

**THE ROLE OF BACTERIAL TRANSLOCATION AND MICROBIOTA IN INTESTINAL BLOCKAGE:
AN EXPERIMENTAL STUDY.**

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ABSTRACT

Background:

The objective of this study is to investigate bacterial typing (BT) in the context of infectious diseases (I.O.), including the identification of pathogens, their transmission routes, and common characteristics.

Methods:

The study included 90 albino male rats in three groups: control (GI), simple ileal blockage (GII), and strangulated (GIII). Various surgical procedures were conducted under intramuscular ketamine anesthesia. Biochemical analysis measured CRP, IL-10, and oxidant and antioxidant activities. Histopathology assessed mucosal injury and inflammatory cell infiltration. Bacteriological examination identified bacterial species and CFU by cultivating tissue and blood samples.

Observation:

Strangulated intestinal obstruction increased bacterial proliferation, oxidative stress indicators, and IL-10 response, according to the study. The mean CFU/g of luminal bacteria count was 8.8×10^8 in GI, 5.6×10^{10} in GII, and 1.4×10^{12} in GIII. Mean MDA levels were 5.5 ± 0.5 in GI, 12.0 ± 1.9 in GII, and 21.9 ± 1.0 in GIII. The mean GPx levels were 125 ± 30 in GI, 165 ± 40 in GII, and 150 ± 35 in GIII. The mean IL-10 levels were 50 ± 15 in GI, 36 ± 12 in GII, and 9 ± 3 in GIII. Bacterial translocation was polymicrobial, with 49% in GII and 60% in GIII. Most enteric bacteria were E. coli, 42.2% in GII and 45.5% in GIII.

Conclusion:

Intestinal obstruction causes bidirectional bacterial translocation through a systemic portal, with a three-hit model primarily responsible for the ensuing inflammatory response. A non-selective marker of possible I.O. cases is the C-reactive protein. The C-reactive protein serves as an indicator of both Impaired blood flow to the tissues and the level of BT.

Recommendations:

Further studies should focus on developing more specific markers for early diagnosis of intestinal obstruction subtypes and exploring potential therapeutic interventions to manage bacterial translocation effectively.

Keywords: Obstruction of the Intestines, Ischemia, Translocation of Bacteria, Rats

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INTRODUCTION

Multorgan dysfunction syndrome is primarily to blame for the high mortality rate associated with intestinal obstruction (IO), a common and deadly abdominal emergency (MODS) [1]. Septic peritonitis and bacterial translocation (BT) are the main causes of MODS in IO [2]. In the past, research on BT served as a unifying theory to explain MODS; however, more recently, other distinct mechanisms—such as immunoinflammatory ones—have come into play [3].

Bacterial overgrowth that upsets the natural ecological balance causes bacterial translocation [4,5], host immune dysfunction triggering a balance between cytokines that are pro- and anti-inflammatory [6], and impairment of the mucosal barrier, which promotes the release of oxidants [7]. There is currently no accurate, expensive, and non-reproducible diagnostic procedure for intestinal strangulation outside of computerized tomography [8]. Lastly, ischemia and neovascularization are thought to be indicated by the inflammatory marker C-reactive protein

[9,10], thus verified in this investigation for IO, IO subtypes, and BT detection.

Objective

The objective of this work is to showcase bacterial typing (BT) in the context of infectious diseases (I.O.), including the identification of pathogens, their transmission routes, and common characteristics. Additionally, this work aims to investigate the local and systemic inflammation associated with BT and I.O., as well as the reliability of C-REACTIVE PROTEIN as a tool for studying BT and I.O. The potential impacts of I.O. on the ecological balance of microorganisms, chemical composition, and immunological defenses that protect B.T. are being considered.

METHODS

Study design

An experimental observational study.

Study setting

This study was conducted at the Histology experimental laboratory unit from April 2022 to March 2024. The study involved 90 albino male rats, which were separated into three categories, with each category consisting of 30 rats. Category I serves as the control category. Category II represents a simple ileal obstruction, where the ileum is tied off 5 cm before the caecum. Category III consists of a strangulated ileal obstruction, where a 5 cm loop of the ileum is tied off along with its mesentery, 5 cm before the caecum.

