

Bacteriological aspects implicated in abdominal surgical emergencies

A.M. Israil¹, C. Delcaru¹, R.S. Palade², C. Chifiriuc¹, C. Iordache¹, D. Vasile², M. Grigoriu², D. Voiculescu²

¹Cantacuzino Institute, Bucharest

²First Surgical Clinic of the University Hospital of Bucharest

Rezumat

Aspecte bacteriologice în urgențele chirurgicale abdominale

Scopul prezentului studiu a fost acela de a stabili atât etiologia microbiană a urgențelor chirurgicale abdominale cât și relația dintre etiologia bacteriană și factorii de virulență sintetizați de respectivele tulpini izolate.

Materiale și metode: Au fost izolate 110 tulpini bacteriene din 100 de cazuri clinice randomizate operate în cursul anilor 2009-2010 în Clinica I Chirurgică a Spitalului Universitar București. Cazurile clinice (raport sexe: 52 M/48 F, vârste între 22-85 ani) au fost clasificate în trei grupe de risc, în raport cu severitatea lor. Tulpinile au fost caracterizate pe baza aspectelor de cultură, microscopice și biochimice. După identificare, tulpinile au fost investigate din punct de vedere al potențialului de virulență (aderența la suprafețe abiotice și producerea de factori solubili de virulență).

Rezultate: Produsele patologice au fost recoltate din diferite cazuri clinice: peritonită acută difuză, infecții ale căilor biliare, pancreatită acută severă, urmate de procese septice etc. Cele 110 tulpini bacteriene (72 aerobe, 38 anaerobe) au fost izolate doar în 70 din cele 100 de cazuri. Din cele 70 de cazuri, în 45 de cazuri, supuse tratamentului empiric preoperator cu antibiotice cu spectru larg, au fost izolate 74 de tulpini, în timp ce din 25 de cazuri fără tratament au fost izolate 36 de tulpini. Etiologia a fost fie monospecifică, fie multispecifică (asociațiile aerobe-anaerobe fiind prezente în

special la persoanele în vârstă). Din 30 de cazuri la care culturile au fost negative, 16 erau deja supuse tratamentului empiric (parenteral) cu antibiotice în momentul colectării probelor. În general, etiologia aerobă a fost dominată de *Enterobacteriaceae*. Dintre speciile anaerobe, cel mai frecvent au fost izolate genurile *Clostridium*, *Peptococcus* și *Bacteroides*. Este de menționat că în 11 (10%) cazuri de urgențe abdominale chirurgicale s-au izolat tulpini din speciile *Bifidobacterium* și *Veillonella*, ceea ce pledează pentru posibilitatea ca în anumite condiții, bacterii din flora enterală comensală să devină patogene cu înalt grad de virulență și invazivitate. Tulpinile aerobe cât și cele anaerobe au prezentat factori de virulență: mucinaza, esculinaza, lecitinaza, enzime proteolitice, capacitate de aderență (factor slime), ceea ce explică severitatea respectivelor cazuri clinice.

În **concluzie:** etiologia bacteriană a urgențelor chirurgicale abdominale a prezentat un spectru foarte larg, numărul cel mai mare de tulpini fiind de origine endogenă (*Enterobacteriaceae* și anaerobi). Tulpinile izolate au prezentat un număr mare de factori de virulență ceea ce poate explica larga diversitate și severitate a patologiei abdominale clinice. Rezultatele pledează pentru reajustarea periodică a tratamentului preoperator empiric cu antibiotice.

Cuvinte cheie: peritonită difuză acută, pancreatită acută severă, factori de virulență, tratament preoperator empiric cu antibiotice, grupuri de risc

Corresponding author:

Israil Michaela-Anca MD
Cantacuzino Institute
Splaiul Independentei 103 Bucharest
E-mail: ancaisrail@yahoo.com

Abstract

The *purpose* of the present study was to establish the microbial etiology of abdominal surgical emergencies as well as the relationship between the bacterial etiology and the virulence factors produced by the respective isolated strains.

110 bacterial strains were isolated from 100 randomized clinical cases, operated during 2009-2010 in the First Surgical Clinic of the University Hospital of Bucharest. The clinical cases (sex ratio 52 M/48F aged between 22-85 years old) were classified into three risk groups, as related to their severity. The isolated strains were characterized by cultural, microscopic and biochemical methods. After identification, the bacterial strains were investigated for their virulence potential (adherence to abiotic surface and production of soluble virulence factors).

