Romania-Republic of Moldova
ENI-CROSS BORDER COOPERATION

NEW PROTOCOL

for antibiotic prophylactic treatment

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A project implemented by
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i. Project identification data	
Title of the Action:	Changes in human colonic microbiome in antibiotic generated stress
Project acromym:	COLONSTRESS
Lead Beneficiary:	PMSI Institute of Oncology, Republic of Moldova
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ii. Information concerning the cross border partnership	
Beneficiary no.1:	Regional Institute of Oncology Iasi

CONTENT

RELEVANCE

Through the proposed objectives and the results obtained, the research in the project "Changes in human colonic microbiome in antibiotic generated stress" COLONSTRESS, focused on sequencing the colonic microbiome using Ion Torrent and Illumina NGS technologies to elucidate how the mode, type, duration and dose of antibiotics administered perioperatively can affect the composition of the gut microbiome.

The COLONSTRESS project is organized in line with the World Health Organization (WHO) Strategy for Antibiotic Therapy,

- ✓ responds to:

Objective 1: "Improve awareness and understanding of antimicrobial resistance through effective communication, education and training" by drawing attention to the liberal use of antibiotics and demonstrating their effect on the human microbiome.

Objective 2: "Strengthening the knowledge and evidence base through surveillance and research" - demonstrating the immediate effect of antibiotics on the microbiome and presenting evidence substantiated by health professionals.

Objective 3: "Optimizing the use of antimicrobial drugs in human health" by presenting and comparing two radically different models/regimens of antibiotic administration, thereby promoting optimization of antibiotic use.

- ✓ and is fit for purpose:

To compare the differences between the microbiome of two groups of cancer patients subjected to different antibiotic protocols and to develop a centre of excellence in molecular biology that addresses a different geographic region, thus extending the diagnostic value of particular techniques to other development areas.

WHO defines optimal antibiotic stewardship as "*the cost-effective use of antimicrobial agents in such a way as to achieve maximum therapeutic effect while both drug toxicity and resistance are minimized*".

The overall objective of the COLONSTRESS project is creating a common network of cross-border cooperation in molecular research focused on establishing the impact of antibiotic treatment on the human colonic microbiome, to create measurable evidence of antibiotic-induced changes and to

promote a new protocol for antibiotic use, as a way to reduce costs and limit the growth of multidrug-resistant germs.

In achieving the main objective, the following opportunities were taken into consideration, which fostered and objectified the research:

Opportunity 1 - Involvement of two cancer institutions in the cross-border cooperation region that treat similar pathologies but have different antibiotic policies. Iasi Regional Institute of Oncology uses a standard antibiotic prophylaxis regimen: a single preoperative dose of Cefuroxime 1.5 g. In PMSI Institute of Oncology, Chisinau so far there is no unified and clear policy on antibiotic administration in pre-, intra- and postoperative periods, in the given study 10 types of antibiotics were used, in different regimens, combinations, doses and periods.

Opportunity 2 - Evaluation of a significant group of patients (400 patients), relatively uniform by geographic area and dietary habits, allows to demonstrate the influence of antibiotics on the microbiome and provides the opportunity to assess the direct different impact of the two antibiotic prophylaxis/therapy policies. Microbiome assessment was performed by DNA sequencing, of the 16s RNA gene (V2, V3, V4 and V8 variable region) by NGS method.

Opportunity 3 - Creation of special molecular biology units to perform DNA sequencing and to increase professional qualification. Based on the new technology, we aimed to produce a cross-border cooperation of excellence group for parallel DNA sequencing in two centres, so as to increase the level and quality of expertise, and as a result - to create the common platform for synergistic work and compatible protocols.

The COLONSTRESS project was based on two main components of actions:

- Comparative analysis of gut microbiome changes after different perioperative antibiotic regimens in two similar study groups of cancer patients;
- the creation of a joint centre of excellence capable of transferring expertise in molecular research and generating new research approaches to stimulate economic development and innovation.

PERIOPERATIVE ANTIBIOTIC PROPHYLAXIS - RESULTS AND PERSPECTIVES

Pre-operative antibiotic prophylaxis is defined as the administration of an antibiotic prior to bacterial contamination during a surgical procedure for the purpose of prophylaxis of healthcare-associated infections. Antibiotic prophylaxis is performed for any surgery associated with a high risk of infection. The objective of antibiotic prophylaxis in surgery is to prevent infection by eradicating transient micro-organisms with colonising potential or slowing the growth of resident germs. Despite the best aseptic and antiseptic techniques, some surgical procedures are at increased risk of infectious complications. The surgical method in oncology is used not only as a treatment method but also for diagnostic purposes. Oncological procedures differ from general surgical operations in that they are not only operations on an organ but also on the lymphatic territory. Cancer surgery necessarily requires ablation and antiblastic principles.