Study protocol

Anesthesia was induced using intramuscular ketamine at a dosage of 5 mg/kg body weight for GII and GIII. Following sterilization, a centerline laparotomy was performed to carry out the intended intraosseous (I.O) procedure. The abdominal closure was done using Vicryl sutures in layers. After recovering, all categories were kept in a room with a semi-controlled temperature of 23°C (±2). They were provided with laboratory chow and tap water over a specific duration of 28 hours until the relaparotomy.

In the second laparotomy, the same anesthetic technique was used for all three categories. A surgical cut was made in the thoracoabdominal region while maintaining a sterile environment. The following procedures were performed: direct sampling of blood from the heart, sampling of blood from the portal vein, resection of the left hepatic lobe, excision of multiple mesenteric lymph nodes, and resection of the ileal segment proximal to the ligature. In addition,

resection of the ileal segment in GI, which included the luminal contents, was done for cases of obstruction in GII and strangulated section of the ileum in Grade III. Finally, animals were euthanized by cervical dislocation.

Laboratory study

Biochemical study

1. The study focused on investigating the oxidant and antioxidant activity of the ileal loop.
2. The levels of serum C-reactive protein and IL-10 were measured.

1)a. Oxidant (Malonyl dialdehyde (MDA)): The samples of tissue were subjected to homogenization using a 0.1 ml/L PBS solution and subsequently subjected to centrifugation at a speed of 2000 rpm. The concentration of MDA was measured at an optical density of 534 nm [11].

1)b. The measurement of the Glutathione peroxidase, an antioxidant, was conducted using the NAD PH oxidation principle and recorded at an optical density of 340nm [12].

(2) a. The C-reactive protein a semiquantitative assay was conducted using a latex agglutination test with a normal cutoff of less than 0.5 mg/L.

(2) b. The serum level of IL-10 (interleukin-10) was determined using available ELISA kits.

Histopathologic study

(1) The ileal segments were analyzed to assess the extent of damage to the mucosal lining [13].

(2) The ileal segment, MLN, and samples were analyzed to determine the severity of inflammatory cell infiltration. The grading system used was as follows: GI: one inflammatory cell per cubic millimeter, GII: two to four inflammatory cells per cubic millimeter, and GIII: more than five inflammatory cells per cubic millimeter [14].

Bacteriologic study

The luminal contents refer to the substances present inside the intestines. The intestinal wall, MLN (mesenteric lymph nodes), and liver tissues are the specific anatomical structures involved. The cardiac and portal blood refers to the blood circulating through the heart and the portal vein, respectively. Samples were collected to determine their colony forming unit (CFU) index and identify the type of bacteria using gram stain, characteristic biochemical reactions, and resistance to antibiotic tests.

The contents inside the lumen were mixed thoroughly in a sterile solution of isotonic saline and then spread onto McConkay and Columbia blood agar plates, which support the growth of both aerobic and anaerobic bacteria. These plates were obtained from Oxid-Germany. The intestinal

wall, liver, and mesenteric lymph nodes (MLN) were also included in the analysis. The tissues were pulverized in PBS and subsequently cultured in the Gas Park system for anaerobic growth. As for the blood samples, they were centrifuged at 3000 rpm for 30 minutes and the resulting sediments were cultured in the same manner as the tissues.

Statistical analyses

The C-reactive protein values are represented as the middle value in a set of data, while other variables are expressed as the average value plus or minus the standard deviation. The CFU values are converted using a logarithmic scale. The Mann-Whitney U test, Chi-square test, and the one-way analysis of variance test were utilized for comparison, when

appropriate. The Pearson correlation test was employed to identify the relationship between C-reactive protein levels and CFU density in various tissues, while logistic regression was utilized to identify the key predictors.

RESULTS

Intestinal obstruction, particularly the strangulated type, is strongly linked to overgrowth of bacteria, an imbalance in oxidative stress markers (specifically, a disproportionate increase in MDA and GPx), and an impaired IL-10 response (marked decrease in IL-10 levels) (Table 1).