Results: The specimens were collected from different clinical pathologies: diffuse acute peritonitis, biliary duct infections, severe acute pancreatitis followed by septic processes etc. The 110 bacterial (72 aerobic and 38 anaerobic) strains were isolated only in 70 out of 100 cases. Out of these 70 cases, in 45 already submitted to pre-operative empiric broad spectrum antibiotic therapy, there were isolated 74 strains, whereas in 25 cases without any treatment, there were isolated 36 strains. The etiology was either mono-specific or multi-specific (aerobic-anaerobic associations, especially in old persons). Out of the 30 negative culture cases, 16 were already submitted to pre-operative parenteral empiric antibiotic therapy at the moment of specimen collection. The aerobic etiology was dominated by *Enterobacteriaceae*. The most frequent anaerobic species belonged to *Clostridium*, *Peptococcus* and *Bacteroides* genera. It is to be mentioned that the isolation of *Bifidobacterium* and *Veillonella* spp. in 11 (10%) severe cases of the studied abdominal surgical emergencies is pleading for the fact that in certain conditions, bacteria belonging usually to commensal gut flora can turn to pathogenic becoming responsive for life-threatening cases. All aerobic and anaerobic strains exhibited some of the following virulence factors: mucinase, esculinase, pore-forming toxins (lecithinase), proteolytic enzymes, adherence ability (slime factor). The presence of these virulence factors (VF) could explain the severity of the clinical aspects.

Conclusions: The bacterial etiology of the abdominal surgical emergencies exhibited a very large spectrum, the highest number of strains being of endogenous origin (*Enterobacteriaceae* and anaerobic strains). It was demonstrated that the isolated strains produced (cell associated and soluble) VF proving in this way their role as important virulence sources in the hospital environment and explaining the large diversity and severity of the clinical abdominal pathology. The results of the present study are also pleading for periodical readjustments of the pre-operative empiric antibiotic therapy.

Key words: diffuse acute peritonitis, severe acute pancreatitis, virulence factors, pre-operative empiric antibiotic therapy, risk groups

Introduction

The surgical abdominal pathology is constituted of very

different clinical forms, such as diffuse acute peritonitis, severe acute pancreatitis, subphrenic and Douglas abscesses, intestinal occlusions, digestive hemorrhage, traumatism with penetrated wounds in the parietal peritoneum, traumatism of the abdominal wall (1) spleen, liver, biliary ducts, pancreas, bowel, mesenter, colon, genito-urinary tract, traumatism complicated with important secondary components etc. (2-6).

Diffuse acute peritonitis with local (abdominal) and general characteristic signs is developing like an inflammatory process as consequence of a septic process at the level of peritoneal cavity.

The primary peritonitis developing in children as well as in adults is at least for the beginning mono-microbial infection and can be due to the inoculation of the peritoneal membrane by haemathogenic way with bacteria coming from a septic extra-abdominal source of infection such as: genital infection (in females), propagation of umbilical infections in newborns, bacterial translocation in patients with ascites, nephritic syndrome or systemic lupus erythematosus. Usually, the frequency of single agent infections is decreasing when submitted to antibiotic therapy.

The secondary acute peritonitis is developing as a complication of abdominal pathological processes in case of massive and continuous peritoneal contamination, by bacterial propagation from an acute septic process, located in the abdominal cavity or by perforation (penetrating abdominal wounds, iatrogenic visceral perforation as consequence of surgical investigations such as endoscopy, puncture etc, or post-operative abdominal complications (7), perforation of digestive segments as consequence of different pathological processes, discharge of a septic collection such as peritoneal abscesses into the abdominal cavity); in these cases, the microbial contamination is multi-microbial, being constituted by aerobic (*E. coli*, *Proteus* sp.) strains (8,9,10).

80% of the acute secondary peritonitis cases are developing as a result of the perforation of cavity organs or of inflammatory processes at the level of intra-peritoneal viscera, whereas 20% are developing as post-operative complications.

In the general context of the abdominal surgical emergencies, the acute diffuse peritonitis (ADP) and especially the severe acute pancreatitis (SAP) accompanied by pancreatic necrosis are frequently recorded, both being characterized by high bacterial aggression (30-40%).

