

Master's Thesis
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**Effect of probiotics on oropharyngeal and
tracheal colonization by pathogenic bacteria
before major surgical procedures under general
anesthesia**

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Summary in Swedish

Populärvetenskaplig sammanfattning

I dagens Sverige tänker många att sjukhusen med all dess personal kan lösa de flesta bekymmer som man kan tänkas råka ut för. Det finns behandlingar och operationer även för det till synes omöjliga. Drabbas man av en infektion får man snabbt och lätt antibiotika utskrivet. På senare tid har dock olika arter av bakterier börjat anpassa sig efter sina nya förutsättningar och påverkas nu inte alltid av samma antibiotikasorter som tidigare. Så kallad antibiotikaresistens har utvecklats.

Det jobbas intensivt med att få fram nya metoder mot angripande bakterier. Det utvecklas ständigt nya typer av antibiotika och andra antibakteriella ämnen. Även nyare varianter finns, där man använder sig av en bakterietyp för att utarma en annan. Att hålla en bra balans i kroppen mellan bra och dåliga bakterier har blivit alltmer uppmärksammat på senare tid. Nya produkter som innehåller så kallade snälla bakterier (=probiotika) säljs i de flesta livsmedelsbutiker. Sådana bakterier finns redan normalt i kroppen och håller tillbaka och motverkar de mekanismer som gör att de dåliga bakterierna angriper den egna kroppen. Ibland händer det att kroppen kommer i obalans och då kan de dåliga bakterierna ta över och angripa kroppen så att en infektion eller andra problem uppstår.

När man genomgår ett kirurgiskt ingrepp belastas kroppens resurser, så att den inte längre kan försvara sig mot påfrestningar den normalt hade klarat av. Kirurgen öppnar upp kroppen och gör ingrepp under den skyddande huden, med risk för att få in både främmande material och bakterier av olika slag. Om man behöver sova under sin operation får man hjälp att andas genom ett plaströr som stoppas ner i luftvägen. Även härigenom kan olika ämnen och bakterier utifrån få hjälp att ta sig in i kroppen. Man stör helt enkelt kroppens naturliga balans. Ett typexempel på detta är att man kan riskera att drabbas av en lunginflammation efter sin operation. Dessa lunginflammationer kan bli mycket allvarliga för den redan försvagade patienten. Som det ser ut idag i Sverige och i många andra delar av världen så använder man, förutom sjukgymnastik, inga förebyggande metoder för att förhindra att dessa lunginflammationer uppkommer. Istället motarbetar man dem först efter att de har fått fäste.

Hur ska man då hantera de patienter som är för sjuka för att kunna medverka till sjukgymnastik? I samband med vissa operationer kan det hända att man får förebyggande antibiotikabehandling, men den är då främst riktad mot de bakterier som kan tänkas angripa operationssåret och inte mot dem som samtidigt kan riskera att angripa lungorna.

I dagens läge med utveckling av bakteriestammar som är resistenta mot antibiotika krävs ett nytt angreppssätt i hanteringen av infektioner. Genom att använda probiotika i förebyggande syfte kanske man kan hålla tillbaka aggressiva bakterier och stötta kroppens eget försvar inför en stor operation. Man har genom forskning kunnat identifiera olika typer av probiotikabakterier och sett att de har god förmåga att fästa till många av kroppens slemhinnor.

I vår studie undersökte vi en metod där man fick skölja munnen med probiotika vid tre tillfällen innan sin operation och därefter svälja ner lösningen i magsäcken. Genom detta skulle det kunna vara möjligt att få rent munhålan och svalget från bakterier av den typ som skulle kunna tänkas angripa lungorna efter operationen. Det skulle också försvåra för tarmbakterier att växa till och ta sig upp i luftvägen från magsäcken. Genom att minska och hålla tillbaka kroppens dåliga bakterier redan före operationen skulle man rent hypotetiskt kunna förhindra en luftvägsinfektion i efterförloppet av sin operation.