Table 1: Factors that increase the probability of bacterial translocation among the categories under study.

	Luminal bacteria count	Intestinal wall mucosal stress		Serum IL-10
	CFU/g mean	MDA nanomol/mg protein	GPx unit/mg protein mean ± SD	Pg/ml mean ± SD
G1	8.8 * 10 ⁸	5.5 ± 0.5	125 ± 30	50 ± 15
G2	5.6* 10 ¹⁰	12.0 ± 1.9	165 ± 40	36 ± 12
G3	1.4* 10 ¹²	21.9 ± 1.0	150 ± 35	9 ± 3
P value	Less than 0.05	Less than 0.05	Less than 0.05	Less than 0.05
P1	Less than 0.05	Less than 0.05	Less than 0.05	Less than 0.05
P2	Less than 0.05	Less than 0.05	Less than 0.05	Less than 0.05
P3	Less than 0.05	Less than 0.05	Greater than 0.05	Less than 0.05

The bacterial translocation exhibited a high prevalence of polymicrobial composition, with rates of 49% in GII and 60% in GIII. Many of the bacteria were of enteric origin,

with a significant proportion being anaerobic. Among the enteric bacteria, E. coli was the most predominant, accounting for 42.2% in GII and 45.5% in GIII (Table 2).

Table 2: Frequency & types of isolated organism.

	GII I. O									GIII I. O								
	Isolates	Enteric				Non- enteric				Isolates	Enteric				Non- enteric			
	134	A	B	C	D	E	F	G	H	168	A	B	C	D	E	F	G	H
Luminal	50	18	2	4	6	4	6	4	6	66	20	6	6	6	6	8	8	6
Wall	42	14	2	2	4	4	6	4	6	40	18	-	4	4	4	4	4	2
MLN	20	10	-	2	2	-	2	2	2	36	16	-	4	2	4	2	6	2
Liver	12	8	-	-	2	-	-	-	2	22	12	2	2	2	-	-	2	2
BL	10	4	-	2	2	-	-	2	-	14	6	2	2	2	-	-	2	-
Portal	0	-	-	-	-	-	-	-	-	8	4	-	2	-	-	-	2	-

DISCUSSION

This study examined the relationship between bacterial overgrowth and intestinal obstruction (I.O), which is known to disrupt the normal functioning of the intestinal tract. The study found that I.O leads to oxidative stress, which causes a depletion of ATP, disruption of the cytoskeleton, and priming of neutrophils. These effects result in dysfunction of the gut barrier. Additionally, the study found that I.O. is associated with impaired IL-10 response, indicating a compromised immune system. These findings are consistent with previous studies conducted [15-18], as well as [19-22]. [23] refutes those associations.

Based on the findings of [22], [24], [25] and [26], it was observed that BT (bacterial translocation) occurred during I.O (intestinal obstruction). Furthermore, the decrease in the occurrence and concentration of the pathogens towards the center supports the idea that tissue colonization is derived from the gut rather than the blood. This study also established that BT, which stands for bidirectional transit, occurs specifically in the ischemic variant of I.O, as reported by [27] and [28]. This bidirectional transit is directly related to injury to the intestinal wall.

Additional evidence supporting the gut origin theory during intraoperative (I.O.) procedures is the prevalence of enteric bacteria, as identified by [30] and [29]. In addition, *E. coli* is more abundant than other pathogens due to its ability to adapt to different environments and its surface structures, such as fimbriae, which help it colonize and spread through the lymphatic system, facilitating bacterial translocation. Moreover, the identification of obligate anaerobic organism isolates, as documented by [1], [31], [32], defines the failure of colonization susceptibility in cases of ischemic I.O.

Bacterial overgrowth, bacterial virulence, and wall integrity collaborate synergistically.

This study supports the gut-origin hypothesis by showing that pathogens are commonly found in the gut. It also suggests that the transmission of these pathogens occurs through local routes such as phagocytes or enterocytes. This is consistent with previous studies by [4], [29], [30], [33], [34], [35], and [26], which found that lymphatic routes are more common in simple I.O and venous portal routes are more common in the ischemic variant.