The perforated gangrenous acute cholecystitis exhibits a high severity as being associated with bacterial multiple resistance as well as with the particular high virulence of the opportunistic infectious agents predominantly implicated in the etiology of these cases.

Concerning the acute pancreatitis it is recorded an incidence of 30-50 cases/ 100.000 persons/year, including all clinical forms, 80 % being characterized by a limited benign evolution, whereas 15-20 % exhibit severe aspects evolving to necrotizing-hemorrhagic forms with 30-40% mortality.

With special reference to the patients with severe forms of acute pancreatitis there are necessary long hospitalization periods (weeks or even months) in the intensive care units, taking into account the fact that the pancreatic necrosis

inducing in the first step multiorgan failure, and in the second step, sepsis and consequently high rate fatalities.

In the last years, the permanent increasing number of SAP cases has urged, in concordance with the international recommendations, the setting up of special new hospital departments called "pancreatic units" (11).

The antibiotic resistance aspects occurred in bacterial strains isolated from intra-abdominal infections signify at present a major problem of therapy in the general context of the surgical pathology (12,13) with serious implications at global level as being responsible for many treatment failures.

Material and Methods

The purpose of the present study was to establish the microbial etiology of 100 clinical cases of abdominal surgical emergencies as well as the relationship between the bacterial etiology and the virulence factors produced by the respective isolated strains.

Bacterial strains

The study was performed on 110 bacterial strains isolated from 100 randomized clinical cases of abdominal surgical emergencies, operated in the First Surgical Clinic of the University Hospital of Bucharest.

The *Staphylococcus aureus* ATCC 25923 reference strain was used for CAMP test.

Methods

The specimens were collected intra-operatory by aspiration using a syringe with needle from intra-abdominal cavity, peritoneal space, ascites liquid, gallbladder liquid, biliary ducts, duodenal liquid, cecal appendix, Douglas space, liver, pancreas and pancreatic necrotic tissue, hepatic and subphrenic abscesses. The aspirates were injected into four different rubber stopped tubes containing different kinds of transport/preservation media (Cary-Blair, nutrient broth, sodium thioglycolate, Schaedler medium). The specimens were injected through the rubber stopper after all air having been expelled from the syringe and needle. The aspirate was also expelled for each specimen on 2 blood agar plates and 2 smears were also prepared for bacterioscopy. All specimens were carried to the laboratory within the first 24 hours and held at room temperature during their processing.

Each specimen was accompanied by a clinical file containing the following data: patient name, surname, age, sex, diagnostic, pre-operative empiric antibiotic therapy, immune status, immunosuppressive factors (radio-, chemotherapy etc), type of samples (pus, bile etc), lesion topographic location (super-mesocolic space: liver biliary ducts etc, infra-mesocolic: bowel, cecal appendix etc, retroperitoneal space: annexes etc), preservation media, smear preparations, identification data of the medical staff implicated in the operatory process and specimens collection, day /month of specimens collection.

These data allowed us to classify the studied cases as

related to their severity level (14) in 3 risk groups as follows:

- first risk group: young persons, without previous contact hospital environment, without treatment, without co-morbidities, with community – acquired infections.
- second risk group: patients with previous contact with the sanitary system, recent antibiotic therapy, aged persons (>65 years) and/or with co-morbidities, with microbial infections produced by bacterial strains with high probability of exhibiting antibiotic resistance, even to more than 3 classes, despite their community origin.
- third risk group: persons with previous long hospitalization, invasive procedures associated with acute severe immunodeficiency syndrome in neoplasm cases, chronic renal failure, diabetes saccharatum, nosocomial infections with hospital multidrug resistant strains.

As soon as arrived to the laboratory, the specimens were inoculated on primary plates (freshly prepared at most 7 days before being used) with appropriate aerobic and respectively anaerobic culture media (for aerobic: 5% sheep blood agar, Mac Conkey agar and for anaerobic: Brucella blood agar with hemin 5 µg/ml and vitamin K (1µg/mL), selective media with bile esculin agar) (15,16). After inoculation, the plates were incubated in aerobic/respectively anaerobic conditions for 48 hours at 37°C. For anaerobic bacteria, the plates were placed in anaerobic bags with reduction substances (pyrogallol) and kept in exicator (after anaerobic conditions having been achieved by burning inside a candle until it extinguished by itself, removing the oxygen) at 35-37°C for 48 hrs until 7 days. Plates were daily examined beginning since 48 hrs of incubation. All colony morphological types from the nonselective aerobic and respectively anaerobic blood agar were subcultured separately for obtaining pure cultures and incubated in available conditions 48-72 hrs. The isolated colonies were used for macroscopic examination of colony morphology, size, pigmentation, hemolysis and thereafter Gram-stained smears were prepared in order to establish the morphological type and Gram staining affinity.