Patienterna som valdes ut till projektet fick inte ha för många grundsjukdomar förutom själva orsaken till operationen. Denna sällning medförde att utvalda patienter var relativt friska, sett till vilka patienter som normalt är inlagda på ett sjukhus. Väldigt få hade därför några dåliga bakterier i mun och svalg från början. De utvalda patienterna drabbades inte i någon betydande omfattning av att bakterierna växte till under eller efter operationen. Om detta berodde på deras normala immunförsvar, antibiotikabehandlingen i samband med operationen eller genom vårt munskölningsprogram med probiotika gick inte att utröna i experimentet.

Forskningsstudien har visat på ett intressant område att undersöka vidare i arbetet för att minska risken för lunginflammation efter operationer. Detta bör studeras ytterligare och kan i framtiden leda till nya, bättre sätt att hantera och förebygga infektioner efter operationer.

Abstract

Background: Postoperative pneumonia is an acknowledged risk following surgical procedures, for which there is currently no prophylactic treatment. Different methods have been tried with varying results, such as the administration of antibiotics or chlorhexidine to reduce pathogens in the oral flora. This study explores a prophylactic oral treatment with a suspension of two lactobacillus-strains, *Lactobacillus plantarum 299* and *Lactobacillus plantarum 299v*, aiming to reduce pathogenic bacteria in the oropharynx before major surgical procedures under general anesthesia.

Methods: Forty-two patients scheduled for surgical procedures with an estimated duration of ≥ 4 hours were randomized into either a study group, rinsing preoperatively with probiotics, or a control group, not rinsing at all. Throat swabs and tracheal secretions were collected at inclusion, immediately after intubation, before extubation and on the first postoperative day.

Results: There were few positive cultures overall, and no trends or apparent connections could be seen. The randomization procedure haphazardly allocated all patients with positive start cultures to the study group so that comparison between the groups was impossible.

Conclusion: We could not draw any conclusions concerning whether or not prophylactic rinsing with probiotics in the oral cavity reduces oropharyngeal or tracheal colonization by pathogenic bacteria. Further observations regarding probiotic prophylaxis would probably have greater potential among other patient populations such as compromised patients with severe underlying illnesses.

Key words: postoperative pneumonia, prophylaxis, probiotics, *Lactobacillus plantarum 299*, *Lactobacillus plantarum 299v*

1. Background

A human being is inhabited by a large number of bacteria constituting the normal flora. The human body itself consists of about 10^{13} cells while the bacteria and fungi harbored in it are reported to number around 10^{14} cells. A large majority of these are found in the colon, with an estimated weight of 1 kg. In a healthy individual these microorganisms exist in symbiosis with each other as well as with the host. Which species form the normal flora differs between individuals and regions of the body. [1]

The normal flora of the upper respiratory tract usually consists of *streptococci*, and occasionally *staphylococci* and *anaerobes*. Potential pathogens such as *Haemophilus influenzae* and *Streptococcus pneumoniae* can also be found in the upper respiratory tract functioning among the normal flora. The lower respiratory tract is usually sterile: pathogens are kept back both through competition with other species of bacteria further up in the oropharynx as well as through the defense mechanisms of the host. [1]

In a healthy individual, bacteria of species normally found in the lower gastrointestinal tract are not usually found in the oropharyngeal flora. Various illnesses increase the risk of colonization of gram-negative bacilli to the oropharynx, most likely due to attenuated defense mechanisms in the oropharynx [2]. Pneumonia with gram-negative bacilli constitutes a severe danger to compromised patients [2].

Postoperative pulmonary complications (PPC) are acknowledged risks following many surgical procedures. Depending on which definition is used, the incidence ranges from 2-40% [3]. The most common PPC's are pneumonia and atelectasis [4]. PPC's often cause severe illness with a mortality rate as high as 20% [3]. The mortality rate of postoperative pneumonia is as high as 30-46% [4]. Numerous surveys have tried to identify patients at risk of developing pulmonary complications as well as to find perioperative factors that could be altered to minimize the risk.

Since most bacterial pneumoniae originate from aspirated pathogens from the oropharyngeal flora [1] it seems appropriate to address this area in order to prevent postoperative pneumonia. Different kinds of prophylactic antibiotic treatments, both systemic and topical, have been assessed with limited success. These treatments probably result in antibiotic-resistant strains of bacteria [5-8].

An additional method to prevent oropharyngeal flora from infecting the lower airways is oral rinsing with chlorhexidine [9-10]. This approach, however, seems to be more efficacious on normal flora than on gram-negative bacilli [10].