The local and systemic immunoinflammatory histopathologic changes observed, as reported by [24], may be associated with the release of cytokines, which leads to an influx of inflammatory cells and tissue injury. This supports the hypothesis that cytokines are responsible for generating inflammation [36]. Therefore, we hypothesize that the three-hit model [2] occurs as follows: first, there is an increase in intestinal pressure or ischemia; second, there is reperfusion injury, which leads to more secretion and less absorption due to prostaglandin release and the opening of collaterals, resulting in gut barrier failure; and finally, there is bacterial and cytokine translocation.

Although there was an increase in C-reactive protein levels during the occurrence of I.O., the cutoff value of C-reactive protein did not accurately predict any specific subtypes of I.O. Therefore, C-reactive protein cannot be considered a selective marker in suspected cases. To determine the presence of I.O subtypes, it is necessary to carefully examine the medical history and physical examination findings, considering whether they support or contradict the cases where C-reactive protein is positive. However, once I.O (ischemic optic neuropathy) is diagnosed, the C-reactive

protein (C-reactive protein) becomes a strong indicator of the ischemic variant [37].

Elevated levels of C-reactive protein were found to be linked to bacterial translocation (B.T) during intraoperative (I.O) procedures, as well as its various subtypes. The cutoff level of C-reactive protein has been found to have an indicator value that has a significant impact according to statistical analysis in accurately and specifically detecting bacterial translocation (BT) during intra-abdominal infections (I.O.) [1]. Therefore, C-reactive protein is considered a solid test for BT detection. The previously discovered parallel relationship between C-reactive protein levels and BT (frequency and density), particularly in the case of the ischemic variant, is primarily associated with the cascades of mediators involved in systemic inflammation [38]. C-reactive protein can serve as an indicator of both vascular compromise and the severity of BT.

CONCLUSION

In conclusion, the various types of intestinal obstruction are linked to precursors of BT. BT operates during I.O. and has routes that allow for movement in both directions. The majority of pathogens are of enteric origin, particularly *E. coli*, with the occasional presence of obligate anaerobes in the ischemic variant.

Bacterial overgrowth, pathogenicity, wall structure, and function synergistically interact. Both the BT (blood-brain barrier) and cytokine synthesis hypotheses are active during immune-oncology (I.O), and the 3-hit model is suitable. The C-reactive protein is a diagnostic marker that is not specific to a particular condition, but it can be used to identify suspected cases of I.O. Once I.O. is confirmed, the C-reactive protein becomes a reliable indicator of its different subtypes. The C-reactive protein is a dependable diagnostic test for bacterial contamination during intraoperative procedures (I.O.). The C-reactive protein serves as an indicator of both Impaired blood flow to the tissues and the level of BT.

LIMITATIONS

One limitation of this study is the use of an animal model, which may not fully replicate the complexities of human intestinal obstruction and bacterial translocation. Additionally, the controlled laboratory setting and the specific conditions applied to the rats might not accurately reflect the variability seen in clinical practice. The relatively small sample size of 90 rats and the short duration of observation might have limited the ability to detect long-term effects and subtle differences between groups.

RECOMMENDATIONS

Further studies should focus on developing more specific markers for early diagnosis of intestinal obstruction subtypes and exploring potential therapeutic interventions to manage bacterial translocation effectively.