The primary plates were reincubated along with purity plates for an additional 48-72 hrs and examined again for slowly growing strains.

After isolation, the colonies grown in aerobic conditions were submitted to biochemical identification using conventional tests (phenylalanine, urease, Simmons citrate, indole production, lysine decarboxylase, ornithin decarboxylase, arginin dihydrolase) (17), multitest media: TSI, MIU (11) and API microtests (API 20E, API 20NE), whereas the anaerobic strains were inoculated on API 20A microtests. The reading and interpretation of results were done after 48 hours incubation at 37°C.

In cases where by the first inoculation of the specimens from the preservation media to the primary plating, it was not possible to isolate any bacteria, a new inoculation from the preservation media into a regenerated thioglycolate broth (in the purpose to enrich the possible small number of anaerobes) was tried and in case of bacterial growth after 24-72 hrs incubation at 35-37°C, the culture was plated on aer-

obic blood agar and respectively on anaerobic Brucella blood agar with hemin 5 µg/ml and vitamin K 1 µg/ml for colony isolation and biochemical identification.

Thereafter, the strains were investigated for their virulence potential (18). In this purpose, the strains were cultured on specific media containing: 5% sheep blood agar (for alpha, beta haemolysins, and CAMP-like factor tests), 1% esculin-iron salts (esculin hydrolysis test), 15% casein agar (caseinase test), 3% gelatin agar (gelatinase test) 10% starch agar (amylase test) 1% porcine gastric mucine in brain heart agar with 2% NaCl (mucinase test), DNA- agar (DNase test), 1% Tween agar (lipase test), 2.5% yolk agar (lecithinase test), Wagatsuma's agar

(Kanagawa hemolysin production) following protocols previously described (19,20). Adherence ability on abiotic surfaces was evidenced by slime test. The tests were performed either in aerobic or anaerobic conditions, depending on the oxygen requirements of the identified bacterial strain. In case of anaerobic strains, the culture media were supplemented with hemin 5 µg/ml and vitamin K 1 µg/ml.

Results

The samples were collected from 100 patients, sex ratio 52 M/48F aged between 22-85 years old (11 patients between 22 and 35, 44 between 35-64, and 45 > 65 years) with the following diagnostics: diffuse acute peritonitis (33 cases), biliary ducts infections (11 cases), acute cholecystopancreatitis (5 cases), septic collections subsequent to acute severe pancreatitis (16 cases), infected digestive neoplasma (12 cases) and different clinical hypostasis with peculiar localizations (postappendectomy stercoralis fistula, hepatic, pelvic, right iliac fossa and right infra hepatic abscesses, umbilical hernia with intestinal ansa necrosis, strangulated eventration with intestinal ansa necrosis) (7 cases).

Out of the 100 studied cases, only in 70 cases were isolated 110 strains (72 aerobic and 38 anaerobic). Among these 70 positive cases in 45 cases submitted to pre-operative empiric antibiotic therapy there were isolated 74 strains, whereas in 25 cases without any treatment, there were isolated 36 strains.

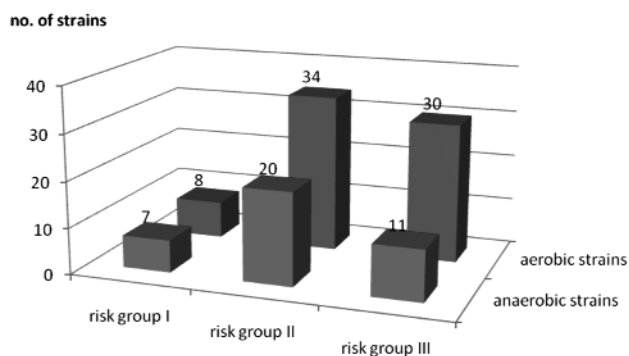


Figure 1. Distribution of aerobic and anaerobic strains in different risk groups

Out of the 30 negative (without any bacterial isolation) cases, 16 were submitted to pre-operative empiric therapy at the moment of specimen collection whereas 14 were not.