The purpose of prophylactic antibiotic administration to cancer patients undergoing surgery are:

- to reduce the incidence of surgical infections;

- the use of antibiotics in a manner that is evidence-based and effective;

- to minimise the effect of antibiotics on patients' normal bacterial flora;

- minimising adverse effects;

- minimal changes to patient defence mechanisms.

Antibiotic prophylaxis is an adjuvant and not a substitute for proper surgical techniques and should be seen as a component of nosocomial infection control policy.

Inadequate antibiotic treatment has multiple causes, which are related to the physician and his professional training, hospital management, the health care system and the medical education system, etc. The current consensus is related to the need to introduce antibiotic use control measures applicable at all levels that would have beneficial effects on costs, antibiotic resistance and clinical outcomes of treatments.

RESULTS OF THE RESEARCH ACTIVITY

As mentioned, if the Regional Institute of Oncology Iasi uses a standard antibiotic prophylaxis regimen: a single preoperative dose of Cefuroxime 1.5 g, in PMSI Oncological Institute, Chisinau so far there is no unified and clear policy on antibiotic administration in pre-, intra- and postoperative periods, in the given study 10 types of antibiotics were used, in different regimens, combinations, doses and periods (Table 1).

Table 1 Diversity of antibiotics in use

No.	Name of Antibiotic	Number of uses	Average number of days	Min. days	Max. days
1	Sol. Metronidazol 0,5% - 100 ml	177	4,41	1	9
2	Sol. Cefoperazon 1g + Sulbactam 1g	148	3,81	1	12
3	Sol. Cefazolin 1g	93	5,47	1	10
4	Sol. Cefoperazon 1g	46	1,28	1	5
5	Sol. Ceftriaxon 1g	24	5,00	1	8
6	Sol. Cefotaxim 1g	9	5,88	3	9
7	Sol. Obrocin 500 mg	9	5,88	5	7
8	Sol. Amikacin 500 mg	8	4,62	2	8
9	Sol. Cefuroxim 750 mg	2	7,00	7	7
10	Sol. Imipenem 500 mg + Cilastatina 500 mg	1	10,00	10	10

Changes in gut microbiome composition before (M) and after (T) perioperative antibiotic administration were analyzed by high-throughput sequencing of the V3-V4 (IRO Iasi) and V2-4-8 (PMSI Institute of Oncology, Chisinau) regions of the 16S rRNA gene in patient stool samples.

Research in the project demonstrates the variation in bacterial diversity and abundance before and after antibiotic administration being investigated using Ion Torrent next generation sequencing (NGS) technology. Three hypervariable regions of the 16S rRNA gene - V2, V4 and V8 - were sequenced. These hypervariable regions can be used to detect microbial communities in a sample with high accuracy down to the gene level. Ion Reporter Software was used to analyze sequencing data and characterize microorganisms, which aligns fragments to the Curated MicroSEQ(R) 16S Reference Library v2013.1 and Curated Greengenes v13.5 databases.

A total of 140 metagenomic DNA samples were sequenced, 70 isolated from samples collected before antibiotic administration and 70 after antibiotic treatment of colorectal cancer patients.

To determine the microbiome composition between samples collected before antibiotic administration (M group) and after administration (T group), the total dataset of M and T samples was

analysed combined. A total of 262 OTUs (Operational Taxonomic Unit, gene level) were identified in both groups, 210 OTUs in the M group and 219 OTUs in the T group, of which 167 OTUs were detected in both research groups, 43 OTUs only in the M group but not in the T and 52 OTUs only in the T group but not in the M group. Alpha diversity or its abundance (number of taxonomic groups) and Chao1 index showed a smaller difference between M samples and a larger difference between T samples. M samples represent an abundance between 20 and 60 taxons (genus level), while in T samples an abundance of taxa between 10 and 70 is observed.

Pooled analysis of samples collected before and after antibiotic administration revealed 26 OTUs with a mean relative abundance > 0.01 (1.00%).