A previous oral care study performed in the Intensive Care Unit (ICU), which compared the treatment of chlorhexidine-rinsing with a probiotic lactobacillus-suspension treatment (*Lactobacillus plantarum 299*), showed promising results in preventing establishment of gram-negative bacteria in the oropharyngeal flora [11].

There is currently no prophylactic treatment shown to prevent postoperative pneumonia. Prophylactic antibiotic regimens are used at some surgical procedures, but focus solely on preventing infections in the area of the procedure. As antibiotic-resistant strains of bacteria emerge there is a need to find other ways to prevent postoperative complications such as pneumonia.

This is a pilot study exploring a prophylactic oral care treatment with a suspension of two lactobacillus-strains, *Lactobacillus plantarum 299* and *Lactobacillus plantarum 299v*, in order to reduce pathogenic bacteria in the oropharynx before major surgical procedures under general anesthesia.

2. Materials and Methods

2.1 Materials

The study product - packages containing freeze dried bacteria, Lp299+Lp299v (each 1×10^{10} cfu) and 1g maltodextrine – was provided by Probi AB, Lund, Sweden, and kept in a freezer (-18°C) until given to the patient.

Most patients were given the bacteria the day before surgery. They were instructed to keep the bacteria at room-temperature until use. If given the bacteria less than a week before surgery patients were told to keep them in a refrigerator at home. If given the bacteria more than a week before surgery patients were told to store the bacteria in the freezer.

2.2 Methods

The study was approved by the Regional Ethical Review Board, Lund, Sweden, and was performed in compliance with the Helsinki Declaration. Good clinical practice and the International Conference on Harmonisation Guidance were applied. Written informed consent was obtained from the patients.

Criteria for inclusion were: age ≥ 18 years, elective surgery or other procedure requiring intubation with an estimated time of anesthesia ≥ 4 hours, and capability of making own informed decisions. Exclusion criteria were: known lesions/injuries in the oral cavity/pharynx/oesophagus or planned surgery in any of those above mentioned areas as well as the stomach, on-going airway infection or infections in the oral cavity/pharynx, lung disease with oxygen treatment or known immunosuppression.

The study was carried out in surgical units or at the radiology department at Lund University Hospital as well as in the hospital wards where the patients had been admitted.

To identify suitable patients the hospital's surgery-planning program (Orbit v. 4.20.7) was used. For each recorded planned intervention with an estimated procedure time of 4 hours or more, the patient's hospital records were checked for any apparent exclusion criterion. If the patient fulfilled inclusion and exclusion criteria, information about the study was given to the patient, and if willing to participate the patient then signed a consent form.

Depending on the type of intervention planned, the patients were assigned to one of four groups; ear surgery (cochlear implant or tumor resections in the middle ear or external ear canal), interventional neuroangiography, liver resections/sarcomas in the abdominal tract or gynecological surgery.

The patients were randomized into groups of six to receive either the study treatment with probiotics or the standard care, which comprised of no specific prophylaxis.

The time of inclusion differed between the different groups as well as for some individual patients. The patients in the ear surgery group were included up to three weeks before their surgery since they had their last preoperative hospital visit at this time. In the gynecological surgery group patients were most often included about a week before surgery. In the interventional neuroangiography group most patients were included a few days before their surgery. Most liver/sarcoma-patients were included the day before surgery and only in a few cases inclusion took place a few days before surgery.

Patients in the study group were instructed to rinse their oral cavity for 1 minute with the study product on three occasions before surgery. The suspension was swallowed on all three occasions. The rinsing procedures took place in the afternoon the day before surgery, before going to bed the night before surgery, and early in the morning on the day of surgery. The control group patients did not rinse their oral cavity.

Preparation:

To prepare the suspension of the study product the patients were instructed to mix the study product with 20 ml of sterile water. To allow the freeze-dried bacteria to recover to a functional state the suspension was kept at room temperature for between 20 and 60 minutes before the rinsing procedure took place.

