, which had 10% rate of infection in this study, is another virus associated with morbidity in intestine transplant recipients with the EBV genome being found in 90% of patients who develop PTLD.[11]

Previously published risk factors for the development of BSI in intestine transplantation include liver inclusion with the intestine graft, age (< 18 years), and bilirubin >5 mg/dL at the time of transplant. In contrast to previous studies, this study found no correlation between liver inclusion and recipient age and the development of BSI. However, inclusion of the large intestine was associated with a reduced risk of BSI development. These findings suggest that more research is needed to identify risk factors and predictors of BSI development that will enable clinicians to better care for this high-risk population. This is a small population, such that the

results are more descriptive of this unique population rather than a statistically driven resource for protocol development.

In conclusion, this study reports the one-year incidence of infection in the high-risk pediatric intestine transplant population, with results stratified by anatomic location and pathogen type (including bacterial, fungal, viral, and individual pathogenic species). The high rate of infection and associated morbidity among this patient population informs the need for better methods of prevention, and the need for improved treatment options to mitigate the detrimental effects of immunosuppression-related post-transplant infections. Additionally, minimization of immunosuppression must be employed in patients identified as lower risk for graft rejection to minimize exposure to and risk from infectious pathogens.

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	Overall
Number	52 (100%)
Recipients	
Age (years, median, range)	1.6 (0.3 - 18)
Gender: male	60%
Race: White	65%
Disease	
Portomesenteric thrombosis	6%
Tumor	2%
Intestine failure / Other	92%
Transplant type	
Intestine only	35%
Modified multivisceral	0%
Full multivisceral	65%
Immunosuppression induction	
Antithymocyte globulin and steroids	25%
Antithymocyte globulin, steroids and	
rituximab	75%
Donors	
Age (year, median, range)	1.5 (0.1 - 22)
Gender: male	46%
Race: White	57%
Cause of death	
Stroke	12%
Trauma	46%
Anoxic brain injury	42%

**Table 1.** Demographics for 52 consecutive pediatric intestine transplant patients.

	Overall
	52
Infection first year post transplant (any)	
Bacterial (any)	90%
Abscess	4%
Urinary	73%
Pulmonary	29%
Blood stream	75%
Wound	23%
Clostridium difficile	23%
Viral (any)	75%
Blood	31%
Upper respiratory	39%
Pulmonary	12%
Gastrointestinal tract	52%
Fungal (any)	25%
Abscess	2%
Urinary	10%
Pulmonary	4%
Blood stream	10%
Wound	2%
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**Table 2.** Site of infection for 52 consecutive pediatric intestine transplant patients.

Gram Positive VRE Enterococcus MRSA	52 12%
VRE Enterococcus MRSA	
Enterococcus MRSA	
MRSA	
	52%
	4%
MSSA	10%
CoNS	48%
Streptococcus	23%
Corynebacterium	4%
Bacillus	4%
Lactobacillus	2%
Clostridium	0%
Other	27%
Gram Negative	
Pseudomonas	37%
Enterobacter	48%
Klebsiella	37%
E. coli	37%
Bacteroides	8%
Citrobacter	8%
Stenotrophomonas	4%
Prevotella	4%
Serratia	2%
Proteus	0%
Other	50%
Aycobacterium	0%

Table 3. Infections by bacterial organisms in pediatric intestine transplant.

	Overall
	52
Fungal	
Candida species	23%
C. glabrata	4%
C. albicans	12%
C. krusei	4%
C. parapsilosis	4%
Aspergillus	2%
Other	6%
Viral	
CMV	17%
Bloostream	15%
Tissue invasive	8%
Adenovirus	29%
EBV	10%
Rotavirus	12%
Norovirus	14%
Herpesvirus 6	10%
Upper Respiratory virus	44%
RSV	6%
Rhinoviurs	27%
Parainfluenza	10%
Other	12%

**Table 4.** Fungal and Viral species isolated from pediatric intestine transplant recipients.

	# of potionta	with BSI	n voluo	Median Days to 1st BSI	n volue
Overall	# of patients 52	40 (77%)	p-value	38	p-value
Overall	52	40(7770)		50	
Age	_		0.72		0.13
$\leq 2$ years old	28	21 (75%)		35	
2 to 18 years old	24	19 (79%)		49	
Gender	_		0.57		0.42
Male	31	23 (74%)		29	
Female	21	17 (81%)		46	
Race	_		0.56		0.21
White	34	27 (79%)		40	
Other	18	13 (72%)		35	
Liver Inclusion	_		0.43		0.43
Liver included	34	25 (74%)		35	
Liver not included	18	15 (83%)		43	
Colon Inclusion	_		0.02		0.74
Colon included	6	2 (33%)		85	
Colon not included	46	38 (83%)		35	
Immunosuppression induction	_				
Antithymocyte globulin and					
steroids	13	11 (85%)	0.48	16	0.02
Antithymocyte globulin,					
steroids, and rituximab	39	28 (72%)		43	

**Table 5.** Risk factors for bloodstream infections in pediatric intestine transplantation.