When classified in concordance with the clinical risk levels in the first risk group, were included 21 cases (15 strains), in the second 44 cases (54 strains) and in the third 35 cases (41 strains).

Concerning the distribution of aerobic/anaerobic strains into the three risk groups: in the first risk group there were 8 aerobic/7 anaerobic, in the second: 34 aerobic/20 anaerobic and in the third: 30 were aerobic/11 anaerobic (Fig. 1).

As it is evidenced in the first risk group, the number of the isolated strains was low (15 strains/ 21 cases) whereas in the second and third risk groups, the number of the isolated strains was much higher (i.d. 54 strains/44 cases and respectively 41 strains/35 cases).

In the above mentioned 70 positive cases, the infections were generated: in 43 cases by 60 aerobic bacterial strains, in 16 cases by 27 anaerobic bacteria and in 11 cases by 23 strains in aerobic-anaerobic associations (mixed infections) (Fig. 2).

It is to be pointed out that the high number of 31 anaerobic bacteria was isolated in the second and third risk group. If it is taken into account the aspect that the patients included in these two risk groups are old persons with a corresponding immuno-depressed status, some of them having been submitted to repeated antibiotic therapies, the respective conditions promoting the selection of certain high pathogenic strains, these aspects can explain the isolation of anaerobic bacteria responsive for the high bacterial aggression. In septic abdominal processes, the location of the infection source in the distal part of the digestive tract explains the presence of mixed association of pathogenic aerobic and anaerobic bacteria.

The frequent association of many pathogenic strains isolated in abdominal infectious processes is signifying a factor of high severity for the evolution and prognostic of the respective clinical cases. The associations of 1-3 aerobic and 1 aerobic/1-2 anaerobic strains were present with the highest frequency in the third risk group.

Concerning the distribution of the 30 negative cases

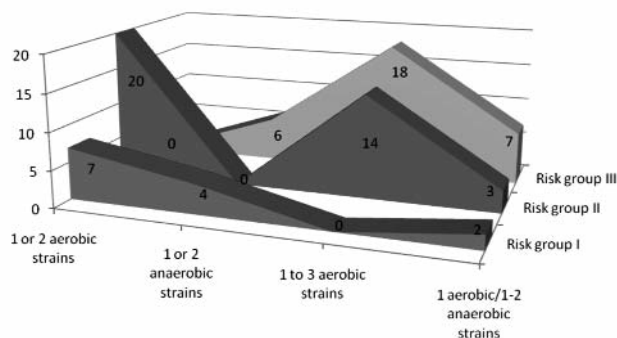


Figure 2. Distribution of aerobic, anaerobic and mixed infections in the three risk group level

into the 3 risk groups, in the first group were included 11 cases (6 with pre-operative empiric antibiotic therapy), in the second 10 cases (5 with pre-operative therapy) and in third 9 cases (5 with pre-operative therapy) (Fig. 3).

It is interesting to mention that in this first risk group among the 6 cases with absence of microbial growth and without pre-operative treatment, it was included one case of a perforated duodenal ulcer early operated in the first 6-8 hrs from the onset of clinical symptoms determined by an acute additional peritoneal process that was proved not to be bacterial, but of chemical (HCL, bile, blood) irritation origin.

For the 16 negative cases with pre-operative empiric antibiotic therapy, the explanation can be given by the efficiency of antibiotic therapy but at the same time by more other aspects related to the pathological substrate of the morbid process as failure of specimen processing with special reference to anaerobic strains.

The high number of the isolated pathogenic strains included in the second and the third risk group is pleading for the fact that the bacterial aggression is directly influenced by the patient age, status of immunodeficiency, precarious reactivity, co-morbidities, contact with the hospital environment and recent antibiotic therapy with role in selection of resistant strains, all these factors generating favorable conditions for bacterial aggression and development of a complex severe infectious process.

Among the aerobic strains there were found 17 bacterial species and one fungal strain: *E. coli* (45.5%), *Klebsiella* (*pneumoniae*, *oxytoca*, *ornyolitica*, *planticola*, *ozenae*) (15.6%), *Morganella sp.* (2.6%), *Enterobacter sp.* (3.9%), *Proteus sp.* (6.5%), *Citrobacter sp.* (2.6%), *Salmonella arizonae* (1.3%), *Pseudomonas aeruginosa* (3.9%), *Acinetobacter baumannii* (1.3%), *S. aureus* (5.2%), *S. epidermidis* (1.3%), *Enterococcus faecium/faecalis* (2.6%), *Streptococcus sp.* (3.9%), *Candida albicans* (3.9%) (Fig. 4).