Six samples for microbiological cultures were collected from all included patients:

1: Culture from the oropharynx preoperatively (at the time of inclusion). -> O1

2 and 3: Culture from the oropharynx and from the trachea (suctioning through the endotracheal tube) immediately after intubation. -> O2, T2

4 and 5: Step 2 and 3 repeated immediately before extubation. -> O3, T3

6: Culture from the oropharynx on the first postoperative day. -> O4

The samples were analysed according to normal routines, as sputum from the oropharynx and as tracheal/bronchial secretions from the trachea, at the Department of Clinical Microbiology, Lund University Hospital. The different species of bacteria and fungi detectable in cultures are shown in **Table 1 (appendix 2)**. In the cases where cultures were collected outside office hours they were kept in the refrigerator (4°C) throughout the night.

Blood samples were collected from all included patients and tested for white blood cell count (WBC) and C-reactive protein (CRP) preoperatively (most often at the time of inclusion, in some cases on the day of surgery) as well as the first three postoperative days. Blood samples postoperatively were only collected as long as the patient was still admitted.

Hospital records were checked to see if any postoperative infection, regarding the respiratory tract, occurred during each patient's length of stay (LOS). Once the patient had been discharged, follow-ups were made through the hospital records on postoperative days 8, 15, and 28 to see how the patients were doing and whether they were alive or deceased on day 28.

3. Results

A total of 42 patients were included. Only three of the patients asked to participate declined, either due to fear of adverse effects from the bacterial suspension or due to difficulty in understanding how to manage the rinsing program. The ear-surgery group consisted of 12 patients, 6 controls and 6 study patients, 10 female/2 male. The interventional neuroangiography group consisted of 6 patients, 3 controls and 3 study patients, 5 female/1 male. One female study patient in this group was removed due to protocol violation. The liver resection/sarcoma group consisted of 18 patients, 9 controls and 9 study patients, 8 female/10 male. The gynecological surgery group consisted of 6 patients, 3 controls and 3 study patients. One control patient in this group was removed due to cancellation of surgery (*Table 2 - appendix 2*). The study group had 13 female and 7 male patients, median age 59.5 years (range: 24-80), compared to the control group with 14 female and 6 male patients, median age 57.5 years (range: 18-77). All of the above-mentioned patients were further grouped as undergoing either abdominal or non-abdominal procedures.

Routine antibiotic prophylaxis differed between the groups depending on the type of surgery. The ear-surgery group most often used cefotaxime, and only occasional clindamycin and ciprofloxacin. The interventional neuroangiography group did not receive any prophylactic antibiotics. Almost all patients in the liver resection/abdominal sarcoma group were treated with metronidazole and trimethoprim/sulfamethoxazole: one was treated with piperacillin/tazobactam instead. In the gynecological surgery group patients were given metronidazole and doxycycline.

Results from the cultures are presented in *Table 3 and 4 (appendix 3)*. Altogether there were few cultures with positive results. Only the study group showed occasional cases of positive start cultures. Moreover, it was not possible to show any trends or differences between groups regarding either emergence of pathogens in the oropharynx/trachea or postoperative pneumonia. Only one suspected case of postoperative pneumonia was encountered: even here the diagnosis was never confirmed and the patient's symptoms may have been caused by a deep wound infection.

No apparent connections were demonstrated regarding differences in prophylactic antibiotic regimens, duration of intubation or site of procedure compared to emergence of bacteria in the oropharynx or trachea. Presence of orogastric/nasogastric tube, or postoperative vomiting could not be connected to increased numbers of positive cultures. None of the six patients who had visited their dentist within the 2 weeks prior to surgery presented with a positive culture.

Of the 11 patients with one or more positive culture, three were smokers (all in the non-abdominal group). In the abdominal group there were two smokers who presented with negative cultures throughout. There were two former smokers in the abdominal group. One study patient, who stopped smoking 2 months prior to surgery, presented with a positive start culture. The other, a control patient, stopped smoking 6 months prior to surgery and presented with negative cultures throughout.

Of the two patients with chronic obstructive pulmonary disease (COPD), both randomized into the study group, the one in the non-abdominal surgery-group presented with a positive start culture, whereas the one in the abdominal group presented with negative cultures throughout. Both of these patients were smokers.

Four patients, including the COPD patients mentioned above, received medication for pulmonary disease. Three of these were in the non-abdominal surgery group: 2 study patients and 1 control patient. One was in the abdominal surgery group, randomized to the probiotic group. All three in the non-abdominal group presented with positive cultures whereas samples from the patient in the abdominal group were all negative.