Only 22 genera (cohort M) out of 210 exceed 1% abundance and 18 genera out of 219 exceed 1% abundance in T samples. Out of 167 OTUs detected in both research groups, 22 OTUs have a mean relative abundance >1.00% in group M and 18 OTUs in group T. 14 OTUs exceed 1% in both groups (M and T) as follows: Prevotella - 14.73%:4.60%, Bacteroides - 14.24%:6.51%, Faecalibacterium - 7.51%:1.02%, Sutterella - 5.25%:3.07%, Bifidobacterium - 4.60%:1.78%, Finegoldia - 3.52%: 3.00%, Porphyromonas - 3.49%:1.15%, Fusobacterium - 2, 95%:1.45%, Ruminococcus - 2.80%:1.17%, Corynebacterium - 2.16%:11.33%, Parabacteroides - 1.91%:2.87%, Peptoniphilus - 1.85% :7.72%, Acinetobacter - 1.63%:1.14% and Streptococcus - 1.54%:1.11%.

Out of 22 OTUs exceeding 1% abundance in group M and found in both cohorts, 8 OTUs have 1% abundance only in group M: M:T Desulfovibrio (2.12%:0.31%), Campylobacter (1.97%: 0.34%), Anaerococcus (1.73%:0.96%), Sporobacterium (1.33%:0.10%), Alistipes (1.20%:0.22%), Parvimonas (1.17%:0.15%), Clostridium (1.12%:0.91%) and Raoultella (1.09%:0.01%). On the other hand, out of 18 OTUs exceeding 1% abundance in group T and found in both cohorts, 4 OTUs have 1% abundance only in group T: M:T Enterococcus (0.15%:34.84%), Pseudomonas (0.25%:2.80%), Actinomyces (0.35%:1.85%) and Morganella (0.36%:1.21%). According to the results, the abundance of Enterococcus genus increased approximately 232-fold after antibiotic administration and Corynebacterium 5-fold. The next largest changes are related to Actinomyces, which increases about 11 times and Morganella - 5 times in terms of abundance less than 1.00% in group M.

All 43 OTUs detected only in M samples but not in T and all 52 OTUs detected only in T samples but not in M do not exceed 1.00% abundance and do not significantly influence the total abundance.

The most abundant genera in the M cohort were Prevotella (14.73%) and Bacteroides (14.24%). The next most common genera were Faecalibacterium (7.51%), Sutterella (5.25%) and Bifidobacterium (4.60%) showing a decrease in relative mean abundance in T samples: Prevotella (4.60%), Bacteroides (6.51%), Faecalibacterium (1.01%), Sutterella (3.06%) and Bifidobacterium (1.78%), while, the most abundant genera in the T cohort were Enterococcus (38.83%) and Corynebacterium (11.32%). These genera constitute 0.15% and 2.16% of the total abundance in the M group, respectively.

Our results in detecting higher levels of bacteria belonging to the Bacteroides-Prevotella group in group M are in agreement with some recent studies, where high abundance of these genes is indicated to be associated with colorectal cancer. Species of the genus Prevotella can cause anaerobic infections and inflammation of the colonic mucosa and some species of Bacteroides spp. are opportunistic human pathogens.

According to our results, Enterococci heavily colonize the gut following antibiotic prophylaxis - which effectively depleted the gastrointestinal tract of protective commensals. Thus, due to the possible multiresistance of enterococci as well as Corynebacterium after antibiotic administration and disruption of the colonic flora, their abundance increased significantly. On the other hand, following the analysis performed, we observe that the abundance of Finegoldia, Parabacteroides, Acinetobacter and Streptococcus genera does not exceed the difference of 1.00% between the M and T group, which indicates that these taxons are not influenced by the antibiotics used, enemas or purgatives (polyethylene glycol).As expected, we found that the use of multiple types of antibiotics and in multiple doses disrupts the diversity and abundance of bacteria in the human colon, providing fertile ground for an explosion of multidrug-resistant bacteria, thus prolonging the time to restore normal microflora.

