

# Effect Of Abdominal Insufflation On Bacterial Growth In An Experimental Model Of Peritonitis - A Randomized Controlled Trial.

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## Citation

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## Abstract

**Background:** Despite the advantages of laparoscopy, studies suggest that gases used for insufflation may adversely affect patients with peritonitis by bacterial proliferation and impair the clearance of bacteria from the peritoneal cavity. **Methods:** This was an experimental study conducted on 33 albino-rats from February 2004 to September 2004 in Department of Surgery N.S.C.B, Government Medical College, Jabalpur (MP), India. This study was done to assess the effect of pneumoperitoneum on peritonitis. The rats were divided in three groups (A, B, and C). In group A (control group) 0.5ml of normal saline (5%) was instilled intraperitoneally. In group B and C, a single infusion of 0.5ml suspension containing  $1 \times 10^9$  Escherichia coli/ml was instilled intraperitoneally. In group A and C, an additional 5ml of atmospheric air was instilled. The colony counts in each group were noted after 72 hours after laparotomy. **Results:** In the control group there was no peritoneal contamination, no bacterial growth, none of the rats had died and there was no evidence of pus or pus pockets seen. In the experimental group the mean colony count was 659.91 ( $\pm 680.34$ ). There were no macroscopic pus pockets or adhesions seen but there was frank pus in one of the rats of this group. The t-value for comparison of colony count was  $t_{A/B} = 3.22$  ( $p < .05$ ). In group C there were no macroscopic pus pockets seen, no adhesions observed and a mean colony count of 840.09 ( $\pm 681.21$ ) was observed. **Conclusion:** There is an increase in average colony count in the pneumoperitoneum group as compared to non-pneumoperitoneum group.

## INTRODUCTION

Laparoscopic surgery was initially introduced at the beginning of the 20th century by Dimitri Ott, Georg Kelling and Hans Christian Jacobaeus. Von Ott inspected the abdominal cavity of a pregnant woman in 1901, afterwards Georg Kelling performed a procedure called "Koelioscopic" and in the same year Jacobaeus published his first report on "Laparothorakoskopie". Advantages of laparoscopic surgery are less postoperative pain, reduced morbidity, shorter hospital stay, reduced recovery period, better cosmetic results and less post-surgical adhesions. The limitations of laparoscopic surgery are lack of hand-eye coordination, arterial bleeding and loss of tactile feedback. The gases used for insufflation may adversely affect patients with peritonitis by increasing bacterial proliferation and impairing the clearance of bacteria from the peritoneal cavity<sup>1</sup>. Preventing and treating these infections are derived in part from white cell dysfunction. In this hostile peritoneal environment that contains aerobic and anaerobic bacteria and inert particulate matter, prevention and treatment is a topic of concern<sup>2,3</sup>.

## MATERIAL AND METHODS

This study includes 33 healthy albino rats (*Mus Norvagicus Albinus*) of both sexes with a weight between 130 and 164g. The study was done to assess the effect of the use of pneumoperitoneum in cases of peritonitis on bacterial count in peritoneal fluid as produced for laparoscopic surgery by assessing frank pus, colony counts of peritoneal fluid, pus pockets and adhesions in the peritoneal cavity. This project was submitted to the Institutional Ethics Committee for Animals and due clearance was obtained. All animals were treated humanely according to the guidelines of Institutional Ethics Committee for Animals. These animals were kept in good hygienic conditions in separate cages with proper labeling.

## MATERIALS USED

Albino rats (*Mus Norvagicus Albinus*), 1ml, 2ml and 5ml sterile syringes, povidone iodine solution, ether, *Escherichia coli* suspension containing  $1 \times 10^9$  *Escherichia coli*/ml suspension.

## PROCEDURE

Rats were randomly divided into three groups:

Group A: The abdomen was cleaned and 0.5ml of normal saline was instilled inside the peritoneal cavity of the rats. A pneumoperitoneum was created using atmospheric air after four hours of normal saline instillation.

Group B: The abdomen of rats was cleaned and 0.5ml of *Escherichia coli* suspension was instilled inside the peritoneal cavity in the right iliac fossa. No pneumoperitoneum was created.

Group C: 0.5ml of *Escherichia coli* suspension was instilled inside the peritoneal cavity in the right iliac fossa after cleaning the abdomen of the rats. After four hours of instillation of *Escherichia coli*, atmospheric air was insufflated inside the peritoneal cavity.

Each group was labeled accordingly and kept in different cages after the procedure.

After 72 hours, laparotomy was performed using ether as anesthetic agent. Mortality was noted separately. A midline abdominal incision was made and the abdominal cavity was examined for frank pus, pus pockets, adhesions and inflammation. There was no evidence of iatrogenic injury to viscera from insertion of the needle instilling *Escherichia coli* and creating the pneumoperitoneum. Peritoneal fluid from the peritoneal cavity was collected and diluted 100 times in normal saline and send to the microbiology laboratory immediately for colony count after proper labeling, where it was analyzed.