Comparison of blood test results between the groups (non-abdominal vs abdominal and study group vs control group) show slight differences between procedures, but not correlated to findings of positive cultures. Open abdominal surgery was associated with higher values of WBC and CRP than procedures confined to a smaller area such as ear-surgery or interventional neuroangiography (*Figure 1, 2 - appendix 1*). In all, there was no substantial difference within the surgery groups between control and study patients. The most prominent differences would appear to be individual and related to the surgical insult.

No differences between sexes were observed. No correlation of positive cultures to age could be noted either. No differences could be seen to be related to body mass index (BMI).

Length of stay (LOS) at the hospital-ward was similar for both the probiotic and the control group patients, median 6 days (range: 2-16) and 6 days (range: 3-13) respectively. There was no apparent difference in LOS between the control and study patients. Two patients were not covered to completion in follow-up. However they were both alive on day 20 and day 21 respectively. All other patients were alive on follow-up, day 28.

One of the excluded patients (due to protocol violation) presented with interesting results concerning emergence of bacteria (*Table 5 - appendix 4*). The patient was randomized to the probiotic group but did not implement the rinsing treatment (this was revealed by the patient on day 3 post procedure). The patient used detachable dentures and

was also a smoker. The procedure the patient was subjected to involved no prophylactic antibiotics. The emergence of bacteria did not require antibiotics since the patient did not present with any symptoms of airway infection.

4. Discussion

We cannot draw any conclusions from the results of this study, regarding whether or not prophylactic rinsing with probiotics in the oral cavity has any advantages over no prophylaxis for reduction of colonization of pathogenic bacteria in the oropharynx or trachea in connection with general anesthesia requiring intubation. No adverse effects of the probiotic treatment were apparent.

Overall few patients presented with positive cultures and those who did were relieved of the pathogens during their hospital stay. The disappearance of bacteria may be due either to concurrent antibiotic prophylaxis, regular defense mechanisms of the host or an effect of the probiotic rinsing protocol. Only patients from the study group presented with positive start cultures, which makes it hard to compare the development of pathogenic flora in the oral cavity during the procedure and the first postoperative day with the control group. Hypothetically patients from the control group would show further development of pathogenic bacteria during their surgical procedure than the study group patients. Due to the reasons mentioned above we were unable to present results supporting this hypothesis.

Previous studies have shown that it is a normal mechanism to aspirate during deep sleep. Moreover, the risk of aspiration increases with decreased consciousness [12]. One way to prevent aspiration during general anesthesia is to inflate the cuff on the endotracheal tube, although this is shown to be less than completely effective: aspiration around the cuff occurs [13]. Another study, performed in a surgical ICU setting, indicates that not all pathogens in the trachea originate from the oropharyngeal flora. They suggest that pathogens may be of exogenous origin, inserted along with the endotracheal tube at intubation. Furthermore almost half of the pathogens found in the trachea after intubation disappear within 48 hours either spontaneously or due to concurrent antibiotics treatment [14].

In our study setting, patients were included only if the estimated time of intubation was 4 hours or longer. Almost all patients in the above-mentioned surgical ICU study were colonized with tracheal pathogens within 24 hours of intubation [14]. The time needed for intubated patients to become colonized is not extensively studied. Most studies addressing this risk factor also evaluates other risk factors, making it hard to ascertain whether ‘tube-time’ alone predisposed to colonization or whether it is a combination of factors working together that leads to the increased risk. A study from 1968 implicates that increased length of surgical procedures does not increase the risk for postoperative pulmonary complications [15]. A review from 2010 on the other hand notes that there is good evidence that surgical procedures lasting for more than three hours is a risk factor [3].

That same review also states that factors such as increased age, COPD, abdominal surgery and general anesthesia are risk factors that have good evidence and also that smoking is a risk factor that has poor evidence. Our study could neither confirm nor dispute these results.