As it can be seen, *E. coli* was the most frequent aerobic bacteria isolated in the abdominal infection but the much boarder spectrum of the isolated strains is pleading for a compulsory bacteriological examination of the intra-operative collected specimens.

Certain bacteria with high invasiveness ability (i.d. *Klebsiella sp.*, *Proteus sp.*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*) were frequently isolated in cases belonging to the third risk group playing an important role in the prognostic of the respective cases.

Among the anaerobic bacteria, there were identified only 12 species such as follows: *Clostridium sp.* (18.7%), *Peptococcus sp.* (14.5%), *Propionibacterium sp.* (10.8%), *Bifidobacterium sp.* (10.8%), *Bacteroides fragilis* (8.1%), *Bacteroides ovatus* (8.1%), *Veillonella sp.* (8.1%), *Fusobacterium sp.* (8.1%), *Actinomyces sp.* (5.4%), *Gemella sp.* (2.7%), *Peptostreptococcus sp.* (2.7%), *Campylobacter sp.* (2.7%) (Fig. 5).

The isolation of anaerobic bacteria in the first risk group was only sporadic whereas in the cases belonging to the second and third risk groups was much higher. This aspect is pleading for the importance of the status of the host organism during the evolution of the infectious process. The precarious reactivity is favouring the development of the

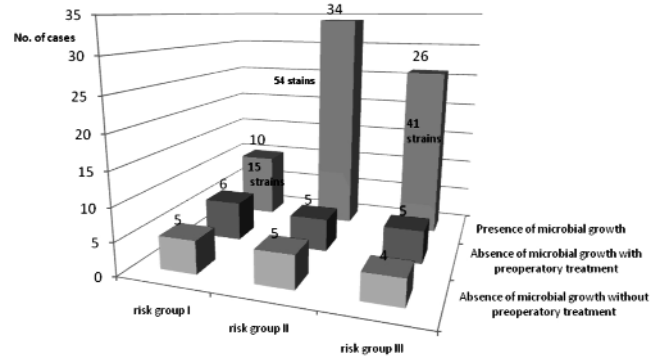


Figure 3. The distribution of clinical cases with and without microbial growth into 3 risk groups

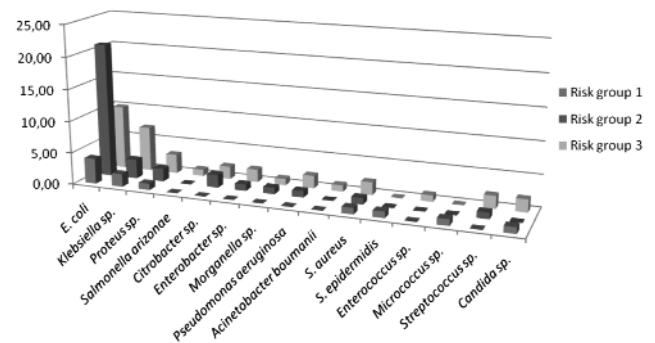


Figure 4. Distribution of different aerobic microbial strains in the 3 risk groups

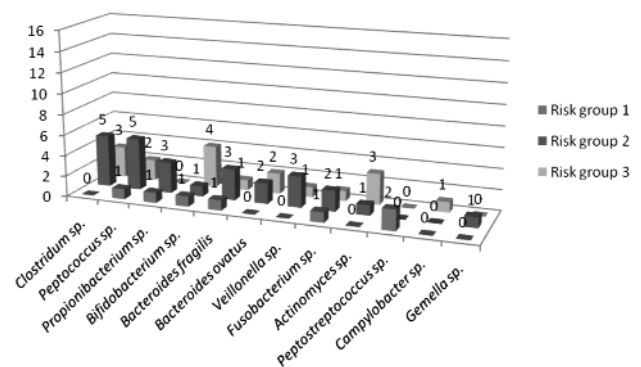


Figure 5. Distribution of different anaerobic microbial strains in the 3 risk groups

septic process by selection of bacterial strains with peculiar aggressivity. In these two special cases, the spectrum of the pathogenic strains was very large. A high incidence of certain anaerobic strains like: *Clostridium sp.* (8), *Peptococcus* (7), *Bifidobacterium sp.* (6), *Bacteroides fragilis* (5) was found out.