As a result of the research, the following conclusions were drawn:

1. The alpha diversity chao1 index (reflecting the number of taxa per sample) varies less between M samples compared to T samples;
2. The most numerous bacterial genera in the M group are Prevotella spp. and Bacteroides spp;
3. The most abundant bacterial genera in group T are Enterococcus spp. and Corynebacterium spp;
4. The average number of taxons per sample decreases after antibiotic administration;
5. The greatest variation in data between study groups is observed for the genera Enterococcus, Corynebacterium, Prevotella, Bacteroides, Sutterella and Faecalibacterium;
6. The high abundance of enterococci in the T cohort may be due to acquired multiresistance following widespread antibiotic administration in the population.
7. Abundance of Finegoldia, Parabacteroides, Acinetobacter and Streptococcus genera do not exceed 1% difference between cohort M and T, possibly, these taxa are not influenced by antibiotics used, enema or polyethylene glycol.

The data and information from the Regional Institute of Oncology Iasi partners, who use antibiotic prophylaxis with Cefuroxime in a single dose of 1.5 g, do not show a significant change in the composition of the gut microbiome one week after administration of the antibiotic, with only some loss of taxonomic variety.

CONCLUSIONS

From the presented data of our study, we can make the following conclusions:

1. Collaboration, unification and standardization of high performance DNA sequencing methods (V3-V4 region (IRO Iasi) and V2-4-8 (PMSI Institute of Oncology, Chisinau) of the 16S rRNA gene, use of Ion Torrent Next Generation Sequencing (NGS) and use of Ion Reporter Software for processing the data obtained, which aligns fragments to the Curated MicroSEQ(R) 16S Reference Library v2013.1 databases and Curated Greengenes v13.5, it can be said that a joint centre of excellence has been created, capable of transferring experience in molecular research and generating new research approaches to boost economic development and innovation.

2. Inappropriate use of antibiotics in pre-, intra- and postoperative periods (a practice still widely used in some surgical departments of PMSI Institute of Oncology, Chisinau) has a severe negative impact on the gut microbiome, but does not seem to significantly mitigate inflammatory complication rates, morbidity and postoperative mortality.

3. The experience of surgical teams in IRO Iasi, which have been using antibiotic prophylaxis with Cefuroxime, administered preoperatively in a single dose of 1.5 g, for more than 10 years, proves the lack of significant stress on the gut microbiome, but there is also no negative impact on postoperative complications, morbidity and mortality.

NEW PROTOCOL FOR ANTIBIOTIC PROPHYLAXIS IN CANCER SURGERY

Demonstrating efficacy in several clinical trials, cephalosporins have become the most widely used drugs for surgical prophylaxis in general surgery, and Cefuroxime, a second-generation cephalosporin, is effective against both gram-positive and gram-negative bacteria and can be given in combination with other antibiotics if necessary. In addition, it is a safe and affordable drug and is the most stable β-lactam antibiotic used to reduce the risk of post-operative infections, sepsis or abscesses.

On the basis of the conclusions drawn from the COLONSTRESS study, we consider that it is rational and propose for wide use in the daily practice of the PMSI Oncological Institute a **NEW PROTOCOL FOR ANTIBIOTIC PROPHYLAXIS IN SURGICAL ONCOLOGY**

The protocol provides antibiotic prophylaxis for surgery by intravenous administration of Cefuroxime 1.5 g, single dose, at induction of anaesthesia or at least 30 minutes before incision.

If surgery lasts more than 4 hours (double the half-life of the antibiotic), in patients weighing more than 100 kg or with a BMI over 35, in case of other risks of septic-inflammatory complications, it is useful to administrate an additional dose of 750 mg 4 hours after the first administration.

The solution for intravenous administration is prepared by dissolving 1.5 g in a minimum of 15.0 ml and 750 mg in a minimum of 6.0 ml of solution for injection. Short-term intravenous perfusion is also possible, when 1.5 g of Cefuroxime is dissolved in a minimum of 50.0 ml of solution for injection and administered as a slow intravenous infusion.

Cefuroxime is combined without adverse effects with Metronidazole, and can be used in this combination if required, simultaneously preoperatively intravenously, infused at a dose of 7 mg/kg body, also at induction of anaesthesia, in a single dose.

Implementing new antibiotic prophylaxis protocol at the institutional level and strictly monitoring compliance might have some benefits:

- Antibiotic prophylaxis strategy and tactics would be unified through a standardised checklist, which would provide additional security for the surgical intervention;

- It would save important financial resources, as antibiotic costs would be significantly reduced;

- It would have a minimal and probably rapid and easily reversible impact on the gut microbiome, thus improving patients' early postoperative recovery;

- It would significantly reduce antibiotic resistance rates of in-hospital germs and others, with no negative effects on surgical infection rates.