A number of studies list additional risk factors for PPC besides those mentioned above: large surgical incisions near the diaphragm can limit lung function due to pain or severed motor or sensory nerves, thus depressing the normal defense mechanisms for clearing mucous and pathogens in the airways [3-4, 16]. Emergency surgery is a risk factor supported by good evidence [3], as is a severe underlying disease such as immune deficiency/immunosuppression, diseases in the respiratory tract or heart disease [3, 16]. Increased severity of illness has also been shown to be associated with findings of gram-negative bacteria in the oropharynx [2]. Further observations regarding prophylaxis with probiotics would probably have greater potential among these compromised patients.

Detecting bacteria only through cultures may have contributed to the low number of positive test-results. Another way of detecting bacteria is to use polymerase chain reaction (PCR). In future studies this method could be used to complement bacterial cultures.

Conclusions

The result of this study was inconclusive regarding whether or not probiotic prophylaxis with *Lactobacillus plantarum* 299 and 299v reduces pathogens in the oropharynx and trachea and thereby also reduces the risk for postoperative pneumonia. Our patient population was most likely too healthy to benefit from the treatment.

As a consequence of the limited number of patients in this pilot study the results are not applicable to a larger population. Due to the limited number of participants the randomization process managed to create an imbalance in the distribution of patients with positive start cultures so that comparisons between study patients and controls were made impossible. We did not see any apparent adverse effects of the probiotic treatment.

Further studies concerning probiotic prophylaxis are needed, taking into consideration group composition and perhaps other risk factors than those which were addressed in this study.

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Figure 1 – WBC the first three postoperative days