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LIST OF ABBREVIATIONS

BT: Bacterial Translocation
CFU: Colony Forming Units
CRP: C-Reactive Protein
G: Group
GI: Control Group
GII: Simple Ileal Obstruction Group
GIII: Strangulated Ileal Obstruction Group
GPx: Glutathione Peroxidase
IL-10: Interleukin-10
IO: Intestinal Obstruction
MDA: Malonyl Dialdehyde
MLN: Mesenteric Lymph Nodes
MODS: Multiorgan Dysfunction Syndrome

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Cevikel MH, Ozgun H, Boylu S, Demirkiran AE, Aydin N, Sari C, Erkus M. C-reactive protein may be a marker of bacterial translocation in experimental intestinal obstruction. *ANZ J Surg.* 2004;74(10):900-4.
2. Deitch EA. Bacterial translocation or lymphatic drainage of toxic products from the gut: what is important in human beings? *Surgery.* 2002;131(3):241-4.
3. Gatt M, Macfie J. Bacterial translocation in surgical patients. *Recent Adv Surg.* 2005;28:23-32.
4. Deitch EA. Simple intestinal obstruction causes bacterial translocation in man. *Arch Surg.* 1989;124(6):699-701.
5. Nieuwenhuijs VB, Verheem A, van Duijvenbode-Beumer H, Visser MR, Verhoef J, Gooszen HG, Akkermans LM. The role of interdigestive small

- bowel motility in the regulation of gut microflora, bacterial overgrowth, and bacterial translocation in rats. *Ann Surg.* 1998;228(2):188-93.
6. Lane JS, Todd KE, Lewis MP, Gloor B, Ashley SW, Reber HA, McFadden DW, Chandler CF. Interleukin-10 reduces the systemic inflammatory response in a murine model of intestinal ischemia/reperfusion. *Surgery.* 1997;122(2):288-94.
 7. Choudhry MA, Fazal N, Goto M, Gamelli RL, Sayeed MM. Gut-associated lymphoid T cell suppression enhances bacterial translocation in alcohol and burn injury. *Am J Physiol Gastrointest Liver Physiol.* 2002;282(6):G937-47.
 8. Kanda T, Fujii H, Tani T, Murakami H, Suda T, Sakai Y, Ono T, Hatakeyama K. Intestinal fatty acid-binding protein is a useful diagnostic marker for mesenteric infarction in humans. *Gastroenterology.* 1996;110(2):339-43.
 9. Johnson HL, Chiou CC, Cho CT. Applications of acute phase reactants in infectious diseases. *J Microbiol Immunol Infect.* 1999;32(2):73-82.
 10. Westhuyzen J, Healy H. Review: biology and relevance of C-reactive protein in cardiovascular and renal disease. *Ann Clin Lab Sci.* 2000;30(2):133-43.
 11. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem.* 1951;193(1):265-75.
 12. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med.* 1967;70(1):158-69.
 13. Shah KA, Shurey S, Green CJ. Characterization of apoptosis in intestinal ischaemia-reperfusion injury: a light and electron microscopic study. *Int J Exp Pathol.* 1997;78(5):355-63.
 14. Nanis ON. Comparative evaluation of the effects of electrical stimulation and therapeutic ultrasound on wound healing in rabbits [MD Thesis]. Mansoura: Mansoura Faculty of Medicine; 1993.
 15. Akin ML, Uluutku H, Erenoglu C, Ilicak EN, Elbuken, Erdemoglu A, Celenk T. Hyperbaric oxygen ameliorates bacterial translocation in rats with mechanical intestinal obstruction. *Dis Colon Rectum.* 2002;45(7):967-72.
 16. Sagar PM, MacFie J, Sedman P, May J, Mancey-Jones B, Johnstone D. Intestinal obstruction promotes gut translocation of bacteria. *Dis Colon Rectum.* 1995;38(6):640-4.
 17. Wilson J, Winter M, Shasby DM. Oxidants, ATP depletion, and endothelial permeability to macromolecules. *Blood.* 1990;76(12):2578-82.
 18. Welsh MJ, Shasby DM, Husted RM. Oxidants increase paracellular permeability in a cultured epithelial cell line. *J Clin Invest.* 1985;76(3):1155-68.
 19. Swank GM, Deitch EA. Role of the gut in multiple organ failure: bacterial translocation and permeability changes. *World J Surg.* 1996;20(4):411-7.
 20. Schoenberg MH, Poch B, Younes M, Schwarz A, Baczako K, Lundberg C, Haglund U, Beger HG. Involvement of neutrophils in postischaemic damage to the small intestine. *Gut.* 1991;32(8):905-12.
 21. Zingarelli B, Yang Z, Hake PW, Denenberg A, Wong HR. Absence of endogenous interleukin 10 enhances early stress response during post-ischaemic injury in mice intestine. *Gut.* 2001;48(5):610-22.
 22. Souza DG, Vieira AT, Soares AC, Pinho V, Nicoli JR, Vieira LQ, Teixeira MM. The essential role of the intestinal microbiota in facilitating acute inflammatory responses. *J Immunol.* 2004;173(6):4137-46.
 23. O'Boyle CJ, MacFie J, Dave K, Sagar PS, Poon P, Mitchell CJ. Alterations in intestinal barrier function do not predispose to translocation of enteric bacteria in gastroenterologic patients. *Nutrition.* 1998;14(4):358-62.
 24. Akcay MN, Capan MY, Gundogdu C, Polat M, Oren D. Bacterial translocation in experimental intestinal obstruction. *J Int Med Res.* 1996;24(1):17-26.
 25. Antequera R, Bretana A, Cirac A, Brito A, Romera MA, Zapata R. Disruption of the intestinal barrier and bacterial translocation in an experimental model of intestinal obstruction. *Acta Cient Venez* 2000;51(1): 18-26
 26. Kocdor MA, Kocdor H, Gulay Z, Gokce O. The effects of pentoxifylline on bacterial translocation after intestinal obstruction. *Shock.* 2002;18(2):148-51.
 27. Boedeker EC. Adherent bacteria: breaching the mucosal barrier? *Gastroenterology.* 1994;106(1):255-7.
 28. Wells CL, Maddaus MA, Reynolds CM, Jechorek RP, Simmons RL. Role of anaerobic flora in the translocation of aerobic and facultatively anaerobic intestinal bacteria. *Infect Immun.* 1987;55(11):2689-94.
 29. O'Boyle CJ, MacFie J, Mitchell CJ, Johnstone D, Sagar PM, Sedman PC. Microbiology of bacterial translocation in humans. *Gut.* 1998;42(1):29-35.
 30. Mainous MR, Tso P, Berg RD, Deitch EA. Studies of the route, magnitude, and time course of