## RESULTS

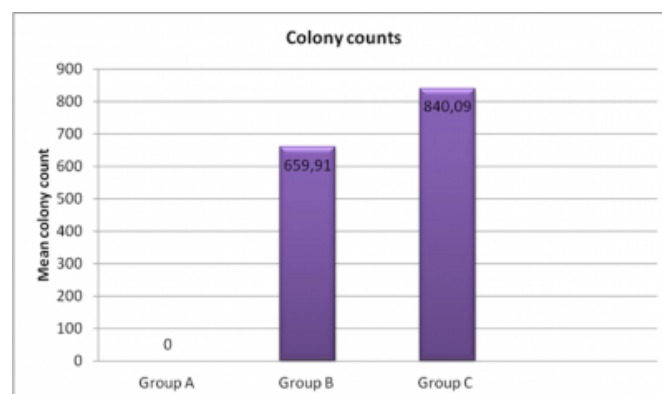
The result of the present study shows that pneumoperitoneum increases bacterial proliferation and peritoneal contamination in an experimental peritonitis model in rats. These findings suggest that intra-abdominal gas insufflation has a negative effect on bacterial clearance from peritonitis; it stimulates bacterial growth or reduces immune responses. In our study it was observed that in group A, the control group, there was no peritoneal contamination and no bacterial growth, none of the rats had died and there was no evidence of pus or pus pockets seen. In the second group, i.e. group B, the mean colony count was 659.91 ( $\pm 680.34$ ). There were no macroscopic pus pockets or adhesions seen but there was frank pus in one of the rats of this group. The t-value for comparison of colony

count was  $tA/B = 3.22$  ( $p < 0.05$ ).

In group C there were no macroscopic pus pockets seen, no adhesions observed, a mean colony count of 840.09 ( $\pm 681.21$ ) was observed, and the t-value for comparison of colony count of group A and C was  $tA/C = 4.09$  ( $p < 0.0001$ ) while the t-value for comparison of colony count of group A and B was  $tA/B = 3.22$  ( $p < 0.005$ ), but the t-value for comparison between group B and C was  $tB/C = 0.62$  ( $p > 0.05$ ). This is not significant. For comparison between mortality in group A and B the t-value was  $tA/B = 0.05$  ( $p > 0.05$ ) and for comparison between group A and C, it was  $tA/C = 1.56$  ( $p > 0.05$ ) - there is no significant t-value as far as mortality is concerned. And for comparison between mortality of group B and C the t-value was  $tB/C = 0.63$  ( $p > 0.05$ , also not significant), but there was one mortality in group B, i.e. 9.1% and there were two mortalities in group C, i.e. 18.2%. We can say that there is a higher incidence of mortality in group C as compared to group B.

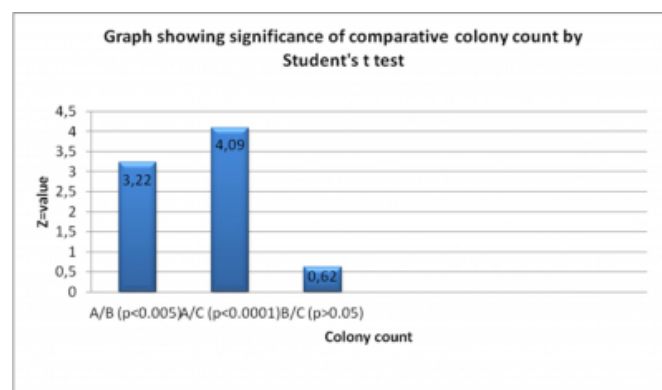
**Figure 1**

Figure 1: Colony counts



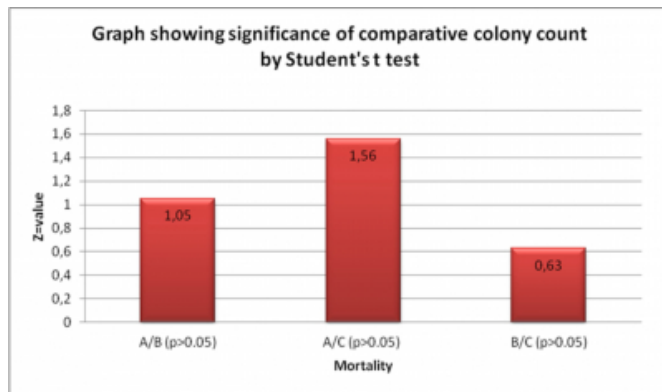
**Figure 2**

Figure 2: Graph showing significance of comparative colony count by Student's t-test



**Figure 3**

Figure 3: Graph showing significance of comparative colony count by Student's t-test