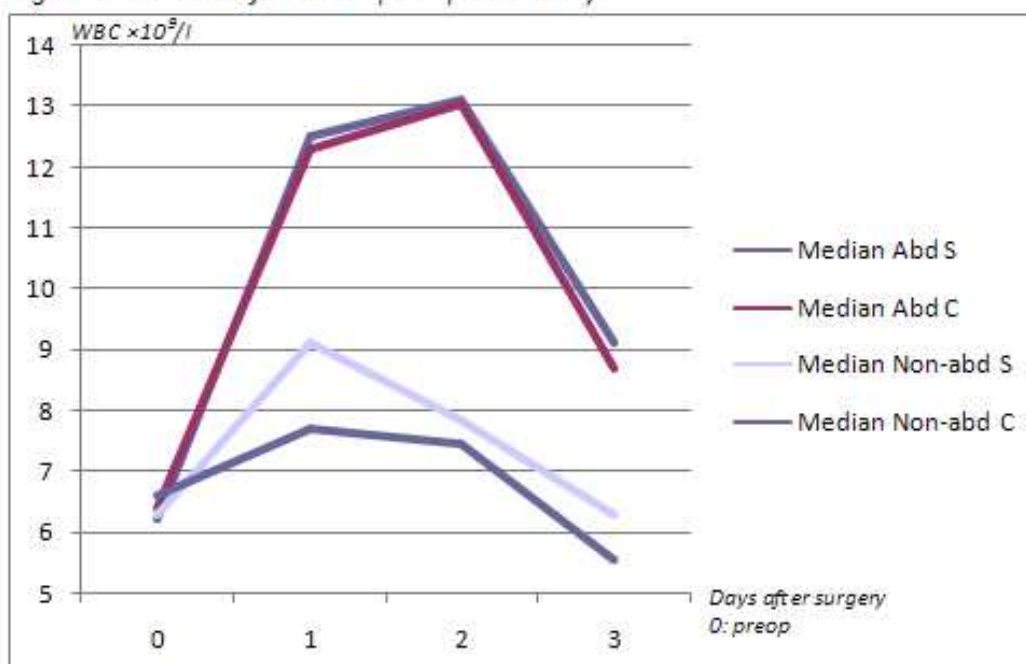


Figure 2 – CRP the first three postoperative days

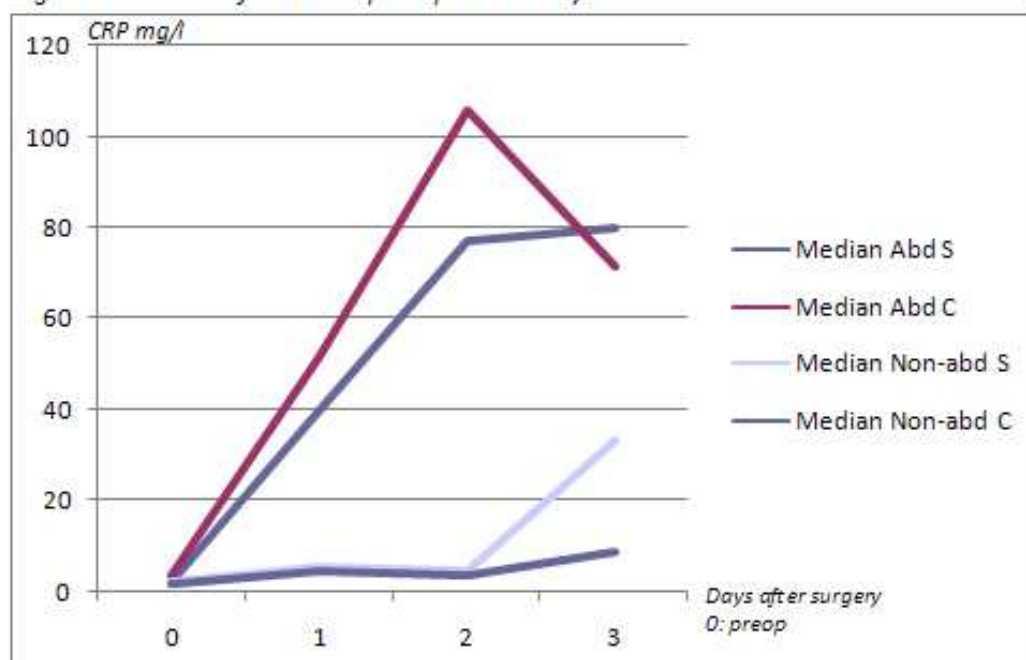


Table 1 - Species of bacteria and fungi detectable in cultures

Mixed anaerobic flora
Candida albicans
Candida glabrata
Candida parapsilosis
Candida tropicalis
Citrobacter (diversus) koseri
Citrobacter spp.
Enterobacter aerogenes
Enterobacter cloacae
Enterococcus faecalis
Enterococcus faecium
Escherichia coli
Haemophilus influenzae
Hafnia alvei
Klebsiella oxytoca
Klebsiella pneumoniae
Moraxella catarrhalis
Morganella (Proteus) morganii
Proteus mirabilis
Proteus vulgaris
Pseudomonas aeruginosa
Serratia marcescens
Serratia spp.
Staphylococcus aureus
Stenotrophomonas (Xanthomonas) maltophilia
Streptococcus group B (S. agalactiae)
Beta-Streptococcus group G
Streptococcus pneumoniae
Streptococcus pyogenes

Table 2 - Patients

Surgery group	Included patients		Study patients		Control patients		Excluded patients
	Female	Male	Female	Male	Female	Male	
Ear-surgery	12	10	2	6	6	-	
Interventional neuroangiography	6	4	1	2	3	1 female, study group patient	
Liver resection/Abdominal sarcoma	18	8	10	9	9	-	
Gynecological surgery	6	5	-	3	2	1 female, control group patient	
Total	42	27	13	20	20		

Table 3 - Non-abdominal surgery: 'tube time', culture results and antibiotic prophylaxis

Patient	Tube time	O1	O2	T2	O3	T3	O4	Antibiotic prophylaxis
E-C	4h 0min	-	-	-	-	-	-	Cefotaxime
E-C	4h 17min	-	-	-	-	-	-	Clindamycin
E-C	4h 30min	-	-	-	-	-	-	Cefotaxime
A-C	4h 30min	-	-	-	-	-	-	-
E-C	4h 50min	-	<i>H. influenzae</i>	<i>H. influenzae</i>	-	-	-	Cefotaxime
E-C	5h 23min	-	-	-	-	-	-	Cefotaxime
E-C	6h 7min	-	-	-	-	-	-	Cefotaxime
A-C	6h 20min	-	-	-	-	-	-	-
A-C	7h 0min	-	-	-	-	-	-	-
A-S	3h 15min	<i>H. influenzae</i>	-	-	-	-	-	-
E-S	4h 20min	<i>H. influenzae</i>	-	-	-	-	-	Clindamycin+Ciprofloxacin
E-S	4h 25min	<i>H. influenzae</i>	-	-	-	-	-	Cefotaxime
A-S	4h 30min	-	-	-	-	-	-	-
E-S	4h 58min	-	-	-	-	-	-	Cefotaxime
E-S	6h 15min	-	-	-	-	-	-	Cefotaxime
E-S	7h 20min	-	<i>H. influenzae</i>	-	-	-	-	Cefotaxime
E-S	11h 5min	-	-	-	-	-	-	Cefotaxime