It is to be mentioned that in 11 severe cases of surgical abdominal emergencies, there were isolated *Bifidobacterium* (in 8 cases) and *Veillonella* (in 4 cases) respectively, out of which

favoring factors for surgical pathology.

The aerobic bacterial etiology was dominated by Gram-negative bacteria (35 strains), the enterobacterial strains being isolated from all risk groups whereas *Pseudomonas* and *Acinetobacter* only from the second and the third groups. A large diversity of *Enterobacteriaceae* species was isolated but the predominant in all three risk groups was *E. coli* sp. (45,%) followed by different *Klebsiella* species (15,6%).

A large range of anaerobic species was also isolated from the second and third risk groups, the most frequent being *Clostridium*, *Peptococcus*, *Bifidobacterium* and *Bacteroides* spp.

The isolation of *Bifidobacterium* and *Veillonella* strains (belonging usually to the commensal gut flora) in 11 severe cases of necrotizing, necrotizing hemorrhagic pancreatitis, acute diffuse purulent peritonitis, neoplasm and acute lithiasic cholecystitis demonstrated that these bacteria in certain conditions (old age, immunodeficiency status, neoplasm, ethilism, metabolic disorders as dislipidemy, diabetes saccharatum) can turn into pathogenic and lead to life-threatening cases. Recent data of the literature are recording similar aspects in a case report of induced necrotizing pancreatitis (21).

The multi-microbial etiology (i.e. aerobic or a mixed aerobic-anaerobic associations) was frequently present in the second and third risk level groups explaining the severity of cases, the difficulties encountered during the eradication of these infections, pleading at the same time, for the necessity of periodical control and readjustment of the pre-operative empiric antibiotic therapy in concordance with the patient risk level and taking into account the fact that these infections are determined frequently by persistent nosocomial agents.

Out of 100 clinical cases, 61 were submitted and 39 not submitted to pre-operative empiric antibiotic therapy. When taking into account that out of 61 cases (with positive bacterioscopy) submitted to pre-operative therapy, only in 16 cases (26,3%) the isolation of bacterial strains became negative, whereas in 45 cases (73,7%) there were isolated 74 bacterial strains, this last aspect could have different explanations such as: the period of time between the moment of implementing the antibiotic therapy and that of the specimens collection (during the intra-operative process) was too short for the antibiotics to exert their therapeutic effects; another explanation could be the non-corresponding pre-operative empiric antibiotic therapy at least in some of these cases.

The pre-operative antibiotic therapy is empiric (of first-intention) but it is necessary to be adapted to the microbial spectrum and to the clinical conditions of the patients, concerning the development of the morbid processes. These aspects are in close relation with the risk level groups and the correct evaluation of the clinical hypostasis. When the therapy is limited by the financial restrictions, its efficiency is seriously affected. However, the bacteriological examination remains the unique and the most appropriate possibility to offer concret data concerning the infection etiology and therapeutic efficiency of certain antimicrobials of large spectrum. In this context, the direct bacterioscopy is playing an important role in orienting the diagnostic and therapy

(gram \pm bacteria/molds, leucocyte afflux, phagocytosed bacteria, tissue necrosis, pleomorphic bacterial aspects, diverse morphology etc).

Out of 39 cases without any pre-operative empiric treatment, in 25 were isolated 36 strains whereas in 14 the cultures remained negative.

In the respective 14 cases (all with positive gram stain bacterioscopy of the corresponding specimens) non-submitted to any pre-operative empiric therapy, the absence of strains isolation (negative culture) could have different explanations and namely on one side: poor transport, excessive exposure to air during processing, failure of the anaerobic incubation system to achieve anaerobic atmosphere and on the other side: specimens minor contamination, strong immunitary status of young persons, specimens collection just in the first hours of the onset of the pathological process, the time being too short to be achieved a massive bacterial contamination (or corresponding multiplication of the infectious agents), acute pathological processes (ischemia, perforation with peritoneal chemical irritating process, unpropitious biliary medium for multiplication of all bacterial species and requiring a certain period of time for selection of the aggressive bacteria strains), absence of optimal conditions for the infectious agent since the onset of the infectious process until the moment of specimens intra-operative collection, to acquire the ability of exerting repressive action upon the defensive armamentarium of the host organism.