**Figure 4**

Table 1: Colony Counts In Each Group

|              | Group A | Group B        | Group C        |
|--------------|---------|----------------|----------------|
| Colony Count | 0       | 659.91 ±680.34 | 840.09 ±681.21 |
| Pus Pockets  | 0       | 0              | 0              |
| Adhesions    | 0       | 0              | 0              |
| Mortality    | 0       | 1              | 2              |

A=Normal Saline (control group)

B=Escherichia coli

C=Escherichia coli + pneumoperitoneum

Colony count= Mean±SD. (standard deviation)

**Figure 5**

Table 2: Comparative t-value of colony count between groups

| GROUPS | t-value | p-value |
|--------|---------|---------|
| tA/B   | 3.22    | <0.005  |
| tA/C   | 4.09    | <0.0001 |
| tB/C   | 0.62    | >0.05   |

## DISCUSSION

In our study on experimental peritonitis in rats there were no pus-pockets or adhesions, but there was frank pus in five out of 33 rats and there was significant increase in colony counts in peritoneal fluid except in the control group. Three rats died on the third day.

A study by Sare et al. showed that there was a significant

increase in the number of bacteria between 8 and 16 hours of peritoneal contamination. In conclusion, this study suggests that in CO<sub>2</sub> insufflation may be the factor promoting intra-abdominal anaerobic bacterial growth, which may lead to intra-abdominal abscess formation or may cause localized peritonitis to develop into generalized peritonitis<sup>6</sup>.

Horratas et al. noticed that there was an increased risk of bacterial translocation in comparing groups that underwent pneumoperitoneum with those that did not. This study shows an effect of presence of gas, but not necessarily the type of gas used for insufflation.<sup>7</sup>

In our study, the colony counts of Escherichia coli in group C was 840.09 (±681.21) and in group B it was 659.91 (±680.34). The t-values for groups B and C were p>0.05 (not significant). But when compared with group A the t-values of group B and C were p<0.005 and p<0.0001, respectively (significant).

In our study there is a difference in colony count between the insufflation and non-insufflation group, but p is not significant (p>0.05). But when compared to control group A, group B and C have significant p-values (p<0.05 and p<0.0001, respectively).

In the study published by Berguer et al.<sup>8</sup>, the three-day postoperative mortality was 36.4% and 22.7% for open and laparoscopic CLP (caecal ligation and puncture), respectively. Gurtner et al. clarified that there was no increased bacteraemia or endotoxaemia and no adverse affect on physiological or laboratory parameters of sepsis compared as with laparotomy in an animal model of peritonitis<sup>9</sup>.

In their animal model study of bacterial removal from the peritoneal cavity, Nystrom et al. used Escherichia coli and Bacteroides fragilis as bacterial agents in which two elimination patterns emerged. The concentration of both species was decreased within 2 to 4 hours following peritoneal contamination with 10 (10) CFU. The elimination of B. fragilis was simultaneous with mobilization of granulocytes into the peritoneal cavity, and was possibly attributable to bactericidal action of the granulocytes<sup>10</sup>.

In our study there were less deaths in the non-insufflation group as compared to the insufflation group (18.2% and 9.1%, respectively), but the difference was not significant (p>0.05). In a study on microbial synergy in experimental intra-abdominal abscess done by Onderdonk et al., intra-

abdominal sepsis was produced in rats by using four microbial species in single or dual combinations and the results were evaluated by mortality rates and intra-abdominal abscesses on autopsy. Mortality was restricted to the recipients of *Escherichia coli*, thus implicating coliforms in the acute lethality associated with this experiment. All animals receiving *Escherichia coli* died within 2 days. Intra-abdominal abscesses were produced in 61 out of 65 (94%) animals that received the combination of anaerobic and facultative organism. No abscesses were detected with single microbial species. This study further clarifies the role of selected bacteria in pathological events<sup>11</sup>. There were one and two deaths in group B and C, respectively; the overall mortality in group B and C was 3 out of 22 rats (13.6%).

Ozguc et al. studied the effect of CO<sub>2</sub>-pneumoperitoneum on bacteremia in experimental peritonitis. The conclusion was that there is no difference in the mortality rates between control and pneumoperitoneum group; 10<sup>9</sup> CFU of *Escherichia coli* represents a lethal amount. It causes 100% mortality in rats and 60% in pigs. Robertson et al. who used rabbits and the same amount of bacteria found no mortality in their study<sup>12</sup>. In our case, there was one death in group B and there were two deaths in group C, mortality rate was 9.1% and 18.2%. The p-value was >0.05 (not significant).

## CONCLUSION

There is only a slight increase in the average colony count in the pneumoperitoneum group as compared to the non-pneumoperitoneum group, which means that there is no gross difference between pneumoperitoneum and non-pneumoperitoneum group.

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