E - Ear surgery, A - Interventional neuroangiography, C - Control patient, S - Study patient

Table 4 - Abdominal surgery: 'tube time', culture results and antibiotic prophylaxis

Patient	Tube time	O1	O2	T2	O3	T3	O4	Antibiotic prophylaxis
L-C	3h 15min	-	-	-	-	-	-	Metronidazole+Trimethoprim/Sulfamethoxazole
L-C	3h 45min	-	-	-	-	-	-	Metronidazole+Trimethoprim/Sulfamethoxazole
G-C	3h 50min	-	-	-	-	-	-	Metronidazole+Doxycycline
L-C	5h 35min	-	-	-	-	-	-	Metronidazole+Trimethoprim/Sulfamethoxazole
G-C	5h 45min	-	-	-	<i>S. aureus</i>	-	-	Metronidazole+Doxycycline
L-C	6h 19min	-	-	-	-	-	-	Metronidazole+Trimethoprim/Sulfamethoxazole
L-C	6h 41min	-	-	-	-	-	-	Metronidazole+Trimethoprim/Sulfamethoxazole
L-C	6h 46min	-	-	-	-	-	-	Metronidazole+Trimethoprim/Sulfamethoxazole
L-C	7h 5min	-	-	-	-	-	-	Metronidazole+Trimethoprim/Sulfamethoxazole
L-C	7h 15min	-	<i>S. aureus</i> + <i>S. agalactiae</i>	-	-	-	-	Metronidazole+Trimethoprim/Sulfamethoxazole
L-C	9h 35min	-	-	-	-	-	-	Metronidazole+Trimethoprim/Sulfamethoxazole
G-S	1h 55min	<i>Pseudomonas</i> species	-	-	-	<i>H. influenzae</i> *	-	Metronidazole+Doxycycline
G-S	1h 55min	<i>Beta</i> <i>streptococci</i> group G	-	-	-	-	-	Metronidazole+Doxycycline
G-S	3h 22min	<i>Pseudomonas</i> species	<i>S. pneumoniae</i>	-	-	-	-	Metronidazole+Doxycycline
L-S	4h 2min	-	-	-	-	-	-	Metronidazole+Trimethoprim/Sulfamethoxazole
L-S	4h 5min	-	-	-	-	-	-	Metronidazole+Trimethoprim/Sulfamethoxazole
L-S	4h 5min	-	-	-	-	-	-	Metronidazole+Trimethoprim/Sulfamethoxazole
L-S	4h 20min	-	-	-	-	-	-	Metronidazole+Trimethoprim/Sulfamethoxazole
L-S	4h 35min	-	-	-	-	-	-	Metronidazole+Trimethoprim/Sulfamethoxazole
L-S	4h 45min	-	-	-	-	-	<i>E. coli</i>	Metronidazole+Trimethoprim/Sulfamethoxazole
L-S	5h 18min	-	-	-	-	-	-	Metronidazole+Trimethoprim/Sulfamethoxazole
L-S	7h 55min	-	-	-	-	-	-	Metronidazole+Trimethoprim/Sulfamethoxazole
L-S	9h 20min	-	<i>S. agalactiae</i>	-	<i>S. agalactiae</i>	-	-	Piperacillin/Tazobactam
L-S	9h 20min	-	<i>S. agalactiae</i>	-	<i>S. agalactiae</i>	-	-	Metronidazole+Trimethoprim/Sulfamethoxazole

L - Liver resection/Sarcoma surgery, G - Gynecological surgery, C - Control patient, S - Study patient

* culture from tube after being removed from trachea and put into a glove

Table 5 - Excluded patient

Tube time	O1	O2	T2	O3	T3	O4
8h 55min	<i>S.pneumoniae</i> <i>H.influenzae</i>	<i>H.influenzae</i>	-	<i>S.pneumoniae</i>	<i>S.pneumoniae</i>	-