The aerobic etiology was dominated by *Enterobacteriaceae*, (78%), the most frequent isolated being *E. coli* strains followed by nosocomial (i.e. *K. pneumonia*, *Ps. aeruginosa*, *S. aureus*, *E. faecalis*) agents. It is to be pointed out the high incidence (41%) of nosocomial isolates in patients of the third risk group.

All microbial agents, aerobic and anaerobic, irrespective from the risk group of cases to which they were belonging, exhibited in their virulence equipment a lot of virulence factors that could explain the severity of the clinical aspects involving tissue damage up to necrosis and severe inflammatory syndrome.

With reference to the VF, the mucinase determines the degradation of the mucus layer lining the intestinal lumen and gives bacteria the access to the receptors of the enterocytes surface, the slime (exopolysaccharide) is necessary to protect cells against bactericidal factors (complement, phagocytosis, antibiotics), whereas esculin hydrolysis implicated in siderophores production, provides bacteria living in free iron limiting conditions.

All strains, especially those isolated from the second and third risk groups enhanced proteolytic enzymes implicated in destruction of the tissue integrity, microbial invasion and pore-forming toxins (i.e. lecithinase) responsible for invasiveness, this last feature being present with high incidence especially in bacterial strains belonging to the third risk group.

Although the anaerobic bacterial strains exhibited a lower number of VF than the aerobic, however esculin hydrolysis, pore-forming toxins, proteases, mucinase and amylase were present in their enzymatic equipment; the anaerobic microbiota demonstrated as being involved in polysaccharide

degradation, this last metabolic ability conferring them a competitive nutritive advantage.

Conclusions

1. The highest number of surgical abdominal emergencies hospitalized during 2009-2010 in First Surgical Clinic of the University Hospital of Bucharest was included in the second (44%) and the third (35%) risk groups of the clinical cases.
2. The highest number of the pathogenic (aerobic as well as anaerobic) strains was isolated in patients belonging to the second and third risk groups.
3. The associations of many pathogenic (aerobic, anaerobic, aerobic/anaerobic) strains were present with the highest frequency in the third risk group signifying a gravity factor for the evolution and prognostic of the respective infectious processes.
4. The bacterial etiology exhibited a very large spectrum, 85% being of endogenous origin (the Enterobacteriaceae and Gram-positive anaerobic strains) demonstrating the role of endogenous microbiota as a source of abdominal infectious processes.
5. In certain conditions, *Bifidobacterium* and *Veillonella* species (usually belonging to commensal gut flora) proved be able to turn into pathogenic as are pleading the aspects of the 11 (10 %) severe cases of the studied abdominal surgical emergencies.
6. The highest level of virulence was pointed out in the bacterial strains isolated from patients belonging to the second and third risk groups of clinical cases.
7. Concerning the equipment of bacterial virulence factors, mucinase determines degradation of the protective mucus layer of the intestinal lumen favoring the close adherence of bacteria to the enterocyte receptors, bacterial exopolysaccharides (slime factor) is playing the role of bacterial cells protectors against the bactericidal factors of the host (complement, phagocytosis) and antimicrobials; lecithinase is implicated in microbial invasiveness whereas the bacterial enzymes responsive for aesculin hydrolysis provide the siderophore production even in free iron limited conditions conferring the invasiveness/virulence ability to the respective bacteria. With special reference to anaerobic strains amylase is responsive for polysaccharides degradation, an important ability of these bacteria conferring them a competitive nutritive advantage.
8. The prevalence of bacterial strains as well as their virulence factors in cases belonging to the second and third risk groups demonstrated that the bacterial aggression is evidently influenced by patient age, immunodeficiency status, precarious reactivity, co-morbidities, contact with the hospital medium, recent antibiotic therapy favoring the selection of resistant strains, all these aspects leading to a severe complex infectious process.
9. When taking into account that at least in a lot of cases even submitted to pre-operative empiric antibiotic treat-

ment, the bacteria isolation was still possible, this aspect put the question of the availability of the present empiric therapeutic choices in certain emergency abdominal cases, making necessary for the future, the susceptibility testings of these kinds of enteral endogenous bacteria in the purpose periodical readjustment of the pre-operative empiric antibiotic associations be effected.

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