bacterial translocation in a model of systemic inflammation. Arch Surg. 1991;126(1):33-7.

31. Brooks SG, May J, Sedman P, Tring I, Johnstone D, Mitchell CJ, MacFie J. Translocation of enteric bacteria in humans. Br J Surg. 1993;80(7):901-2.
32. Moore FA, Moore EE, Poggetti R, McAnena OJ, Peterson VM, Abernathy CM, Parsons PE. Gut bacterial translocation via the portal vein: a clinical perspective with major torso trauma. J Trauma. 1991;31(5):629-36.
33. MacFie J, O'Boyle C, Mitchell CJ, Buckley PM, Johnstone D, Sudworth P. Gut origin of sepsis: a prospective study investigating associations between bacterial translocation, gastric microflora, and septic morbidity. Gut. 1999;45(2):223-8.
34. Lemaire LC, van Lanschot JB, Stoutenbeek CP, van Deventer SJ, Dankert J, Oosting H, Gouma DJ. Thoracic duct in patients with multiple organ failure: no major route of bacterial translocation. Ann Surg. 1999;229(1):128-36.
35. Adams CA Jr, Xu DZ, Lu Q, Deitch EA. Factors larger than 100 kd in post-hemorrhagic shock mesenteric lymph are toxic for endothelial cells. Surgery. 2001;129(3):351-63.
36. Deitch EA, Xu D, Franko L, Ayala A, Chaudry IH. Evidence favoring the role of the gut as a cytokine-generating organ in rats subjected to hemorrhagic shock. Shock. 1994;1(2):141-5.
37. Willetts IE, Kite P, Barclay GR, Banks RE, Rumley A, Allgar V, Stringer D. Endotoxin, cytokines and lipid peroxides in children with intussusception. Br J Surg. 2001;88(6):878-83.
38. Moore FA. The role of the gastrointestinal tract in postinjury multiple organ failure. Am J Surg. 1999;178(6)

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